Abstract #1205 Chart

	Toxicity Profile RAPA-TAC (Group A) (n=41)	RAPA-MMF (Group B) (n=27)
Patients with any infectious	41%	44%
PTDM	28%	13%
Wound complications	15%	4%
Lymphocele	7%	7%
% increase in cholesterol from baseline	25%	30%
% increase in triglycerides from baseline	100%	97%

p=ns for all variables

Conclusions: The main differences in efficacy parameters between these 2 regimens were an increased occurrence of subclinical rejection in RAPA-MMF patients and better creatinine clearance in RAPA-MMF patients. Although not statistical significant, the incidences of PTDM and wound complications were higher in RAPA-TAC patients. Long-term results from this study will be useful in determining the overall benefit-risk ratios from these 2 regimens.

Abstract# 1206

12 MONTH RESULTS OF A PHASE III PROSPECTIVE, RANDOMISED STUDY TO EVALUATE CONCENTRATION CONTROLLED RAPAMUNE WITH CYCLOSPORIN DOSE MINIMIZATION OR ELIMINATION IN DE NOVO RENAL ALLOGRAFT RECIPIENTS. Alan G. Jardine. *[1]Department of Medicine, Western Infirmary, Glasgow, United Kingdom.*

Objective: To assess the safety and efficacy of sirolimus (SRL) plus low dose steroids as maintenance regimen with or without low dose cyclosporin (CsA) in kidney transplant recipients. **Methods:** We present a twelve-month interim analysis of data for this international study . 280 recipients of kidney allografts from cadaveric or living donors have been enrolled; 172 patients are presented in this analysis. All patients received CsA (125-250 ng/mL) + SRL (4-12 ng/mL) + low dose steroids daily after transplantation. At three months, eligible patients were randomized 1:1 to CsA elimination (eCsA) or minimization (mCsA). In the mCsA group, drug levels were maintained between 50-100 ng/mL. In both arms, SRL trough levels were increased to achieve maintenance levels of 8-16 ng/mL. Antibody induction was prohibited while steroids were generally tapered to reach 5 mg at six months. **Results:** Patient and graft survival at 12 months were 98.3% and 95.9% respectively (n = 172). The overall first biopsy proven acute rejection rate at 12 months was 25.0% (43 episodes in 172 patients). This comprised 34 episodes (19.8%) during the first 3 months of the study and 9 episodes (5.2%) following randomization. 117 patients were randomized to receive eCsA (n=59) and mCsA (n= 58) of which 102 completed 12 months study participation. Pretransplant demographic and donor variables were similar between groups. Following randomization, 4 patients experienced acute rejection in the mCsA group and 8 in the eCsA group. 3 of the 8 eCsA patients had already experienced acute rejection whilst on combination treatment. All acute rejections in the post-randomisation period were mild or moderate. At twelve months, creatinine clearance was significantly higher in the eCsA group vs mCsA group; 70.9 mL/min vs 54.6 mL/min (p= 0.0001). Mean serum creatinine at 12 months was significantly lower in the eCsA group vs mCsA group 124.6 v 153.4 umol/l (p= 0.0032). There was no significant difference in serum cholesterol, triglycerides, LDL, or HDL between the groups. No malignancies have been reported to date. **Conclusions:** Sirolimus permits the elimination of cyclosporin from maintenance immunotherapy and this is associated with improved renal function.

Abstract# 1207

PROTECTIVE EFFECTS OF SIROLIMUS ON HUMAN MESANGIAL CELL (HMC) CHOLESTEROL HOMEOSTASIS. Zac Varghese,[1] Ray Fernando,[1] John F. Moorhead,[1] David C. Wheeler,[1] Stephen H. Powis,[1] Xiong Z. Ruan.[1] *[1]Renal Research Unit, Royal Free Hospital, London, United Kingdom.*

Ross established the concept that atherosclerosis is an inflammatory disease (1). We have previously reported that inflammatory cytokines TNFα and IL-1β increase lipid accumulation in HMCs by increasing lipid uptake through LDL receptors (2), scavenger receptors (3), and by reducing cholesterol efflux pathways via ATP binding cassette transporter A1(ABCA1). The antiproliferative properties of sirolimus have recently found clinical application in the sirolimus-eluting coronary stent (4) and a more specific antiatherosclerotic effect of sirolimus is suggested by results from the ApoE-knockout mouse model of hyperlipidaemia (5). We investigated the effect of sirolimus on cholesterol homeostasis in human mesangial cells in culture to determine possible mechanisms for an antiatherosclerotic effect. Our study demonstrated that sirolimus reduced lipid accumulation in HMCs in culture in the presence of inflammatory cytokine IL-1β; shown as a reduction in Oil Red O lipid droplet staining. Since intracellular lipid content is governed by influx and efflux mechanisms, the balance between lipid uptake through lipoprotein receptors and cholesterol efflux mechanisms is important. Using real-time PCR, we screened the mRNA expression of lipoprotein receptors, which mediate lipid uptake. Sirolimus significantly suppressed LDL receptor, VLDL receptor, and CD36 gene expression. Furthermore, sirolimus also increased cholesterol efflux from HMCs by increasing PPARs, LXRα and ABCA1 gene expression. More interestingly, sirolimus overrode the suppression of cholesterol efflux and ABCA1 gene expression induced by inflammatory cytokine IL-1β. In a separate study, we demonstrated that sirolimus significantly inhibited pro-inflammatory cytokine IL-6 production in human THP-1 cell lines. These results provide a possible basis for the quantitative reduction in atherosclerotic plaque formation that has been observed in sirolimus fed ApoE knock out mice. Sirolimus may have a significant effect in preventing cholesterol accumulation, even in the presence of hyperlipidaemia and inflammation, by regulating both cholesterol influx and efflux pathways. References 1. Ross. N.Engl.J.Med 340:115-126,1999 2. Ruan XZ, Varghese Z, Powis SH, Moorhead JF. Kidney Int. 60:1716-25. 2001 3. Ruan XZ, Varghese Z, Powis SH, Moorhead JF. Kidney Int 56:440-451, 1999 4.Morice MC, Serruys PW, Sousa JE, et al. N.Engl.J.Med. 346: 1773-1780, 2002 5. Adleman SJ, Sehgal SN, Hsu PL, et al. Transplant 2001(abstract), Chicago May,2001

Abstract# 1208

RAPAMYCINE BUT NOT CYCLOSPORIN FACILITATES THE EXPRESSION OF THE DEATH RECEPTOR FAS. Iza van Riemsdijk,[1] Carla Baan,[1] Franciska Hoekstra,[1] Sandra van den Engel,[1] Saskia Postma,[1] Jan IJzermans,[2] Willem Weimar.[1] *[1]Internal Medicine; [2]Surgery, Erasmus Medical Center, Rotterdam, Netherlands.*

Due to its unique mechanisms of action, rapamycin may find its own specific place in our immunosuppressive arsenal. Animal studies showed that allograft tolerance may be facilitated by rapamycin and not by calcineurin inhibitors, while both calcineurin inhibitors and rapamycin block the proliferation of activated T cells in vitro, they may have differential effect on T-cell apoptosis. Cyclosporin and tacrolimus but not rapamycin inhibit IL-2 transcription. In immune responses IL-2 serves a dual role: it is not only required for T-cell expansion but also sensitizes activated T-cells for apoptosis by upregulating the death receptor Fas. To determine whether cyclosporin and rapamycin differ in their effect on T-cell apoptosis and T-cell proliferation, we measured gene transcription levels of Fas, FasL, FLIP, Bcl-2, Bax, survivin, and the mitogen and IL-2 induced proliferation of peripheral blood cells of 8 de novo renal allograft recipients (6 men, 2 women; mean age: 50±13 years; 6 LURD, 2 LRD transplantations) on combination therapy with cyclosporin/rapamycin/corticosteroids (3 months after transplantation) and, after discontinuation of cyclosporin, on rapamycin/corticosteroids (6 mo after transplantation). In all patients the transplant function was good. No rejection periods or graft losses were seen during triple therapy or after discontinuation of cyclosporin. Target trough levels of rapamycin were 10 ng/ml and of cyclosporin 150 ng/ml. After discontinuation of cyclosporin, in rapamycin treated patients, the mRNA expression level of Fas significantly increased in 7/8 patients compared to the level during the combination therapy with cyclosporin and rapamycin (p=0.03). Stopping cyclosporin treatment did not significantly affect the mRNA expression levels of FasL, FLIP, Bcl-2, Bax and survivin. Also, the ratio Bcl-2/Bax, the so-called "death-switch" did not change after discontinuation of cyclosporin. But, using whole blood proliferation assays, discontinuation of cyclosporin treatment positively influenced the mitogen (PHA, 1 mg/ml), but not the IL-2 (10 ng/ml) derived T-cell proliferation (p=0.009). Conclusion: Cyclosporin impairs the expression of signals needed for the apoptotic death of alloreactive T-cells, while rapamycin may facilitate the mechanisms required for the induction of allograft tolerance.

KIDNEY: COMPARATIVE TRIALS

Abstract# 1209

THE EFFECT OF MAINTENANCE IMMUNOSUPPRESSION ON ALLOGRAFT FUNCTION IN LONG-TERM KIDNEY TRANSPLANT RECIPEITNS. John S. Gill,[1,3] Marcello Tonelli,[2] Chris Mix,[3] Brian J. G. Pereira.[3] *[1]Nephrology, St.Paul's Hospital, Vancouver, BC, Canada; [2]Nephrology, University of Alberta, Edmonton, AB, Canada; [3]Nephrology, New England Medical Center, Boston, MA.*

The effect of maintenance immunosuppressive therapy on long-term allograft function is uncertain. The purpose of this study was to determine the effect of baseline maintenance immunosuppressive treatment on the annualized change in GFR among long-term kidney transplant recipients. We studied 40,963 transplant recipients between 1987 and 1996 with graft survival of at least two years in the United States Renal Data System. To determine the annualized change in GFR, linear regression methods were applied to serial GFR estimates (using the MDRD equation) performed at 6 months and then yearly after the time of transplantation until death, graft loss or end of follow up (September, 1998). A multivariate regression analysis that included recipient age, gender, race and cause of ESRD, donor age and type, HLA match, PRA, delayed graft function, acute rejection, duration of transplantation and baseline GFR at one year after transplantation was used to determine the effect of maintenance immunosuppression on the annualized change in GFR. During a median follow-up of 5.3 years, the mean ± SD of the annualized change in GFR was –1.7 ± 6.5 ml/min/1.73m²/year. The table shows that patients who received tacrolimus compared to cyclosporine, and mycophenolate mofetil (MMF) compared to azathioprine had slower rates of renal loss. We conclude that baseline maintenance immunosuppression appears to have a clinically significant effect on allograft function in long-term kidney transplant recipients.

Maintenance Immunosuppressant	Unadjusted Annualized Change in GFR (ml/min/1.73m²/year) (mean ± sd)	Adjusted Annualized Change in GFR (ml/min/1.73m²/year) (β ± sem	P
Cyclosporine	-1.8 ± 6.4	reference	
Tacrolimus	0.1 ± 7.1	2.5 ± 0.2	< 0.001
Azathioprine	-1.7 ± 6.3	reference	
MMF	-1.0 ± 9.0	2.1 ± 0.2	<0.001

Abstract# 1210

EFFICACY AND SAFETY OF 2 DOSES OF EVEROLIMUS COMBINED WITH REDUCED DOSE NEORAL® IN *DE NOVO* KIDNEY TRANSPLANT RECIPIENTS: 6 MONTHS ANALYSIS.

Helio Tedesco,[1] Julio Pascual,[2] Giovanni Civati,[3] Gentil Filho,[4] Valter Garcia,[5] Tomas Haas,[6] Peter Bernhardt,[6] Johanna Geissler.[6] *[1]Division of Nephrology, Hospital do Rim e Hipertensao, Sao Paulo, Brazil; [2]Servicio de Nefrologia, Hospital Ramon y Cajal, Madrid, Spain; [3]Divisione Nefrologia e Dialisi, Az. Osp. Niguarda Ca' Granda, Milano, Italy; [4]Internal Medicine Department, Hospital das Clincas - UNICAMP, Campinas, Brazil; [5]Servicio de Nefrologia, Santa Casa de Porto Alegre, Porto Alegre, Brazil; [6]Business Unit Transplantation, Novartis Pharma AG, Basel, Switzerland.*

Everolimus (Certican™, RAD) is an investigational proliferation inhibitor for the prevention of rejection in *de novo* kidney and heart transplants. This class of compounds has been reported to allow for the reduction of calcineurin inhibitor exposure. The purpose of this report is to evaluate renal function and to quantify the incidence of biopsy-proven acute rejection epidsodes, graft loss, death or lost to follow-up in this randomized, open label, multicenter 1-year trial. **Methods:** Patients (N=122) were randomized to either everolimus 1.5 mg/day (N=60), or everolimus 3 mg/day (N=62), in combination with steroids and reduced dose of NEORAL®. NEORAL® was tapered using C_2-monitoring: target blood levels 1200 ng/ml (week 0-4), 800 ng/ml (week 5-8), 600 ng/ml (week 9-12) and 400 ng/ml (after week 12). This abstract reports on partial 6-month interim results. **Results:** The incidence of primary efficacy failure (biopsy-proven acute rejection, graft loss, death or loss to follow-up) was comparable between everolimus 1.5 mg and everolimus 3 mg groups (21% and 20%, p=NS), as was the incidence of biopsy-proven acute rejection (17.7% and 15%, p=NS). Renal function at 6 months was comparable between both treatment arms with a mean (median) serum creatinine of 1.66 (1.47) mg/dL in the everolimus 1.5 mg group and 1.49 (1.39) mg/dL in the everolimus 3 mg group. Mean creatinine clearance (Cockcroft-Gault) was 67.3 and 63 ml/min respectively. CMV infections were reported in 3.3% and 6.5% in the 1.5 mg and 3 mg groups respectively. Thrombocytopenia was lower in the 1.5 mg everolimus group (3.2% and 8.3%). The incidence of hyperlipidemia reported as adverse event was lower in the 1.5 mg everolimus treatment group (17.7% versus 30%, p=NS). **Conclusions:** Renal transplant patients treated with everolimus in combination with steroids and reduced dose of NEORAL® maintained good efficacy results as well as excellent renal function. This type of regimen may be considered as a new option to individualize patient care after sucessfull kidney transplantation, although long-term follow-up is required to confirm this interim analysis.

Abstract# 1211

BIOEQUIVALENCE BETWEEN CYCLOSPORINE MICROEMULSION FORMULATIONS MAY NOT TRANSLATE INTO EQUIVALENT CLINICAL OUTCOMES.

David J. Taber,[1,2] G. Mark Baillie,[1,2] Elizabeth Ashcraft,[1,2] Christopher Berman,[2] Jeffrey Rogers,[1] Angello Lin,[1] Fuad Afzal,[1] Osemwegie Emovon,[1] Prabhakar Baliga,[1] P. R. Rajagopalan,[1] Kenneth D. Chavin.[1] *[1]Division of Transplant Surgery, Medical University of South Carolina, Charleston, SC; [2]Department of Pharmacy Services, Medical University of South Carolina, Charleston, SC.*

Rising drug costs have encouraged many hospitals such as ours to replace Neoral® with a generic cyclosporine microemulsion (CyaME) formulation to reduce costs. However, most studies comparing CyaME formulations have included only small numbers of subjects and only reported steady-state pharmacokinetic profiles in either healthy volunteers or stable transplant recipients. Trials reporting actual clinical outcomes with generic CyaME formulations are lacking. The aim of this study was to compare biopsy proven acute rejection at 6 months between a generic CyaME (Gengraf™) and the 'innovator' CyaME (Neoral®). METHODS: We conducted a retrospective review of all de novo kidney transplant patients at our center between Jan 1999 and June 2002. The hospital switch to Gengraf™ occurred in May 2001. Standard immunosuppression consisted of CyaME, MMF, steroids ± induction therapy. Pediatric recipients and patients who received either tacrolimus, sirolimus, or investigational drugs, and technical failures were excluded. Endpoints included graft and patient survival, acute rejection, and Cya concentrations. RESULTS: One-hundred and eighty-eight patients were included for comparison (Gengraf™=88; Neoral®=100). Patient demographics are in Table 1; both groups were well matched. There were no differences between the 2 groups in use of induction or patient and graft survival .

Table 1: Patient Demographics

Group	Male/ Female	Age (yrs)	Race W/B/O	Mean PRA	HLA Mismatch	CIT (min)	WIT (min)
Gengraf (n=88)	56/32	51±13	37/47/3	4±17	4±2	770±533	36±16
Neoral (n=100)	56/44	49±13	38/60/2	3±12	4±2	819±513	35±10

Biopsy-proven acute rejecction at 6 months was significantly higher in the Gengraf™ group as compared to the Neoral® group (39% vs 25%; p<0.05).

Table 2: Mean Cyclosporine Concentrations

	Day 2	Day 7	Days 8-14	Days 15-30	Days 31-60	Days 61-90
Gengraf	306±173	439±164	415±156	415±152	332±122	318±131
Neoral	279±151	353±147	372±134	375±148	318±126	292±174

Although Cya trough concentrations were similar between the two groups, there appears to be more variability in the Gengraf™ group early post-transplant . CONCLUSION: This retrospective study demonstrated a significant difference in biopsy-proven acute rejection at 6 months post-transplant between two different CyaME formulations. Further prospective randomized trials are warranted to confirm these results.

Abstract# 1212

A SEQUENTIAL PROTOCOL USING SIMULECT® VS THYMOGLOBULIN® IN LOW IMMUNOLOGICAL RISK RENAL TRANSPLANT RECIPIENTS : SIX-MONTH RESULTS OF A FRENCH MULTICENTER, RANDOMIZED TRIAL.

Georges J. Mourad,[1] Lionel Rostaing,[2] Christophe Legendre,[3] Valérie Garrigue,[1] Eric Thervet,[2] Dominique Durand.[3] *[1]Nephrology, Hopital Lapeyronie, Montpellier; [2]Hopital Rangueil, Toulouse; [3]Hopital Saint Louis, Paris.*

Background : Induction therapy with delayed introduction of calcineurin antagonists (sequential protocols) has the advantage of avoiding nephrotoxicity while providing effective anti-rejection therapy. Induction with Simulect and immediate introduction of cyclosporine was shown to significantly reduce acute rejection. We investigated safety and efficacy of a sequential protocol using Simulect, Cellcept and steroïds, with delayed Neoral. Methods : In an open-label, multicenter study, recipients 18-65 year-old of cadaveric or non HLA-identical living related renal transplants were randomized to Simulect (S-group) or Thymoglobulin (T-group) induction. Patients with panel reactive antibodies ≥ 20 % or those who had lost their first graft within 1 year due to rejection were excluded. Patients received Prednisolone (20 mg/d) and Cellcept (2g/d) ; Neoral (6 mg/kg/day) was introduced when serum creatinine reached 250 µmol/l. The study major end-points were the incidence of adverse events (leucopenia, thrombopenia, infections) and of biopsy-proven acute rejection (BPAR). Results : From 102 randomized patients, 89 reached 6-month follow-up (S.group : 46 ; T. group : 43). There was no difference in patient demographics : mean age 45 ±11 vs 46 ± 11 years (mean ± SD), cadaver donor 97 vs 100 %, retransplants 7 % vs 14 %, cold ischemia 20.4 ± 7 vs 20.2 ± 8 hours in groups S vs T respectively. Neoral was introduced at 6 ± 2.8 vs 6.6 ± 4.7 post-operative day; first dose was 207 ±80 vs 220±86 mg/day in S vs T groups respectively. There was no difference in Cellcept and steroïd doses at 1, 3 and 6 months. Incidence of BPAR was 7.5 % in the S and 10.2 % in the T group (NS). Incidence of side effects was significantly lower in the S. group: leucopenia 20 vs 43.6 % (p <0.05) ; thrombopenia 0 vs 36 % (p <0.001), CMV infection 17.5 vs 41 % (< 0.005). There was no difference in the incidence of delayed graft function (41 vs 43 %) or serum creatinine levels at 1, 3 and 6 months. In the S.group, one patient died and 2 lost their grafts, all from surgical reasons. In the T-group, there was only 1 graft loss (vascular thrombosis). Conclusion : Sequential protocols using Simulect, Cellcept and steroids with delayed Neoral are as efficacious as thymoglobulin for prevention of BPAR in low immunological risk recipients. Furthermore, they are associated with significantly lower side effects.

Abstract# 1213

SHORT-TERM BENEFIT OF TACROLIMUS VS CYCLOSPORINE THERAPY AFTER RENAL TRANSPLANTATION: AN ANALYSIS OF UNOS/OPTN DATABASE.

Barbara A. Bresnahan,[1] Wida S. Cherikh,[2] Yulin Cheng,[2] Nauman A. Siddiqi,[1] Sundaram Hariharan.[1] *[1]Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]UNOS, Richmond, VA.*

Background: There has been a progressive increase in kidney half-life with each transplant (Tx) year. During the last 10 yrs there have been several newer medications added to the immunosuppressive regimen. The current study analyzed the effect of two different calcineurin inhibitors: tacrolimus (TAC) and cyclosporin (CSA) on short-term endpoints (EP) and composite endpoint (defined as occurrence of at least one of the EPS) . **Methods**: All adult kidney transplants from 1995-2000 (Cad=30,549 and LD=15,579) reported to UNOS/OPTN were analyzed by discharge immunosuppression (CSA=46,128 and TAC=13,026). Recipient and donor demographics, transplant (immunologic and non-immunologic) and post-transplant variables (such as DGF, use of MMF vs Aza, Tx year) were used in the logistic regression model to calculate the odds ratio (OR). The impact of TAC over CSA was assessed for various short-term EPS at 1 yr: graft loss, patient death, acute rejection (AR), S Cr>1.5 mg/dl and the composite end point (CEP: combination of at least one of the 1-yr EPS) **Results**: There were higher proportion of recipients with induction therapy (45% vs 40%), PRA>10% (26% vs 11%), previous Txs (20% vs 10%) in the TAC vs CSA based regimens. With each Tx year there was an increase in proportion of patients on TAC. After correcting for various variables the OR's for short-term EPS (graft loss, AR, S Cr>1.5 mg/dl and CEP) were lower with TAC compared to CSA therapy as shown below:

Outcomes at 1 yr	OR	95% CI	p- value
Graft Loss	0.902	(0.832, 0.977)	**0.0117**
Patient Death	0.949	(0.841, 1.071)	0.3958
AR	0.926	(0.883, 0.972)	**0.0019**
Cr> 1.5 mg/dl	0.716	(0.682, 0.752)	**< 0.0001**
CEP	0.751	(0.718, 0.786)	**< 0.0001**

Other variables such as donor and recipient (age and race), PRA, DGF, Re Txn, use of MMF and Tx year were also significant. The 1, 3 and 5 year unadjusted actuarial graft survival for cadaveric recipients with TAC based regimen was 91.8%, 81.1% and 69.8%. The corresponding values for CSA based therapy was 90.3%, 79.9% and 67.5% respectively, p< 0.0001. **Conclusions**: The use of TAC based regimens was associated with a decrease in 1-yr EPS such as graft loss, AR and Cr>1.5 mg/dl and 1-yr CEP. This effect was independent of other risk variables such as Tx year, PRA, DGF, and MMF use.

Abstract# 1214

LONG-TERM IMMUNOLOGICAL EFFECTS OF ATG INDUCTION THERAPY IN RENAL ALLOGRAFT RECIPIENTS.

Rolf Weimer,[1] Sevgi Yildiz,[1] Anne Staak,[1] Volker Daniel,[2] Lucy Rainer,[1] Hartmut Dietrich,[1] Shirin Kamali-Ernst,[3] Wolfgang Ernst,[3] Winfried Padberg,[4] Gerhard Opelz.[2] *[1]Department of Internal Medicine, University of Giessen, Giessen, Germany; [2]Institute of Immunology, University of Heidelberg, Heidelberg, Germany; [3]Dialysis centers, Wetzlar and Langenselbold, Germany; [4]Department of Surgery, University of Giessen, Giessen, Germany.*

It has been shown that ATG induction therapy induces a long-lasting decrease of the CD4/CD8 ratio. To analyze long-term effects of ATG induction on lymphocyte function, we prospectively assessed CD4 helper function, B cell/monocyte and cytokine responses in 84 renal transplant recipients up to 1 year (1y) posttransplant. 44 of the 84 patients received prophylactic rabbit ATG induction due to an increased immunological risk profile (acute renal failure, PRA>5% or retransplantation; n=37) or simultaneous islet kidney transplantation (n=8). Basic immunosuppression and drug trough levels were comparable in the ATG and non-ATG groups. A PWM-driven allogeneic coculture system was used to assess helper function of CD4+ T cells and T cell-dependent B cell responses. SAC I was used for T cell-independent stimulation of B cell cultures. B cell differentiation was assessed in a reverse hemolytic plaque assay. ELISAs were used to determine in-vitro cytokine secretion (IL-2, IL-4, IL-6, IL-10). 1-year graft survival (93% vs. 98%), acute rejection incidence (25 vs. 28%) and 1-year graft function (creatinine clearance: 44±3 vs. 52±6 ml/min) were not significantly different between the ATG and non-ATG groups. ATG induced a persistent decrease of PBMC counts compared to non-ATG treatment (4 months (4m) and 1y; p<.0005) due to a predominant decrease of CD4+ T cells (4m and 1y; p<.0005) which was associated with decreased CD4 cell IL-2 responses (4m; p<.0005). However, previously identified predictors of acute rejection (helper activity, IL-4 and IL-10 responses of CD4+ T cells) were not affected by ATG induction therapy. Adhesion molecule expression increased in ATG-treated compared to non-ATG treated patients (p<.05; CD54+ T cells and monocytes (4m,1y), CD11a/CD18+ T cells (1y), CD58+ monocytes (4m)) and coincided with late acute rejections in 4 ATG-treated patients. Our data show that ATG induction therapy in immunological high-risk patients induces a profound long-term decrease in cell counts and IL-2 responses of CD4+ T cells, whereas their helper activity as well as IL-4 and IL-10 responses, which were previously shown to be predictors of acute rejection, were not affected. Increasing adhesion molecule expression 4 months post-transplant might favor late acute rejections in ATG-treated patients with elevated immune responsiveness.

Abstract# 1215

PHASE 2, RANDOMIZED, MULTICENTER, OPEN-LABEL STUDY OF ISA247 AND NEORAL® IN POST-RENAL TRANSPLANT PATIENTS.

Randall W. Yatscoff,[1] Mark D. Abel,[1] Launa J. Aspeslet,[1] Robert T. Foster,[1] Derrick G. Freitag,[1] Robert B. Huizinga,[1] Patrick R. Mayo,[1] Daniel J. Trepanier.[1] *[1]Isotechnika Inc., Edmonton, AB, Canada.*

Purpose: The purpose of this 12 week study was three fold: To evaluate the calculated creatinine clearance of post-renal transplant patients receiving ISA247, to monitor safety parameters in post-renal transplant patients receiving ISA247, and to measure calcineurin inhibition and pharmacokinetics of ISA247 in post-renal transplant patients. Methods: Renal transplant patients (greater than 6 months post transplantation) with stable renal function (calculated creatinine clearance > 30 mL/min/1.73m²) and on a stable dose of cyclosporine A were recruited to participate. Patients were randomized to receive either Neoral® or ISA247, a new generation calcineurin inhibitor. Patients were evaluated at regular intervals for rejection, renal function, clinical biochemistry, side effects, drug pharmacokinetics and calcineurin inhibition as a pharmacodynamic marker. ISA247 and Neoral® doses were titrated using C_0. Results: This data analysis is based on n= 45 ISA247 and n = 51 Neoral® patients having completed through day 70 of the study. A complete summary of all patients enrolled in the trial will be presented. Patient groups were similar for all demographic parameters. Calculated creatinine clearance has remained stable in both treatment groups with mean values at day 70 of 57.2 ± 16.7 and 58.0 ± 20.3 mL/min/1.73 m² for ISA247 and Neoral®, respectively. The most common adverse events for ISA247 and Neoral® were gastrointestinal disorders (15% and 5%) and nervous system disorders (14% and 8%) of which headache was most common. The mean dose of ISA247 was 0.65 ± 0.29 mg/kg compared with 1.1 ± 0.2 mg/kg for Neoral®. ISA247 Cmax and AUC were 171 ± 96 ng/mL and 703 ± 362 ng.h/mL, respectively. Neoral® Cmax and AUC were 650 ± 237 ng/mL and 2330 ± 696 ng.h/mL. Less ISA247 exposure was associated with identical calcineurin inhibition to Neoral® (ISA247 43 ± 17% and Neoral® 49 ± 12%). Correlations between ISA247 C_0, C_2, and $AUC_{(0-4)}$ were $r^2 = 0.51$ and $r^2 = 0.89$, compared to Neoral® of $r^2 = 0.05$ and $r^2 = 0.47$. ISA247 C_0 directly correlated with calcineurin inhibition ($r^2 = 0.34$) while Neoral® did not ($r^2 = 0.01$). Conclusions: Renal function has remained stable in both ISA247 and Neoral® groups. ISA247 has a similar incidence of adverse events to Neoral® despite 3-fold greater potency. ISA247 C_0 strongly correlates with AUC and calcineurin inhibition suggesting C_0 can be used for therapeutic drug monitoring.

Abstract# 1216

CYCLOSPORINE VS. TACROLIMUS IN RENAL TRANSPLANTATION: FIVE-YEAR RECIPIENT OUTCOMES.

Sali Aswad,[1] Steven R. Potter,[1] Rafael G. Mendez,[1] Shirley Mirador,[1] Ziad Haddad,[1] Hamid Shidban,[1] Robert Mendez.[1] *[1]National Institute of Transplantation, Los Angeles, CA.*

Introduction: Cyclosporine (CYA) and tacrolimus (FK) have revolutionized renal transplantation. Potential differences in efficacy and side-effect profiles of these agents are a source of ongoing debate. **Objective:** To compare the efficacy and side effects of CYA and FK used in combination with mycophenolate (MMF) and prednisone in a large cohort of renal transplant recipients with long follow-up. **Methods:** We reviewed the courses of 1420 consecutive patients receiving renal transplants at one center between January 1996 and January 2001. Mean follow-up time was 40.1±22 months. CYA was used in 688 (48.6%) recipients, while FK was used in 379 (26.7%). 353 (24.9%) recipients had incomplete records or follow-up and were excluded, leaving 1067 recipients for analysis. Univariate analysis of demographic data including donor and recipient gender, age, weight, race, ratio of cadaveric and living donors, haplotype match, cold-ischemia time and CMV status revealed no significant differences between patients receiving CYA and FK. Recipients receiving FK were more likely to have panel reactive antibody (PRA) levels >10 (p=0.045). **Results:** Recipients on FK were more likely than those on CYA to have elevated serum glucose levels (>150 mg/dl) at 1 month (27% vs. 11%), 6 months (26% vs.13%; p=0.0273), 1 year (25% vs. 17%), 3 years (23% vs. 9%; p=0.0001), and 5 yrs (23%; p=.017). Recipients on CYA were more likely to have cholesterol levels >240 mg/dl at 1 mo. (16% vs. 6.1%; p<0.0001) and at 5 years. (17%), but not significant differences between CYA and FK arms at other time-points. Delayed graft function (DGF) was more common in patients receiving FK (6.7% vs. 2.3%; p=0.0085). CYA and FK arms had equivalent serum creatinine levels at 1 month, 1year, and 3 years. Graft survival rates at 1, 3, and 5 years for CYA and FK were 98% and 96%, 92% and 86%, and 86 and 75% respectively. Patient survival rates were equivalent for FK and CYA at all time-points. **Conclusions:** The use of FK was associated with a higher likelihood of elevated serum glucose levels and equivalent cholesterol levels during most of follow-up. With all donor and recipient demographic factors equivalent except for a higher likelihood of PRA>10, FK was associated with higher DGF rates and lower graft function rates at 5 years after transplant. Subset analysis to identify patients likely to benefit from CYA or FK use is ongoing.

Abstract# 1217

A PROSPECTIVE RANDOMIZED SINGLE CENTER STUDY OF RAPID CORTICOSTEROID ELIMINATION COMPARING TWO MAINTENANCE IMMUNOSUPPRESSIVE PROTOCOLS: TACROLIMUS(FK506)/MYCOPHENOLATE MOFETIL (MMF) VERSUS TACROLIMUS/SIROLIMUS.

Lorenzo G. Gallon,[1] Joseph R. Leventhal,[2] Dixon B. Kaufman,[2] Alan J. Koffron,[2] Jonathan P. Fryer,[2] Michael M. Abecassis,[2] Talia Spainer,[2] Paolo Salvalaggio,[2] Frank P. Stuart.[2] *[1]Medicine, Division of Nephrology, Northwestern University, Chicago, IL; [2]Surgery, Division of Transplantation, Northwestern University, Chicago, IL.*

We have previously reported the feasibility of rapid corticosteroid elimination (3 days posttransplant) using a FK506/MMF based maintanance immunosuppression regimen in renal transplantation. The aim of this study was to further investigate rapid corticosteroid elimination by using two maintenance immunosuppressive protocols: FK506/MMF vs FK506/Sirolimus. **Methods:** Prospective study of rapid corticosteroid elimination in kidney transplant recipients recieving induction therapy with IL2 receptor antagonist and randomized to recieved either FK506/MMF (n=45) or FK506/Sirolimus (n=41). The results of the two treatment arms were compared to an historical control group of kidney transplant recipients maintained on FK506/MMF/Prednisone (n=80). **Results:** The mean follow-up was 21.2 months for the FK506/MMF group, 20.5 months for the FK506/Sirolimus group and 42.3 months for the control group. Demographics (age, gender, race, HLA match, PRA, CMV status) were nor different between the 3 groups. At 24 months there were no statistically or clinically significant differences in outcome based on patient survival, graft survival, or risk of rejection. See table.

	Patient Survival	Graft Survival	Rejection-free
Control (n=80)	98.8%	96.3%	83.7%
FK506/MMF (n=45)	100%	100%	80%
FK506/Sirolimus (n-41)	97.6%	87.8%	73.2%

Conclusions: In our prospective study, rapid corticosteroid elimination in kidney transplant recipients is feasible when using both maintenance immunosuppressive protocols. The FK506/MMF group showed a trend (no statistically significant) of better outcome in regards to graft survival and risk of rejection when compared to the FK506/Sirolimus group.

Abstract# 1218

A REGIMEN OF SIROLIMUS AND *REDUCED-DOSE* TACROLIMUS RESULTS IN IMPROVED RENAL ALLOGRAFT FUNCTION: COMBINED ANALYSIS OF THE NORTH AMERICAN *TARGET*, EUROPEAN AND AUSTRALIAN SIROLIMUS-TACROLIMUS TRIALS. J. Whelchel,[1] L. Paczek,[2] W. O. Bechstein,[3] G. Russ.[4] *[1]Organ Transplant Services, Piedmont Hospital, Atlanta, GA; [2]Clinic of Immunotherapy and Internal Diseases, Institute of Transplantation, Warsaw, Poland; [3]Department of Surgery, Ruhr-Universitat, Bochum, Germany; [4]Renal Unit, The Queen Elizabeth Hospital, South Australia, Australia.*

Objective To evaluate renal allograft function, patient and graft survival, and the incidence of AR in patients receiving sirolimus (SRL) in combination with *reduced-dose* tacrolimus (rTAC) or *standard-dose* tacrolimus (sTAC). **Methods** We report pooled 6-month interim results of the comparative, open-label, randomized, multicenter North American, European, and Australian Sirolimus-Tacrolimus trials. 361 *de novo* patients receiving primary or secondary renal allografts from cadaveric or living donors were randomized 1:1 to rTAC+SRL (n=184, TAC 3-7ng/mL) or sTAC+SRL (n=177, TAC 8-12ng/mL). All patients received steroids. Planned antibody induction was prohibited. **Results** Demographics were similar between groups: rTAC+SRL (65.8% male, 88.0% caucasian, 70.1% CAD) and sTAC+SRL (63.8% male, caucasian 84.7%, CAD 77.4%). PS and GS were 96.2% and 94.6% (rTAC+SRL) and 98.3% and 96.6% (sTAC+SRL, P=NS). Mean serum creatinine (umol/L±SEM) was 124.0±3.85 (rTAC+SRL) and 148.6±3.94 (sTAC+SRL, P<0.001). Mean creatinine clearance (mL/min±SEM) was 70.2±1.52 (rTAC+SRL) and 58.9±1.59 (sTAC+SRL, P<0.001). Protocol amendment was enacted in response to an imbalance in early AR [rTAC+SRL: n=9/30 (30%) vs. sTAC+SRL: n=3/29 (10.3%), P=NS]. Post-amendment the incidence of AR was similar between groups [rTAC+SRL: n=23/154 (14.9%) vs. sTAC=SRL: n=15/148 (10.1%), P=NS). The overall incidence of AR (including episodes which occured prior to amendment) was 17.4%, (n=32/184) rTAC+SRL and 10.2% (n=18/177) sTAC+SRL (P=0.049). Mean SRL trough levels (ng/mL±SEM) were 9.5±0.3 (rTAC+SRL) and 8.2±0.3 (sTAC+SRL, p=0.002), and mean TAC trough levels (ng/mL±SEM) were 5.9±0.2 (rTAC+SRL) and 9.2±0.2 (sTAC+SRL, P<0.001). There was no significant difference in mean serum cholesterol, triglycerides, LDL, or HDL between groups. The rate of discontinuation was 29.3%(rTAC+SRL) and 29.9%(sTAC+SRL, P=NS), with AE the most common reason for discontinuation. Three malignancies were reported: 2 skin CA (rTAC+SRL) and PTLD (sTAC+SRL). **Conclusions** These preliminary data support the safety and efficacy of SRL and *reduced-dose* TAC. In patients receiving SRL-based immunosuppression, reduced exposure to TAC early after transplantation is associated with improved renal function. Adequate immunosuppressant exposure must be achieved early to minimize risk of AR.

Abstract# 1219

AN OPEN-LABEL, CONCENTRATION-CONTROLLED, RANDOMISED 6-MONTH STUDY OF *STANDARD-DOSE* TACROLIMUS + SIROLIMUS + STEROIDS COMPARED TO *REDUCED-DOSE* TACROLIMUS + SIROLIMUS + STEROIDS IN RENAL ALLOGRAFT RECIPIENTS. L. Paczek,[1] W. O. Bechstein,[2] L. Wramner,[3] J. P. Squifflet,[4] the European Sirolimus-Tacrolimus Study Group. *[1]Institute of Transplantation, Warsaw, Poland; [2]Ruhr-Universitat, Bochum, Germany; [3]Sahlgrenska University Hospital, Goteborg, Sweden; [4]Saint Luc Hospital, Brussels, Belgium.*

Objective To evaluate renal allograft function, patient and graft survival, and the incidence of AR in patients receiving sirolimus (SRL) with *reduced-dose* tacrolimus (rTAC) or *standard-dose* tacrolimus (sTAC). **Methods** In this comparative, open-label, randomised, multicenter, 6-month trial 128 *de novo* patients receiving primary or secondary cadaveric renal allografts were randomised 1:1 to rTAC+SRL or sTAC+SRL. Steroid taper was standardised. Planned antibody induction was prohibited. **Results** Final 6-month analysis includes 128 patients. Demographic/donor variables were similar between the rTAC+SRL (n=63) and sTAC+SRL (n=65) groups. PS and GS were 95.2% and 92.1% (rTAC+SRL) and 96.9% and 95.4% (sTAC+SRL, P=NS). Mean serum creatinine (umol/L±SD) was 136.3±45.28 (rTAC+SRL) and 153.0±47.27 (sTAC+SRL, P=0.085). Mean creatinine clearance (mL/min±SD) was 63.8±17.32 (rTAC+SRL) and 52.7±18.89 (sTAC+SRL, P=0.005). Suboptimal immunosuppressant exposure contributed to 5 early ARs in the rTAC+SRL group. Following protocol amendment to optimise early SRL and TAC exposure, the incidence of AR was similar between rTAC+SRL and sTAC+SRL groups [13.6%, n=6/44 vs. 10.4%, n=5/48 respectively, p=NS]. The overall incidence of biopsy confirmed AR was 17.5% (n=11) in the rTAC+SRL group and 7.7% (n=5) in the sTAC+SRL group (P=NS). Mean SRL troughs (ng/mL±SD) were 10.9±4.92 (rTAC+SRL) and 6.8±2.48 (sTAC+SRL, p<0.001). Mean TAC troughs (ng/mL±SD) were 5.9±2.04 (rTAC+SRL) and 9.2±2.01 (sTAC+SRL, P<0.001). There was no significant difference in mean serum cholesterol, triglycerides, LDL, or HDL between groups. The most common noninfectious AEs with rTAC+SRL were anemia (28.6%), hyperlipemia (27.0%), and increased creatinine (19.0%), and with sTAC+SRL were anemia (26.2%), diarrhea (26.2%), and hyperlipemia (23.1%). The most common infections in the rTAC+SRL group were UTI (30.2%) cystitis (4.8%) and pharyngitis (4.8%), and in the sTAC+SRL group were UTI (35.4%), URI (6.2%), bronchitis (6.2%) and pneumonia (6.2%). Two malignancies were reported: PTLD (sTAC+SRL) and basal cell CA of the lip (rTAC+SRL). **Conclusions** These data support the safety and efficacy rTAC+SRL in renal allograft recipients. rTAC+SRL provides adequate immunosuppression and is associated with improved creatinine clearance and a trend toward improved creatinine.

Abstract# 1220

TACROLIMUS VS. CYCLOSPORIN MICROEMULSION: 3-YEAR FOLLOW-UP OF A LARGE, MULTICENTRE TRIAL. Bernhard K. Kraemer,[1] Giuseppe Montagnino, Manuel Arias, Julio Pascual, the Tacrolimus vs. Cyclosporin Follow-Up Study Group. *[1]Klinik und Poliklinik für Innere Medizin II, Klinikum der Universtität Regensburg, Regensburg, Germany.*

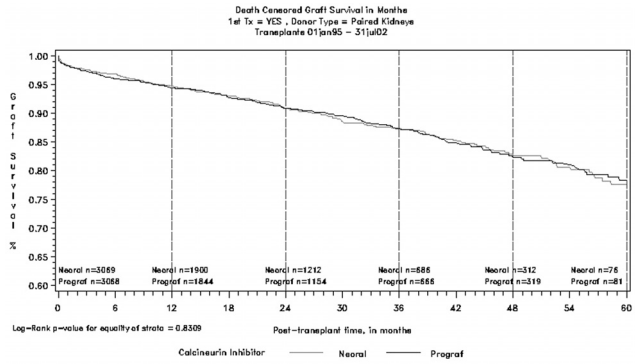
Background: This is a follow-up study of a large, multicentre study that compared tacrolimus and microemulsified cyclosporin after renal transplantation. A total of 557 patients had been recruited into the original 6-month study with 286 patients randomized to tacrolimus (Tac) and 271 patients to cyclosporin microemulsion (CyA), concomitantly with steroids and azathioprine. After 6 months, there was a significant difference (p<0.001) in the incidence of biopsy-proven acute rejection with 19.6% (Tac) and 37.3% (CyA). **Methods**: All centres were asked to provide follow-up information of all patients whether withdrawn during the main study or not at 1, 2, and 3 years post transplant. Data were entered and analysed by an independent clinical research organisation. **Results**: At the 3-year follow-up, data were available for 217 (Tac) and 203 (CyA) patients (76.2% of total cohort). Up to the 3-year follow-up 9 patients had died in each group, in the Tac group 25 grafts and in the CyA group 32 grafts were lost since transplantation. Median calculated creatinine clearance was 64.5 mL/min in the Tac group compared with 60.3 mL/min in the CyA group. The incidence of acute rejection between 6 months and 3 years was low in both groups (Tac 11 vs. CyA 16), preserving the difference observed after 6 months. The mean Tac dose was 0.08 mg/kg/day and the mean trough level was 8.5 ng/mL. The mean CyA dose was 2.9 mg/kg/day and the mean trough level was 137.3 ng/mL. Mean blood pressure was similar in both groups (SBP 135.5 vs.138.4 mmHg, DBP 83.2 vs. 83.1 mmHg), however, the use of antihypertensive medication was higher in the CyA group (73.7% vs. 80.7%, p=ns.). Despite the significantly higher use of lipid lowering medication (17.5% vs. 32.7%, p<0.01), the mean cholesterol level was significantly higher (5.13 mmol/L vs. 5.47 mmol/L, p<0.05) in the CyA group. The administration of MMF differed between the groups (Tac 8.8% vs. CyA 15.8%, p<0.05). In the Tac group 2.8% and in the CyA group 18.2% of patients had switched their baseline immunosuppressant (p<0.01). More patients in the CyA group (67.7% vs. 79.3%, p<0.01) received steroids, the mean doses were 4.9 mg/day (Tac) and 5.5 mg/day (CyA). **Conclusion**: At 3 years post transplantation the advantages of Tac therapy seen in the first 6 months were preserved. There was a tendency towards better graft survival and better renal function in the tacrolimus group. Concomitant medication load was lower in tacrolimus treated patients.

Abstract# 1221

LONG-TERM GRAFT SURVIVAL COMPARISON IN PATIENTS TREATED WITH NEORAL VS PROGRAF: A LIVING DONOR AND PAIRED KIDNEY ANALYSIS. Bruce Kaplan, Jesse Schold, Herwig-Ulf Meier-Kriesche. *[1]Medicine, University of Florida, Gainesville, FL.*

Neoral and Prograf are the cornerstones of most immunosuppressive protocols. Studies have shown differences in acute rejection rates between the two calcineurin inhibitors (CI) but no differences in graft survival. Live donor kidney transplants are growing in number. Analysis of this segment of patients can decrease selection bias in comparing two agents. Therefore, we conducted a retrospective study to investigate long term graft survival associated with different CI's, in renal transplant recipients of a live donor kidney. In addition, we looked at paired cadaveric donor kidneys to minimize potential donor variability. Utilizing the SRTR, we analyzed patients who received a live donor transplant and who experienced no delayed graft function (DGF). In addition, to avoid donor bias we analyzed paired cadaveric kidneys where one kidney went to a patient initiated on Neoral and the other on Tacrolimus. Kaplan Meier and Cox models were utilized for graft and patient loss. Graft survival in living transplant recipients was not statistically different between Neoral vs. Prograf based regimens (95.6% vs. 95.6% survival at one year, and 80.5% vs. 78.2% at five years of follow up for Neoral and Prograf respectively). In the multivariate analysis, Neoral and Prograf were equal for death censored graft loss (RR 0.71 and 0.70 respectively as compared to Sandimmune). Five year death censored graft survival was almost identical among paired cadaveric donor kidneys for Neoral vs. Prograf based immunosuppressive regimen. (Figure 1) In Summary, in living transplant recipients, long term graft and patient survival were not statistically different between Neoral and Prograf based immunosuppression. The paired kidney analysis also revealed no difference in 5-years graft and patient survival. At this time, there is no registry evidence for an association of either CI's with superior patient or graft survival.

Abstract #1221 Figure



Abstract# 1222

RANDOMIZED TRIAL OF THREE DIFFERENT IMMUNOSUPPRESSIVE REGIMENS TO PREVENT CHRONIC RENAL ALLOGRAFT REJECTION. Joshua Miller,[1] George W. Burke,[1] Gaetano Ciancio,[1] Bonnie B. Blomberg,[1] Anne Rosen,[1] Jeff Gaynor,[1] David Roth,[1] Warren L. Kupin.[1] *[1]Depts. of Surgery-Div of Transplantation & Microbiology/Immunology & Medicine-Div of Nephrology, Univ Miami Med Sch & Miami VA Medical Center, Miami, FL.*

At the present time, chronic renal allograft nephropathy remains the primary cause of graft loss after the first transplant year in spite of a significant reduction in early acute rejection episodes. Both Rapamycin (RAPA) and Mycophenolate (MMF) have theoretical advantages in preventing chronic allograft vasculopathy and interstitial fibrosis. The clinical benefit of these agents in combination with decreased dosaging of calcineurin inhibitors has not been defined. Our study consists of a long-term (3 yr) single center, prospective, randomized trial of 150 (CAD or mismatched LD) recipients equally divided into 3 arms: Group I: Tacrolimus (FK) + RAPA; Group II: FK + MMF; and Group III: Neoral (NEO) + RAPA. All patients received induction therapy with Daclizumab (5 doses) and steroids. Target trough levels for FK were 10 ng/ml for the first 3 mo and 6-8 ng/ml to 1 yr. Target trough RAPA levels were 8 ng/ml throughout, and NEO target trough levels were 200-250 ng/ml for the first 3 mo, decreasing by 25 ng/ml after this. MMF was to be dosed at 2 gm/day. Protocol biopsies at implant and at 1 and 3 yr (were and) are being performed. All rejection episodes were biopsy proven. Pharmacokinetic studies were done at 3 mo. At interim analysis performed November 1, 2002, 138 patients had reached at least 12 mo of follow-up.

	Group I (n=50)*	Group II (n=50)*	Group III (n=50)*
Patient survival**	96%	94%	98%
Graft survival**	96%	92%	92%
Death-censored graft survival**	100%	98%	94%***
% Acute rejection**	4%	4%	21%***
Serum creatinine (mg/dl) (@ 12 mo)	1.48 (n=43)	1.29 (n=39)	1.69*** (n=40)
% HMG CoA (@ 12 mo)	54.3%*** (n=46)	17.4%*** (n=46)	82.6%*** (n=46)
FK Tmax (minutes)	120 (n=16)	75*** (n=21)	—
RAPA AUC (ng/hr/ml)	141 (n=16)	—	181*** (n=15)

*No difference in LD or CAD recipient # between groups; **Actuarial 1 yr; ***p<.05

In this interim analysis, a significantly greater incidence of acute rejection was seen in Group III (p=.013 by the logrank test) along with a greater incidence of graft failure and a higher mean creatinine level. The use of HMG CoA for hyperlipidemia was significantly different among each of the groups, and the highest use was seen in Group III. Significant interactions between NEO, MMF, FK, and RAPA, demonstrated by pK studies, require careful monitoring in these drug combinations. Differences in acute (& chronic) rejection now begin to appear (and will be updated with 1 yr biopsy data at presentation).

Abstract# 1223

A COMPARATIVE, OPEN-LABEL STUDY TO EVALUATE GRAFT FUNCTION IN DE NOVO RENAL ALLOGRAFT RECIPIENTS TREATED WITH REDUCED-DOSE OR STANDARD-DOSE CYCLOSPORINE IN COMBINATION WITH SIROLIMUS AND CORTICOSTEROIDS. David J. Cohen,[1] Flavio Vincenti,[2] the US Rapamune-CSA Study Group. *[1]Nephrology, Columbia Presbyterian Medeical Center, New York, NY; [2]Transplant Surgery, University of California, San Fransisco, CA.*

Objective: This study evaluated renal allograft function and the incidence of acute rejection in patients receiving sirolimus (SRL) in combination with reduced-dose (rCSA) or standard-dose cyclosporine (sCSA). **Methods:** 309 patients were enrolled in this 1 year, comparative, open-label, randomized, multicenter trial. Non-African American renal allograft recipients of cadaveric or living organs were eligible. Patients were randomized to receive rCSA (50-125 ng/mL)+ SRL (10-20 ng/mL) or sCSA (150-300 ng/mL) + SRL (5-15 ng/mL). Target CSA levels were achieved by month 3. Patients received corticosteroids according to local practice. Antibody induction was permitted only in patients with DGF. **Results**: At the final 12 month analysis, demographic and donor source characteristics were similar between patients receiving rCSA+SRL (n=154) and sCSA+SRL (n=142). In both groups, patient survival was 96%, graft survival 98%. Mean serum creatinine was 1.58±0.06 mg/dL in patients receiving rCSA+SRL, and 1.76±0.07 mg/dL in those receiving sCSA+SRL (p<0.05). Mean calculated creatinine clearance was 66±1.9 mL/min (rCSA+SRL) and 62±3.3 mL/min (sCSA+SRL) (p=NS). There was no significant difference in the incidence of biopsy-confirmed acute rejection between groups (rCSA+SRL,14.9%; sCSA+SRL,12.7%). Mean whole blood SRL trough levels at 12 months were 10±0.44 ng/mL (rCSA) and 8.4±0.4 ng/mL (sCSA). Mean CSA trough levels were 103±5.5 ng/mL (rCSA) and 177±15 ng/mL (sCSA). Total cholesterol in patients receiving rCSA+SRL was 220 ±5.8 mg/dL, and 301±92 mg/dL in patients receiving sCSA+SRL (p=NS). Two malignancies occurred in the rCSA+SRL arm (1 skin, 1 PTLD) and 4 in the sCSA+SRL arm (2 skin, 1 melanoma, 1 PTLD). Adverse events most commonly reported with rCSA+SRL were peripheral edema (66.9%)and hyperlipemia (40.3%); with sCSA+SRL, peripheral edema (68.3%), and constipation (39.4%). The most common infectious complications were of the urinary tract (rCSA+SRL, 28%; sCSA+SRL, 29%) and upper respiratory system (rCSA+SRL, 23%; sCSA+SRL, 21%). **Conclusions**: These data support the safety and efficacy of an immunosuppressive regimen of SRL and rCSA. Excellent patient and graft survival and low rates of acute rejection were observed. The impact of this combination on long-term renal allograft function remains to be determined.

Abstract# 1224

AN OPEN-LABEL STUDY TO EVALUATE THE EFFICACY & SAFETY OF CYCLOSPORINE REDUCTION IN *DE NOVO* RENAL ALLOGRAFT RECIPIENTS RECEIVING SIROLIMUS: A DOSE COMPARATIVE STUDY. F. Muehlbacher,[1] H. H. Neumayer,[2] D. del Castillo,[3] S. Stefoni,[4] the European Sirolimus CsA Minimisation Study Group. *[1]University Clinic of Surgery, Vienna, Austria; [2]Universitatsklinikum Charite-Mitte, Berlin, Germany; [3]Hospital Reina Sofia, Cordoba, Spain; [4]Azienda Ospedaliera S. Orsola, Malphigi, Bologna, Italy.*

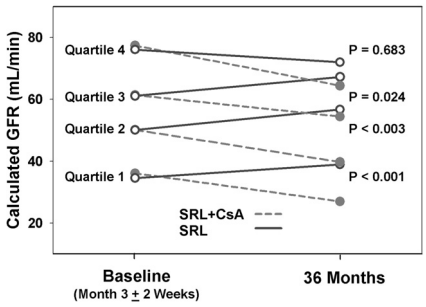
Objective This study evaluated whether a regimen of *reduced-dose* cyclosporine (rCsA)+sirolimus (SRL) would provide adequate immunosuppression while reducing the potential for nephrotoxicity associated with *full-dose* cyclosporine (fCsA). **Methods** 420 primary or secondary renal allograft recipients were enrolled in this one-year, multicenter, randomised, comparative study. Patients were randomised 1:1 to rCsA or fCsA; SRL was administered daily to maintain trough levels 4-12 ng/ml (HPLC); corticosteroids (CCS) were administered according to local practice. Planned antibody induction was prohibited. **Results** This 12-month interim analysis includes 341 randomised patients: rCsA (n=170) and fCsA (n=171). Demographic and donor variables were similar between the rCsA and fCsA groups. PS and GS were 100% (n=170) and 99.4% (n=169) rCsA and 98.2% (n=168) and 97.1% fCsA respectively (P=NS). The incidence of biopsy confirmed AR was 10.0% (n=17) in the rCsA group vs 14.0% (n=24) in the fCsA group (p=0.318). Mean serum creatinine (umol/L±SEM) was 158.6±10.30 (rCsA) vs 173.1±6.82 (fCsA, p=0.0085). Mean creatinine clearance (mL/min±SEM) was 57.0±2.07 (rCsA) vs 49.8±3.01 (fCsA, p=0.0027). Mean SRL trough levels (ng/mL±SEM) were 9.1±0.42 (rCsA) and 8.2±0.38 (fCsA, P=0.139), and mean CsA trough levels were 85.7±2.98 (rCsA) and 135.2±5.45 (fCsA, P<0.001). There was no significant difference in mean cholesterol, LDL or HDL between groups. Fasting serum triglycerides (mmol/L±SEM) were significantly different at only the 12-month time-point: 2.3±0.10 (rCsA) vs 2.6±0.17 (fCsA, P=0.0440). The most common noninfectious AEs with rCsA were hyperlipemia (29.4%), hypertension (16.5%) anemia (15.3%) and hypercholesterolemia (15.3%), and with fCsA were hyperlipemia (28.1%), lymphocele (17.0%) and hypertension (15.2%). The most common infections with rCsA were UTI (21.8%), herpes simplex and bronchitis (5.3%) and with fCsA were UTI (19.9%), pneumonia (8.8%) and oral moniliasis (4.7%). Three malignancies were reported: PTLD (rCsA), lymphoma (fCsA), and gastric malignancy (fCsA). **Conclusions** Sirolimus in combination with rCsA and CCS results in excellent patient and graft survival, low AR, and improved renal function. Sirolimus administered with reduced-dose CsA and CCS provides adequate immunosuppression while reducing the potential for CsA's nephrotoxic effects.

Abstract# 1225

SUPERIOR RENAL FUNCTION AFTER EARLY CYCLOSPORINE WITHDRAWAL AND SIROLIMUS MAINTENANCE THERAPY, REGARDLESS OF BASELINE RENAL FUNCTION: INTENT-TO-TREAT RESULTS AT 3 YEARS.

Christophe Legendre,[1] Alfredo Mota, Peter Friend, Giuseppe Segoloni, Josep M. Grinyo, Brian Hutchison, Jose M. Morales, Joseph Lawen, Javier Martinez, Anders Hartmann, Karine Doyen, the Rapamune Maintenance Regimen Study Group. *[1]Hopital St Louis, Paris, France.*

Purpose: To determine the impact of baseline renal function when withdrawing cyclosporine (CsA) at 3 months in renal transplant recipients receiving sirolimus (Rapamune®, SRL), CsA, and steroids (ST). **Methods:** This open-label study was conducted in Europe, Australia, and Canada. 525 renal allograft recipients were enrolled and received SRL 2-mg tablets, CsA, and ST from the time of transplantation. At 3 months ± 2 weeks, 430 patients were eligible to be randomly assigned to remain on triple therapy (SRL+CsA, n=215) or to have CsA withdrawn (SRL, n=215) and SRL trough concentrations increased (troughs 16-24 ng/mL, chromatographic assay). Patients were divided into 4 equal groups (quartiles) according to their baseline calculated GFR (the last value before randomization). ANCOVA was used to compare changes from baseline. All data were included, whether or not the patient remained on assigned therapy (ITT analysis). **Results:** 52% vs 62% (SRL+CsA vs SRL, p = 0.041) of patients remained on therapy 36 months after transplantation. Although the differences were greater for the on-therapy analysis, the ITT analysis showed that patients in the lowest 3 quartiles (GFR ≤ 67 mL/min) undergoing CsA withdrawal had markedly and significantly better renal function outcomes (see Figure). **Conclusion:** The results from the quartile analysis indicate that renal function improves with CsA elimination and SRL-based therapy. The difference in calculated GFR was most prominent in patients with moderately impaired renal function.



Abstract# 1226

AN OPEN STUDY TO EVALUATE THE EFFECT OF SIROLIMUS AND STEROIDS FOLLOWING CYCLOSPORINE REDUCTION OR ELIMINATION IN RENAL ALLOGRAFT RECIPIENTS WITH PROGRESSIVE IMPAIRMENT OF RENAL FUNCTION.

V. Sparacino,[1] S. Federico,[2] F. P. Schena,[3] P. Altieri,[4] D. Donati.[5] *[1]Civic Hospital, Palermo, Italy; [2]II Policlinic Hospital, Naples, Italy; [3]Policlinic Hospital, Bari, Italy; [4]Civic Hospital, Cagliari, Italy; [5]Macchi Hospital, Varese, Italy.*

Purpose: To evaluate the safety and efficacy of converting (totally or partially) renal transplant patients from a cyclosporine (CsA) based therapy to Sirolimus (SRL) and the effect on renal function (serum creatinine, sCr). **Methods**: 87 patients were enrolled in this 6 months, multicenter study. Main inclusion criteria were: 1 to 10 years post transplant, screening creatinine 1.8-3.5 mg/dl, increase ≥30% of the creatinine nadir at any time post transplant. On day 1 patients received SRL 6 mg loading dose and discontinued MMF or AZA. From day 2 to 6 SRL dose was 2 mg . If on day 7 the SRL trough level (HPLC) was between 6-12 ng/ml CsA dose was reduced to reach a trough level between 50-100 ng/ml. **Results**: 70 patients completed, 17 dropped. Screening CsA trough level mean was 128.2 ng/ml (±50.1), 6 months CsA level mean was 16.8 ng/ml (±30.3), 6 months SRL trough level mean was 10.0 ng/ml (±4.1). Eighteen patients (26.0%) completely eliminated CsA at the end of the first month. Mean screening creatinine was 2.24 mg/dl (±0.46), at 6 months 2.37 mg/dl (±0.95), not statistically significant (Paired T-Test, p=0.15). Four clusters came out of the analysis of the delta creatinine (final–screening): in 30 patients creatinine decreased (4-37%), in 12 patients remained the same, 9 patients observed a limited increase (5-11%) and 19 reported an increase (12-180%). A multivariate analysis was conducted on: time from transplant (TFTx, median 54 months) and screening creatinine (median 2.2 mg/dl). Results at 6 months were: patients with a TFTx < 54 months had a sCr mean = 2.11 mg/dl (±0.62) vs 2.64 mg/dl (± 1.14) with a p = 0.02 (ANOVA); patients with a sCr < 2.2 mg/dl had a sCr mean = 1.92 mg/dl (±0.54) vs 2.83 mg/dl (±1.06) with p<0.001. The relationship between CsA trough level at month 2 and sCr at month 6 shows a correlation coefficient r=0.13 (p=0.3), i.e. higher Csa trough predicts worst creatinine. The best predictor in the multivariate analysis was screening sCr (p<0.001). No rejection occurred. **Conclusion:** This data support the feasibility of converting chronic patients from CsA based regimen to SRL with an improvement/stabilization in 60% of patients. The subjects who will mostly benefit from this conversion are the one with the following characteristics: screening sCr < 2.2 mg/dl, TFTx < 54 months, rapid elimination of CsA (within 2 months from screening).

Abstract# 1227

PROTECTIVE EFFECT OF BCL-XL FUSED TO A PROTEIN TRANSDUCTION DOMAIN IN PANCREATIC ISLETS.

Dagmar Klein,[1] Sundararajan Jayaraman,[1] Melina Ribeiro,[1] Norma S. Kenyon,[1] Camillo Ricordi,[1] Ricardo L. Pastori.[1] *[1]Diabetes Research Institute, University of Miami School of Medicine, Miami, FL.*

OBJECTIVE: Transplantation of islets is becoming an established method to treat type 1 diabetes. However many recipients do not achieve normoglycemia after only a single transplant because during the islet isolation and subsequent culture many of the functioning insulin producing cells are lost due to apoptosis induced by oxidative stress and other insults. Unlike in healthy cells, in isolated islet cells the pro- and anti-apoptotic proteins are not strictly in equilibrium thus enhancing the proclivity for cell death. The balance between the pro-apoptotic and anti-apoptotic members of Bcl-2 protein family controls mitochondrial function and induction of cell death. Temporary overexpression of anti apoptotic proteins such as Bcl-XL in transplantable islets will enhance their viability. To avoid the complications associated with long term expression of anti-apoptotic proteins, we investigated possibility of Bcl-XL delivery to islets by protein transduction. In this method proteins fused to cell penetrating peptides such as penetratin, TAT peptide (an 11-aa derived from TAT/HIV protein) or Arg9, are readily transduced in to living cells. METHODS: The recombinant TAT-Bcl-XL was generated by subcloning of the coding region cDNA in frame with the TAT peptide (YGRKKRRQRRR) using the pTAT bacterial expression vector. The TAT-fusion protein was expressed and purified from BL21 E. coli cells. A 14-mer TAT peptide control (GYGRKKRRQRRRGC) was synthesized and the correct sequence was confirmed by mass spectrometry. RESULTS: We observed a reduction of enzymatically-assessed caspase-6 activation during islet culture. Caspase-6 activity was reduced 20%-45% in both human islets and non human primate islets transduced with 200-400 nM Bcl-Xl, compared to either untransduced islets or islets transduced with 200-400nM TAT peptide alone. The cell death assessed by PI staining was reduced in islets cultured with either TAT-Bcl-XL or TAT-BH4 (an antiapoptotic BH4 domain peptide fused to pTAT). Percentage of dead cells after 24hs in culture was as follows: control (untransfected): 22%; TAT-BH4: 200 nM, 14% and 400 nM, 11%; TAT-Bcl-XL: 200 nM, 15% and 400 nM, 7,5%. CONCLUSIONS: Our data suggest that TAT-Bcl-XL and TAT-BH4 fusion proteins protect islet cells from induction of apoptosis. Fusion proteins technology might represent a suitable approach for the improvement of quality/function of transplantable islets.

Abstract# 1228

CASPASE INHIBITION DECREASES APOPTOSIS AND LEADS TO IMPROVED LUNG FUNCTION IN A RAT MODEL OF LUNG TRANSPLANTATION.

Syed M. Quadri,[1] Lorne Segall,[1] Andre Dutly,[1] Patricia Wegrynowski,[2] Vern Edwards,[3] Brendan Mullen,[2] Nicola Jones,[3] Thomas K. Waddell,[1] Mingyao Liu,[1] Shaf Keshavjee.[1] *[1]Thoracic Surgery, Toronto General Hospital, Toronto, ON, Canada; [2]Pathology, Mt. Sinai Hospital, Toronto, ON, Canada; [3]Gastroenterology, Hospital for Sick Children, Toronto, ON, Canada.*

Previously, we have described significant cell death through apoptosis and necrosis—up to 30% of cells—in the lung following transplantation and reperfusion in humans and animals. Multiple pathways lead to programmed cell death; it is not known which are relevant in lung transplantation. Furthermore, the clinical significance of this cell death is not known. **Methods:** In randomised blinded studies, syngeneic left single lung transplantation (Lewis rats) was performed after 6h (n=15) and 18h (n=8) of cold ischemic storage (CIT). Animals and storage solutions were treated with caspase inhibitors (IDN6556 n=6, or zVAD-fmk n=3 in 6h CIT and IDN6556 n=4 in 18h CIT) or control (DMSO n=6 and n=4 in 6h and 18h CIT, respectively). After implantation and 2h reperfusion, lung function was assessed by evaluating the pO_2 level of the transplanted lung at FiO_2 1.0. Caspase 3 activity in lung tissue lysates was measured through a fluorometric assay with DEVD-AFC. Lung samples were subjected to electron microscopy. Tdt mediated nick-end labelling (TUNEL) was used to evaluate apoptosis in paraffin embedded lung sections. **Results:** After 6h CIT, transplantation and 2h reperfusion, the paO_2 levels, at FiO_2 1.0, were not significantly different. However, after 18h CIT, transplantation and 2h reperfusion, the paO_2 levels at FiO_2 1.0 were significantly higher in the caspase inhibitor group as compared to control: DMSO 232±18 versus IDN 368±43 mmHg, *p=0.03*. Caspase 3 activity rose dramatically from a baseline of 1769±112 (units / 100µg protein) immediately after harvest to 11252±668 after 6h CIT and then dropped to 4488±1674 after 18h CIT in control animals. Animals treated with caspase inhibitor did not exhibit any significant increases in caspase 3 activities. Electron microscopy demonstrated apoptotic cell death in endothelial cells and lymphocytes in control animals. TUNEL studies show that apoptosis was markedly reduced in animals treated with caspase inhibitor. **Conclusions:** Activation of caspase 3 during cold ischemia contributes significantly to ischemia-reperfusion induced apoptosis of endothelial cells and lymphocytes in lung transplantation. A systemically administered pan-caspase inhibitor successfully inhibited caspase 3 activity in the lung, led to decreased TUNEL positivity, and improved lung function after 18h CIT, transplantation and 2h reperfusion.

Abstract# 1229

STAT6 TRANSCRIPTION IS REQUIRED FOR IL-13 CYTOPROTECTION AND DOWNREGULATION OF INFLAMMATORY INJURY INDUCED BY HEPATIC ISCHEMIA/ REPERFUSION. Bibo Ke, Xiu-Da Shen, Feng Gao, Ronald W. Busuttil, Jerzy W. Kupiec-Weglinski. *[1]Dumont-UCLA Transplant Ctr, UCLA School of Medicine, Los Angeles, CA.*

Background: Antigen-independent inflammatory injury caused by ischemia/ reperfusion (I/R) is detrimental to both early and long-term function of orthotopic liver transplants (OLTs). We have shown that activation of Stat4 transcription pathway plays a key role in the hepatic inflammatory response triggered by I/R injury, whereas Stat4 disruption or local induction of Th2-type IL-13 are cytoprotective. This study was designed to test a hypothesis that Stat6 is required for cytoproptection rendered by Ad-IL-13 gene transfer during hepatic I/R injury. **Methods**: Using a partial lobar liver ischemia model, male Balb/c wide-type (WT) and Stat6-deficient (KO) mice were used (n=4/gr). Animals were injected with Ad-IL-13 or Ad-βgal reporter gene ($2.5x10^9$ pfu i.v.). 48 h later, mice were heparinized, and an atraumatic clip was used to interrupt blood supply to the cephalad liver lobes. After 90 min of partial hepatic ischemia, the clip was removed, initiating hepatic reperfusion. Sham controls underwent the same procedure, but without vascular occlusion. Mice were sacrificed after 6 h of reperfusion; liver tissue and blood samples were harvested for analyses. **Results**: Serum ALT levels (IU/L), a measure of hepatocellular damage, were markedly decreased in WT mice treated with Ad-IL-13, as compared with WT, Stat6 KO and Stat6 KO treated with Ad-IL13/ Ad-βgal (220 vs. 2071, 2123, 1881 and 2097, respectively; $p<0.005$). This functional data correlated with histologic Suzuki's grading of hepatic I/R injury. Indeed, WT, Stat6 KO and Stat6 KO treated with Ad-IL-13/Ad-βgal showed significant edema, sinusoidal congestion/cytoplasmic vacuolization, and severe hepatocellular necrosis (30-50%), whereas WT mice given Ad-IL-13 revealed minimal sinusoidal congestion without edema/vacuolization or necrosis. WT mice conditioned with Ad-IL-13 had significantly decreased liver neutrophil accumulation (MPO activity assay), and markedly upregulated expression of anti-oxidant HO-1, as compared with WT, Stat6 KO, or Stat6 KO + Ad-IL-13/Ad-βgal (Western blots). Unlike in WT, Stat6 KO, or Stat6 KO + Ad-IL-13/Ad-βgal mice, the expression of mRNA coding for TNF-α/IL-1β and MIP-2 remained depressed, whereas that of IL-13/IL-4 reciprocally increased selectively in WT + Ad-IL13 gene transfer group (competitive-template RT-PCR). **Conclusion**: This study provides evidence that Stat6 transcription pathway is required for Ad-IL-13 gene tranfer to exert cytoprotective functions during hepatic I/R injury.

Abstract# 1230

TACROLIMUS AMELIORATES RENAL ISCHEMIA/ REPERFUSION INJURY IN HYPERTENSIVE TRANSGENIC mREN-2 RATS. Ondrej Viklicky,[1] Radka Bohmova,[1] Eva Honsova,[1] Jan Rajnoch,[1] Vaclav Mandys,[2] Uwe W. Heemann,[3] Stefan Vitko.[1] *[1]Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]3rd Medical Faculty, Charles University, Prague, Czech Republic; [3]Technical University, Munich, Germany.*

Background: Renal ischemia/reperfusion injury (I/R) and hypertension represent major alloantigen-independent risk factors contributing to the development of chronic rejection of renal allografts. Recently, a critical role of T-cells in I/R has been discussed. In this study we evaluated the long-term effect of immunosuppressant tacrolimus (TAC) in a model of accelerated renal injury where high-renin hypertension aggravates functional and morphological changes induced by I/R. *Methods:* 30 anesthetized uninephrectomised hypertensive transgenic (m-REN-2)-27 rats (TGR) and 30 normotensive Han-SD rats (SD) as genetic controls received clip on renal pedicle for 45 minutes. In both TGR and SD groups, animals were treated with either TAC (0.10mg/ kg/day i.m.; n=10), enalapril (ENA, 50mg/L in the drinking water, n=10) or placebo (PLA, n=10) for 12 weeks. Proteinuria and blood pressure were evaluated throughout the experiment. At the end of the experiment, kidney function was evaluated and kidneys harvested for morphological analysis. *Results:* At week 12, TAC-treated TGR had lower proteinuria as compared to PLA-treated TGR (41.1±21.5 vs. 126.7±35.8; $p<0.01$; ENA-TGR: 19.6±4.5mg/day) and reduced % of obsolete glomeruli (4.4±2.3 vs. 20.9±10.9; $p<0.01$; ENA-TGR: 2.7±0.9%). There were no differences in systolic blood pressure and serum urea levels between TAC- and PLA-treated TGR. TAC-treated SD had lower proteinuria as compared to PLA-treated SD (7.0±3.8 vs. 19.8±5.6; $p<0.05$; ENA-SD: 13.3±3.9mg/day) but there were no difference in the % of obsolete glomeruli (1.4±1.3 vs. 0,5±0.8; ENA-SD: 0.4±0.6%). We observed no differences in systolic blood pressure and serum urea levels between TAC-SD and PLA-SD groups. Both ENA-treated TGR and SD had lower systolic blood pressure than TAC- and PLA-treated animals. *Conclusion:* Tacrolimus treatment ameliorated late functional and morphological changes induced by I/R injury in hypertensive TGR. Our observation thus supports the hypothesis about the key role of immune system in the progressive renal injury. *Supported by Grant No. ND6641/3 – 2001.*

Abstract# 1231

17β-ESTRADIOL DIFFERENTIALLY ACTIVATES THE MITOGEN-ACTIVATED PROTEIN KINASES AND IMPROVES SURVIVAL FOLLOWING REPERFUSION INJURY OF REDUCED-SIZE LIVER. Devin E. Eckhoff,[1] Guadalupe Bilbao,[1] Christopher Eckstein,[1] Cheryl A. Smyth,[1] Juan L. Contreras.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL.*

Ischemia/reperfusion-injury (I/R-I) of the liver is a common occurrence in resectional surgery and liver transplantation and may impair regeneration, ultimately leading to liver failure. The three major mitogen-activated protein kinase (MAPK) signaling pathways, including extracellular signal-regulated protein kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK), are critical in the transmission of signals triggered by proinflammatory cytokines, stress, and growth factors. Previous studies demonstrated that estradiol reduces I/R-I. In this study, we assessed the effects of estradiol on liver function, survival, and activation of MAPK in a murine model of reduced-size liver I/R-I. Methods: 70% of the liver mass in C57BL/6 mice were subjected to ischemia for 45 minutes. After reperfusion, the non-ischemic lobes were resected. estradiol or the estrogen antagonist ICI-182780 were given 1 hour before the injury (n=7). ALT was assessed at 6 hours and apoptosis by ELISA at 24 hours. The the activation of JNK, p38 and ERK was assesed by western blots. Results: Females presented lower initial hepatocellular injury (ALT=714±110) and 70% had indefinite survival after a reduced-size I/R-I, whereas all males died within 5 days (ALT=1808±327, $P<0.05$). Ovariectomy reduced the mean survival time to 3 days and estradiol improved survival in 60% of males. The liver mass in females and males treated with estradiol increased steadily after the injury, whereas no significant changes were observed in males or ovariectomized mice. Higher incidence of apoptosis was observed in male animals given saline (enrichment factor =7.22± 0.8) *versus* males treated with estradiol (5.85±0.3, $P<0.05$). JNK activation following I/R-I was attenuated by estradiol, effect reversed by ICI-182780. Conversely, estradiol promoted p38β (5.8±1.2 normalized to actin) and ERK (7.12±2.6 normalized to actin) activation compared with controls (1.28±0.8 and 3.36±1.12 normalized to actin, respectively, $P<0.05$). Conclusions: Estradiol limited hepatocellular injury and promoted survival following reduced-size I/R-I to the liver. Estradiol inhibited the activation of the pro-apoptotic JNK pathway and induced the activation of the anti-apoptotic p38β pathway, and proliferation through ERK. Estrogen therapy may be important in clinical conditions associated with I/R-I, specially split or living donor liver transplantation.

Abstract# 1232

CRITICAL ROLES OF CALCINEURIN AND CHOLESTEROL FOR RENAL ISCHEMIC PRECONDITIONING. Takeshi F. Andoh,[1] Seung Ok Choi,[1] Richard A. Zager,[2] John F. Valente,[1] William M. Bennett.[1] *[1]Solid Organ and Cellular Transplant Services, Legacy Good Samaritan Hospital, Portland, OR; [2]Univ of Washington, Seattle, WA.*

Cholesterol is a major constituent of plasma membrane. Recently we reported that renal cholesterol levels rise after various renal insults including calcineurin inhibitor (CNI) therapy. Theses increases serve to protect the kidney from subsequent ischemia reperfusion (I/R) injury. We hypothesized that preconditioning of donors with CNI would prevent renal dysfunction in a rat kidney transplant (KTx) model. **Methods:** F344 rat donor kidneys were perfused with UW solution and exposed to 2 h of cold ischemia and orthotopic KTx was performed in syngeneic F344 rats with a warm ischemia time of 20 min. Experimental groups included: 1. control animals with uni-nephrectomy (Con); 2. KTx from donors treated with saline (KTx); 3. KTx from donors treated with tacrolimus (FK, 1 mg/kg) 24 h before Tx (KTx + FKacu); 4. KTx from donors treated with FK 7 days before Tx (KTx+FKdelay); 5. KTx from donors treated with cyclosporine (CsA, 10 mg/kg) 24 h before Tx (KTx+CsAacu). GFR (inulin clearance, ml/min/100 g bw) was measured 3 and 7 days after Tx and structural injury, namely tubular dilation, vacuolization, atrophy and interstitial inflammation was semi-quantified (0-3+) using digital image analysis. Free cholesterol was determined in the contralateral kidney of donor animals (nMole cholesterol / microMole total phosphate). **Results:** Data are shown as mean ± SEM (n=5~7). * P < 0.05 vs Con, # P < 0.05 vs KTx by ANOVA/Tukey test.

(Day 3 or 7)	Con	KTx	KTx+FKacu	KTx+FKdelay	KTx+CsAacu
GFR (7)	0.58±0.03	0.25±0.04*	0.48±0.03#	0.53±0.02#	0.41±0.02#
Histology (7)	0.01±0.01	2.2±0.2*	1.3±0.1#	1.0±0.2#	1.3±0.1#
Creatinine (3)	0.6±0.02	1.9±0.1*	0.9±0.05#	0.8±0.03#	1.0±0.03#
Cholesterol		215±2	223±4	228±2#	

Both acute and delayed preconditioning with FK significantly improved graft function and I/R structural injury associated with Tx. The tissue cholesterol of donor kidney increased progressively with time, reaching statistical significance by 7 days. The protective effect of CsA seemed to be the same as FK. **Conclusion:** These data demonstrate protective effect of donor preconditioning with CNI on kidney graft function and structure. This effect is transferable from donors to recipients after Tx and can be sustained for at least 7 days. Although the underlying mechanism remains to be elucidated, a renal stress response including cholesterol accumulation may be involved.

Abstract# 1233

DELTA OPIOID AGONISTS PROTECT RODENT LIVER FROM COLD STORAGE, ISCHEMIA/REPERFUSION INJURY. Steven M. Rudich,[1] Wenjian Chang.[2] *[1]Surgery, University of Cincinnati College of Medicine, Cincinnati, OH; [2]Surgery, University of Michigan Health System, Ann Arbor, MI.*

Background: The quality of the liver allograft after cold storage and ischemia/reperfusion is of major importance in minimizing the incidence of primary graft failure and organ dysfunction upon transplantation. It has been found that some delta opioid agonists, acting as hibernation induction factors, can decrease injury in lung and cardiac animal transplantation models. **Objective:** To determine if synthetic hibernation induction factors, in the form of the delta-2 specific peptide DPDPE (d-phenylalanine, d-phenylalanine enkephalin) can protect the rodent liver from cold storage/reperfusion injury. **Methods:** Sprague-Dawley rats were given a single i.v. injection of either saline (control) or DPDPE (2 mg/kg) at various times prior to surgery. In other experiments, the delta-2 opioid inhibitor BNTX was injected 30 mins prior to DPDPE. After 30 mins of global warm hepatic ischemia, the liver was removed, perfused and cold stored in UW solution for 6 hrs. Upon removal, the liver was placed into an ex-vivo perfusion circuit and perfused with Krebs-Hanseleit buffer at 37°C for 3 hrs. Variables monitored included: quantity and length of time of bile flow, intra-parenchymal vascular resistance, wet-to-dry liver weight ratios, oxygen consumption, perfusate liver function enzymes and cytokines (TNF-$^{\alpha}$9 IL-1, and IL-6), and histology, including TUNEL staining. **Results:** Rats treated with DPDPE prior to global hepatic ischemia, followed by cold storage and reperfusion, exhibited significantly prolonged bile production compared to control animals. The ability of DPDPE to protect the stressed liver was also noted by a significant decrease in hepatic enzyme release, potassium, lactate, and inflammatory cytokines in the perfusate; decreased wet-to-dry liver weight ratio, and a significant decrease in the degree of apoptosis, vs animals treated with saline alone. The specific inhibitor BNTX abolished these protective effects, yielding results upon ex-vivo liver perfusion comparable to those observed with saline injection. These results were markedly improved compared to similar experiments performed using the more delta-1 specific opioid agonist, DADLE. **Conclusions:** The delta-2 specific opioid agonist DPDPE, in a receptor specific fashion, was able to manifest significant protection upon the rodent liver following a cold storage/reperfusion insult. Protection was demonstrated biochemically, mechanically, and histologically. These (and similar) agents may find use in developing new strategies to further enhance transplant outcomes and perhaps even donor usage.

Abstract# 1234

NEAR TOTAL SUPPRESSION OF COLD ISCHEMIC INJURY BY AN OPTIMIZED TROPHIC FACTOR SUPPLEMENT TO THE UW PRESERVATION SOLUTION. Jonathan F. McAnulty,[1] Kenneth R. Waller,[1] Murray K. Clayton,[2] Christopher J. Murphy.[1] *[1]Dept. of Surgical Sciences, University of Wisconsin, Madison, WI; [2]Dept. of Statistics, University of Wisconsin, Madison, WI.*

PURPOSE: We have reported that use of trophic factors doubled the feasible duration of preservation and reduced the time to return to normal serum creatinines to one-third of that with unmodified UW solution. The factors used in that study were Substance P (SP), insulin-like growth factor-1 (IGF), epidermal growth factor (EGF), nerve growth factor (NGF) and bactenecin (B). In this study, we examine combinations of these factors to ascertain their interactions and contributions to preservation. METHODS: Dog kidneys (n=40) were cold stored 4 days with UW solution +/- combinations of trophic factors. The combinatorial groups were 1) no factors, 2) B-SP-IGF-EGF-NGF, 3) SP-IGF-EGF-NGF, 4) B-IGF-EGF-NGF-, 5) B-SP-EGF-NGF, 6) B-SP-IGF-EGF, 7) B-SP-IGF, 8) B-SP-IGF-NGF, 9) B-SP, 10) B-SP-NGF, 11) SP-IGF-NGF, 12) B, 13) IGF, 14) SP, and 15) NGF. After storage, kidneys were autotransplanted with immediate contralateral nephrectomy. Logarithmic transformed area under the serum creatinine curve was used as an integrated index for analysis of immediate graft function. Data were analyzed using the GLM procedure of SAS, treating the data as an unbalanced, incomplete factorial design whereby all main effects and the majority of two-way interactions could be estimated. RESULTS: Significant interactions were detected between IGF and NGF and between EGF and NGF, the latter being a negative interaction, where the benefits of NGF were suppressed by EGF. Immediate function was best ($p<.05$) with B-SP-IGF-NGF (group 8; n=4). In this group, 4 day storage yielded average peak serum creatinine values of 2.2 +/- .17 mg/dl (mean +/- S.E.) This was in contrast to unmodified UW solution (mean peak creatinine- 4.9 +/- 0.6 mg/dl) or all trophic factors together (group 2; mean peak creatinine- 2.9 +/-0.2). Group 8 dogs had more rapid normalization of serum creatinines (3.5 +/- 0.3 days) compared to UW solution (19.5 +/- 0.8) or group 2 dogs (6.3 +/-1.0 days). CONCLUSIONS: Trophic factors markedly reduce cold storage injury. It is known that many factors interact to provide unique effects at warm temperature but this has not previously been shown in cold stored organs. Optimizing the mix of factors further reduces cold ischemic injury over previous reports. The ability to suppress cold ischemic injury has far-reaching implications in terms of expanding the organ donor pool, organ sharing and in reducing allograft pathology that affects premature graft loss.

Abstract# 1235

EFFECT OF ISCHEMIA/REPERFUSION AND ANTI-LFA 1 INHIBITION ON TELOMERE LENGTHS AND EXPRESSION OF CDKI GENES IN A CONCORDANT EX-VIVO HEMOPERFUSION OF PRIMATE KIDNEYS. Archil B. Chkhotua∗,[1] Peter Wiegand∗,[2] Stefan Grosse,[3] Simone Reis,[2] Hubert Schelzig,[3] Dietmar Abendroth.[3] *[1]National Centre of Urology, Tbilisi, Georgia; [2]Dept. of Legal Medicine; [3]Transplantation Center, University Clinic Ulm, Ulm, Germany.*

Increased endothelial activation and cellular adhesion due to the LFA-1/ICAM-1 interaction are the major pathogenetic mechanisms of ischemia/reperfusion (I/R) injury. Telomere (T) length, p21 $^{(WAF1/CIP1)}$ and p27$^{(Kip1)}$ CDKI genes are considered the markers of cell senescence and DNA damage. The aim of the current study was to evaluate the influence of I/R and anti LFA-1 mAb administration on the value of abovementioned markers. An experimental model of ex-vivo hemoperfusion of primate kidneys was used as described earlier (Transplant Proc, 1996). 13 Macaque cynomolgus monkey kidneys were harvested after in vivo cold perfusion and placed in Eurocollins solution. 9 kidneys were ex-vivo perfused with human blood, with (n=5) or without (n=4) addition of anti LFA-1 mAb (study group); and 4 kidneys were not perfused at all (control group). Tissue expression of p21 $^{(WAF1/CIP1)}$ and p27$^{(Kip1)}$ CDKI genes was evaluated immunohistochemically and telomere lengths were measured by southern blotting technique. The marker levels have been compared between the groups and subgroups. Significantly higher levels of p21 and p27 were expressed by the glomeruli (p=0.001 and 0.0001), tubules (p=0.0065 and 0.0006) and interstitial cells (p=0.0017 and 0.0022, respectively) of the xenoperfused kidneys. The mean T length was higher in the control group (5.56±0.60 Kbp), than in the study group kidneys (5.46±0.36 Kbp) (P=NS). Addition of anti LFA-1 mAb did not influence the gene expression profile in glomerular, tubular, interstitial and vascular renal cells. The mean T length in the kidneys with anti LFA-1 treatment was longer than without the antibody administration (5.7±0.11 vs. 5.13±0.31 Kbp) (p=0.0661). For the first time to our knowledge, it has been shown by the results of the current study that renal ischemia followed by concordant hemoperfusion is associated with telomere shortening and over-expression of p21 and p27 CDKI genes, indicating on the DNA damage and/or accelerated tissue senescence. Although anti LFA-1 mAb treatment had a protective effect on the telomeres, it did not influence the gene expression profile in our study.

Shared First Authorship∗

Abstract# 1236

INHIBITION OF THROMBOSIS IN TRANSGENIC MICE EXPRESSING HUMAN CD39. Karen M. Dwyer,[1] Simon C. Robson,[3] Harshal H. Nandurkar,[2] Tharun B. Mysore,[1] Elzbieta Kaczmarek,[3] Peter J. Cowan,[1] Anthony J. F. D'Apice.[1] *[1]Immunology Research Centre, St.Vincent's Hospital, Melbourne, VIC, Australia; [2]Department of Haematology, St.Vincent's Hospital, Melbourne, VIC, Australia; [3]Liver Center, Medicine, Beth Israel Deaconess Hospital and Harvard Medical School, Boston, MA.*

Background & Aims: Rejection of pig-to-primate xenografts is almost invariably associated with intragraft thrombosis. Unregulated platelet activation is an early event in this process. Contributing mechanisms include inactivation of CD39/NTPDase1, a critical regulator of platelet function, on the endothelial cell surface. Transgenic over-expression of CD39 in the donor pig may solve this problem. We therefore produced mice transgenic for human CD39 to assess the ability of this molecule to prevent thrombosis in small animal xenograft models. Methods: Transgenic mice were generated by microinjection of an H2-K^{b} promoter-driven human CD39 construct and analysed for CD39 expression by flow cytometry, immunohistochemistry and ATPDase activity assay. CD39-expressing mice were compared to non-transgenic mice by measuring: (i) bleeding times, (ii) response to collagen/ADP injection in a systemic thromboembolism model, and (iii) response of isolated pancreatic islets to *ex vivo* incubation with human blood in a model of xenograft-induced thrombosis. Results: Two lines of CD39-expressing transgenic mice were generated. In addition to expression on peripheral blood leucocytes and platelets, both lines exhibited widespread tissue expression that was particularly strong on endothelium. The transgenic mice were healthy and reproduced normally, but exhibited prolonged bleeding times and increased resistance to intravascular coagulation induced by collagen and ADP. Furthermore, the stable thrombus precipitated by incubation of non-transgenic mouse islets with human blood was not observed when CD39-expressing islets were used. Conclusions: Expression of human CD39 in transgenic mice produced a distinct anticoagulant phenotype, with prolonged bleeding times and resistance to induced thrombosis, without apparent deleterious effects. Importantly, CD39 also prevented thrombosis in an *ex vivo* xenograft model, suggesting that organs or tissues from CD39 transgenic pigs may be resistant to intravascular thrombosis.

Abstract# 1237

IDENTIFICATION OF AN ANTI-IDIOTYPIC ANTIBODY THAT DEFINES B CELL SUBSET(S) PRODUCING FUNCTIONALLY ACTIVE XENOANTIBODIES. Mary K. Kearns-Jonker,[1] Jacqueline Fischer-Lougheed,[1] Alan Xu,[1] Carol Lah,[1] Lora Barsky,[2] Vaughn Starnes,[1] Donald V. Cramer.[1] *[1]Cardiothoracic Surgery, Children's Hospital of Los Angeles and University of Southern California, Los Angeles, CA; [2]Division of Research Immunology/BMT, Children's Hospital of Los Angeles, Los Angeles, CA.*

Anti-idiotypic antibodies that define B cell subsets represent a potentially valuable tool for the characterization and depletion of B cells producing xenoantibodies. The 2G10/IgG1k anti-idiotype is a mouse monoclonal antibody with specificity for human immunoglobulins encoded by Ig genes in the V_H3 and V_H4 families. Reactivity has been mapped to residues 74-79 of framework 3. Previous studies in our laboratory have shown that xenoantibodies in humans are encoded by a small number of germline progenitors within the V_H3 and V_H4 families. We cloned and sequenced the germline progenitors encoding antibodies in B cells identified by this anti-idiotype to determine if this reagent could be used to define B cells producing xenoantibodies. cDNA libraries were prepared from human peripheral blood lymphocytes labeled with the 2G10 antibody and sorted using the FACSVantage. The immunoglobulin genes expressed in these cells were identified by cloning and sequencing genes encoding antibodies in this group of B cells. The results indicate that antibodies expressed by B cells identified with the anti-idiotypic antibody 2G10 are encoded by V_H3 germline genes IGHV3-11, V3-74, V3-7, V3-23, V3-21 and V3-30. Twenty percent of the IGV_H genes sequenced from the sorted B cells utilized the IGHV3-11 germline progenitor, 27% utilized the V3-7 germline progenitor and 7% utilized the V3-74 germline gene to encode the expressed antibody. The antibodies encoded by IGHV3-11, V3-74, V3-7, and V3-23 germline progenitors have all been demonstrated to bind to the αgal antigen in humans. Multi-color flow cytometry was used to sort B cells using CD5, CD11b, FITC-gal, and the anti-idiotype 2G10. In humans, 8-12% of B cells bind to 2G10. Multi-color flow cytometry indicated that 2G10 positive cells were predominantly $CD5^-$ and express low levels of CD11b. The cells were sorted on the basis of phenotype into $CD11b(mac-1)^+$ $CD5^-$ $2G10^+$; $CD11b^-$, $CD5^-$, $2G10^+$; and $CD11b^+$, $CD5^+$, $2G10^+$ cells using the FacsVantage for the preparation of Ig gene libraries and sequencing of V_H genes expressed in each of the sorted groups. Nucleic acid sequencing of Ig gene libraries from sorted 2G10 positive cells demonstrates that the xenoantibody–producing subset of B cells identified with this anti-idiotypic antibody in humans is $2G10^+$, $CD11b^+$, $CD5^-$. This antibody may be useful in methods requiring depletion of pre-existing xenoantibody-producing B cells.

Abstract# 1238

VERY ACUTE REJECTION (VAR): A NOVEL FORM OF XENOGRAFT REJECTION MEDIATED BY ANTI-GAL IgG1 MONOCLONAL ANTIBODIES. Jikun Shen,[1] Dengping Yin,[1] Huasong Zeng,[1] Lianli Ma,[1] Hui Xu,[2] Guerard W. Byrne,[2] Anita S. Chong.[1] *[1]Department of Surgery, The University of Chicago, Chicago, IL; [2]Nextran, Princeton, NJ.*

There is increasing evidence that anti-Gal Abs play a dominant role in xenograft rejection. Hyperacute rejection (HAR) of xenografts mediated by anti-Gal IgM Abs depends absolutely on the activation of complement and the assembly of terminal complement complexes on the surface of endothelial cells. We recently reported that complement-fixing anti-Gal IgG3 mAbs can elicit classic HAR Unexpectedly, poorly complement-fixing anti-Gal IgG1 mAbs also elicited xenograft rejection within 2 hours of mAb administration. We have labeled this IgG1-mediated rejection very acute rejection (VAR). VAR shares many histological features with HAR including minimal myocyte damage and macrophage infiltration, platelet microthrombi in the microvessels of the graft, fibrin deposition, C3 deposition, P-selectin and von Willebrand factor. One notable histological difference was an absence of C5 deposition in VAR. This observation is consistent with in vitro observations that anti-Gal IgG3 and IgG1 mAbs could induce mouse C3 deposition (IgG3 more efficiently that IgG1), but only IgG3 effectively induce C5 deposition and membrane attack complex (MAC)-dependent lysis. In vivo studies comparing the mechanism of IgG1-mediated VAR to IgG3-mediated HA revealed both VAR and HAR were inhibited by cobra venom factor, which depletes serum C3. However anti-asialo GM1, which classically depletes natural killer (NK) cells, was able to only inhibit VAR, but not HAR. To address the possibility that anti-asialo GM1 may have consumed serum complement for the depletion of NK cells, we co-administered rabbit complement (0.2 ml baby rabbit sera/recipient) with anti-asialo GM1. Again, anti-asialo GM1 inhibited VAR under these conditions, suggesting that the inhibition of VAR was due to the specific depletion of asialo GM1-expressing cells. To test the hypothesis that NK cells may be mediating their effects via the FcγRIII, we tested the ability of blocking anti-FcγRII/III mAbs (2.4G2) to inhibit VAR. Anti-FcγRII/III mAbs (0.5 mg/mouse) administered 2 hours before anti-Gal IgG1 prevented VAR. Collectively these observations support the conclusion that anti-Gal IgG1 mediates a unique form of xenograft rejection that has overlapping kinetics with HAR but is mechanistically distinct in its dependence on both C3 deposition and FcγRIII-expressing NK cells.

Abstract# 1239

THE USE OF LENTIVIRAL VECTORS TO INDUCE LONG-TERM TOLERANCE TO GAL$^+$ HEART GRAFTS. Mary Kearns-Jonker,[1] Jacqueline Fischer-Lougheed,[1] Irina Shulkin,[1] Karli Edholm,[1] Meguru Watanabe,[1] Donald B. Kohn,[2] Anthony d'Apice,[3] Vaughn Starnes,[1] Donald Cramer.[1] *[1]Cardiothoracic Surgery, Children's Hospital of Los Angeles and University of Southern California, Los Angeles, CA; [2]Research Immunology/BMT, Children's Hospital of Los Angeles and University of Southern California, Los Angeles, CA; [3]Immunology Research Centre, St.Vincent's Hospital, Melbourne, Australia.*

Tolerance to the gal carbohydrate can be induced in knockout mice following transplantation with bone marrow cells expressing the gal epitope. The application of gene therapy to induce tolerance to porcine xenografts following transplantation of autologous bone marrow represents a clinically-feasible approach of inducing tolerance to pig grafts in patients. The ability to induce tolerance is limited, however, by the vectors and methodology associated with transducing pluripotent stem cells. Lentiviral vectors represent a novel and promising approach to achieve high level, long term expression of the gal carbohydrate in autologous bone marrow as they integrate into dividing and non-dividing cells. We transduced autologous gal $^{-/-}$ bone marrow with lentiviral vectors expressing the porcine α1,3 galactosyltransferase gene, constructed with and without the WPRE regulatory element, and examined expression in knockout mice. Transduced bone marrow cells were transplanted into lethally-irradiated gal^-/gal^- mice and chimerism was examined at 7 weeks –5 months post-transplantation. Chimerism in T,B, erythroid and granulocyte lineages in peripheral blood, spleen and bone marrow was examined by flow cytometry using two color labeling for the gal carbohydrate and lineage-specific markers, including anti-CD19, anti-CD3, anti-Gr-1 and Ter 119. Stable chimerism was demonstrated in all mice, as demonstrated by gal^+ expression on 49-63% of T cells, 41-70% of B cells, 37-47% of granulocytes in the spleen five months post-transplantation. Xenoantibodies directed at purified α-gal carbohydrate and pig cells were examined by ELISA prior to and following bone marrow transplantation, RRBC immunization, and transplantation with gal^+ hearts. Anti-gal xenoantibodies dropped to background levels in mice that were 2-6 months of age at transplant, and antibodies to pig cells were not detected by flow cytometric cytotoxicity. Solid organ gal^+ heart grafts transplanted into knockout mice transplanted 12 weeks earlier with gal^+ transduced stem cells (from both primary and secondary transplants) have not rejected to date (48 days post-transplantation), whereas controls rejected within 12 days. These results demonstrate that the high levels of stable chimerism achieved by the use of lentiviral vectors to transduce bone marrow cells is associated with long-term tolerance to gal^+ hearts in knockout mice.

Abstract# 1240

INDEFINITE XENOGENEIC CARDIAC SURVIVAL IN INTERLEUKIN-12 AND B CELL KNOCKOUT MICE: A DUAL ROLE FOR INTERLEUKIN-12 IN XENOGENEIC TRANSPLANTATION REJECTION. Karoline A. Hosiawa,[1,3] Hao Wang,[2] Mark E. DeVries,[1,3] Bertha Garcia,[2,3] Robert Zhong,[2,3] David J. Kelvin.[1,3] *[1]Experimental Therapeutics, Toronto General Hospital-University Health Network, Toronto, ON, Canada; [2]Pathology and Surgery, London Health Sciences Centre, London, ON, Canada; [3]Microbiology and Immunology, University of Western Ontario, London, ON, Canada.*

INTRODUCTION: We have previously reported that IL-12 has beneficial effects in protecting against acute vascular rejection (AVR) in a rat-to-mouse cardiac transplant model. Heart xenografts in C57BL/6 mice are rejected in 21 days, whereas, heart xenografts in C57BL/6-*IL-12*$^{-/-}$ mice are rejected in 6 days. The precise mechanism of action remains unknown. The present studies were undertaken to determine how IL-12 regulates xenogeneic rejection. **MATERIALS & METHODS:** Hearts from two-week-old Lewis rat hearts were transplanted heterotopically into twelve week old mice. B cell activation was examined by flow cytometry. mRNA expression was quantitated by Real-Time PCR. **RESULTS:** The number of activated ($IgM^+/CD69^+$) splenic B cells is elevated by 42% in C57BL/6-*IL-12*$^{-/-}$ mice vs. C57BL/6 mice on POD6. Xenograft hearts in C57BL/6-*Igh-6*$^{-/-}$ (B cell deficient) recipients are rejected in 51 days, in contrast, xenografts survive past 100 days in C57BL/6-*IL-12*$^{-/-}$ *Igh-6*$^{-/-}$ double knockout recipients.

Strain	N	Mean Survival + SD	Rejection Type
C57BL/6	15	21+4.5	AVR + CMR
C57BL/6-IL-12-/-	10	6+ 1	AVR
C57BL/6-Igh-6-/-	11	51 + 6	CMR
C57BL/6-IL-12-/-Igh-6-/-	7	>100	-

H/E staining of xenograft heart tissue at POD100 in double knock mice shows no evidence of thrombosis, hemorrhage, infarction, vasculitis, or inflammatory cell influx. Xenograft hearts in C57BL/6- *Igh-6*$^{-/-}$ recipients show increased infiltration with $CD8^+$ and $CD4^+$ T cells than in C57BL/6-*IL-12*$^{-/-}$ *Igh-6*$^{-/-}$ recipients at POD45. Xenograft hearts in C57BL/6- *Igh-6*$^{-/-}$ recipients show elevated Granzyme A/B mRNA expression indicative of cytotoxic T lymphocyte (CTL) activity, in comparison to C57BL/6-*IL-12*$^{-/-}$ *Igh-6*$^{-/-}$ recipients. **CONCLUSION:** IL-12 has a dual role in regulating xenograft rejection: 1) IL-12 suppresses xeno B cell responses which drive rapid AVR; and 2) IL-12 promotes a cell mediated rejection in absence of B cell responses. Xenograft rejection in absence of B cells is mediated by CTL. In summary, IL-12 protects against xenograft rejection by blocking xeno-specific B cell activation, however, this protection is counterbalanced by IL-12 promoting xeno-specific CTL responses. Understanding the role of IL-12 action in xenograft survival may lead to new potential therapeutic measures. This work was supported by grants from CIHR, MOTS, ORDCF, and CANVAC.

Abstract# 1241

DISTINCT SUBSETS OF DENDRITIC CELLS REGULATE THE PATTERN OF ACUTE VASCULAR XENOGENEIC REJECTION. Hao Wang,[1,2] Nobuyuki Kanai,[2] Weiping Min,[2] Bertha Garcia,[3] Robert Zhong.[1-3] *[1]Department of Surgery, London Health Sciences Center, London, ON, Canada; [2]Immunology and Transplantation Group, John P. Robarts Research Institute, London, ON, Canada; [3]Department of Pathology, The University of Western Ontario, London, ON, Canada.*

Dendritic cells (DCs) play a critical role in dictating immune responses. However, the role of DCs in regulating acute graft rejection following xenotransplantation remains unknown. We previously reported that rat heart grafts in C57BL/6 mice were rejected with cell-mediated rejection (CMR) in 21 days, whereas same grafts in BALB/c mice were rejected with acute vascular rejection (AVR) in 6 days (*Nature Medicine* 2000). The present study was undertaken to determine whether distinct subclasses of DCs direct Th1/Th2 cytokine production and regulate pattern of xenograft rejection. Hearts from two-week-old Lewis rats were heterotopically transplanted into mice. At endpoint rejection, heart grafts in C57BL/6 recipients showed predominant expression of IL-2, IFN-γ and IL-12 (Th1 cytokines), while BALB/c recipients displayed predominant intragraft expression of IL-4 and IL-10 (Th2 cytokines). Splenic DCs measured by flow cytometry demonstrated that population of $CD11c^+CD8\alpha^+$ cells (DC1-type) and $CD11c^+IL\text{-}12^+$ cells in C57BL/6 recipients were significantly higher than those of BALB/c recipients. Notably, adoptive transfer of bone marrow DCs from $IL\text{-}12^{-/-}$ C57BL/6 mice, in which $CD11c^+CD11b^+$ cells (DC2-type) were predominant, to wild-type C57BL/6 recipients significantly reduced the graft survival time from 21 ± 4.5 days to 9.5 ± 0.6 days. The pattern of graft rejection was also shifted from CMR to typical AVR, characterized by interstitial hemorrhage, intravascular thrombosis, fibrin deposition and increased levels of IL-4 and IL-10 in these mice. We conclude that 1) high population of DC1-type in C57BL/6 mice facilitates Th1 cytokine expression and attenuates AVR; 2) low population of DC1-type in BALB/c mice is associated with Th2 cytokine expression and the development of AVR; 3) transfer of DC2-type cells accelerates xenograft rejection in C57BL/6 recipients. These data suggest that distinct subsets of DCs play an essential role in regulating cytokine profiles and immune responses in a rat-to-mouse heart transplantation model.

Abstract# 1242

CELLULAR IMMUNITY TO XENOGRAFTS DOES NOT DEPEND ON RECIPIENT TOLL-LIKE RECEPTORS OR SECONDARY LYMPHOID ORGANS. Daniel R. Goldstein,[1] Bethany M. Tesar,[1] Fadi G. Lakkis.[1] *[1]Internal Medicine, Yale University, New Haven, CT.*

Introduction It is not completely understood how cellular immune responses to xenografts are initiated. Therefore, we utilized a porcine to murine skin xenograft model to examine whether acute cellular xenograft rejection is dependent on the presence of recipient Toll-Like Receptors (TLRs, critical innate immune receptors for pathogen recognition) and secondary lymphoid organs (required for initiating an immune response). Split porcine skin grafts were transplanted onto the lateral thorax in the following groups. **Results**

Table 1 Experimental groups + results

Recipient	Survival time (days)	P value vs. WT
Wild Type (WT)	13,14,17,17,19,19,19,20,21,21,22,22,23,25	
MyD88 KO	12,17,19,19,19,22,23,24,26	0.35
Aly/Aly	17,20,21,29,29	0.44
Aly/Aly -no spleen	15,17,19,21,23	0.77
WT -spc	15,18,19,20,21	0.5
RAG KO	>100 x 5	0.0001
Aly/Aly no spleen + (NK1.1)	14,20,21,23	0.89

All recipients B6 strain background, **Aly/Aly**= Alymphoplasia mutation:mice lack Peyers patches + lymph nodes, **MyD88 KO**= universal TLR signal adaptor deficient, **NK1.1**= NK depleting Ab.

All groups rejected porcine xenografts in the same tempo except RAG KO (B + T cell deficient). Naive B6 animals did not demonstrate the presence of preformed anti porcine IgM or IgG Abs. WT animals demonstrated an induced IgG donor specific response. In contrast, splenectomized Aly/Aly (i.e. no secondary lymphoid organs) did not demonstrate an IgG or IgM response (see table 2 below).

Table 2 Presence of Donor Specific Xeno Ab by Flow cytometric Crossmatch

Group	IgM	IgG	P value vs. Isotype
Isotype control	19+/-13	12+/-3	
Naive B6	24+/-10	19+/-6	IgM =0.77, IgG=0.39
WT	40+/-3	115+/-22	**IgG=0.01**, IgM=0.33
Aly/Aly -Spc	10+/-1	10+/-1	IgM=0.8, IgG=0.51

All measurements = median fluorescent intensity. Levels determined at day +14 post transplantation.

Conclusion Acute cell mediated xenograft rejection is dependent on an adaptive immune response. However, adaptive immunity to xenografts does not depend on recipient TLR expression, NK cells or secondary lymphoid organs and can occur at a normal tempo in the absence of donor specific antibodies. This work demonstrates, unlike the immune response to allografts, that the cellular immune response to xenografts occurs outside secondary lymphoid organs.

Abstract# 1243

ROLE OF DN REGULATORY T CELLS IN LONG-TERM CARDIAC XENOGRAFT SURVIVAL. Wenhao Chen, Megan S. Ford, Kevin J. Young, Myron I. Cybulsky, Li Zhang. *[1]Department of Laboratory Medicine and Pathobiology, Mutiple Organ Transplantation Program, University Health Network, University of Toronto, Toronto, ON, Canada.*

A novel subset of $CD3^+CD4^-CD8^-$ (double negative; DN) regulatory T cells has recently been shown to induce donor-specific skin allograft acceptance following donor lymphocytes infusion (DLI). In this study, we investigated the effect of DLI on rat-to-mouse cardiac xenotransplant survival and the ability of DN T cells to regulate xenoreactive T cells. METHODS: The C57BL/6 mice were given either DLI from Lewis rats, a short course of depleting anti-CD4 monoclonal antibody (mAb), both DLI and anti-CD4 treatment together, or left untreated. All mice were transplanted with a Lewis heart. The survival of grafts were monitored daily. The numbers of $CD4^+$, $CD8^+$, and DN T cells were monitored in the spleens and grafts of recipient mice. Furthermore, the ability of splenic and graft infiltrating DN T cells to suppress anti-donor T cells was assessed. RESULTS: DLI alone did not prolong graft survival when compared to untreated controls. Although anti-CD4 depleting mAb alone significantly prolonged graft survival, the grafts were eventually rejected by all recipients. Interestingly, the combination of DLI and anti-CD4 treatment induced permanent (>200 days) cardiac xenograft survival. Furthermore, we demonstrate that recipients given both DLI and anti-CD4 treatment have a significant increase in the number of DN T cells in both spleens and grafts, and a significant decrease in the number of $CD8^+$ T cells in grafts after transplantation when compared to recipients with the other treatments. More importantly, Both splenic and xenograft infiltrating DN T cells from DLI plus anti-CD4 treated mice could inhibit the proliferation of anti-donor T cells in a dose-dependent and antigen-specific manner. These results demonstrate that a combination of pretransplant DLI and anti-CD4 depleting mAb can induce permanent survival of rat-to-mouse cardiac xenografts. Both peripheral and graft infiltrating DN T cells can suppress the proliferation of anti-donor T cells, which may play an important role in maintaining the survival of concordant cardiac xenografts.

Abstract# 1244

XENOGENEIC VASCULARIZED THYMIC LOBE TRANSPLANTATION AS A POTENTIAL METHOD OF TOLERANCE INDUCTION IN A LARGE ANIMAL MODEL OF PIG-TO-PRIMATE TRANSPLANTATION. Parsia A. Vagefi, Shin Yamamoto, Chisako Kamano, Hitoshi Arakawa, Katsuhito Teranishi, Megan Sykes, David H. Sachs, Kazuhiko Yamada. *[1]Department of Surgery, Transplantation Biology Research Center, Massachusetts General Hospital/Harvard Medical School, Boston, MA.*

Background: We have previously reported a technique for vascularized thymic lobe (VTL) transplantation in miniature swine, and have now investigated whether or not VTL grafts can support thymopoeisis and induce donor-specific unresponsiveness across xenogeneic (pig-to-baboon) transplantation barriers. **Methods:** Donor thymus was meticulously harvested from pigs transgenic for human Decay Accelerating Factor. Experimental baboons (n=3) received VTL grafts and omental thymic tissue implants after undergoing thymectomy and splenectomy. Immunosuppression consisted of a 28-day regimen of cyclophosphamide/ anti-CD40L/ ATG/ MMF/ Methylpredinisolone/ FK506/ antibody immunoadsorption/ and cobra venom factor. VTL grafts were biopsied for histological and FACS analyses. Control (n=2) baboons received the same immunosuppressive regimen. One control underwent sham transplantation without receiving porcine tissue, and a second control received an IV infusion of porcine thymocytes (5x10^8 thymocytes/kg) on Day 0. Recipient immunological status was assessed by MLR. **Results:** All three recipients of xenogeneic VTL grafts developed donor-specific unresponsiveness by POD 30; in contrast, control recipients demonstrated normal anti-pig MLR responses (sham), or even sensitization to the donor (thymocyte infusion). Xenogeneic VTL recipients demonstrated immature baboon thymopoiesis with $CD3^-CD44^+CD25^+$and $CD44^+CD4^{low}$ cells. In addition, VTL recipients demonstrated peripheral reconstitution of CD45RA/CD4 DP native T-cells, while control recipients did not. **Conclusions:** This study demonstrates that VTL transplantation is feasible across a discordant xenogeneic barrier in large animals. To our knowledge, this is the first demonstration of thymopoiesis and donor-specific unresponsiveness by VTL transplantation in a large animal xenograft model. If used in combination with strategies aimed at inhibiting humoral rejection, VTL transplantation offers a promising strategy for tolerance induction across xenogeneic barriers.

Abstract# 1245 Poster Board #-Session: P1-III

MINIMIZING RISK OF ACUTE RENAL REJECTION IN SENSITIZED PATIENTS: PREEMPTIVE THERAPY WITH PLASMAPHERESIS, ANTI-TNFa mAb AND LOW-DOSE OKT3. Nina Babel,[1] Georg Cherepnev,[2] Constanze Schoenemann,[3] Hans D. Volk,[2] Petra Reinke.[1] *[1]Nephrology and Intensive Care, Humboldt University, Charite, Campus Virchow, Berlin, Germany; [2]Immunology, Humboldt University, Charite, Campus Mitte, Berlin, Germany; [3]Blood transfusion, Humboldt University, Charite, Campus Virchow, Berlin, Germany.*

Background: In kidney transplantation, acute vascular rejection (AVR) represents a challenging clinicopathological entity carrying a poor prognosis. Approximately 10-15% of kidney transplant recipients are highly sensitized. In 50-80 % of cases, allograft failure due to AVR occurs within the first three months postransplant even if no donor-specific antibodies were detectable before Tx.. About 50% of patients with AVR are resistant to both steroid and antilymphocyte therapy. The best treatment of this condition remains undefined. The involvement of both humoral and cellular immune processes are discussed. Previously it has been demonstrated that combination of OKT-3 and plasmapheresis is very effective in reversing steroid-resistant acute rejections in high-risk patients. We hypothesized that a preemptive approach rather than therapeutic rejection-related use of plasmapheresis and OKT-3 might improve outcomes and decrease the risk of overimmunosuppression. Methods: So we developed a protocol with preemptive low-dose OKT 3 combined with plasmapheresis and anti-TNF mAb (to reduce initial NFkB related-activation of APC in sensitizied patients). We treated 8 highly sensitized patients (high rate of pretransplant PRA, repeated loss of grafts in history) with OKT-3 at d 0+1 (2.5 mg/d), plasmapheresis before transplantation and during the first 5 days post-Tx. In 3 cases, the patients additionally received 1x400 mg anti-TNF-a mAb. All patients received triple-drug maintenance immunosuppression (FK506, MMF, methylprednisolone). Results: All patients showed normalization of creatinine (0.9+/-0.2 mg/dl) within 2-20 weeks (mean 5.6+/-7). An episode of acute rejection was observed in one patient only, which was succsessfully treated with steroid pulse. The patients showed stable creatinine level in the follow up of one year. Conclusion: The preliminary results of the current study demonstrate the value of combined preemptive use of low-dose OKT-3, plasmapheresis and anti-TNF-alpha therapy in the prevention of acute graft failure in sensitized high-risk recepients. Elimination of memory/effector T cells by OKT3, removal of PRA and DRA by plasmapheresis, and prevention of local non-specific factors of innate response by anti-TNF-a-Ab contribute to the improvement of the clinical outcome in the sensitized patient group.

Abstract# 1246 Poster Board #-Session: P2-III

THE THERAPY OF DOUBLE FILTRATION PLASMAPHERESIS IN THE SENSITIZED RECIPIENTS OF CADAVERIC KIDNEY TRANSPLANTATION. Hongfeng Huang, Jianghua Chen, Yimin Wang, Jianyong Wu, Jianguo Zhang. *[1]The Nephrology Center,The 1st Affiliated Hospital, Medical College,Zhejiang University, Hangzhou, Zhejiang, China.*

Objective To evaluate therapeutic effect of double filtration plasmapheresis(DFPP) for sensitized recipients. **Methods** Penal reactive antibody (PRA) was examined with ELISA method. 50 highly sensitive recipients(PRA>40%) was carried out before kidney transplantation. **Result** After the treatmant of DFPP,the PRA level of 19 recipients turned negative(PRA<10%).In 50 recipients , the level of PRA of 50 patients decreased the range from 63.1%±20.8%% to 19.2%±21.2%, decreased by an average of 43.4%±19.2% (P< 0.001). The 50 sensitized recipients had received kidney transplantation.The incidence rate of hyperacute rejection(HAR) was 2%(1/50); the incidence rate of acute rejection(AR) was 28%(14/50). In the 15 rejected patients ,12 cases reversed with anti-rejection therapy including DFPP. The graft/patient 1 year survival rate of 50 sensitive recipients was 98%(49/50) and 92%(46/50). **Conclusion** DFPP can remove the sensitive antibody and renders highly sensitive recipients gaining the chance of transplantation. It not only reduces the incidence of rejection but also increased the success of anti-rejection therapy. The graft/patient 1 year survival rate is satisfactory.

Abstract# 1247 Poster Board #-Session: P3-III

IMMUNOADSORPTION, IMMUNOMODULATION, SELECTIVE TARGETING OF B-LYMPHOCYTES AND QUADRUPLE MAINTENANCE IMMUNOSUPPRESSION IN THE MANAGEMENT OF HIGHLY SENSITIZED LIVING DONOR KIDNEY TRANSPLANT WITH POSITIVE CROSS-MATCH. Khalid A. Almeshari,[1] Khalid Shaibani,[1] Ibrahim Al Ahmadi,[1] Ahmed Chaballot,[1] Khalid Hamawi,[1] Abdulghani Tabakhi,[2] Arla Manser, Samhar Akash,[1] Hazem El Gamal,[1] Magdy Salhy,[1] Syed Raza,[1] Kesavamuthy Mohan.[1] *[1]Renal Transplant Program, King Faisal Specialist Hospital and Research Centre, Riyadh, Central Province, Saudi Arabia; [2]Department of Pathology and Laboratory, King Faisal Specialist Hospital and Research Centre, Riyadh, Central Province, Saudi Arabia.*

Introduction: Allosensitization represents a major barrier to kidney transplantation. The aim of this study was to examine the feasibility of living donor positive cross-match (XM) kidney transplantation in highly sensitized individuals, after desensitization with a new protocol. **Methods:** We performed three positive cross-match kidney transplants by complement dependent cytotoxicity (CDC) and flow cytometry (FC), in three highly sensitized individuals (one male and two females, PRA was persistently ³ 65% by FC). HLA class I specific antibodies were determined by ELISA and FC. Immunosuppression consisted of: 1) Pre-transplant protein A immunoadsorption (IA) and intravenous immunoglobulin (IVIG). The transplant was performed if XM was negative by CDC and FC, 2) Peri-transplant induction with ATG and rituximab (single dose of 375 mg/m2 BSA), 3) Post transplant quadruple maintenance immunosuppression consisting of tacrolimus, rapamycin, mycophenolate mofetil and prednisone. Tacrolimus and rapamycin doses were concentration – controlled to keep trough levels of 5 – 10 and 10 – 15 m g/L for tacrolimus and rapamycin respectively, during the first 3 months post-engraftment. **Results:** The three patients were successfully desensitized and transplanted from one haplotype-matched donor.

Patients	PRA (baseline)	PRA (Pre)	F/U (weeks)	Cr (µmol/L)
1	85%	40%	10	89
2	65%	12%	4	200
3	92%	44%	1	140

Conclusion: Living donor kidney transplantation can be performed successfully in highly sensitized, positive cross-match recipients with a regimen consisting of pre transplant IA and IVIG, peri-transplant induction with ATG and rituximab, and post-transplant quadruple maintenance immunosuppression. Although long-term follow up is needed, this protocol represents a promising option for individuals who otherwise are unlikely to receive a transplant.

Abstract# 1248 Poster Board #-Session: P4-III

3 YEAR RESULTS OF A PROSPECTIVE TRIAL USING DEPLETING ANTIBODY INDUCTION IN COMBINATION WITH TACROLIMUS BASED IMMUNOSUPPRESION IN HIGHLY SENSITIZED/HIGH RISK RENAL TRANSPLANT RECIPIENTS. Jeffrey S. Zaltzman,[1] Vivian C. McAlistar,[2] David Russell,[3] Phillip Halloran,[4] David N. Landsberg,[5] Stephan Busque,[6] Ahmed Shoker,[7] Anne Boucher,[8] Jean I. Tchervenkov,[9] Jean Shapiro.[10] *[1]Medicine, Division of Nephrology, St. Michael's Hospital, Toronto, ON, Canada; [2]Surgery, QEll Health Sciences centre, Halifax, NS, Canada; [3]Medicine, Division of Nephrology, St. Joseph's Hospital, Hamilton, ON, Canada; [4]Medicine, Division of Nephrology, University of Alberta Hospital, Edmonton, AB, Canada; [5]Medicine, Division of Nephrology, St. Paul's Hospital, Vancouver, BC, Canada; [6]Surgery, Hopital Notre Dame, Montreal, QC, Canada; [7]Medicine, Division of Nephrology, Royal university Hospital, Saskatoon, SK, Canada; [8]Medicine, Division of Nephrology, Hopital Maisonneuve-Rosemount, Montreal, QC, Canada; [9]Surgery, Royal Victoria Hospital, Montreal, QC, Canada; [10]Medicine, Division of Nephrology, Vancouver General Hospital, Vancouver, BC, Canada.*

Backround: Increased risk of rejection and allograft loss complicates the management of high immunological risk renal transplantation and is a significant challenge for clinicians.We report the 3 year follow-up data from a prospective study designed to assess the potential of a combination of immunosuppressive agents to improve outcomes in such patients. **Methods:** Fifty-nine "high risk" renal allograft recipients were enrolled in 10 Canadian sites and given a regimen of: depleting antibody induction, tacrolimus, mycophenolate mofetil, and corticosteroids. Patients included: 10(17%) who had lost a previous graft to rejection <1 year, 31(53%) with a current PRA>30%, 47 (80%) with a historic PRA>50%, 4(7%) who had a previous positive cross-match with the current donor, and 6(10%) with a current positive B-cell cross-match. **Results:** At 3 years post-transplant, patient and allograft survival was 88.5% and 73.4%. Over 3 years there were 15 graft loss in total, 10 in the first 6 months, 3 losses between months 12-24, and 2 between month 24-36. There were 11 graft losses other than death with functioning graft, of which 6 were preceded by delayed graft function (p=0.01, X^2). Sixteen(27%) recipients experienced at least 1 biopsy-confirmed acute rejection, 5 Banff l,7 Banff ll, 4 Banff lll. CMV infection was seen in 16 patients(27%). Over 36 months 8 patients who were non-diabetic at baseline required therapy for diabetes(13.5%).**Conclusions:**An aggressive immunosuppresive strategy is warranted for high immunologic risk renal transplant recipients, and demonstrates improved long-term results, not previously described.

Abstract# 1249 Poster Board #-Session: P5-III

OPTIMIZATION OF NEORAL® THERAPY COMBINED WITH BASILIXIMAB INDUCTION PRODUCES VERY LOW ACUTE REJECTION RATE IN RENAL TRANSPANT RECIPIENTS IN THE EARLY POST-TRANSPLANT PERIOD. Robert Mendez,[1] Jimmy Light,[2] Tom Pearson,[3] You Min Wu,[4] John Curtis,[5] Flavio Vincenti.[6] *[1]National Institute of Transplantation, Los Angeles, CA; [2]Washington Hospital Center, Washington, DC; [3]Emory University, Atlanta, GA; [4]University of Iowa Hospitals and Clinics, Iowa City, IA; [5]University of Alabama at Birmingham, Birmingham, AL; [6]University of California at San Francisco, San Francisco, CA.*

Purpose: Cyclosporine-based immunosuppression utilizing 2-hour cyclosporine (CsA) concentrations (C2) as a guide for Neoral® dose optimization, in addition to basiliximab induction, is being evaluated in this prospective multi-center study of *de novo* renal transplant recipients. Two month interim analysis is presented. **Methods:** *De novo* adult renal transplant recipients were recruited at 12 U.S. centers. All subjects were scheduled to receive quadruple immunosuppressive therapy. Neoral was initiated at 10 mg/kg/day and titrated to C2 targets of 1.7 and 1.5 µg/ml for months 1 and 2, respectively. Additional immunosuppression included basiliximab 20 mg on days 0 and 4, mycophenolate mofetil 1 gm BID, and prednisone per center protocol. Non-IL-2-receptor antibody therapy was permitted peri-operatively in patients with delayed graft function (DGF). At two months, patients are randomized to one of two groups with different C2 target ranges for the duration of the 6 month study. **Results:** This interim report includes 84 patients with a mean age of 47 ± 13 years in which 76% were male, 25% African American, and 61% recipients of cadaveric grafts. Mean follow-up was 73 days. Cold ischemia time (CIT) was a median 14.1 hours. DGF developed in 24% of subjects. DGF and immediate graft function (IGF) patients had median CITs of 19.9 and 9.1 hours, respectively. Biposy proven acute rejection developed in 4% of patients (1 DGF and 2 IGF). There has been 1 patient death and 1 graft failure. C2 levels, Neoral dose, and serum creatinine (SrCr) during the 2 month follow-up were as follows (mean ± SD):

	Day 3	Day 7	Day 14	Day 28	Day 60
C2 Level(µ/ml)	1.2 ±0.6	1.3±0.5	1.6±0.6	1.5±0.6	1.6±0.6
Neoral Dose (mg/kg/d)	8.9±2.6	10.0±3.6	9.8±3.3	8.6±3.2	6.9±2.7
SrCr (mg/dl)	4.6±2.9	3.2±2.6	2.3±1.8	1.7±0.6	1.5±0.7

Safety and tolerability were excellent with only 12 serious adverse events related to cyclosporine in which 6 were gastrointestinal disorders; none were related to CsA nephrotoxicity. **Conclusions:** In renal transplant recipients, optimization of Neoral therapy with C2 monitoring combined with basiliximab induction, mycophenolate mofetil, and prednisone provides very good early post-transplant acute rejection prophylaxis in a representative U.S. transplant population.

Abstract# 1250 Poster Board #-Session: P6-III

AFRICAN-AMERICANS (AA) HAVE IMPROVED LONG-TERM RENAL ALLOGRAFT SURVIVAL WITH MYCOPHENOLATE MOFETIL (MMF) AND DACLIZUMAB (D). Carlton J. Young,[1] Clifton Kew,[2] Sharon Hudson,[1] Michael Gallichio,[1] Bruce Julian,[2] John Curtis,[2] Mark Deierhoi,[1] Robert Gaston.[2] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL; [2]Medicine, University of Alabama at Birmingham, Birmingham, AL.*

AA have worse long-term renal allograft survival compared to Caucasians (C). The aim of this retrospective study was to determine the impact of MMF and D on long-term graft survival as well as determine risk factors influencing that survival. **METHODS:** From 1/95 to 6/02, 1289 CAD renal allografts were transplanted at our institution (AA, N=659; C, N=625). Immunosuppressive therapy varied among three eras. ERA 1 (1/95-5/95) Sandimmune/Aza/Steroids(S)/OKT3 (O) or ATGAM (AG); ERA 2 (5/95-5/98) Neoral (N)/MMF/S/ O or AG; and ERA 3 (5/98-6/02) N or FK/MMF/S/Daclizumab (D-two dose). Data was retrieved from our transplant database. There were no significant demographic differences between AA and C. Actuarial analysis was produced by Kaplan-Meier. Survivorship and a hazard function was provided by a three phase parametric model that identified an early phase (<6 months) and a constant phase (> 6 months) to demonstrate risk factors. **RESULTS:** 1-yr graft survival improved for AA and C from ERA 1 to ERA 3 (AA, 79% ⇒ 91%; p=0.005); (C, 81% ⇒ 95%; p=0.003). But, 3-year graft survival for ERA 3 was statistically better for C compared to AA, (87% v. 80%; p= 0.02). 12 month mean serum creatinine was significantly better for C versus AA in all eras (ERA 1: 1.41 v. 1.75; p=0.02; ERA 2: 1.52 v. 2.14; p=0.03; ERA 3: 1.58 v. 1.93; p=0.04). Significant risk factors in Table 1.

TABLE 1: RISK FACTORS FOR GRAFT LOSS

Early Phase (< 6 months)	p value	Constant Phase (> 6 months)	p value
Donor Age >60	0.02	African-American	0.0001
ERA 1 Therapy	0.01	ERA 1 Therapy	0.0001
ABDRMM>0	0.03	Donor Age >60	0.02

From ERA 1 to ERA 3, half-life improved for both AA and C.

TABLE 2: HALF-LIFE IN MONTHS BY ERA

	AA	C	p value AA v. C
ERA 1	53.7	99	0.01
ERA 2	93	151	0.003
ERA 3	97	180	0.004

CONCLUSIONS: (1) MMF and D significantly improved graft survival for AA and C. (2) Mean serum creatinine at 12 months was higher for AA. (3) ERA 1 therapy was a significant risk factor for graft loss for AA and C. (4) Donor age >60 was a risk factor for both early and late graft loss. (5) AA while improving graft survival and half-life with ERA 2 and ERA 3 therapy, they continue to lag significantly behind C for long-term graft survival. (6) Additional studies are needed to determine reasons for the continued poor long-term graft survival in AA.

Abstract# 1251 Poster Board #-Session: P7-III

RAPID STEROID ELIMINATION PROTOCOL (RSEP) IN LIVING DONOR RENAL TRANSPLANT RECIPIENTS (LRTS) USING BASILIXIMAB INDUCTION THERAPY. Anne M. Wiland,[1] Benjamin Philosophe,[1] Jeffrey C. Fink,[2] Stephen T. Bartlett.[1] *[1]Transplant Services, University of Maryland Medical Center, Baltimore, MD; [2]Nephrology, University of Maryland School of Medicine, Baltimore, MD.*

Antibody induction is controversial in LRTs due to the low incidence of delayed graft function and high cost of induction, but may be required to eliminate the need for prednisone maintenance therapy without increasing acute rejection rates. Early steroid elimination or avoidance is desirable to avert the long-term adverse effects associated with steroid use. In this study, we compared LRTs who received no antibody induction maintained on triple drug therapy with tacrolimus (FK506), mycophenolate mofetil (MMF) and a standard prednisone taper (standard therapy group) with LRTs induced with basiliximab, 3 doses of methylprednisolone and maintenance therapy of FK506 and MMF only (RSEP). The primary outcome measures were 6-month and overall rejection rates. 35 LRTs (22 living related and 13 living unrelated) followed the RSEP and 72 LRTs (49 living related and 23 living unrelated) received standard therapy. The median follow-up for the RSEP LRTs was significantly shorter than the LRTs receiving standard therapy (7.1±0.54 vs.17.8±0.54 months, p=0.001, respectively). There was no difference in age, sex, peak PRA, CMV status or AB and DR mismatch between the groups. The mean initial hospitalization length of stay (LOS) was lower in the RSEP LRTs (6.8±3.7 vs. 10.1±21.7 days, p=0.06) although there was no difference in the median LOS between groups (5.0 days each group). There was no difference in overall rejection rates between groups although rejection occurred earlier in the RSEP protocol LRTs. Thirty (85.7%) RSEP LRTs currently remain off prednisone.

	RSEP	Standard Therapy	p-value
#LRTs with rejection	7	15	0.92
6-month rejection rate	17.1%	13.9%	0.62
Overall rejection rate	20%	20.8%	0.66
Mean time to rejection (days)	31.9±66	101.1±142	0.015

One graft loss occurred in the standard therapy group. The most recent mean serum creatinine was 1.35±0.36 mg/dL in the RSEP group and 1.57±0.88 mg/dL in the standard therapy group (p=0.16). Hospital readmissions for new onset glucose intolerance or diabetes post-transplant occurred in none of the RSEP LRTs and 4 standard therapy LRTs. This preliminary analysis indicates that RSEP with basiliximab induction therapy provides the ability for steroid-free maintenance immunosuppression without increasing rejection rates or compromising short-term graft survival. Other beneficial effects such as reduced incidence of post-transplant glucose intolerance or diabetes were also observed.

Abstract# 1252 Poster Board #-Session: P8-III

SIMULTANEOUS CORTICOSTEROID AVOIDANCE AND MINIMIZATION OF CALCINEURIN INHIBITORS. J. W. Alexander,[1] T. J. Metze,[1] H. Goodman,[1] M. Cardi,[2] J. Austin,[2] S. Goel,[2] S. Safdar,[2] J. Fidler,[1] R. Alloway,[1] J. Buell,[1] M. Hanaway,[1] B. Suskind,[1] N. Greenburg,[3] E. S. Woodle.[1] *[1]Department of Transplantion, The University of Cincinnati, Cincinnati, OH; [2]Department of Transplantation, The Christ Hospital, Cincinnati, OH; [3]Novartis Nutrition, Novartis Incorporated, St Louis Park, MN.*

The purpose of this study was to evaluate a unique immunosuppressive protocol which utilizes immunonutrients. **Methods:** This protocol for renal transplantation (TX) Thymoglobulin 1.5 mg/kg (avg 2.7 doses), rapamycin (RAPA) 2 mg/day, mycophenolate mofetil (MMF) 2 gm/day, low dose cyclosporin (CsA)[Phase one (18pts) 4 mg/kg (200 ng/ml), Phase 2 (36 pts) 2mg/kg(100 ng/ml), Phase 3 (20 pts) 1 mg/kg (target n/a)], L. Arginine and supplemental omega 3 / omega 9 fatty acids. **Results:** 74 pts enrolled before 11/25/2002 included 62 Cauc/11 AA/1 other; 37 LRD/12 LUD/25 CAD pts; 69 1st TX, 5 2nd TX; and 34 diabetics. One EBV neg pt received an EBV + graft and died from PTLD on day 108. Two first CAD TXS died from MI on post transplant day 3 and day 9. (overall patient survival 96 %) Two pts have undergone TX nephrectomy, one after transplantation of a S. marcescens contaminated kidney and the second in an and EBV neg pt who developed PTLD after reveiving and EBV pos kidney. (overall graft survival 93 %). The mean follow-up was 459 days (std 245.2). Currently 87.5 % of the pts are rejection free. One pt is currently taking maintenance steroids due to a reoccurrence of original disease but the remaining pts are steroid free. 50/53 patients that have reached six months have discontinued CsA per protocol. The patients that have been followed for 2 years have discontinued MMF and remain on RAPA and dietary

supplements only. None of the patients at the time of this report have developed post transplant diabetes mellitus. **Conclusion:** The initial experience with this corticosteroids free/calcineurin inhibitor sparing regimen is associated with 1) infrequent rejections, 2) good blood pressure control, 3) excellent renal function, 4) freedom from PTDM, 5) reduced renal toxicity, and 6) reduction to single dose therapy after 2 years.

Time (# of pts)	Avg B/P (mm/hg)(# of Meds)	MMF Dose (mg/Day)	Rapa Trough (ng/ml)	CsA Trough (ng/ml) (in order of phase)			WBC (10,000/ul)	SCr (mg/dl)	Chol/TG (mg/dl)
Pre-op (n=68)	145/82 (1.7)	n/a	n/a	P-1	P-2	P-3	n/a	n/a	n/a
1 month (n=62)	137/78 (1.7)	1476.1	9.6	209.9	109.6	105.3	4.7	1.8	232 / 224
3 month (n=59)	133/78 (1.5)	1338.5	11.1	182.7	113.6	92.8	4.4	1.4	212 / 197
6 month (n=53)	132/77 (1.5)	1208.6	9.6	132.9	92.7	0	4.8	1.3	199 / 251
12 month (n=47)	130/76 (1.6)	1187.5	8.7	109.5	0	0	5.7	1.5	222 / 189

Abstract# 1253 Poster Board #-Session: P9-III

STUDY DESIGN AND BASELINE CHARACTERISTICS OF A MULTIPLE DOSE, RANDOMIZED, CONTROLLED OPEN-LABEL STUDY COMPARING A COSTIMULATION BLOCKER BASED REGIMEN OF BMS-224818 (LEA29Y) VS. CYCLOSPORINE IN RENAL TRANSPLANT. Flavio Vincenti,[1] Antoine Durbach,[2] Christian Larsen,[3] Ferdinand Muhlbacher,[4] Bjorn Nashan,[5] Gilles Blancho,[6] Thomas Pearson,[3] Josep Grinyo,[7] Evren Atillasoy,[8] Kannan Natarajan,[8] David Hagerty,[8] James Burdick,[9] Jonathan Bromberg,[10] Bernard Charpentier,[2] the LEA29Y Study Group. *[1]UCSF; [2]Bicetre Nephrologie, France; [3]Emory; [4]General Hospital Vienna, Austria; [5]Klinik fur Viszeral-und Transplantations-Chirurgie, Germany; [6]Centre Hospitalo-Universitaire Nephrologie, France; [7]Hosptial de Bellvitge, Spain; [8]Bristol-Myers Squibb; [9]Johns Hopkins; [10]Mount Sinai.*

BACKGROUND: LEA29Y is a second-generation version of BMS-188667 (CTLA4Ig) that has increased avidity for CD80/CD86. LEA29Y blocks the second signal for T cell activation as a result of binding to CD28 ligands CD80 and CD86. Primate transplant experiments show that LEA29Y is superior to CTLA4Ig for prevention of acute rejection. OBJECTIVE: Assess the relative efficacy and safety of LEA29Y in renal transplant recipients compared to Neoral using a non-inferiority design. DESIGN: Cadaver or living renal allograft recipients receive background therapy with MMF 2gm/day, corticosteroids and 2 doses of basiliximab and are randomized 1:1:1 to maintenance treatment with LEA29Y (more intensive), LEA29Y (less intensive) or Neoral. Subjects were initially enrolled in 3 groups of 18 subjects with pauses in enrollment between groups, in order to observe subjects for safety and efficacy. Study personnel and subjects are blinded to the dose and schedule of LEA29Y but are not blinded to assignment of LEA29Y or Neoral. Primary endpoint is the incidence of clinically suspected and biopsy proven rejection at 6 months. Secondary endpoints are renal function, incidence of HTN, DM, hyperlipidemia, as well as graft and patient survival. An external safety board monitors the unblinded efficacy and safety of the study. RESULTS: Enrollment is complete and 228 subjects were randomized and treated. The study is ongoing. Preliminary characteristics are comparable in the two groups(see table). Characteristics of patients and donors will be discussed. CONCLUSION:LEA29Y represents a novel costimulation blocker with potential utility in non-calcineurin based regimens. This Phase II study will ultimately provide important information on the comparative efficacy and safety of LEA29Y vs. cyclosporine-based regimens.. As of December 2002, the safety board supports continuation of the study.

Preliminary Demographics of LEA29Y Study

Variable	LEA29Y	CsA
Mean Age	44.7	44.4
Male Sex	71	68
Race C(%) B (%)	C 81% B 9%	C 78% B 10%
% Patients with PRA > 20% ever	8%	7%

Abstract# 1254 Poster Board #-Session: P10-III

TACROLIMUS, BUT NOT CYCLOSPORINE, DOSE REQUIREMENT IS CORRELATED WITH CYP3A5 AND CYP3A4 GENOTYPE. Dennis A. Hesselink,[1] Ron H. N. van Schaik,[2] Peter J. H. Smak Gregoor,[1] Willem Weimar,[1] Teun van Gelder.[1,3] *[1]Internal Medicine; [2]Clinical Chemistry; [3]Pharmacy, Erasmus MC, Rotterdam, Netherlands.*

Background: The calcineurin inhibitors (CNI) cyclosporine A (CsA) and tacrolimus (TRL) are standard immunosuppressive drugs in many transplantation centers. However, both drugs have a narrow therapeutic index and show considerable interindividual variation in their pharmacokinetics (PK). Therefore, therapeutic drug monitoring is essential to avoid over -or underimmunosuppression. The poor oral bioavailability of CNI is thought to result from the actions of the main CNI metabolizing enzymes CYP3A4 and CYP3A5 and the multi-drug efflux pump, P-gp, encoded by the MDR1 gene. Recently identified genetic polymorphisms of these 3 enzymes could explain the observed interindividual variability in CNI PK. **Aim**: To determine the role of genetic polymorphisms in CYP3A4, CYP3A5 and MDR-1 with respect to interindividual variability in CsA and TRL PK. **Methods**: Kidney transplant patients receiving CsA (n=110) or TRL (n=64) were genotyped for CYP3A5∗3 and ∗6, CYP3A4∗1B and MDR-1 C3435T. CsA and TRL concentrations were analyzed in whole blood using the EMIT 2000 assay at 3 and 12 months after transplantation. Dose-adjusted pre-dose (C0) concentrations were calculated and correlated with genotype. **Results**: TRL dose-adjusted C0 was significantly higher in CYP3A5 ∗3/∗3 patients (n = 45 or 73%) when compared to ∗1/∗3 plus ∗1/∗1 (wild-type) patients (n = 17 or 27%): Median (range): 94 (34-398) vs. 61 (37-163) ng/ml per mg/kg; $p<0.0001$, Mann-Whitney U test. CYP3A4∗1B allele carriers (n = 10 or 16%) showed a significantly lower TRL dose-adjusted C0 compared to patients with the wildtype (∗1/∗1) genotype (n = 54 or 84%): Median (range): 57 (40-163) vs. 89 (34-398) ng/ml per mg/kg; $p<0.003$, Mann Whitney U test. No evidence was found supporting a role for the MDR-1 C3435T mutation in TRL PK. For CsA, neither of the 3 polymorphisms studied correlated with PK. **Conclusion**: CYP3A5 genotype is strongly correlated with TRL PK. Patients carrying the CYP3A5 ∗3/∗3 genotype need significantly less TRL to reach target concentrations and have a higher likelihood of reaching toxic drug concentrations. CYP3A4∗1B carriers need more TRL to reach adequate whole blood levels and are at risk of developing acute rejection.

Abstract# 1255 Poster Board #-Session: P11-III

VALIDATION OF THE PREDICTIVE VALUE OF LOW DONOR-SPECIFIC CYTOTOXIC T-LYMPHOCYTES FREQUENCIES TO SAFELY REDUCE IMMUNOSUPPRESSION AFTER KIDNEY-TRANSPLANTATION AT A ROUTINE BASIS. Jacqueline Rischen,[1] Barbara J. van der Mast,[1] Petronella de Kuiper,[1] Nicole M. van Besouw,[1] Peter J. H. Smak Gregoor,[1] Jan. N. M. IJzermans,[2] Carla C. Baan,[1] Willem Weimar.[1] *[1]Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands; [2]Surgery, Erasmus Medical Center, Rotterdam, Netherlands.*

Introduction: Prolonged use of immunosuppression can cause serious side effects. The severity and incidence of these side effects are directly related to the immunosuppressive load and therefore patients would benefit from tapering immunosuppression. At our center we previously demonstrated that in patients on calcineurin-inhibitors (CNI) with a low frequency of donor-specific cytotoxic T- lymphocytes (CTLpf) i.e. $< 10/10^6$ PBMC, conversion to mycofenolate mofetil (MMF) or Azathioprine (Aza), followed by a 50% dose reduction is a safe procedure one year after kidney transplantation. **Methods:** In January 2001 we started a prospective study to confirm these findings in patients who were already switched from CNI to MMF/Aza therapy. Patients with a stable graftfunction after conversion, no proteinuria and at least 2 years after kidneytransplantation were included. The median followup period after conversion was 3 months (range 1-12). The immunosuppression consisted of 2000 mg MMF or 2 mg/kg Aza and 10 mg prednisone. At this moment the donor-specific CTLpf was measured in a limiting dilution assay. At 4 and 8 months after inclusion the dose of MMF/Aza was reduced with 1/3rd. **Results:** In 71 patients the CTLpf was measured. In 43 patients the donor-specific CTLpf was low ($< 10/10^6$ PBMC). The CTLpf against a 3rd party was normal. Of these patients 34 had a living donor kidney and 9 has a postmortal donor kidney. The immunosuppression consisted in 24 patients of MMF and in 19 patients of Aza. At four month the immunosuppression was lowered in all 43 patients with a 1/3rd. At 8 month a second reduction has been performed in 35 patients. Twentyfive of the 43 patients have reached the endpoint of 12 months after inclusion and they all have a stable creatinine level without proteinuria. During the reduction period only one acute reversible rejection occurred. **Conclusion**: These results validate the predictive value of a low donor-specific CTLpf in kidneytransplant patients to safely reduce immunosuppression at a routine basis.

Abstract# 1256 **Poster Board #-Session: P12-III**

SIROLIMUS (SIR) AND TACROLIMUS (FK) IN RENAL TRANSPLANT: RESULTS OF A RANDOMIZED TRIAL OF A MAINTENANCE THERAPY BASED IN SIROLIMUS VERSUS THE COMBINATION OF SIROLIMUS AND TACROLIMUS. Josep M. Grinyo,[1] Josep M. Campistol,[2] Javier García,[3] Javier Paul,[4] José M. Morales,[5] Manuel Arias,[6] Dolores Prats.[7] *[1]Nefrología, Hospital Bellvitge, Barcelona, Spain; [2]H. Clínic, Barcelona; [3]H. La Fe, Valencia; [4]H. Miguel Servet, Zaragoza; [5]H.Doce Octubre, Madrid; [6]H.Valdecilla, Santander; [7]H.Clínico, Madrid.*

Background: The clinical experience with the concurrent use of SIR and FK in de novo renal allograft recipients is scarce. Aim: To compare renal function at 12th month post graft achieved with two different immunosuppressive regimens both with FK, SIR and steroids (STE) in the post operation period, one with long term maintenance with FK, SIR and STE and the other with FK withdrawal from the 3rd month and STE. Methods: Group I: SIR 2 mg/d po following a single loading dose of 6 mg on day 1, trough HPLC levels 4-8 ng/ml, FK 0.1 mg/kg/d trough levels 8-12 ng/ml first 3 months, and 5 to 10 ng/ml after 3rd month. Group II SIR 15 mg on day 1, following 5mg/d trough HPLC levels 8-16 ng/ml, FK 0.05 mg/kg/d trough levels 3-8 ng/ml with FK elimination during the fourth month. Due to the difficulties to achieve the target SIR and FK levels in both groups, an amendment was performed concerning loading doses: Group I SIR 3 mg/day following a triple dose of 6 mg on days 1-3, FK 0.2 mg/kg/d. Group II SIR 15 mg on days 1-3, followed by 6 mg/d, FK 0.1mg/kg/d. Results: 86 patients (72% male) were randomized. Amendment was applied in 55 patients (68%). In group II, FK was eliminated in 31 (70%) patients in a median of 5 months. The median FK and SRL levels in the moment of 1st rejection were 5,4 and 4,2 ng/ml, respectively. Two patients developed acute rejection after FK withdrawal, while being with SRL levels of 7,9 and 10,2 ng/ml. There were no differences in patient and graft survival. Thrombocytopenia was more frequently reported in group II (27% vs 4%, p<0.05). Conclusion: The combination of SIR and FK attains adequate immunosuppression after renal transplant, and achieves good renal function at 6 months. Difficulties to get the target levels may have accounted in the early AR episodes observed. Data at 12 months will be presented at the meeting.

	Group I (n=44)	Group II (n=42)	p
Recipient age (years)	49 (11)	50 (13)	N.S.
Donor age (years)	45 (16)	43 (15)	N.S.
Cold ischemia time (hours)	18,6 (3,8)	17,9 (5,4)	N.S.
"On protocol" serum creatinine (mg/dl) at 6 m	1,5	1,3	<0,05
"On protocol" cholesterol (mg/dl) at 6 m	205,6	231,6	<0,05
Biopsy proved acute rejection (AR) (%)	9,5	22,7	N.S.
AR post amendment (%)	10,7	11,1	N.S.
Days to 1st AR	6,5	9	N.S.

Median (Standard deviation). Six month data shown

Abstract# 1257 **Poster Board #-Session: P13-III**

TWELVE-MONTH PHARMACOKINETIC STUDY OF THE NOVEL COMBINATION OF TACROLIMUS AND RAPAMYCIN IN DE NOVO RENAL ALLOGRAFT RECIPIENTS: Dirk R. J. Kuypers,[1] Kathleen Claes,[1] Pieter Evenepoel,[1] Bart Maes,[1] Yves Vanrenterghem.[1] *[1]Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium.*

Recent phase II trials confirmed the efficacy of combining rapamycin (R) and tacrolimus (T). Little is known about the long-term pharmacokinetics (PK) of this combination and possible interactions. We performed AUC measurements for R (HPLC) and T (immunoassay) at 1, 3 and 12 months post-Tx in 9 *de novo* recipients. A reduced dose of T (target trough: 3-7 ng/ml) was combined with a standard dose R (10-20 ng/ml) or a standard dose of T (10-15 ng/ml) was combined with a reduced dose R (5-10 ng/ml). Dose changes of R and T were reflected by PK exposure parameters and differed significantly between groups (table). Dose-corrected AUC of R and T did not change significantly over time (not shown). However, at 6 and 12 months post-Tx, the daily dose of R corrected for body weight of patients on a low R regimen did not differ from that of patients on a high R regimen (6 m: 0.07 vs 0.075 mg/kg/d -12 m: 0.06 vs 0.07 mg/kg/d, ns) despite significant differences in dose-corrected exposure parameters. Conclusion: recipients on a low dose of R with a standard dose of T seem to require dose increments of R over time in order to maintain constant R exposure.

Rapamycin and Tacrolimus pharmacokinetics

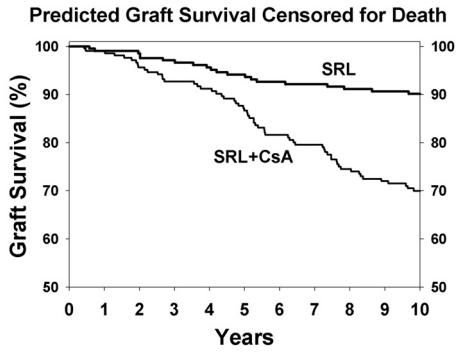
Mean (+/- SD)	Time a/ Tx	Dose (mg/day)	Trough (ng/ml)	Tmax (hr)	Cmax (ng/ml)	AUC4h (ng*hr/ml)	AUC24h (ng*hr/ml)
Rapamycin PK (reduced dose T)	1 month	4.25[a]	16.1 (5.9)[b]	1.77 (0.97)	49.3 (10)[b]	152.1 (29.9)[b]	476.2 (185.6)[b]
	6 months	6	20.2 (0.9)[b]	1.76 (0.33)	43.1 (1.6)	140.9 (4.95)	607.8 (429.1)
	12 months	6	23 (5.5)	1.52 (0.69)	64 (0.4)[b]	200.9 (8.4)[b]	658.9 (45.4)[a]
Rapamycin PK (standard dose T)	1 month	2.8	7.6 (2.2)	1.81 (1.33)	25.2 (14)	75.9 (42.5)	202.5 (105.4)
	6 months	5.4	13.9 (4.7)	1.21 (0.59)	42 (9.1)	122.4 (21.8)	250.6 (55.7)
	12 months	4.75	14.5 (4)	1.37 (0.4)	45.1 (6.8)	145.6 (28.4)	310 (121.9)
Tacrolimus PK (reduced dose T)	1 month	3.75[a]	5.4 (2.1)[b]	2.01 (1.15)	11 (2.95)[b]	33.2 (9.7)[a]	76 (20.4)
	6 months	3[a]	4.5 (0.4)[a]	2.26 (1.03)	9.1 (0.2)[b]	29 (1.8)[b]	68.3 (10.9)[b]
	12 months	2.25[a]	5.2 (0.4)[b]	1 (1.42)	6.7 (2.5)[b]	21.3 (6.7)[a]	59.8 (23.8)[b]
Tacrolimus PK (standard dose T)	1month	9.6	9.8 (2.6)	2.92 (1.15)	19.5 (2)	59.3 (3.8)	120.6 (39.7)
	6 months	7.8	9.4 (1.8)	2.21 (1.26)	19.5 (6.2)	52.3 (11.4)	133.9 (24.2)
	12 months	7.6	7.5 (1.5)	2.84 (1.63)	14.8 (3.6)	45.9 (2.9)	102.9 (16.8)

[a]p<0.01 and [b]p<0.05 between reduced-dose tacrolimus and standard-dose tacrolimus group

Abstract# 1258 **Poster Board #-Session: P14-III**

PREDICTING 10-YEAR GRAFT SURVIVAL WITH SIROLIMUS USED EITHER AS BASE THERAPY OR IN ASSOCIATION WITH CYCLOSPORINE. Josep M. Campistol,[1] Rainer Oberbauer, Henri Kreis, Yves Brault, James T. Burke, the Rapamune Maintenance Regimen (RMR) Study Group. *[1]Hospital Clinic i Provincial, Barcelona, Spain.*

Purpose: Retrospective analyses have indicated that renal function at 6 or 12 months is predictive of long-term graft survival (GS). In the RMR Study, renal function at 12 months was significantly better in sirolimus (SRL)-treated patients undergoing cyclosporine (CsA) withdrawal. This paper proposes a model to estimate the difference in GS through 10 years based on 3-4 years of follow-up. **Methods:** 525 renal allograft patients received SRL 2 mg (tablets), CsA, and steroids. At 3 months ± 2 weeks, 430 eligible patients were randomized to remain on triple therapy (SRL+CsA group), or to have CsA withdrawn and SRL troughs increased (SRL group). Random coefficient regression analysis of calculated GFR over 6-36 months was used to calculate changes in renal function. Individual estimates were made for all patients; a patient was considered to have a functional graft loss when calculated GFR was 10 mL/min. A Weibull model was developed to predict death through 10 years, and this rate was randomly applied to the studied population using a 1000 simulations. **Results:** Based on ≥ 36 months follow-up (n=430), graft loss censored for death (7.9% vs 3.3%; difference [95%CIs], 4.7% [0.3%, 9.0%]) was significantly lower with SRL-based therapy. The difference in death (7.4% vs 4.2%, SRL+CsA vs SRL) was not significant. The slope of GFR was negative for SRL+CsA (-3.02 mL/min per year, p<0.001) and positive for SRL (0.77 mL/min per year, p=0.086); the difference between treatments was significant (-3.79 ml/min per year, p<0.001). At 10 years, the predicted difference in favor of the SRL group was 20.2% (figure). **Conclusion:** This predictive model of GS allowed us to demonstrate the potentially dramatic differences in outcome over 10 years for this resource-restrained population. Based on this model, treatment with SRL could potentially ease organ shortage and extend the life of the kidney.



Abstract# 1259 **Poster Board #-Session: P15-III**

EVALUATION OF THE ACCURACY AND PRECISION OF THE INTELLIGENT DOSING SYSTEM™ FOR OPTIMIZING SIROLIMUS THERAPY IN TRANSPLANTATION. John P. McMichael,[1] Dietmar K. Abendroth,[3] Carolin Dame,[4] Ulrich Weigel,[5] Steven H. Mishkind,[1] Wolfgang Arns.[2] *[1]The RxFiles Corporation, Nokomis, FL; [2]Stadt. Klinikum Koln Merheim, Koln, Germany; [3]Visceral and Transplantational Surgery, University Ulm, Ulm, Germany; [4]General Surgery, Westfalische Wilhelms-University Munster, Munster, Germany; [5]Wyeth Pharma GmbH, Munster, Germany.*

Purpose: In this study we used the Intelligent Dosing System™ (IDS™), which is a simple dosing algorithm that can be used to facilitate standardization of patient sirolimus dose management, to correlate changes in dose and resulting levels. Methods: Patient data was collected both prospectively and retrospectively from 3 clinical sites on 94 kidney transplant patients. There were 44 male patients in this study (mean age = 44.1 years, range = 18 to 70 years). There were 50 females in this study (mean age = 46.2 years, range = 26 to 69 years). The data included sirolimus doses and corresponding levels obtained at the start of sirolimus treatment. Given the current dose and the current level, the IDS can calculate the next dose (to achieve a desired level) or the expected level (given the next dose). Our predictions of the next level (interval = 1 to 4 days) were made when there was at least a 10% change in the dose. Results: In 47 patients where the criteria for analysis was met, we calculated the actual dose to suggested dose % agreement (average % agreement = 100.9, StdDev = 12.2, correlation coefficient = 0.937, $p < 0.01$). We calculated the observed level to predicted level (average % agreement = 100.6, StdDev = 13.8, correlation coefficient = 0.899, $p < 0.01$). We stratified the group between early doses (day 2 to day 5) and later doses (day 6 to day 14). The early doses (n = 19) were calculated by actual dose to the suggested dose % agreement (average % agreement = 99.4, StdDev = 11.2, correlation coefficient = 0.907, $p < 0.01$). We calculated the observed level to predicted level (average % agreement = 102.0, StdDev = 13.3, correlation coefficient = 0.953, $p < 0.01$). The later doses (n = 28) were calculated by actual dose to the suggested dose % agreement (average % agreement = 103.8, StdDev = 13.8, correlation coefficient = 0.941, $p < 0.01$). We calculated the observed level to predicted level (average % agreement = 97.8, StdDev = 14.8, correlation coefficient = 0.861, $p < 0.01$). Conclusions: The IDS showed excellent correlation between change in dose and resulting change in level. The IDS is potentially useful technology that can standardize sirolimus dosing and assist in the attainment of the immunosuppressive goals. Further prospective evaluation of the IDS is underway.

Abstract# 1260 **Poster Board #-Session: P16-III**

PHARMACOKINETICS OF EVEROLIMUS IN HISPANIC DE NOVO RENAL TRANSPLANT PATIENTS. F. Juarez,[1] J. M. Kovarik,[2] L. McMahon.[2] *[1]Hospital de Especialidades, Coahuila, Mexico; [2]Novartis Pharmaceuticals, Basel, Switzerland and East Hanover, NJ.*

The pharmacokinetics of several transplant immunosuppressants are known to be altered in blacks and occasionally in hispanics compared with whites. For everolimus (RAD, Certican), clearance was 20% higher in blacks and they had a significantly higher acute rejection rate in phase 3 studies compared with whites. These observations prompted us to explore further for ethnic differences in everolimus pharmacokinetics. **Study design:** This was an open-label, 6-month study in 20 hispanic *de novo* renal allograft recipients resident in Mexico. They received everolimus 1.5 mg bid in addition to Neoral and corticosteroids. Blood samples for determination of everolimus and cyclosporine trough concentrations (Cmin) were obtained in weeks 1, 2 and months 1, 2, 3, 6. Pharmacokinetic profiles of both drugs over the 12-h dose interval were obtained at month 1. These data were compared with those from white patients (n = 41) in a previous study conducted in North and South America using the same dose of everolimus. **Results:** Hispanic patients weighed significantly less than white patients (65.8 ± 13.9 vs 78.3 ± 19.2 kg, $p = 0.01$) resulting in a higher weight-adjusted everolimus dose in hispanics (0.024 ± 0.005 vs 0.020 ± 0.005 mg/kg, $p = 0.01$). Everolimus Cmins in hispanics were significantly lower at week 1 (3.7 ± 2.1 ng/ml) and week 2 (5.1 ± 2.1 ng/ml) compared with month 1 (7.5 ± 2.8 ng/ml); thereafter values remained stable to month 6. By comparison, everolimus Cmins in white patients were stable throughout the 6-month period: 6.8 ± 5.4 ng/ml (week 1), 6.7 ± 4.8 ng/ml (week 2), 8.2 ± 4.5 ng/ml (month 1 and thereafter). The month 1 pharmacokinetics are tabulated below. None of the everolimus parameters were significantly different between ethnic groups. The correlation between everolimus Cmin and AUC was likewise comparable: slope 14.9, $r = 0.93$, $p < 0.001$ in hispanics and slope 11.3, $r = 0.81$, $p < 0.001$ in whites. As shown in the table, there were no differences in concurrent exposure to cyclosporine in the two groups.

Drug	Patient group	Cmin (ng/ml)	Cmax (ng/ml)	AUC (ng.h/ml)
Everolimus	Hispanic	7.5 ± 2.8	23.7 ± 6.6	149 ± 45
Everolimus	White	8.2 ± 4.5	21.2 ± 8.3	138 ± 63
Cyclosporine	Hispanic	232 ± 143	1418 ± 564	6550 ± 2750
Cyclosporine	White	269 ± 162	1383 ± 497	6174 ± 2325

Conclusions: With the exception of modestly lower everolimus Cmins in the first two weeks posttransplant, the disposition of everolimus in hispanic de novo renal transplant patients resident in Mexico was comparable to that of white patients from North and South America.

Abstract# 1261 **Poster Board #-Session: P17-III**

AVOIDING CALCINEURIN-INHIBITORS IN RENAL TRANSPLANTATION: A RANDOMIZED TRIAL OF SIROLIMUS VS TACROLIMUS. Timothy S. Larson,[1] Matthew D. Griffin,[1] Mikel Prieto,[2] Thomas R. Schwab,[1] William J. Lund,[2] Scott L. Nyberg,[2] Stephen C. Textor,[1] James M. Gloor,[1] Sandra J. Taler,[1] Mark D. Stegall.[2] *[1]Department of Internal Medicine, Division of Nephrology; [2]Department of Surgery, Division of Transplantation Surgery, Mayo Clinic, Rochester, MN.*

Introduction. To evaluate whether an immunosuppression regimen following kidney transplantation free of calcineurin-inhibitors (CI) is tolerable, has low rejection rates and results in improved renal function, we are conducting an open labeled randomized prospective trial of sirolimus (SRL) vs tacrolimus (TAC), with mycophenolate mofetil, prednisone and thymoglobulin induction. **Methods.** From April 2001 to December 2002 one hundred ten recipients entered the trial of which 99 (51 randomized to TAC, 48 to SRL) have been followed for 3 months (mean 13 ± 5 mo; range 3-20 months) and were evaluated for this report. 83% received living donor allografts. Glomerular filtration rate (GFR) was assessed by non-radiolabeled iothalamate, and surveillance allograft biopsies obtained 4 and 12 months post transplant. **Results.** Patient and graft survivals were comparable over the study period (see table).

Group	Pt./Graft Survival	Acute Rejection	Cr-mg/dl/GFR ml/min/BSA (1 mo)
SRL (n=48)	100%/98%	12%	1.5±0.4/60±19
TAC (n=51)	92%/89%	8%	1.8±0.8/53±16

All rejections occurred within 5 months following transplantation. Rejections were detected on 4 month surveillance biopsies (subclinical) in 1 TAC and 3 SRL recipients whereas clinically evident rejections occurred in 3 TAC and 3 SRL recipients. Serum creatinine was lower and GFR tended to be higher at one month in the SRL group compared to the TAC group ($p=0.03$ and $p=0.07$, respectively); however at 1 year there was no difference in renal function between groups (Cr 1.6 ± 0.6 vs 1.7 ± 0.4 mg/dl and 57 ± 17 vs 56 ± 19 ml/min/BSA, SRL vs TAC, respectively). Significantly fewer patients in the TAC group required treatment for hyperlipidemia but there was no difference in blood pressure or the number of antihypertensive agents prescribed to recipients in the 2 groups. To date 8 recipients have switched from TAC to cyclosporine or SRL, while 9 have been switched from SRL to TAC. **Conclusion.** Use of a CI-free immunosuppression regimen following kidney transplantation results in low acute rejection rates, excellent renal function and acceptable tolerability. The lack of a difference in renal function at one year may be due to the overall excellent graft function in these living donor kidneys.

Abstract# 1262 **Poster Board #-Session: P18-III**

EARLY ADEQUATE IMMUNOSUPPRESSION EXPOSURE IS REQUIRED TO PREVENT ACUTE REJECTION IN KIDNEY TRANSPLANTATION. Bryce A. Kiberd,[1] Joseph Lawen,[2] Albert Fraser,[3] Tammy Keough Ryan,[1] Roman Panek,[1] Philip Belitsky.[2] *[1]Medicine, Dalhousie University, Halifax, NS, Canada; [2]Urology, Dalhousie University, Halifax, NS, Canada; [3]Pathology, Dalhousie University, Halifax, NS, Canada.*

Early adequate exposure to cyclosporine is associated with low acute rejection rates. MMF exposure has been associated with low rejection rates but the importance of early exposure has not been studied. We prospectively evaluated 73 first solitary kidney transplants treated with Neoral, MMF (2 gm/d), and prednisone. Simulect was also given to 59 of the recipients. Neoral C2 levels and 5 point/4hour MPA AUC levels (measured by HPLC on samples at C0, C1, C2, C3, and C4 post dose) were measured on days 3 and 5. The primary outcome was biopsy proven rejection within the first 3 months post transplantation. Statistical analysis for the 2x2 contingency tables to test the association of early exposue to rejection was by the Chi-square/Fischer exact test (SAS). Patients were 49±13 years old, 70% were cadaver grafts, and 15% had diabetes mellitus. Acute rejection was seen in 11 (15.0%) and delayed graft function (requiring dialysis) in 7 (10%). Low cyclosporine exposure defined as a C2<1.70 ug/L was observed in 38 (52%) of the patients. The odds ratio for rejection in the low versus adequate exposure groups was 1.75 (95% CI 0.5-8.5, p=0.52). Low MPA exposure, defined as 5 point MPA AUC of <14 mg*hr/L, was observed in 37 (51%) of the patients. The odds ratio for rejection in the low versus adequate MPA exposure groups was 5.3 (95% CI 1.1-27, p=0.0468). Low exposure to both MPA and cyclosporine was observed in 23 (32%) of the patients. The odds ratio for rejection in the low vs adequate exposure for one or both drugs was 3.2 (95% CI 0.9-12, p=0.0895). The correlation was weak between cyclosporine and MPA AUC exposure (r=0.29, p=0.019). The odds ratio for rejection in patients not receiving Simulect versus the Simulect group was 4.9 (95% CI 1.2-20, p=0.03). The lack of adequate exposure of any drug (no Simulect, low cyclosporine and low MPA) occurred in 8 patients with rejection in 4 (50%). The odds ratio for rejection in these patients compared to adequate exposure of any one of the three medications (n=65) was 8.3 (95% CI 1.7-41). As with cyclosporine, early adequate exposure to MPA is important for preventing acute rejection. In the absence of Simulect inadequate exposure to cyclosporine and MPA is associated with a high rejection rate. Combination therapy likely provides benefit by increasing the chance of early adequate exposure of at least one potent immunosuppressive medication.

Abstract# 1263 **Poster Board #-Session: P19-III**

RITUXIMAB:A HIGHLY SUCCESSFUL THERAPY FOR TREATMENT OF VASCULAR REJECTION. Yolanda T. Becker,[1] John D. Pirsch,[2] Hans W. Sollinger.[1] *[1]Department of Surgery, University of Wisconsin, Madison, WI; [2]Department of Medicine, University of Wisconsin, Madison, WI.*

Rituximab is a high-affinity CD20 specific antibody that inhibits B-cell proliferation and induces apoptosis. Thus, it is a rational choice for therapy in transplantation to abrogate B-cell mediated events. Vascular rejection may be B-cell mediated. Therefore, we hypothesized that rituximab would be an effective means of treating this very difficult type of rejection. 27 patients (pts) were diagnosed with vascular rejection between 2/99 and 2/02. The pts were treated with a single dose of rituximab IV at 375 mg/M². In addition to rituximab, pts received various other therapy in an effort to reverse the vascular rejection. 24/27 received additional steroids. 22 pts were also treated with combinations of plasmapheresis and ATG. In successully treated patients, the admission creatinine at the time of therapy was 5.6 ± 1.0 mg/dl and decreased to 0.95 ± .07 mg/dl at the time of discharge. Only 3 of the 27 patients had treatment failure. 2 patients died with functioning allografts during the period of follow-up. 3 patients who had initial reversal of rejection ultimately succumbed to chronic rejection. Vascular rejection is difficult to treat. To date, several different treatment modalities including monoclonal antibodies, anti T-cell therapy and plasmapheresis have been relatively unsuccessful. The addition of rituximab to our regimen significantly increased the treatment success for vascular rejection. Clinical trials to assess the use of rituximab in transplantation are clearly warranted.

Abstract# 1264 **Poster Board #-Session: P20-III**

PREDICTIVE VALUE OF URINARY RETINOL BINDING PROTEIN (RBPu) FOR THE DEVELOPMENT OF GRAFT DYSFUNCTION AFTER KIDNEY TRANSPLANTATION. Beatriz H. Hosaka,[1] Cláudia R. Felipe,[1] Sung I. Park,[1] Luciana Kamura,[1] Riberto Garcia,[1] Paula G. Machado,[1] Hélio Tedesco-Silva,[1] José O. Medina-Pestana.[1] *[1]Hospital do Rim e Hipertensão–Nephrology Division, Universidade Federal de São Paulo, São Paulo, SP, Brazil.*

RBPu is a low molecular mass protein, filtered in the renal glomeruli, and very efficiently reabsorbed by the proximal convoluted tubules. The normal value is < 0.400 mg/L. In tubular dysfunction, which reflects tubulointerstitial injury, high concentrations of RBP are found in urine. We evaluated the predicitve value of RBPu, measured during the first 6 months after kidney transplantation, for subsequent development of graft dysfunction at one year. **Methods**: 235 kidney transplant patients receiving different immunosuppressive regimens were prospectively monitored regarding creatinine, calculated creatinine clearance, and RBPu during the first year after transplantation. Baseline graft function was determined as the best creatinine during the first 3 months after transplantation. Graft dysfunction was assessed at one year as a > 20% or > 30% increase on the 1/Cr, the current best predictor of graft loss. The predicitve value of increasing cut-off concentrations of RBPu, measured betwen 3 and 6 months, for subsequent graft dysfuction was assessed using receiver operating characteristics (ROC) analysis. **Results**: Mean age of the patients was 38.2±10.3 years, 62% male, and mean BMI was 21.3±3.4 kg/m². Mean nadir creatinine at month 3 and mean creatinine at month 12 after transplantation were 1.2±0.2 (0.5-1.6) vs. 1.6±0.7 (0.8-6.1) mg/dL ($p<0.001$). There was no correlation between RBPu and delta 1/Cr at one year ($r^2=0.06$). ROC analysis did not find a predicitve cut off concentration showing better association with graft dysfunction than the normal cut-off RBPu value of <0.4 mg/dL. Mean % increase in 1/Cr from month 3 to 12 was 12% for those with RBP <0.4 vs. 20% in those with RBP>0.4 mg/L ($p=0.022$). The percentage of patients with > 20% or >30% increase in 1/Cr was higher among patients with RBP > 0.4 (33% vs. 45%, $p=0.048$; 21% vs. 33%, $p=0.05$), respectivelly. **Conclusion**: In patients with normal graft function (Cr between 0.5 to 1.6 mg/dL) during the first 3 months, RBPu higher than 0.4 mg/dL was associated with graft dysfunction at one year measured by delta 1/Cr. Long-term follow-up is necessary to define the clinical utility of RBPu in predicting patients at higher risk to develop chronic allograft dysfunction and subsequent graft loss.

Abstract# 1265 **Poster Board #-Session: P21-III**

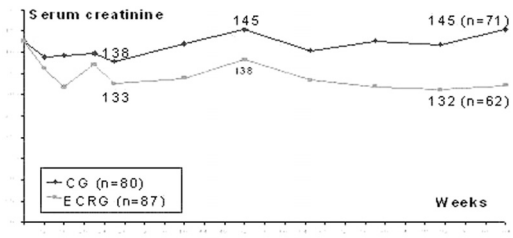
POST-TRANSPLANT DETECTION OF DONOR-REACTIVE ALLOANTIBODIES BY ELISA CORRELATES WITH ADVERSE CLINICAL OUTCOME. Ronald P. Pelletier,[1] Patrick Adams,[4] Ronald M. Ferguson,[1] Charles G. Orosz.[1,2,3] *[1]General Surgery, Ohio State University College of Medicine, Columbus, OH; [2]Pathology, Ohio State University College of Medicine, Columbus, OH; [3]Molecular Virology, Immunology, and Medical Genetics, Ohio State University College of Medicine, Columbus, OH; [4]Tissue Typing Laboratory, Ohio State University College of Medicine, Columbus, OH.*

We have previously demonstrated that renal Tx patients who exhibit post-Tx alloantibodies which react with MHC class II, but not MHC class I molecules are at increased risk for acute rejection (AR) and chronic rejection (CR) (AJT 2: 134, 2002). That study used flow bead PRA analysis (flowPRA) to detect alloantibodies, but this method detects alloantibodies that are not necessarily donor-reactive. We have recently employed an ELISA-based assay that measures the binding of IgG to independently captured HLA class I or class II molecules derived from donor leukocytes. We used this assay to re-evaluate the incidence, specificity, and clinical significance of donor HLA-reactive antibodies detected both before and after tx in K and SPK recipients. To date we have tested 337 recipients (mean follow-up 3.3 +/- 2.0 years, range 1month – 7 years) at least once post-tx (median of 2 tests, range 1 to 7). Of these, 245 were also tested pre-tx. For pre-tx alloantibodies, the ELISA data was similar to the previously reported flowPRA data with regards to a) the incidence of alloantibody production (12% vs 17%), b) the proportions of patients with alloantibodies directed at HLA class I, HLA class II, or both, and c) the lack of correlation with AR, CR or graft loss. For post-tx alloantibodies, however, there were some notable differences between the flowPRA and the ELISA results. Although the overall incidence of antibody detection was similar for both methods (18% vs 21%, respectively), the ELISA detected fewer patients with isolated donor HLA class II-reactivity (8% versus 15% by flowPRA). ELISA detected a similar proportion of patients with isolated donor HLA class I-reactivity (5% versus 2% by flowPRA) or combined donor HLA class I and II (5% versus 5%). Importantly, there was a strong correlation between the display of ELISA-detectable donor HLA class I-reactive antibodies and AR ($p<0.001$) , CR ($p=0.003$), and graft loss ($p<0.001$) that was not demonstrable using flowPRA analysis. The detection of HLA class II-reactive antibodies with either ELISA or flowPRA methods correlated very strongly with AR and CR (AR-$p=0.008$ versus $p<0.001$, respectively; CR-$p<0.001$ for both). We conclude that post-tx expression of donor HLA class I or II-reactive alloantibodies is a serious risk factor for poor graft outcome, and that the ELISA method is superior to the flowPRA method for detecting clinically relevant donor HLA-reactive antibodies during the post-tx period.

Abstract# 1266 **Poster Board #-Session: P22-III**

DACLIZUMAB AND MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANT RECIPIENTS: 2-YEAR OUTCOME AFTER EARLY REDUCTION OF CYCLOSPORINE. Henri Kreis,[1] Tatiana Miloradovich, Georges Mourad, Olivier Cointault, François Berthoux, Michel Delahousse, Elisabeth Cassuto, Jean-Marc Chalopin, Denis Glotz, Yvon Lebranchu, Christophe Legendre, Luc Potaux, Jean-Louis Touraine, Paul Vialtel, Philippe Wolf, Bruno Moulin, Rajsingh Purgus. *[1]Renal Transplantation, Hôpital Necker, Paris, France.*

CAN and long-term outcome could be improved by reducing IS regimen nephrotoxicity. A multicenter, open-label, randomized, French clinical trial compares 2-year efficacy (graft function) and safety of two IS strategies. 1st or 2nd renal allograft recipients (169) were given a same combination of daclizumab, mycophenolate mofetil (MMF), CsA, and steroids from surgery to week (W) 8. Then they were allocated to either continue the same regimen (Control Group, CG, n=80) with CsA reduction after 1 year*, or to enter Early CsA Reduction Group (ECRG, n=78)**. HHDemographic patterns were similar in the 2 groups. Mean year-2 T0 CsA were 75±17ng/ml in ECRG vs122± 27 in CG. Over the 2 years, Scr improved in ECRG, but difference between groups was not significant (p=0.84).



Overall BPAR incidence was 5.1% before W8, and 10.3% in ECRG (8 pts,10 BPAR) vs 7.5% in CG (6 pts, 8 BPAR) after W8 (p=0.59). BPAR mainly occurred during the 1st 6 months in ECRG (9.0%) and CG (6.3%) (p=0.78). Graft survival was 96.2% in ECRG vs 98.8% in CG (NS). Pt survival was 97.4% in ECRG vs 98.8% in CG (NS). Conclusion: These data suggest that the combination of daclizumab and MMF may allow early 50% reduction of T0 CsA without raising significantly the incidence of acute rejection and graft loss after 1st or 2nd renal transplant. The observed improvement of renal function, although not reaching statistical significance, is promising. Centralized review of the 2-year biopsies will determine the exact impact of CsA dose reduction on CAN. *T0 (ng/ml): W9-12: 150-200, W12-M12: 150-200, M12-24: 100-150 **T0 (ng/ml): W9-12: 100-150, W12-M12: 75-100, M12-24 : <75

Abstract# 1267 **Poster Board #-Session: P23-III**

ROLE OF INTERCELLULAR JUNCTIONAL MOLUCULES, VE-CADHERINS, IN CYCLOSPORIN A MEDIATED MICROVASCULAR INJURY. Chumpon Wilasrusmee,[1] Gaurang Shah,[1] David Bruch,[1] Dilip S. Kittur.[1] *[1]Surgery, SUNY Upstate Medical University, Syracuse, NY.*

Glomerular endothelial injury can lead to chronic allograft nephropathy. The increased glomerular capillary permeability from endothelial injury causes increased glomerular capillary permeability is generally due to weakening of intercellular junctions normally maintained by VE-cadherin molecules. We have previously shown in an in vitro capillary model that cyclosporin A (CyA) disrupts intercellular junctions, and that this injury is associated with endothelin-1 (ET-1) release. In this study we determined if CyA mediated endothelial injury is due to impairment in the expression of the intercellular junctional molucules, VE-cadherin. Endothelial capillaries formed by SVEC4-10 mouse endothelial cells on Matrigel were treated with CyA to disrupt the intercellular junctions between endothelial cells (ECs). VE-cadherin gene expression was studied by a RT-PCR assay in RNA isolated from the in vitro capillaries under three conditions: 1) ECs that formed capillary-tube like structures on Matrigel, 2) ECs that failed to form capillary tubes with CyA (2-10 m g/ml), and 3) ECs in capillary tubes injuried by high dose CyA (10-20 m g/ml). VE-cadherin mRNA was undetectable 1 hour after plating the ECs on Matrigel but reappeared after 3 hours when the ECs started to make cell-cell contacts and formed capillary tubes. The VE-cadherin expression in the ECs continued to increase as the cells made more capillary networks at 24 and 48 hours. In contrast to this expression in normal capillary networks, the VE-cadherin expression was inhibited in ECs that failed to form capillaries in the present of CyA, and in the capillaries that were disrupted by CyA treatment. Our results suggest a mechanism by which CyA weakens the intercellular junctions between endothelial cells. In conjunction with our previous results indicating that CyA induces ET-1 release, and with those by others indicating that ET-1 suppresses cadherin expression, the present results suggest that CyA mediated ET-1 release downregulated the expression of VE-cadherins, thus weakening the intercellular junctions between endothelial cells. Our results also suggest a mechanism by which endothelial integrity could be damaged in allografts thus leading to chronic allograft vasculopathy.

Abstract# 1268 **Poster Board #-Session: P24-III**

PREDICTIVE FACTORS FOR LONG TERM CD4 T CELL LYMPHOPENIA AFTER TREATMENT WITH THYMOGLOBULIN. Jean-Francois Valentin,[1,2] Azmi Al-Najjar,[1] Gilles Thibault,[2] Mathias Buchler,[1] Jean-Michel Halimi,[1,2] Hubert Nivet,[1] Lebranchu Yvon.[1,2] *[1]Nephrologie Immunologie Clinique, CHU de Tours, Tours, France; [2]Laboratoire d'immunologie, CHU de Tours, Tours, France.*

Background Little is known about long-term changes in lymphocytes subsets induced by Thymoglobulin in kidney transplantation and the predictive factors for long-term CD4 lymphopenia Methods Between 1994 and 1998, 216 cadaveric kidney transplantations were performed in our institution, data was available for 175 patients, mean age was 44.2 (15-69) years, sex ratio was M/F 107/68. 144 (82.28%) patients were treated with Thymoglobulin, and 18 (17.71%) had no Thymoglobulin therapy. Lymphocytes subsets were analyzed by FACS before transplantation, at 6 months, and every year for five years. Clinical data, immunossuppression and greft survival were collected for tje entire period. Patients who lost their graft in the first year were nor included. Results Lymphocyte T CD4 follow-up is markedly decreased in patients trteated with Thymoglobulin as compared to the No-Thymoglobulin group as shown in table I. Graft survival at five years in the two groups was not different. In the Thymoglobulin group, Thymoglobulin duration (11.48±0.31 vs 10.97±0.84 days, p=ns) and Thymoglobulin total amount (715.5±82 vs 653±73 mg, p=ns) were not predictive factors for patients who had and important T CD4 lymphopenia (<200 cell/mm3) from year 1 to year 5 vs patients who had more than 200 T CD4/mm3 . Patients with profound long term T CD4 lymphopenia (<200 cell/mm3) are older than those who have more than 200 T CD4/mm3 (56.73±7.32 vs45.17±0.76 years, p=0.03). Other factors as sex, CMV disease or etiology of kidney disease were not statistically significative. Conclusion We conclude that Thymoglobulin induces long-term lymphopenia and T CD4 lymphopenia with and important delay in lymphocyte restore in kidney transplant recipients. Predictive factors for long term T CD4 lymphopenia (200 cell/mm3) include age at transplantation. Thymoglobulin duration and amount are not predictive factors for long term lymphopenia. Although, T CD4 restore is more delayed in patients with higher amounts of Thymoglobulin.

Table I

	Before Tx	year 1	year 2	year 3	year 4	year 5
Thymoglobulin	825.0±36	294.9±15	389.8±19	455.0±19	478.4±22	515.2±29
NoThymoglobulin	888.5±72	899.2±64	877.7±69	886.1±70	924.8±88	840.2±115
p	ns	<0.0001	<0.0001	<0.0001	<0.0001	=0.0003

Abstract# 1269 **Poster Board #-Session: P25-III**

OPEN-LABEL, MULTICENTER STUDY OF TWO - DOSE ZENAPAX IN COMBINATION WITH MYCOPHENOLATE MOFETIL, CYCLOSPORINE AND STEROIDS IN THE PREVENTION OF ACUTE REJECTION IN RENAL ALLOGRAFT RECIPIENTS. Li-xin Yu, Yun Miao. *[1]Kidney Transplantation Department, Nan Fang Hospital, Guangzhou, Guangdong, China; [2]Kidney Transplantation Department, Nan Fang Hospital, Guangzhou, Guangdong, China.*

Objectives: To evaluate the efficacy and safety of two-dose Zenapax in combination with new triple therapy for the prevention of acute rejection and the improvement of graft/patient survival. Methods: 213 patients received their first cadaveric renal allograft in 14 centers in China from September 2000 to December 2001. All of them received 2 dose Zenapax in combination with Cellcept, cyclosporine and steroids. The first dose Zenapax was given within 24 hours prior to transplant (If body weight < 75kg, the dosage would be 50mg; if body weight >75kg, the dosage would be 75mg). The second dose was given on postoperative day 14. In 97 recipients and the control group of one center, count of CD25+ cells was performed before and after transplantation at 1day, 1 week, 2 weeks, 4 weeks and 6 weeks, using Beckman Coulter flow cytometer and monoclonal antibodies of CD25+. Results: The number of acute rejection episode during six months follow-up was 12 (all diagnosed with clinical symptom without biopsy proven) and the acute rejection rate is 5.63% (95% CI: 0 - 10.9%). After methylprednisolone pulse therapy, nine cases were easily reversed while the other three required ATG therapy. Mean numbers of CD25+ cells after transplantation were significantly lower in comparison with the control group in one center's study. At 3, 6 months, and 1 year after transplantation, mean serum creatinine were 117.9 ± 35.4, 113.6 ± 47.7, 121.2 ± 37.4 umol/L; graft survivalgraft survival and patient survival were 100%, 96.7% and 94.8%. Eleven patients died during 1 years after transplantation. Two died of cerebral hemorrhage of hypertension, five died of severe infection, one is acute cardiac infarction, one got HBV relapsed and died of liver failure, one was severe insufficiency of lung function and one accidental death. No graft loss censored for patient death. Onset of delayed graft function was 11 cases (5.16%) and no acute rejection was observed among these patients. During this study, Zenapax was well tolerated; no cytokine release syndrome or other significant adverse event was observed. Conclusion: 2 dose Zenapax combined with tripe therapy can reduce acute rejection in renal transplantation effectively as same as 5-dose protocol, and it is well tolerated without any significant adverse effects. The patient and graft survival of 3,6 months and 1 year are satisfactory.

Abstract# 1270 **Poster Board #-Session: P26-III**

ALTERNATE-DAY THYMOGLOBULIN INDUCTION DOSING SIMPLIFIES CLINICAL MANAGEMENT AND REDUCES COST IN KIDNEY AND PANCREAS TRANSPLANTATION. A. K. Sundberg,[1] P. L. Adams,[2] M. S. Rohr,[3] R. J. Stratta.[3] *[1]Pharmacy; [2]Medicine; [3]Surgery, Wake Forest Univ., Winston-Salem, NC.*

Background: Thymoglobulin (Thymo) induction therapy is effective for preventing acute allograft rejection (REJ), however a 7-10 day course may involve extensive clinical monitoring and is costly. The purpose of this study was to evaluate the safety and efficacy of an abbreviated Thymo dosing regimen for induction therapy in kidney (KTX) and pancreas transplant (PTX) patients (pts). **Methods:** Data were collected retrospectively for all pts who received a KTX and/or PTX with Thymo induction since 10/1/01. Pts with immediate graft function (IGF) received 3 doses (1.5 mg/kg) intra-operatively and on post-operative days 2 and 4. Patients with slow graft function (SGF) received up to 7 doses (1.5 mg/kg) on alternate days until therapeutic tacrolimus (TAC) levels were reached. Lymphocyte subsets were not monitored. Cost was analyzed using the average wholesale price of Thymo. **Results:** Ninety pts received Thymo induction, including 71 cadaveric KTXs, 12 simulaneous K-PTXs, 4 living donor KTXs, and 3 sequential PTX after KTXs. A total of 56 pts had IGF (serum creatinine <3.0 mg/dL at median of 3 days) and received a median of 3 doses of Thymo. The remaining 31 pts (34%) had SGF (serum creatinine <3.0 mg/dL at median of 12 days) and received a median of 5 doses of Thymo. The mean total white blood cell nadir was 6300/mm³(range 2400-12,400), and the mean platelet count nadir was 124,000/mm³ (range 25,000-233,000). 21 pts (23%) required dosage adjustments (19 due to thrombocytopenia). Dose-limiting adverse events occurred in 4 pts (4.4%), and included 3 with respiratory distress and 1 with bleeding. The mean total treatment dose was 214 mg in the IGF group (mean cost $2,929) and 426 mg in the SGF group (mean cost $5,831). Patient, kidney, and pancreas graft survival rates were 99%, 94%, and 85%, respectively. Two pts (2%) experienced graft loss due to acute humoral rejection with thrombotic microangiopathy; there were no other acute REJ episodes. The incidence of major infection was 11%, and included 3 urosepsis, 2 complicated wound infections, 2 pneumonias, 2 sepsis, 1 CMV, and 1 polyomavirus. No malignancies were noted. **Conclusions:** An abbreviated Thymo dosing regimen based on initial graft function was both safe and effective as induction therapy in KTX and PTX recipients. Using alternate-day dosing resulted in few adverse events, was less resource-intensive, and was economical. A considerable cost savings is possible without an apparent reduction in efficacy using this alternative regimen.

Abstract# 1271 Poster Board #-Session: P27-III

INDUCTION IMMUNOSUPPRESSION FOR RENAL TRANSPLANTATION USING THYMOGLOBULIN, FK506 AND MYCOPHENOLATE MOFETIL ALLOWS FOR SAFE STEROID WITHDRAWAL AND ELIMINATES THE NEED FOR EARLY PROTOCOL BIOPSIES. Oleh G. Pankewycz,[1] Rabie Stephan,[2] Barbara Stefanick,[3] Andrea Rubino,[4] Romesh Kohli,[1] Inkee Min,[1] Khalid Mahran,[1] Nagaraja R. Sridhar,[1] Mary Applegate,[2] Mark R. Laftavi.[2] *[1]Medicine; [2]Surgery; [3]Pathology; [4]Pharmacy, University at Buffalo, Buffalo, NY.*

Given the availability of many new and potent immunosuppressive agents, steroid avoidance following renal transplantation is now an attainable goal. However, the efficacy and safety of steroid-free therapy remains unresolved. In this clinical trial of steroid-free therapy, we performed protocol biopsies after kidney transplantation and report on the clinical usefulness of the one-month protocol biopsy. Patients were prospectively randomized into 2 groups. Both groups received Thymoglobulin (total dose 3-5 mg/kg), FK506, mycophenolate mofetil (MMF) and steroids (250 mg/iv day 0, 125 mg/iv day 1 and 30 mg/po day 2). The control group (C, n=11) received prednisone starting at 30 m/d, tapering to 5 mg/d by day 30 and continuing indefinitely. In the steroid withdrawal group, steroids were tapered by 5 mg/d and discontinued on day 8 (SW, n=10). All patients in the SW group received cadaveric transplants and 50% were African American. Patients were followed for 6 months after transplantation. Only one patient in each group (C=9%) (SW=10%) was found to have subclinical rejection on protocol biopsy. The one control patient subsequently experienced a clinical rejection episode. Renal function was compared in both groups.

	Renal Function (Cockcroft-Gault)		
	1 month	3 months	6 months
Control	66.3 ± 19.7	68.4 ± 22.3	61.5 ± 25.6
Steroid Withdrawal	62.0 ± 21.7 p=0.88	67.3 ± 27.1 p=0.92	80.7 ± 29.3 p=0.20

No patient lost renal allograft function due to rejection or infection. Despite the presence of a large percentage of high-risk patients in the SW group, the combination of thymoglobulin, FK506 and MMF reduced the rate of clinical (0%) and subclinical rejection (10%) to very low levels in the absence of steroids. These results indicate that the present immunosuppressive regimen using Thymoglobulin induction allows for safe steroid withdrawal in a broad patient population. The low incidence of subclinical rejection in this study calls into question the value of performing this invasive monitoring procedure soon after transplantation. The long-term safety and efficacy of this protocol will require further study.

Abstract# 1272 Poster Board #-Session: P28-III

THYMOGLOBULIN FOR INDUCTION AND FOR TREATMENT OF REJECTION IN RENAL TRANSPLANT RECIPIENTS. A. Lo,[1] B. Olson,[1] L. W. Gaber,[2] A. O. Gaber.[3] *[1]Pharmacy, University of Tennessee, Memphis, TN; [2]Pathology, University of Tennessee, Memphis, TN; [3]Surgery, University of Tennessee, Memphis, TN.*

Introduction: Thymoglobulin (Thymo) induction is widely used in kidney transplantation raising the concerns regarding the advisability of retreatment in patients who developed acute rejection (AR). This study describes the outcomes of Thymo induction in a fairly large transplant population and focuses on the reuse of Thymo in these patients. **Methods:** We preformed a retrospective chart review of all kidney transplants performed between 01/98 and 06/02. Primary renal transplant recipients who received Thymo for induction, had biopsy-proven AR, and treated with Thymo were included. Data collected were patients' characteristics, Thymo dose, hematological profiles, serum creatinine, renal biopsy findings, and the incidences of adverse events. **Results:** 207 primary renal transplant recipients received Thymo induction, of which, only 10(4.8%) had biopsy-proven AR and were treated with Thymo. These 10 patients (9 male, 7 African-American, 8 cadaveric donors, mean age 43 yrs, PRA 0%, delayed graft function 40%) were the study subjects. The mean follow-up time was 374 (70 to 799) days posttransplant (post-tx). The mean cumulative induction Thymo dose was 5.7±2.3 mg/kg. There were significant reductions in white blood cell, lymphocyte, and platelet counts during therapy and for at least a month post-tx. The time to AR was 122 (17 to 333) days post-tx. The Banff grades of the AR were IA (1), IB (2), IIA (1), IIB (2), and III (4). At the time of AR, the mean serum creatinine was 5.3 (1.6 to 12.9) mg/dL and at 1 week after treatment, declined to 3.3 mg/dL (p=0.04). The cumulative treatment Thymo dose was higher (9.9±3.0 mg/kg) than the induction dose (p<0.01). The same degrees of reductions in hematological parameters were observed during retreatment. Early (14 days) posttreatment biopsies were performed in 7/10 patients; 4/7 had complete resolution of AR, 2/7 had mild focal interstitial nephritis, and 1/7 had residual inflammatory infiltrates. Overall, AR was successfully treated in 7/10 patients. Four patients lost their grafts (3 due to progressive chronic rejection and 1 due to death with functioning graft). Other than 5 episodes of bacterial infection, there were no serious adverse events. **Conclusions:** The incidence of biopsy-proven, steroid-resistant AR following Thymo induction was low (5%). In patients who developed AR after Thymo induction, successful treatment with Thymo was high (70%), but was associated with an increased risk of bacterial infection indicating the need for appropriate prophylaxis during reuse of Thymo.

Abstract# 1273 Poster Board #-Session: P29-III

INDUCTION THERAPY WITH ATG VS. BASILIXIMAB (SIMULECT) IN RENAL ALLOGRAFT RECIPIENTS: 1-YEAR RESULTS OF A PROSPECTIVE RANDOMIZED, SINGLE CENTER STUDY. Stefan G. Tullius,[1] Johann Pratschke,[1] Volker Strobelt,[1] Andreas Kahl,[2] Petra Reinke,[2] Gottfried May,[1] Ulrich Frei,[2] Peter Neuhaus.[1] *[1]Dept. of Surgery, Virchow-Clinic, CharitéHumboldt-University, Berlin, Germany; [2]Dept. of Nephrology, Virchow-Clinic, CharitéHumboldt-University, Berlin, Germany.*

Induction therapies are applied for an adequate immunosuppression at the time of transplantation. There are currently no data comparing an induction therapy with ATG and Simulect in combination with a dual immunosuppressive regimen. 124 recipients of first or repetitive renal cadaver allografts received FK 506 (0.2 mg/kg/ trough levels 10 ng/ml) and Methylprednisolon and were prospectively randomized to receive ATG (Fresenius; 9 mg/kg perioperatively/n=62) or Basiliximab (Simulect®, 20 mg on days 0 and 4/n=62). There were no significant differences in regard to age, time on dialysis, gender, distribution of first or repetitive allografts (2nd NTx: ATG: n=8/Simulect: n=16; 3rd NTx: ATG: n=1/Simulect: n=6, p=n.s.), high risk CMV constellation (D+/R-: ATG: 5/Simulect 2, p=n.s.) or cold ischemia. Both groups demonstrated differences in regard to previous panel reactive antibodies > 50%: (ATG: n=4; Simulect: n=13; p= 0.034), while PRA at the time of Tx were not significantly different. All patients in the ATG group survived the observation period while 4 patients (6.5%) receiving Simulect died (1x sepsis, 3x cardiological causes). 6 grafts (9.6%) in the Simulect group vs. 2 grafts in the ATG group discontinued to function during the 1st year (Simulect: 3x vascular rejection, 1x thrombosis, 2x non-function/ATG: 1x non-function, 1x vascular rejection, p=0.039). Acute rejections were observed in 19 patients receiving ATG (30%; 16x steroid sensitive, 3x non-steroid sensitive), and in 15 patients receiving Simulect (24%: 11x steroid-sensitive, 4x non-steroid sensitive, p=n.s.). Serum creatinine was not different between both groups by 12 months: (ATG vs. Simulect: 1st grafts: 1.5 vs. 1.4 mg/dl; 2nd grafts: 1.6 vs. 1.5 mg/dl; 3rd grafts: 1.6 vs. 1.4 mg/dl, p=n.s.). Patients receiving Simulect demonstrated more surgical complications (Simulect: n=14 vs. ATG: n=8, p=n.s.) while increased rates of CMV infection and de-novo Diabetes were observed in the ATG group (CMV: ATG: n=7 vs. Simulect: n=2; de-novo Diabetes ATG: n=2 vs. Simulect: n=0, p=n.s). In summary, we did observe a minor improvement of transplant survival in the ATG group. However, patients in the Simulect did also show a tendency for an increased immunological risk. Future clinical studies testing the effects of induction therapies may be particular relevant in high risk patients receiving grafts with reduced quality.

KIDNEY: LONG TERM OUTCOMES & COMPLICATIONS

Abstract# 1274 Poster Board #-Session: P30-III

ONE-YEAR POST-TRANSPLANT RENAL FUNCTION IS A STRONG PREDICTOR OF LONG TERM KIDNEY FUNCTION: RESULTS FROM THE NEORAL-MOST OBSERVATIONAL STUDY. Maurizio Salvadori,[1] Alberto Rosati,[2] Andreas Bock,[2] Jeremy Chapman,[2] Bertrand Dussol,[2] Lutz Fritsche,[2] John Jeffery,[2] Volker Kliem,[2] Yvon Lebranchu,[2] Federico Oppenheimer,[2] Erich Pohanka,[2] Gunnar Tufveson.[2] *[1]Renal Unit Dept of Renal Transplantation, Careggi University Hospital, Florence, Tuscany, Italy; [2]The Neoral MOST Study Group.*

Purpose: To assess the influence of demographic and transplantation-related risk factors on both 1-year and 5-year graft function, as well as the influence of 1-year graft function on the 5-year graft function in patients receiving Neoral for 5 years. Methods: MOST is a multi-national study in transplant recipients receiving cyclosporine based regimens under conditions of normal clinical practice. The present analysis is based on observation of 1820 de novo and 4630 maintenance kidney transplant recipients from 34 different countries. Graft function was evaluated by both serum creatinine levels and estimated glomerular filtration rate (GFR).We assessed risk factors for which sufficient data at 5 years were available.The patients were stratified according to their serum creatinine (SCr) at year 1,<130 µmol/l and 130-259 µmol/l. Results: The 1-year graft function,analyzed by a chi² test for the relative risk (RR) and the multifactorial analysis of variance, was influenced by donor age >60yrs,p<0.0001;RR for having a one year SCr >130 µmol/l 3.17; delayed graft function, p<0.0001,RR=1.57; acute rejection, p<0.0001,RR=1.50; PRA>50%, p<0.02,RR=1.29; occurrence of CMV infection p<0.04,RR=1.28.The 5-year graft function, analyzed by the unpaired t test and ANOVA followed by post Hoc testing when appropriate, was on average stable and independent of the above-mentioned risk factors in patients with a SCr <130 µmol/l at 1-year.Amongst patients with a 1-year SCr>130 µmol/l, average GFR at year 5 was similar to the earlier determined GFR if they were free from other risk factors, while in those with risk factors, GFR at year 5 declined compared to earlier timepoints. The strongest factors determining a GFR decrease were: donor age >60yrs (p<0.001), PRA >50% (p<0.001),DGF (p<0.001), CMV infection (p<0.001), acute rejection (p=0.006). Conclusion: Peritransplant risk factors have a relevant effect on the 1-year graft function.An ongoing effect on further decline of graft function can be noted in patients whose 1-year SCr is above 130 µmol/l. Despite the inherent limitations of observational studies such as this, the finding is in line with recent observations from registries (UNOS,analyzed by Hariharan, CTS) demonstrating the influence of 1-year renal function on long term graft survival.

Abstract# 1275 **Poster Board #-Session: P31-III**

FAVORABLE CARDIOVASCULAR RISK PROFILE WHEN SIROLIMUS IS USED WITHOUT CYCLOSPORINE COMPARED TO COMBINATION THERAPY: 3-YEAR RESULTS OF THE RAPAMUNE MAINTENANCE REGIMEN TRIAL. Francesco P. Schena,[1] Neville Jamieson, José Colon, Ahmed Shoker, Joao Pena, Javier Martinez, Rowan Walker, Wolfgang Arns, Robert Johnson, Martine Gioud-Paquet, the Rapamune Maintenance Regimen Study Group. *[1]Division of Nephrology, University of Bari, Bari, Italy.*

Purpose: This paper examines whether the higher sirolimus (Rapamune®, SRL) doses used with SRL-based therapy affect cardiovascular risk factors compared with continuous SRL+ cyclosporine (CsA) therapy. **Methods:** 525 renal allograft recipients were enrolled and received SRL 2 mg (tablets), CsA, and steroids. SRL blood levels were maintained >4 ng/mL (chromatographic assay). At 3 months ± 2 weeks, 430 eligible patients were randomly assigned to remain on triple therapy or to have CsA withdrawn and SRL troughs increased to 16-24 ng/mL until month 12, then 12-20 ng/mL, thereafter. **Results:** Based on follow-up ≥ 36 months in the 430 randomized patients, graft loss censored for deaths (7.9% vs 3.3%) was significantly lower with SRL-based therapy; deaths (7.4% vs 4.2%) and postrandomization acute rejection (6.0% vs 10.2%) were not statistically different, SRL+CsA vs SRL, respectively. Treatment-emergent hypertension after randomization was more frequent in patients remaining on CsA (24.2% vs 10.2%, p <0.001), and these patients received significantly more antihypertensive medication. Diastolic (D-BP), systolic (S-BP), and mean arterial blood pressures (MAP) were significantly lower in patients who had CsA withdrawn (see Table). Renal function was significantly better in the SRL group (168 vs 149 µmol/L, p<0.001, ITT analysis). There were no significant differences in the incidence of fatal cardiovascular events (1.4 % vs 1.4%), treatment-emergent diabetes mellitus (6.5% vs 6.0%, SRL+CsA vs SRL), or any of the lipid parameters (see Table). **Conclusion:** There are no significant differences with regard to serum lipids and incidence of diabetes, whereas renal function, blood pressure, and graft survival are improved after CsA withdrawal. Thus, the cardiovascular profile appears favorable for SRL-based therapy compared with continuous SRL+CsA therapy.

Lipid and Blood Pressure Results (mean ±SE) at 36 Months

Regimen	T-Chol (mM)	Trig (mM)	LDL-C (mM)	HDL-C (mM)	D-BP (mm Hg)	SP (mm Hg)	MAP (mm Hg)
SRL+CsA	5.9±0.2	2.3±0.1	3.5±0.1	1.6±0.05	81.2±0.9	140.0±1.9	100.8
SRL	6.3±0.1	2.4±0.1	3.6±0.1	1.7±0.06	76.3±1.0	131.3±1.6	94.7
p	0.059	0.403	0.251	0.952	0.006	0.002	<0.001

Abstract# 1276 **Poster Board #-Session: P32-III**

FIVE-YEAR FOLLOW-UP OF A PROSPECTIVE, RANDOMISED STUDY ON MINIMISING IMMUNOSUPPRESSIVE MEDICATION FROM ONE YEAR AFTER KIDNEY TRANSPLANTATION. Peter J. H. Smak Gregoor,[1] Teun van Gelder,[1] Nicole M. van Besouw,[1] Barbara J. van der Mast,[1] Petronella de Kuiper,[1] Jan N. M. IJzermans,[2] Willem Weimar.[1] *[1]Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands; [2]Surgery, Erasmus Medical Center, Rotterdam, Netherlands.*

Purpose In Rotterdam renal transplant patients have been routinely converted from cyclosporine (CsA) and prednisone (pred) maintenance therapy to azathioprine (AZA)+pred 1 year after transplantation. We published data demonstrating a superior efficacy of mycophenolate mofetil (MMF) compared to AZA, in terms of preventing acute rejection after conversion from CsA+pred to MMF+pred or to AZA+pred, 1 year posttransplantation. A beneficial effect on renal function was noted for both groups. We now report the long-term results. **Methods** All patients with stable graft function at one year post-transplantation, transplanted between September 1995 and January 1997, and on maintenance treatment with CsA+pred were randomised for conversion to either MMF+pred (n=34) or to AZA+pred (n=30). After starting MMF 1 gram bid or AZA 2 mg/kg/day the CsA-dose was gradually tapered and stopped after 4 weeks. To further reduce the total immunosuppressive load, a 25% reduction in the AZA and MMF dose was performed at 4 and again at 8 months after conversion. Patient and graft survival, and renal function for both groups at the end of follow-up (January 1st 2002), was analysed according to intention-to-treat principle. **Results** The average follow-up after transplantation was 59±17 and 58±18 months for the MMF and AZA group (p=ns), respectively. There was no statistical significant difference between graft and patient survival at the end of the study period between the MMF or AZA group. Graft survival (excluding patient death) was 82% vs 90% in the MMF and AZA group, respectively. Patient survival with functioning graft was 94% and 87% in the MMF and AZA group, respectively. A total of 77% compared to 76% of patients in the MMF and AZA group with functioning graft were still on the assigned treatment at the end of follow-up. The median serum creatinine at the time of randomisation was comparable for both groups. This did not differ significantly from the serum creatinine at the end of the study period; 131 vs 106 umol/l in the MMF and AZA group, respectively. **Conclusion** Conversion of CsA+pred to MMF or AZA+pred 1-year after kidney transplantation results in an acceptable renal function at longer follow-up, with comparable patient and graft survival.

Abstract# 1277 **Poster Board #-Session: P33-III**

LONG-TERM CARDIOVASCULAR RISK FOLLOWING KIDNEY TRANSPLANTATION. M. R. First.[1] *[1]For the Transplant Therapy Outcomes Study Group, University of Cincinnati Medical Center, Cincinnati, OH.*

Purpose: Long-term outcomes following kidney transplantation are the focus of post-transplant patient (pt) management. However, few recent prospective clinical trials follow pts for more than 1 year. This retrospective study aims to examine outcomes at 3 years in a projected population of 2000 consecutive pts from 20 centers who underwent primary renal transplantation between 1/97 and 9/98. **Methods:** We report on secondary endpoints representing cardiovascular risk factors from 648 pts in 6 centers. We used electronic Synapse AXON technology to enter data and to generate immediate data reports and statistics. **Results:** Recipient characteristics are described in a separate abstract submitted to ATC. Interim reports from 1/3 of centers capture sites that predominantly prescribe CSA. Therefore, analysis by calcineurin inhibitor treatment group awaits database completion. At data collection time points, SCr values were available for a minimum of 88% of pts. Blood pressure measurements were reported for a maximum of 89% and a minimum of 65% of pts; serum lipid levels for a maximum of 51% and a minimum of 10% of pts. Mean SCr (mg/dL) was 1.61±0.70 at 6 months, 1.63±0.85 at 1 year, and 1.82±1.21 at 3 years. Mean systolic and diastolic blood pressure did not change significantly over the follow-up period (136/80 mmHg at 3 years). However, the proportion of pts receiving antihypertensives increased from 64% at day 0 to 86% at 3 years. Total cholesterol (C) and LDL-C levels increased between day 0 and 6 months. However, levels were maintained within normal limits after month 6. Values at 3 years were: Total C 198±46 mg/dL, LDL-C 114±36 mg/dL. Mean triglyceride levels remained elevated throughout follow-up; at 3 years the level was 186±128 mg/dL. The proportion of pts receiving antihyperlipidemic agents increased from 10% at day 0 to 51% at 3 years. **Conclusions:** We present interim long-term outcome results for 648 pts of a potential 2000 kidney transplant pts studied in a retrospective multicenter study of routine clinical practice. Pt survival was 94.8%, with 32.3% of deaths due to cardiovascular events. Despite concerns about cardiovascular-related deaths in kidney transplant pts, lipid profiles were infrequently monitored. An increasing proportion of pts required antihypertensive and antihyperlipidemic therapy over time. Updated data reflecting the relative influence of calcineurin inhibitors will be presented at ATC.

Abstract# 1278 **Poster Board #-Session: P34-III**

LONG-TERM OUTCOMES FOLLOWING KIDNEY TRANSPLANTATION IN ROUTINE CLINICAL PRACTICE. M. R. First,[1] for the Transplant Therapy Outcomes Study Group. *[1]University of Cincinnati Medical Center, Cincinnati, OH.*

Purpose: Current post-transplant patient (pt) management focuses on long-term outcomes following kidney transplantation. However, few recent prospective clinical trials follow pts for more than 1 year. This retrospective study aims to examine long-term outcomes in 2000 consecutive pts from 20 centers who underwent primary renal transplantation between 1/97 and 9/98. **Methods:** We report on 648 pts from 6 centers followed to 3 years post-transplant, graft loss, or death. We used Synapse AXON technology to enter data and generate reports and statistics. The primary endpoints are long-term renal function (LTRF), acute rejection, graft survival, and pt survival. **Results:** Pt characteristics corroborate data reported to UNOS/SRTR—26% African Americans, 63% cadaveric organ recipients, 60% male, and 27% with DGF (dialysis in 1st wk). Hypertension—27%, diabetic nephropathy—18%, and glomerulonephritis—17%, constituted the majority of pre-transplant diagnoses. Induction therapy was used in 45% of pts. Initial maintenance regimens included CSA—78%, TAC—18%. At 3 years, 28% of pts are receiving TAC; 5% are on no calcineurin inhibitor (CNI). All pts received oral corticosteroids throughout follow-up; 93% are on MMF. Interim reports from 1/3 of centers capture sites that predominantly prescribe CSA. Therefore, analysis by CNI treatment group awaits database completion. There are no reports of chronic rejection. The incidence of acute rejection over the first year was 26%—8.9% of pts required antilymphocyte antibodies.

	1 Year	3 Years
Acute Rejection %		
- Borderline	2.8	3.9
- Mild (Gr I)	10.0	13.1
- Mod.-Severe (Gr II-III)	8.5	14.7
- No biopsy/score not reported	4.5	6.0
Mean SCr (mg/dL)	1.63±0.85	1.82±1.20

Over 3 years, 6% of grafts were lost (death-censored). In pts who discontinued CNIs after the first year, 9/34 grafts were lost. Pt survival was 94.8%; 2.8% of pts died within the first year post-transplant. Cardiovascular events accounted for 32% of deaths; infection—18%, malignancy—12%. Adverse events recorded for >20% pts include GI disorder and bacterial infection. No cases of PTDM have been reported. **Conclusions:** We present interim long-term outcome results for 648 of a potential 2000 kidney transplant pts studied in routine practice. Mean SCr levels at 1 year are above the 1.5 mg/dL level recently reported to correlate with good LTRF. Pt and graft survival corroborate data collected in prospective trials. However, graft loss appears to be greater when CNIs are withdrawn after the first year. Updated data will be presented at ATC.

Abstract# 1279 **Poster Board #-Session: P35-III**

PROBLEMS AND PITFALLS OF CYCLOSPORINE ABSORPTION PROFILING USING C2-MONITORING. K. Budde,[1] G. Einecke,[1] M. Schütz,[1] I. Mai,[1] J. Waiser,[1] L. Fritsche,[1] H. H. Neumayer.[1] [1]*Nephrology, CharitéBerlin, Germany.*

The cyclosporineA (CyA) concentration 2hr postdose (C2) is supposed to be a better method of estimating the extent of CyA absorption. It has been proposed that dose changes lead to proportional changes in C2 levels. We aimed to validate this concept in 29 consecutive de-novo renal transplant patients (pts) treated initially with Neoral (4,5mg/kg BID), mycophenolatesodium, steroids and basiliximab. Pts were monitored by C2 and trough levels (C0). In addition, we analyzed 38 Neoral dose changes in 27 stable pts (>6mo after transplant) monitored by C2 and C0. Results: After 6mo pt and graft survival was 100%, rejection rate was 17%. Over the first 14d mean CyA C0 was between 250-300ng/ml, C2 rose from 693±391 to 1222±510ng/ml (p<0,05). Only a few pts achieved the suggested C2 levels (19% >1500, 50% >1200ng/ml) in the first week despite increasing dose (623±175 to 672±240mg/day, n.s.). After 14d 63% (83%) of pts had reached C2 >1500ng/ml (>1200) despite decreased dose (483±160mg/d; p<0,05). All variables showed a high interpatient variability. Surprisingly, 35% of pts had intermittent high C0 (>300), but low C2 (<800), suggesting poor and slow absorption; most of these pts suffered from CyA toxicity. We observed a significant (p<0,05) change of absorption as measured by C2/C0 (d1: 2,8±2,5 to 5,3±2,1 on d11) leading to an increase of C2/dose (d1: 1,0±0,9 to 3,8±3,1 on d17). Finally, we investigated, whether 193 dose changes within the first 6mo had lead to proportional changes in C0 and C2. C2 correlated only poorly with dose changes (d1-7: r=0,40; d7-30: r=0,22; d30-mo6: r=0,22), whereas C0 correlated a little bit better over the different time periods (d1-7: r=0,53; d7-30: r=0,25; d30-mo6: r=0,55). Even stable pts exhibited only a poor correlation between the percentage of dose change and the percentage of change in C2 levels (r=0,34). Again, the change in C0 correlated better with dose changes (r=0,54). We conclude that 1.) there is high variabiliy in CyA absorption as detected by C2 monitoring; 2.) there is a natural increase of CyA absorption in the initial postoperative period; 3.) despite increase of dosage many pts do not reach the proposed levels; 4.) with the use of basiliximab and mycophenolatesodium lower target levels seem to be sufficient; 5.) a significant proportion of pts are poor and slow absorbers and CyA toxicity may not be detected by C2 monitoring alone; 6.) CyA pharmacokinetics and C2 levels are not dose proportional; 7.) differences between low and high absorbers need further investigation.

Abstract# 1280 **Poster Board #-Session: P36-III**

LONG-TERM OUTCOME AND ANALYSIS OF FACTORS INFLUENCING GRAFT AND PATIENT SURVIVAL AFTER STEROID WITHDRAWAL. Jae S. Chung,[1] Douglas J. Norman,[1] Jonathan C. Prather,[1] Murali S. Golconda,[1] Ann M. Rivinus,[1] Angelo M. de Mattos.[1] [1]*Transplant Medicine Program, Oregon Health and Science University, Portland, OR.*

Background: Due to adverse effects, steroid withdrawal (SW) following renal transplant (tx) has been attempted with variable success rates. Few studies have examined the effects of SW on long-term outcome. This is an analysis of long-term outcomes for patients transplanted at our center between 1993 and 1998 who have undergone SW. **Methods:** 368 adult renal-only recipients were followed until September 2002; 182 patients were enrolled for SW while SW was not attempted in 184 patients (No SW group). SW was attempted for selected patients based on their first year outcome. Inclusion criteria were recipients of serum creatinine lower than 2.1mg/dl; maximum of 2 acute rejections (AR); adequate doses of azathioprine or mycophenolate mofetil and cyclosporine or tacrolimus; no AR in the 6months preceding SW. Groups were analyzed on an intention-to-treat basis. Any variable which could be considered as potential confounders of graft and patient survival were analyzed in a Cox proportional hazards regression model. **Results:** 18 patients failed SW. The causes of SW failure were 9 AR, 5 renal dysfunction, 5 arthralgia, 4 leukopenia and 3 other. After 1 year post-tx, the incidence of acute rejection, total cholesterol, and infection rate were comparable between both groups. However, the SW group had lower incidence of cardiac events (p=.01), and trends toward a more patients requiring less antihypertensive agents at 3 years post-tx (p=.09) and lower incidence of PTDM (p=.09). The factors influencing graft and patient survival were determined by Cox regression analysis. SW (RR=0.21, p<0.01), delayed graft function (DGF) (RR=2.76, p<0.01), and diabetes as original kidney disease (RR=2.65, p=.02) were significantly associated with graft failure. SW (RR=0.26, p<0.01) and delayed graft function (RR=2.76, p=0.01) were significant risk factors of patient death. Peak PRA, HLA mismatch, flow crossmatch, serum creatinine at 1 year post-tx, number of AR, number of tx, gender, age, race, induction and maintenance immunosuppressant were not significantly associated with graft and patient survival. **Conclusion:** After SW, the incidences of cardiac event, hypertension and PTDM were decreased compared with the No SW group. The SW group showed better long-term graft and patient survival after adjusting for potential confounders. DGF was a significant risk factor of both graft and patient survival, and diabetes as original kidney disease was a significant risk factor of graft survival regardless of SW.

Abstract# 1281 **Poster Board #-Session: P37-III**

THE IMMUNOSUPPRESSION IN KIDNEY TRANSPLANT RECIPIENTS CHANGES THE RELATIONSHIP BETWEEN CYTOKINE GENE POLYMORPHISMS AND CYTOKINE PRODUCTION WHICH IS SEEN IN HEALTHY BLOOD DONORS. Nina Babel,[1] Athanasios Vergopoulos,[2] Robert Sabat,[2] Ian Hutchinson,[3] Hans-Dieter Volk,[2] Petra Reinke.[1] [1]*Nephrology and Internal Intensive Care, CharitéBerlin, Germany;* [2]*Medical Immunology, CharitéBerlin, Germany;* [3]*School of Biological Sciences, Univ. Manchester, Manchester, United Kingdom.*

Background: For many cytokine genes a relationship between genotype (gene polymorphisms) and phenotype (high/low responder)was described. Moreover, several studies found an association between cytokine gene polymorphisms and the outcome of allotransplantation (Tx). However, the influence of immunosuppression (IS) on the genetically determined phenotype was unknown. Here we studied the relation between gene polymorphisms of TNFa (-308), IFNg (+874), and IL-10 (-1082) and ex vivo cytokine secretion in kidney Tx patients. **Material and Methods:** DNA was prepared from 31 healthy volunteers and 26 kidney Tx patients with stable graft function. The patients were on standard triple drug maintenance immunosuppression. For measurement of ex vivo cytokine secretion capacity by ELISA, whole blood was stimulated with LPS (monocyte stimulation) or ConA (T cell stimulation) for 4 and 24 hrs. **Results:** We observed that in healthy individuals the GG (-308) TNFa, the AA (+874) IFNg, and the AA (-1082) IL-10 genotypes were associated with significantly higher ex vivo TNF-a, IFNg, and IL-10 production, respectively, compared with the corresponding genotypes (e.g.: TNFa 4h LPS: GG 6536 pg/ml vs. AG/AA 3740 pg/ml, p=0.02; IFNg 24h ConA: TT 51.8 IU/ml vs. AT/AA 78.5 IU/ml, p=0.05; IL-10 24h LPS: AA 319 pg/ml vs. AG/GG 192 pg/ml, p=0.006). Tx patients produced less TNFa and IFNg but, surprisingly, similar amounts of IL-10. Analysing the genotype/phenotype relation in Tx patients, we found that TNFa high-responders (GG) produced similar low amounts of TNF (3005 pg/ml) as healthy low responders, whereas the levels in Tx low responders (AA/AG) did not differ from both groups (3719 pg/ml). Tx patients produced significantly less IFNg than healthy low/high-responders without differences between the two genotypic groups (18.3 vs. 20.4 IU/ml in TT vs. AT/AA, respectively). Surprisingly, IL-10 high response was not significantly changed in Tx (288 pg/ml), whereas low responders produced even more IL-10 (316 pg/ml) than healthy low responder controls. **Conclusion:** IS changes the genotype/phenotype relation seen in controls. T-cell response (IFNg) is strongly suppressed and the high/low responder difference is abolished. IS also equalizes the TNF/IL-10 ratio following monocytic stimulation. It has to be proofed whether steroids can be tappered in TNF low/IL-10 high Tx patients.

Abstract# 1282 **Poster Board #-Session: P38-III**

THE PREDICTIVE VALUE OF SUBCLINICAL REJECTION IN A STEROID FREE IMMUNOSUPPRESSIVE REGIMEN. Kosunarty Fa,[1] Mark R. Laftavi,[2] Elizabeth Ferry,[3] Anil M. S. Kumar,[3] Billie Fyfe,[4] Oleh G. Pankewycz.[2] [1]*Medicine, University of Pennsylvania, Philadelphia, PA;* [2]*Surgery and Medicine, University at Buffalo, Buffalo, NY;* [3]*Surgery, Drexel University, Philadelphia, PA;* [4]*Pathology, Drexel University, Philadelphia, PA.*

Steroid avoidance (SA) following kidney transplantation offers many potential advantages, however, the safety and efficacy of steroid-free immunosuppression is not fully defined. In this study, we assess whether protocol biopsies aid in the evaluation of renal transplant recipients in whom steroids were rapidly discontinued. We performed protocol biopsies one month after transplantation and correlated histological findings with clinical outcome at one year. In this randomized prospective trial, two groups of patients were treated with basiliximab (20 mg/iv on day 0 and 4), cyclosporin (Neoral), mycophenolate mofetil and steroids 250 iv day 0. The control group (C, n=10) received steroids 125 mg/iv day 1, prednisone 30 mg/po on day 2, which was tapered to 5 mg/d/po by day 30 and continued indefinitely. In the experimental group (SA, n=17), prednisone (30 mg day 1) was decreased by 5 mg/d until day 7 and discontinued. Twenty four of 27 patients underwent a protocol biopsy. Both groups were equivalent for demographic and medical characteristics. Only one subclinical rejection in the SA group (Grade 1A) was treated with supplemental steroids. All patients completed one year of clinical observation. Renal function was compared for both C vs. SA groups as well as for those with vs. without subclinical rejection. Two of 10 C patients had subclinical rejection (Borderline, Grade 1B), whereas, 5 of 14 SA patients had subclinical rejection (2 Borderline, 3 Grade 1A) (p=0.40). All 7 patients with subclinical rejection later developed clinical rejection. Only one clinical rejection occurred following a negative protocol biopsy (p<0.001). One graft was lost in the SA group. The C and SA groups had equivalent renal function at one year. In our study, subclinical rejection failed to predict renal function at one year.

	GFR (Cockcroft-Gault) ml/min		
	1 month	6 months	12 months
Control (LDS)	49.8 ± 23.5	57.7 ± 20.7	63.1 ± 18.7
Experimental (SA)	52.5 ± 18.6 p=0.75	55.7 ± 23.0 p=0.82	54.8 ± 20.1 p= 0.30
No Protocol Rejection	52.0 ± 20.9	58.1 ± 20.6	57.7 ± 19.8
Positive Protocol Rejection	55.3 ± 22.6 p=0.64	49.0 ± 26.4 p=0.43	58.2 ± 24.4 p=0.96

Thus, subclinical rejection provides an early assessment of patient risk for clinical rejection. Patients with subclinical rejection may benefit from therapy or an increase in immunosuppression. The long-term impact of subclinical rejection requires further study.

Abstract# 1283 Poster Board #-Session: P39-III

RISK FACTORS FOR IMPAIRED WOUND HEALING IN SIROLIMUS TREATED RENAL TRANSPLANT RECIPIENTS.

Richard J. Knight,[1] Martin Villa,[1] Maria Welsh,[1] Ronald Kerman,[1] Charles T. Van Buren,[1] Stephen M. Katz,[1] Barry D. Kahan.[1] *[1]Division of Immunology and Organ Transplantation, University of Texas Medical School at Houston, Houston, TX.*

Background: Sirolimus has been associated with impaired wound healing.The aim of this study was to determine risk factors for the development of incisional hernia after renal transplantation in patients treated *de novo* with sirolimus **Methods:** A retrospective review of 106 renal transplant recipients from 1/3/2001 through 12/25/2001. All patients received sirolimus beginning on POD#1 titrated to achieve a trough concentration of 10-20 ng/ml and steroids. Cyclosporine was introduced at a dose of 50 mg bid when the serum creatinine was below 2.5 mg/dl and titrated to achieve a Cav of 200 mg/ml (trough 75-125 ng/ml). Additionally, most patients received either basiliximab or thymoglobulin. Potential risk factors for impaired wound healing included recipient age, diagnosis of diabetes, type of transplant (living vs. cadaveric donor), body mass index (BMI) anti-T cell induction agent, total dose of sirolimus over the first 5 post-transplant days and sirolimus trough level at one week post-transplantation. **Results:** After a mean follow-up of 16±4 months, 16 of 106 patients (15%) were diagnosed with an incisional hernia. Seven of 21 (33%) recipients with a BMI >32 developed this complication compared to 9/85 (10%) of recipients with a BMI <32(p: 0.02). Among cadaveric recipients, one of 33 patients (3%) receiving a dose <35 mg over the first 5 days developed an incisional hernia compared to 10/52 patients (19%) with a 5-day dose >35 mg (p: 0.04). Additionally, the use of thymoglobulin vs. basiliximab increased the risk of this complication, 6/20 (30%) vs. 8/78 (10%, p: 0.04). Exposure to high (>15 ng/ml) sirolimus trough concentrations within the first post-transplant week, the type of transplant (living vs. cadaveric), recipient age, and the diagnosis of diabetes had no influence on this complication. **Conclusion:** Recipient BMI >32, thymoglobulin induction, and an initial 5-dose >35 mg were found to be independent risk factors for impaired wound healing in sirolimus treated renal transplant recipients.

Abstract# 1284 Poster Board #-Session: P40-III

SIROLIMUS AND DELAYED GRAFT FUNCTION AFTER RENAL TRANSPLANTATION.

Richard J. Knight,[1] Martin Villa,[1] Maria Welsh,[1] Ronald Kerman,[1] Charles T. Van Buren,[1] Stephen M. Katz,[1] Barry D. Kahan.[1] *[1]Division of Immunology and Organ Transplantation, University of Texas Medical School at Houston, Houston, TX.*

Background: We sought to determine if elevated sirolimus levels generally impaired recovery of renal function after cadaveric transplantation during the early post-transplant period. **Methods:** A retrospective review of 133 rejection-free cadaveric renal transplant recipients from January 2000 through December 2001. All patients received sirolimus (SRL) beginning on POD#1 titrated to achieve a trough concentration of 10-20 ng/ml and steroids. Cyclosporine was introduced at a dose of 50 mg bid when the serum creatinine was below 2.5 mg/dl and titrated to achieve a Cav of 200 mg/ml (trough 75-125 ng/ml). Additionally, all patients received either basiliximab (70%) or rabbit anti-thymocyte globulin (30%). Patients were stratified into 3 groups based on sirolimus trough concentration at one week post-transplantation. Low (<7.0 ng/ml), intermediate (7.1-15.0 ng/ml) and high (>15.0 ng/ml) level. Recovery of renal function was determined by the incidence of delayed graft function (DGF, need for dialysis), and the mean serum creatinine (Cr) at one and 4 weeks post-transplantation. **Results**: The mean cold ischemia time (CIT) was 15±8 hours, the mean donor age was 29±16 years, and the incidence of DGF was 26%. Both prolonged CIT (>24 hours) and older donor age (>50 years) were associated with an increased incidence of DGF (p<0.05). The relationship between sirolimus exposure and recovery of renal function is shown in the table below.

	Group 1 n=22	Group 2 n=64	Group 3 n=47	
SRL trough at 1 wk (ng/ml)	<7.0	7.1-15.0	>15.0	
Donor age (years)	28±18	27±16	33±16	ns
CIT (hours)	14±6	14±8	15±6	ns
DGF	41%	26%	19%	ns
Mean Cr (mg/dl) at 1 wk	6.2±4.1	5.1±3.6	3.2±2.8	<0.01 vs. 1,2
Mean Cr (mg/dl) at 4 wks	1.6±1.0	1.6±0.9	1.2±0.5	<0.01 vs. 1,2

The best renal function was observed among patients who had the greatest exposure to sirolimus. Conclusion: Exposure to high sirolimus levels in the early post-transplant period did not impair recovery from ischemia-reperfusion injury after cadaveric renal transplantation.

Abstract# 1285 Poster Board #-Session: P41-III

THE REJECTION-THROMBOTIC MICROANGIOPATHY ASSOCIATION: IS THERE NEED TO RE-EVALUATE CURRENT IMMUNOSUPPRESSION STRATEGIES?

Rajiv D. Poduval,[1] Pradeep V. Kadambi,[1] Amandeep S. Khurana,[1] Shane M. Meehan,[2] Robert C. Harland,[3] J. Richard Thistlethwaite,[3] Basit Javaid,[1] Michelle A. Josephson.[1] *[1]Department of Medicine, Section of Nephrology; [2]Department of Pathology; [3]Department of Surgery, Section of Transplantation, University of Chicago, Chicago, IL.*

Aim: To delineate the etiology and clinical course of post-transplantation thrombotic microangiopathy (TMA). **Methods:** All transplant (TXP) biopsies done at the University of Chicago between 1997 and 2002 were reviewed for the presence of TMA. Pertinent patient data were collected by review of medical records. **Results:** 17 patients (PTS) with TMA were identified. Mean age was 42±11 years. 8/17 (47%) had a kidney TXP, and 9/17 (53%) had a pancreas and kidney TXP (SPK). Initial immunosuppression included steroids in 17 (100%) PTS, MMF in 13 (76%), FK in 14 (82%), CsA in 3 (18%), sirolimus in 3 (18%), and induction therapy with an IL 2 receptor blocker in 11 (65%). Baseline creatinine (SCr) was 1.9±1.3 mg/dL. SCr at diagnosis of TMA was 3.1±1.4 mg/dL. The cause of TMA was calcineurin inhibitor toxicity (CIT) in 14 (82%), and acute rejection (AR) in 3 (18%). AR group included 2 PTS with humoral, and one with both cellular and humoral rejection. None of the PTS had HUS/TTP, lupus, Hepatitis C, anti-phospholipid antibody syndrome, malignant hypertension, DIC, malignancy or chemotherapy. Of the PTS with CIT related TMA (CIT-TMA), 11/14 (79%) were on FK, and 3/14 (21%) were on CsA. All 3 PTS with AR related TMA (AR-TMA) were on FK. PTS with CIT-TMA had higher FK troughs compared to PTS with AR-TMA (18.2±9.3 ng/mL vs. 5.5±3.3 ng/mL, p=0.003). Mean CsA trough in the 3 PTS with CIT-TMA was 242±37.5 ng/mL. PTS with SPK had higher FK and CsA levels compared to PTS with a kidney alone (FK trough: 17.9±10 ng/mL vs. 12.1±9.7 ng/mL; CsA trough: 242±37.6 ng/mL vs. 223±24 ng/mL). Interestingly, 9/14 (64%) PTS with CIT-TMA had prior AR episodes, warranting increased doses of calcineurin inhibitors (CI). Treatment of TMA included CI discontinuation in 2 PTS, dose reduction in 12, pulse steroids in 4, plasmapheresis in 2, and IVIG in 1. Prognosis was poor, with death-censored graft loss in 3/13 (23%) PTS, and mortality in 4/17 (24%) PTS. **Conclusion: 1.** CIT is a major cause of post-TXP TMA. **2.** Post-TXP TMA is associated with high rates of graft failure and mortality. **3.** The higher incidence of TMA in SPK, compared to kidney TXP, is likely due to the higher CI levels used in SPK. **4.** The association of CIT-TMA with prior AR episodes suggests that the use of high levels of CI for treating AR is an important etiological factor for post-TXP TMA. There is, therefore, need to re-evaluate our CI-based treatment strategies.

Abstract# 1286 Poster Board #-Session: P42-III

ASSESSMENT OF INTERLEUKIN-2 AND INTERLEUKIN 2 RECEPTOR DURING CHRONIC IMMUNOSUPPRESSION IN RENAL TRANSPLANTATION.

Kathleen M. Tornatore,[1,2] Robin DiFrancesco,[1] Kristin Johnson,[1] Andrea Rubino,[1] Alan Forrest,[1] Rocco C. Venuto.[2] *[1]Pharmacy and Medicine, University at Buffalo, Buffalo, NY; [2]Pharmacy and Medicine, Erie County Medical Center, Buffalo, NY.*

Interleukin-2 (IL-2) and interleukin 2 receptor (IL-2R) are a cytokine receptor system responsible for amplification of the immunologic transplant rejection. Minimal data is available correlating drug exposure with IL-2 and IL-2R in renal transplant recipients (RTR) receiving chronic combination immunosuppressives. Methods: Twenty-one female RTR (13 pre-menopausal {PMS} with a mean time post-transplant {TPT}: 58.1 ± 53.0 mos with mean age: 38 years; 8 menopausal {MEN}{mean TPT: 71 ± 92 mos} with mean age: 57 years) were evaluated with a stable serum creatinine {SrCr) of 1.6 mg/dL. All RTR received cyclosporine, mycophenolate mofetil or azathioprine and low-dose methlyprednisolone (MEPN) with cyclosporine trough concentrations (CYAT) targeted between 75-300 ng/ml by monoclonal assay. Serum was collected for IL-2R and plasma analyzed for IL-2 at 8AM prior to immunosuppressive drugs. Concurrent serial collection of serum analysis of MEPN and cortisol was by HPLC with generation of pharmacokinetic parameters: MEPN Area Under the Curve (AUC), Dose Normalized MEPN AUC (AUC*), MEPN clearance (CL), Cortisol AUC. Quantitation of IL-2 and IL-2R was completed in duplicate by ELISA. Results: Il-2 was below the limit of quantitation (< 5.1 pg/ml) in all patients. However, IL-2R was detectable and ranged from 173 to 858 pg/ml in the combined group. No difference in IL-2R was noted between the PMS (349 ± 182 pg/ml) and MEN (320 ± 138 pg/ml). A significant difference was noted in MEPN CL/ total body weight with PMS = 218 ± 94 ml/hr/kg and MEN = 143 ± 31 ml/hr/kg (p = 0.04) and MEPN AUC in PMS = 468 ± 219 ng* hr/ml and MEN = 631 ± 297 ng*hr/ml (p = 0.17). A significant difference was noted in AUC* for PMS = 70 ± 23 ng*hr/ml and MEN = 102 ± 21 ng*hr/ml (p = .004). Multiple linear regression was performed with independent variables: age, SrCr, creatinine clearance, AUC*, MEPN AUC, MEPN CL, Cortisol AUC, CYAT and the dependent variable: IL-2R resulting in significant correlations between IL-2R and SrCr (p< 0.005) and AUC* (p< 0.04). Conclusion: Clinically stable patients with undetectable IL-2 may be due to adequate chemical immunosuppression. However, detectable IL-2R suggests that immune activation is present with a potential association with renal function and steroid drug exposure (AUC*). Presence of IL-2R may be stimulated by alternative chronic immunologic stress mechanisms.

Abstract# 1287 **Poster Board #-Session: P43-III**

EXPANSION OF HLA-SPECIFIC ANTIBODIES FOLLOWING TREATMENT WITH ANTI-THYMOCYTE GLOBULIN. Andrea A. Zachary,[1] Donna P. Lucas,[1] Robert A. Montgomery,[2] Mary S. Leffell.[1] *[1]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.*

Thymoglobulin® is gamma globulin (>90%) purified from the sera of rabbits immunized with human thymocytes. In controlled, multicenter studies, Thymoglobulin® has been shown to be effective in treating acute rejection. One mechanism of action of this drug is depletion of T cells, the numbers of which are drastically reduced within 48 hours of treatment. This mechanism would be efficacious in abrogating rejection mediated by cytotoxic T cells and in preventing de novo activation of B cells by T helper cells. However, in sensitized patients who have undergone immunomodulation, it may have a negative effect by eliminating regulatory cells that are suppressing activation of memory B cells. We have examined sequential serum samples from 7 sensitized patients, 5 of whom were being treated in a protocol to reduce sensitization (plasmapheresis + CMV hyperimmune globulin). Following treatment with Thymoglobulin®, 6 of the patients showed increases in HLA-specific antibody detected by ELISA as shown below. Expanded reactivity was specific for donor HLA antigens in 5 patients and for 3rd party HLA in 3patients. Removal of Thymoglobulin® by absorption did not change the *in vitro* reactivity of sera, indicating that the increased reactivity was not due to an effect of the agent on the test. The increased reactivity may be an epiphenomenon since all the patients were experiencing cellular rejection. It may be due to interaction with B cells resulting in a polyclonal activation However, in flow cytometric assays, we have not detected any binding of this agent to B cells. Finally, it may have resulted from depletion of regulatory T cells, in turn, permitting activation of memory B cells.

Changes in Antibody Following Treatment with Thymoglobulin®

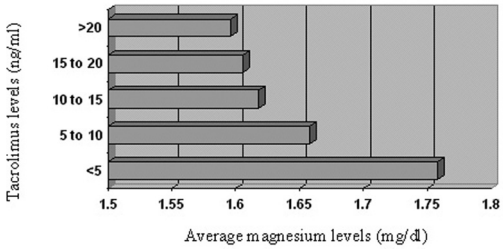
Patient	1	2	3	4	5	6	7
OD pre-treatment	8.4	3.1	2.6	1.5	12	2.3	4.5
OD post-treatment	32.3	26.2	9.1	6.4	22	20.4	6.6
PRA pre-treatment	45	0	ND	2	28	0	2
PRA post-treatment	52	38	ND	12	92	38	5

OD: optical density, ELISA value measuring breadth of HLA-specific antibody

Abstract# 1288 **Poster Board #-Session: P44-III**

ASSOCIATION OF SERUM TACROLIMUS AND MAGNESIUM LEVELS IN RENAL TRANSPLANT PATIENTS. Adhish Agarwal,[1] Lisa Pappas,[1] Troy Somerville,[2] Fuad Shihab,[1] Alexander Goldfarb-Rumyantzev.[1] *[1]Division of Nephrology, University of Utah, Salt Lake City, UT; [2]Transplant Program, University of Utah, Salt Lake City, UT.*

Background: Hypomagnesemia is prevalent in post-renal transplant patients and has been described to have a role in augmenting the nephrotoxic effect of calcineurin inhibitors. Tacrolimus use has been associated with hypomagnesemia, however whether this association is dose (serum level) dependent has never been reported. **Methods**: We retrospectively evaluated serum magnesium levels in 196 post-renal transplant patients who were receiving tacrolimus and were not on magnesium supplementation. There were a total of 2812 observations, comprised of multiple observations for each patient (up to 84) over time. Each observation consisted of a 12-hour trough tacrolimus, serum creatinine and a serum magnesium level all obtained from the same serum sample. A repeated measures statistical analysis was performed to determine the relationship between tacrolimus and magnesium levels. Compound symmetry was assumed to be the structure of the within subject covariance and the statistical model was designed to adjust for serum creatinine. **Results**: Serum tacrolimus levels had a significant negative relationship with serum magnesium levels. A 1ng/ml increase in tacrolimus corresponded to a 0.008mg/dl decrease in serum magnesium ($p<0.0001$). If repeated measures analysis was not used and within subject covariance not adjusted for, this negative relationship remained evident as shown in the figure.



Conclusion: This study suggests that changing tacrolimus dose (and subsequently serum level) is likely to affect serum magnesium levels in renal transplant patients. With the growing amount of data on the role of hypomagnesemia in the pathogenesis of chronic calcineurin inhibitor nephrotoxicity, monitoring of magnesium levels whenever tacrolimus dose is changed should be the standard of care.

Abstract# 1289 **Poster Board #-Session: P45-III**

MYCOPHENOLIC ACID PHARMACOKINETICS (MPA) IN LONG-TERM RENAL TRANSPLANT. Elias David-Neto,[1] Lilian M. P. Araujo,[1] Maria Cristina R. Castro,[1] Cristiane F. Alves,[1] Erica Kakehashi,[1] Nairo M. Sumita,[2] Maria Elizabeth Mendes,[2] Pascoalina Romano,[2] William C. Nahas,[1] Luiz E. Ianhez.[1] *[1]Divisions of Urology and Nephrology -Hospital Das Clinicas, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil; [2]Division of Central Laboratory - Hospital das Clinicas, University of Sao Paulo - School of Medicine, Sao Paulo, SP, Brazil.*

It has been recognized that mycophenolic acid (MPA) should be monitored in the immediate post-renal transplant (RTx) period because MPA target levels for prevention of acute rejection take a few weeks to be reached when fixed-doses of MMFare used. However, little is known about MPA-PK in patients with stable renal graft finction under long term MMF therapy. We studied the MPA-PK in 35 pts (15F/20M) with stable graft function (SCr=1.6±0.6 mg/dL), with a mean age of 42±13 years. The MPA-PKs were performed at a median of 39 mo (24–54) after RTx and 29±19 mo of MMF therapy. Mean serum albumin was 4.1±0.4 g/dL. A complete 12-hour MPA-AUC (EMIT©-MPA test, Dade Behring) was drawn after the MMF morning oral dose. 11pts were under CsA/MMF/Pred, 12 Tacrolimus/MMF/PRED and 12 MMF/PRED. Mean CsA, Tacrolimus and Pred doses were 3.7±1.1, 0.11±0.07 and 0.15±0.04 mg/kg/day, respectively. Mean Tacrolimus trough level and CsA (C2) blood concentration were 8.6±1ug/ml and 615±272ng/ml, respectively. Mean MMF dose was 25.8±8.9 mg/kg/day or 1528±419 mg/day. The mean MPA trough level (C0) was 2.6±1.7 ug/ml, Cmax was 12.6±6.5 ug/ml and trapezoidal AUC_{0-12} was 49.5±21.0 ug.h/ml. The MPA peak level occurred at 1.2±0.5 hours. 27 (77%) patients presented a second MPA peak (5.4±2.9ug/ml), 7.5±2.2 hours after the oral dose, representing 54±42% of the Cmax. 14 (40%) patients were considered to have an adequate exposure (AUC_{0-12} =36-60ug.h/ml) to MPA (49±6.7ug.hr/ml), 9 patients (25%) presented an AUC_{0-12}>60 ug.hr/ml (77.9±14.4 ug.hr/ml) while 12 (34%) showed an AUC_{0-12}<36 ug.hr/ml (28.8±6.4 ug.hr/ml). A Cmax ≥10 ug/ml, which is presumed to be associated with side effects, was seen in 20 (57%) patients. There was no correlation between the MMF dose and dose-normalized MPA-AUC_{0-12}. The MPA trough level (C0) was the isolate parameter that best predicted AUC_{0-12} (R^2= 0.67, $p<0.001$). Mean calculated AUC_{0-12} using the C0 equation, where AUC_{0-12}=23.68+(9.85xC0), was not statistically different from the trapezoidal AUC_{0-12} (49.5±17.2 vs 49.5±21.0 ug.hr/ml). We conclude that 60% of the long-term RTx patients have MPA-AUC_{0-12} out of the presently recommended target range, with 25% over-exposed to the drug. MPA-AUC_{0-12} should be monitored and the C0 abbreviated equation is a useful tool for such monitoring.

Abstract# 1290 **Poster Board #-Session: P46-III**

TACROLIMUS DOSE REQUIREMENT IN RENAL TRANSPLANT RECIPIENTS IS SIGNIFICANTLY HIGHER WHEN USED IN COMBINATION WITH CORTICOSTEROIDS. Dennis A. Hesselink,[1] Hien Ngyuen,[1] Marike Wabbijn,[1] Peter J. H. Smak Gregoor,[1] Ewout W. Steyerberg,[2] Iza C. van Riemsdijk,[1] Willem Weimar,[1] Teun van Gelder.[1,3] *[1]Internal Medicine; [2]Public Health; [3]Pharmacy, Erasmus MC, Rotterdam, Netherlands.*

Background: Tacrolimus is one of the standard immunosuppressive drugs for the prevention of acute rejection after solid organ transplantation. Tacrolimus has a narrow therapeutic index and shows considerable interindividual variability in pharmacokinetics (PK), necessitating therapeutic drug monitoring. Corticosteroids are known inducers of the cytochrome P450 3A4, the main tacrolimus metabolizing enzyme and may thus interfere with tacrolimus PK. We had the opportunity to investigate the effects of corticosteroids on tacrolimus PK in a randomised controlled trial in which one study arm was treated with a corticosteroid-free immunosuppressive regimen and the other arm received prednisone for 3 months. **Patients and Methods**: 65 patients were treated with tacrolimus and mycophenolate mofetil in combination with either daclizumab (n=31) or a 3-month course of prednisone (n=34). Whole blood, pre-dose concentrations (C0) of tacrolimus were measured using the Emit 2000 assay. Pharmacokinetic parameters between month 1-6 were compared between the two groups and within the corticosteroid group before (month 1-3) and after prednisone withdrawal (month 4-6). **Results**: At month 1 the dose-adjusted tacrolimus C0 in the corticosteroid group was 82.7 (± 7.6) compared to 118.8 (±17.2) ng/ml per mg/kg in the daclizumab group ($p = 0.05$). Patients in the corticosteroid group who were treated with calcium channel blockers (CCB) needed a mean tacrolimus dose of 0.18 (±0.013) mg/kg compared to 0.13 (±0.014) mg/kg in the corticosteroid-free group to reach the same C0 at month 1 ($p = 0.013$). The dose-adjusted tacrolimus C0 within the corticosteroid group at month 1 and 2 was 42% and 29% lower compared to month 4 ($p < 0.001$). Renal function was comparable for both groups and did not deteriorate after corticosteroid withdrawal. **Conclusion**: When tacrolimus is used in combination with corticosteroids, a higher dose is required to reach similar target concentrations. The effect of corticosteroids on tacrolimus PK appears smaller when tacrolimus is used in combination with CCBs. Close monitoring of tacrolimus whole blood, pre-dose concentrations is therefore necessary in corticosteroid weaning protocols.

Abstract# 1291 Poster Board #-Session: P47-III

LONG TERM MAINTENANCE WITH LOW CYCLOSPORINE DOSE VERSUS WITHDRAWAL IN KIDNEY TRANSPLANTS RECIPIENTS FROM LIVING RELATED DONOR. Alfredo Chew-Wong,[1] Oscar Ron,[1] Luis Romo,[1] Rafael Reyes-Acevedo.[1] *[1]Nephrology and Transplants, Hospital de Especialidades Miguel Hidalgo, Aguascalientes, Aguascalientes, Mexico.*

Background: Both immune and non-immune factors such as CsA toxicity are important in the pathogenesis of chronic allograft nephropathy (CAN). Controversial evidence exists regarding long term use of CsA in kidney transplant recipients (KTR). **Objetive:** To evaluate long term outcome of KTR with stable and normal graft function with CsA withdrawal as compared to patients with CsA dose reduction. **Patients and Methods:** Retrospective analysis in 156 consecutive patients who received a living related donor kidney transplant from January 1995 to December 2000. Analyzed variables: date of transplant, age, gender, HLA match, CsA dose at month, CsA dose at last visit, frequency of acute rejection (AR), frequency of CAN, serum creatinine (SCr) at month, first year and last visit, and time of follow-up. **Results:** All KTR received CsA+AZA+PDN, 159 living kidney transplants were performed in this period, 148 were included for analysis, 11 were excluded because of incomplete information in the clinical records or ocurrence of any surgical complication that could affect outcome. CsA discontinuation was indicated between 1 and 3 years postransplant in patients with stable and normal graft function at least during the last 6 months. **Conclusions:** Patients maintained with low dose CsA exhibit reduced frequency of CAN and better graft function. Our study shows that complete CsA withdrawal may be associated with more adverse prognosis in patients treated with CsA+AZA+PDN, suggesting that minimal doses of CsA may exert an important immunosuppressive effect in the long term. Graft survival is not different during this time of follow-up, however further evaluation will be requiered in order to evaluate this issue.

Comparison of variables between study groups

	CsA withdrawal (n=32)	CsA low dose (n=116)	p
Age (years)	27 ± 10	29 ± 14	0.37
Male (%)	59	62	0.80
HLA 2, 1, 0 (%)	34, 50, 16	9, 49, 42	0.01
CsA dose at month	5.8 ± 0.9 mg/kg/day	5.3 ± 1.2 mg/kg/day	0.08
CsA dose at last visit	0	3.3 ± 1 mg/kg/day	
SrC at month	1.5 ± 0.3 mg/dl	1.5 ± 0.4 mg/dl	0.67
SrC first year	1.6 ± 0.3 mg/dl	1.4 ± 0.4 mg/dl	0.19
SrC last visit	1.9 ± 1.5 mg/dl	1.5 ± 0.9 mg/dl	0.01
Hypertension (%)	38	45	0.58
Acute rejection (%)	24	23	0.94
CAN (%)	26	5	0.01
Time of follow-up (months)	42 ± 15	40 ± 14	0.10
Graft Survival (%)	81	95	0.72

Abstract# 1292 Poster Board #-Session: P48-III

SCREENING FOR A DRUG INTERACTION OF FTY720 ON CYCLOSPORINE IN RENAL TRANSPLANT PATIENTS. J. M. Kovarik,[1] A. Skerjanec,[1] A. Zimmerlin,[1] B. D. Kahan,[2] H. Tedesco-Silva,[3] G. J. Riviere,[1] R. Schmouder.[1] *[1]Novartis Pharmaceuticals, Basel, Switzerland and East Hanover, NJ; [2]University of Texas Medical School, Houston, TX; [3]Hospital do Rim e Hipertensao, Sao Paulo, Brazil.*

Many drugs used in transplant medicine are known to interact with cyclosporine by competition for metabolism via cytochrome CYP3A4, countertransport via P-glycoprotein, or biliary clearance. Given the high frequency with which adjunct agents are used in cyclosporine-based regimens, all new transplant immunosuppressants should be evaluated both in vitro and clinically for potential drug interactions with cyclosporine during development. A variety of studies addressed this question for FTY720, a novel lymphocyte homing agent. **In vitro studies:** Human liver microsome studies indicated a low potential of FTY at therapeutic blood concentrations to reduce the clearance of CYP3A substrates. **Crossover study in maintenance kidney transplant patients:** 67 patients on a cyclosporine-based regimen randomly received either placebo (n = 15) or FTY at doses of 0.125, 0.25, 0.5, 1, 2.5, or 5 mg/day (n = 6 - 10 per group) for 1 month. Cyclosporine doses were unchanged over the study course. As tabulated below, cyclosporine C0 (trough), C2, Cmax, and AUC did not differ on the last day of treatment (day 28) compared with prestudy (day 0) among the FTY dose levels or placebo (p = NS). Moreover, FTY AUC (range 2 - 809 ng.h/ml pooled across all dose cohorts) was not correlated with cyclosporine AUC (range 2097 - 7323 ng.h/ml) on day 28 (r = 0.16, p = 0.27) further indicating that exposures were independent of each other.

Group	Day	C0 (ng/ml)	C2 (ng/ml)	Cmax (ng/ml)	AUC (ng.h/ml)
FTY720	0	180 ± 73	817 ± 316	1046 ± 356	4579 ± 1349
FTY720	28	185 ± 78	875 ± 334	1047 ± 340	4633 ± 1435
Placebo	0	162 ± 58	834 ± 226	1093 ± 284	4493 ± 937
Placebo	28	204 ± 178	917 ± 297	1141 ± 344	4629 ± 1453

De novo kidney transplant patients: 208 patients were randomized to receive FTY 0.125 mg/day (n = 41), 0.25 mg/day (n = 41), 1 mg/day (n = 38), or 2.5 mg/day (n = 38) or MMF (n = 50) for 3 months posttransplant. A total of 2,306 cyclosporine troughs were obtained at 13 visits. Neither cyclosporine doses nor troughs differed among FTY and MMF treated patients over the study course (p = NS). Troughs at week 1 and month 3 were 327 ± 157 and 259 ± 174 ng/ml in FTY patients vs 291 ± 136 and 247 ± 148 ng/ml in MMF patients. **Conclusions:** No clinically-relevant influences of FTY on cyclosporine pharmacokinetics were detected in extensive in vitro and clinical evaluations.

Abstract# 1293 Poster Board #-Session: P49-III

INFLUENCE OF HYPERLIPIDEMIA ON CYCLOSPORINE PHARMACOKINETICS IN THE CONTEXT OF AN EVEROLIMUS IMMUNOSUPPRESSIVE REGIMEN. J. M. Kovarik,[1] C. Rordorf.[1] *[1]Novartis Pharmaceuticals, Basel, Switzerland.*

In the phase 3 everolimus (Certican) kidney transplant trials everolimus-treated patients received modestly, but significantly, lower cyclosporine (CsA) doses in order to achieve similar CsA trough levels (Cmin) compared with MMF-treated patients. Given the fact that CsA binds to blood lipoproteins and everolimus can elevate cholesterol and triglycerides, we explored whether everolimus-related hyperlipidemia may have contributed to the differential CsA dosing pattern. **Pharmacokinetic and clinical observations**: We examined a total of 3,194 lipid-Cmin data pairs from months 1, 2, 3, and 6 posttransplant in 595 everolimus-treated patients and 323 MMF-treated patients. CsA Cmins were similar at each visit between treatment groups; whereas, the corresponding CsA doses were about 10% lower in everolimus patients. Consequently, CsA Cmin/Dose was significantly higher in everolimus vs MMF patients: 1.39 ± 0.93 vs 1.19 ± 0.57 ng/ml/mg ($p < 0.001$). In everolimus patients, both cholesterol and triglycerides were significantly higher than in MMF patients: 7.1 ± 1.7 vs 6.1 ± 1.3 mmol/L and 3.0 ± 1.9 vs 2.3 ± 1.3 mmol/L (both, $p < 0.001$). **Relationship between lipids and CsA Cmin/Dose:** CsA Cmin/Dose increased with increasing cholesterol and triglyceride levels in everolimus-treated patients when lipids were divided into distribution quartiles as tabulated below. Similar patterns were observed for MMF-treated patients.

Parameter	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Cholesterol (mmol/L)	< 5.8	5.8 - 6.8	6.9 - 8.0	> 8.0
CsA Cmin/Dose (ng/ml/mg)	1.29 ± 0.85	1.35 ± 1.05	1.42 ± 0.80	1.51 ± 1.01
Triglycerides (mmol/L)	< 1.8	1.8 - 2.6	2.7 - 3.7	> 3.7
CsA Cmin/Dose (ng/ml/mg)	1.26 ± 0.94	1.39 ± 0.93	1.34 ± 0.79	1.49 ± 1.02

There was a significant positive correlation between cholesterol and CsA Cmin/Dose ($p < 0.001$) indicating that for each 1 mmol/L increase in cholesterol, Cmin/Dose increased modestly by 4%. Likewise for triglycerides, a significant positive correlation was noted ($p < 0.001$) indicating that for each 1 mmol/L rise in triglycerides, Cmin/Dose increased by 2%. **Conclusions:** Binding of CsA to elevated cholesterol and triglyceride levels in blood may contribute, in part, to the observation that everolimus-treated patients need slightly lower CsA doses to achieve similar CsA Cmins compared with MMF-treated patients. These modest effects on CsA concentration and dose would likely be adjusted for in the context of routine CsA therapeutic drug monitoring.

Abstract# 1294 Poster Board #-Session: P50-III

BAYSESIAN ESTIMATION AND THERAPEUTIC DRUG MONITORING FOR CYCLOSPORINE A AND MYCOPHENOLATE MOFETIL. Yannick Le Meur,[1] Jean-Christophe Szelag,[1] Aurelie Premaud,[2] Franck Saint-Marcoux,[2] Jean Debord,[2] Pierre Marquet.[2] *[1]Department of Nephrology, University Hospital, Limoges, France; [2]Department of Pharmacology and Toxicology, University Hospital, Limoges, France.*

The area under the concentration-time (AUC) better correlates with efficacy and toxicity of immunosuppressive drugs but its use for therapeutic drug monitoring (TDM) is limited by the number of blood samples required. Methods: We first developed a new pharmacokinetic model for cyclosporine A (Csa) and mycophenolic acid (MPA) in a population of 45 renal transplant patients treated with Csa and MMF. We used a convolution of a gamma distribution model (for the absorption rate) with a bi-exponential model. A Bayesian estimator was then developed using a limited sampling strategy (LLS: 3 sampling times) at different periods after transplantation (week 1, 2, M1, M6). Csa and MPA were measured by EMIT. Results : The gamma model yielded a good fit for Csa and MPA in all the patients. The prediction of AUC_{12} provided by the Bayesian estimators was good using the following sampling times : T0, T1, T3 h, (r^2 =0.985) for CsA and T 0.3, 1, 3h (r^2 =0.982) for MMF, giving a non-significant bias. This was confirmed and validated in the 4 post-transplant periods. Compared with classical LSS based on multiple linear regression, the Bayesian estimators are more flexible, as sampling times can be slightly different from the theoretical ones. Furthermore the advantage of a Bayesian model is to provide estimates of other pharmacokinetic parameters like C_{max}, t_{max}, or AUC_4. It also provides a concentration-time curve allowing visual checking of the results. These two algorithms are now being used in two French multicentre prospective studies: DICAM is studying the clinical effects of a reduction of Csa exposure (50% reduction of AUC) in stable renal transplant; and APOMYGRE is addressing the question of the usefulness of MMF TDM in de novo kidney graft recipients. Conclusion. The Bayesian estimators developed in the present study proved to be useful for the TDM of CsA and MMF and are easily applicable in routine clinical practice since they only require 3 blood samples. It should be one option to consider for the TDM of associated immunosuppressive drugs.

Abstract# 1295 **Poster Board #-Session: P51-III**

SURGICAL COMPLICATIONS AFTER KIDNEY TRANSPLANTATION: COMPARING THREE DIFFERENT IMMUNOSUPPRESIVE REGIMENES. Adela Mattiazzi,[1] George W. Burke,[1] Joshua Miller,[1] Gaetano Ciancio.[1] *[1]Department of Surgery, Division of Transplantation, University of Miami School of Medicine, Miami, FL.*

Objective: The aim of this study was to compare the surgical complications after kidney transplantation with three different immunosuppressive regimens. **Methods**: Our study consists of a single center, prospective, randomized trial of 150 patients equally divided in 3 arms: Group I: Tacrolimus (FK) + Rapamycin (RAPA); Group II: FK + Mofetil Mycophenolate (MMF); and Group III: Neoral + RAPA. All patients received induction therapy with Daclizumab (1mg/kg) on the day of surgery, and every other week for a total of 5 doses and maintenance corticosteroids. Target trough levels for FK were 10 ng/ml for the first 3 months then 6-8 ng/ml and 6 ng/ml at 1 year. Target trough RAPA level were 8 ng/ml throughout, and Neoral target trough levels were 200-250 ng/ml for the first 3 months with minimal decrease thereafter. MMF was dosed at 2 gm/day. **Results:** After 1 year follow-up, in **Group I**, 13 of 50 patients (26%) had surgical complications: 3 wound infections and 2 complicated with dehiscence within the first month post-transplant. Nine lymphoceles (8 required percutaneous drainage) were within the first month (5), second (2) and third (2) month post-transplantation respectively; 1 uretheral fistula, and 1 rupture of diverticuli (2 weeks posttransplantation). One patient developed a deadly necrotizing fascitis 2 months post-transplantation. In **Group II**, 4 of 50 patients (8%) had surgical complication: 1 Gastrocnemius abscess and 3 lymphoceles within the first month (1) and the second month (2) post-transplant respectively. In **Group III** 11 of 50 patients (22%) had surgical complications: 2 wound dehiscences (within the first month posttransplantation); 2 inguinal hernias within 3 and 10 months post-transplantation respectively; and 7 lymphoceles within the first month (6) and 3 months (1) after transplantation respectively. The graft survival was 98%, 94% and 92% in the Group I, II and III respectively.

	Group I N=50	Group II N=50	Group III N=50
Wound dehiscence	2	0	2
Lymphoceles	9	3	7
Others	3	0	2
Total surgical complications*	26%	8%	22%
*P value	0.005	0.99	0.01

Conclusion: In this study, patients treated with RAPA show a high incidence of surgical complication compared to the patients treated with MMF. The finding may be associated with the antiproliferative action of the RAPA and further evaluation must be done to correlate these data. Key words: surgical complications, rapamycin, kidney transplantation, immunosuppression.

Abstract# 1296 **Poster Board #-Session: P52-III**

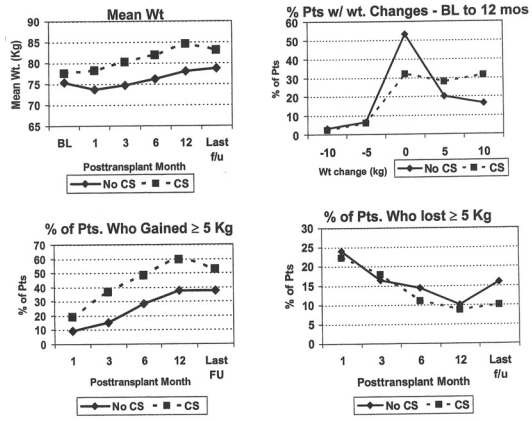
STRATEGIES TO IMPROVE OUTCOME FOR PATIENTS UNDERGOING KIDNEY RETRANSPLANTATION. Benjamin Philosophe,[1] Anne B. Wiland,[1] Eugene J. Schweitzer,[1] Alan C. Farney,[1] Clarence E. Foster,[1] Stephen T. Bartlett.[1] *[1]Surgery, University of Maryland, Baltimore, MD.*

Patients with a failed primary renal transplant reportedly have lower patient survival. Furthermore, retransplantation is typically associated with a higher rejection rate and lower graft survival. Strategies to improve survival following retransplantation are therefore critically needed. We have retransplanted 270 patients in a modern immunosuppression era using mycophenolate mofetil. 221 patients were retransplanted once, 40 underwent their 3rd transplant, 6 underwent their 4th and 1 underwent 5 transplants. The overall 5 year patient survival was 91% and was similar to primary transplants. Retransplants with living donor kidneys have a 95% 5 year patient survival compared to 82% for cadaveric ($p<0.0001$). 5 year graft survival following retransplantation is 63% compared to 71% for primary transplants ($p=0.0009$). However, retransplantation with living donor kidneys have a similar 5 year graft survival compared to primary transplants, 82% vs. 80% ($p=0.799$). Retransplantation with cadaveric kidneys had a 5 year graft survival of 56% compared to 65% for primary transplants ($p=0.0023$). Race, HLA matching, hepatitis C, and PRA had no impact on graft survival following retransplantation. Rejection rates were higher overall following retransplantation. African Americans however, had a higher 5 year rejection rate (36%) than caucasians (15%, $p=0.002$). Retransplants receiving tacrolimus-based immunosuppression had a lower rejection rate compared to cyclosporine (22% vs. 48%, $p=0.002$). This translated to an improved 5 year graft survival for retransplants receiving tacrolimus (75% vs. 36%, $p=0.006$). Failed primary transplant recipients have excellent patient survival following retransplantation. Strategies to improve outcome following retransplantation included finding a living donor and using tacrolimus-based immunosuppression. Although African Americans had a higher rejection rate following retransplantation, this did not impact significantly on graft survival.

Abstract# 1297 **Poster Board #-Session: P53-III**

BODY WEIGHT ALTERATIONS DEMONSTRATE DISTINCT PATTERNS IN RENAL TRANSPLANT RECIPIENTS TREATED WITH STEROID-FREE AND STEROID-BASED IMMUNOSUPPRESSION. C. C. Rogers,[1] R. R. Alloway,[1] J. F. Buell,[1] J. W. Alexander,[1] M. Gupta,[1] G. Sethuraman,[1] T. Metze,[1] H. Goodman,[1] M. A. Alonzo,[1] M. Cardi,[2] R. Munda,[1] E. S. Woodle.[1] *[1]Division of Transplantation, Univ. of Cincinnati; [2]The Christ Hospital, Cincinnati, OH.*

The adverse impact of corticosteroid (CS) therapy on weight (wt) gain is a well-known complication post-transplant (Tx). Moreover, obesity has been identified by the American Heart Association as a risk factor for cardiovascular disease (CVD). Since CVD is the leading cause of death with a functioning graft in renal Tx patients (pts), minimization of cardiovascular risk should be of high priority. The objective of this study was to compare body wt changes in renal Tx pts treated with modern immunosuppressive therapy with either early steroid discontinuation (ESD) or chronic steroid maintenance (CSM). **METHODS:** A retrospective analysis of 164 pts who underwent ESD (ie, within 7 days postTx) were compared to a control group of 148 pts with CSM. ESD pts received calcineurin inhibitor (CI) therapy (100%), sirolimus (68%), mycophenolate mofetil (MMF) (90%), 15% received induction with IL-2 receptor antibody and 50% received thymoglobulin. CSM pts received CS, CI (95%), SRL (4%), MMF (95%), azathioprine (4%). **RESULTS:** Median follow-up for the ESD and CSM groups were 378 and 1096 days. The following figure presents mean wt, the proportion of pts with wt changes in the first yr postTx and time points where wt changes occurred.



CONCLUSIONS: ESD pts demonstrated : 1) statistically significant lower mean wts at 1,3,6 and 12 mos postTx, 2) wt stability in a higher proportion of pts than in CSM group at 1 yr, 3) wt gain in a lower proportion of pts than CSM group, 4) same proportion of pts with wt loss as CSM group. A significant proportion of the difference in wt gain by CSM occurred in the first postTx mo, and thereafter, both groups gained wt at similar rates, and both stopped gaining wt at one yr postTx. A long-term analysis of wt is necessary to determine if the initial wt gain may be lost beyond the first yr postTx.

Abstract# 1298 **Poster Board #-Session: P54-III**

CORTICOSTEROID AVOIDANCE AMELIORATES LYMPHOCELE FORMATION AND WOUND HEALING COMPLICATIONS ASSOCIATED WITH SIROLIMUS THERAPY. C. Rogers,[1] J. Alexander,[1] R. Alloway,[1] T. Metze,[1] R. Boardman,[1] J. Trofe,[1] M. Gupta,[1] T. Merchen,[1] M. Hanaway,[1] J. F. Buell,[1] M. Cardi,[1] P. Roy-Chaudhury,[1] V. Peddi,[1] H. Goodman,[1] G. Sethuraman,[1] E. Woodle.[1] *[1]Division of Transplantation, Univ. of Cincinnati, Cincinnati, OH.*

Both sirolimus (RAPA) and corticosteroids (CS) have been shown to adversely impact wound healing. The availability of new immunosuppressive agents has allowed corticosteroid avoidance (CSAV). The objective of this study was to determine whether CSAV would ameliorate the wound healing complications associated with RAPA. **METHODS**: 59 patients (pts) treated with a CSAV regimen (no pre or post transplant CS) were compared to a historical control group (n=68) that received cyclosporine (CsA), mycophenolate mofetil (MMF) and CS. The CSAV regimen included thymoglobulin (mean 2.6 doses), RAPA (8-12 ng/ml), MMF (2 grams/day), low-dose CsA, (trough 100 ng/ml, discontinued at 4-6 months) along with arginine and canola oil nutritional supplements. Complications were classified as: wound healing complications (WHC) or infectious wound complications (IWC). WHC include lymphocele, wound hernia, dehiscence, and skin edge separation. IWC include wound abscess and empiric antibiotic therapy for wound erythema. **RESULTS:** The CSAV group was largely CS free: 8% of pts received CS for rejection, 3% received CS for recurrent disease and 97% of pts are currently off CS.

	CSAV (n=59)	Control (n=68)	p-value
Mean Age at Transplant	48.3	46.7	0.4
Pre-transplant diabetes	19 (47.5%)	21 (53%)	0.87
Males:Females	39:20	38:30	0.24
Mean BMI	25.3	26.8	0.14
Time to WHC (days)	18.5	31	0.74
Time to IWC (days)	23	29	0.37
WHC Incidence	5 (8%)	13 (19%)	0.07
IWC Incidence	7 (11%)	5 (7%)	0.37
Lymphocele	**3 (5%)**	**11 (16%)**	**0.04**
Empiric Antibiotics for Wound Erythema	5 (8%)	1 (1%)	0.06

The following data is expressed as CSAV vs. control. Additional analyses showed reduced WHC with CSAV primarily observed in pts with BMI > 30 (0% vs. 29%, p=0.07) not in pts with BMI < 30 (10% vs. 15%, p=0.44). Similarly, lymphocele formation with CSAV was observed primarily in pts with a BMI > 30 (0% vs. 29%, p= 0.07) not in pts with BMI< 30, (6% vs. 10%, p=0.41). Finally, wound erythema requiring empiric antibiotic therapy showed a more pronounced effect in patients with BMI < 30 (10% vs. 0%, p=0.03). **CONCLUSIONS:** CSAV in a RAPA based regimen results in: 1) marked reduction in lymphoceles and WHC primarily in obese pts, and 2) more frequent wound erythema requiring empiric antibiotics. CSAV provides a promising approach for addressing WHC associated with RAPA therapy.

Abstract# 1299 **Poster Board #-Session: P55-III**

CYTOMEGALOVIRUS ASSOCIATED PERIODONITIS MAY CONTRIBUTE TO RENAL TRANSPLANT COMPLICATIONS. Hessam Nowzari,[2] Sali Aswad,[1] Nasreen S. Khan,[1] Michael Jorgensen,[2] Edna Osorio,[1] Androush Safarian,[2] Hamid Shidban,[1] Stephen Munroe,[2] Robert Mendez.[1] [1]*National Institute of Transplantation, Los Angeles, CA;* [2]*USC School of Dentistry, Los Angeles, CA.*

Background: Human cytomegalovirus (HCMV) is an opportunistic pathogen and may lead to transplant complications including transplant failure. Recent studies have identified HCMV in the pathogenesis of periodontal disease in immune comprised individuals affected by Trisomy 21 and Fanconi anemia. **Objective:** To determine if active CMV infection detected by the amplification of CMV pp67 mRNA in saliva and gingival crevicular fluid is associated with transplant complications in renal allograft recipients. **Methods:** CMV pp67 mRNA was analyzed in oral fluids of 52 renal transplant recipients 6 months post transplantation. Patients received 200 - 400mg of Zovirax, Acyclovir or Gancyclovir as prophylactic antiviral therapy until 3 mos. after transplant and were receiving cyclosporine at time of study. Nucleic acids were extracted from samples using automated NucliSens Extractor protocol (bioMerieux, NC). Amplification and detection of CMV pp67 mRNA was achieved following the protocol of CMV pp67 assay. Gingival overgrowth was diagnosed by clinical examination. Serum CMV IgG and IgM antibodies were analyzed to differentiate between recent or latent infection in serum. **Results:** Overall incidence of gingival overgrowth was 67.3% (n=35), affecting 72.2% women and 64.7% of men. HCMV pp67 gene transcripts were detected in saliva and crevicular fluid of 30.8% (16) of the patients analyzed. Serum CMV IgM antibodies were detected in 7.7% patients, however, association between active CMV replication and prevalence of CMV IgM was non significant. In contrast, 65.4% of patients were CMV IgG+, however, association between CMV and mRNA amplification and IgG was also non significant. Major clinical complications occurred in 16 (30.8%) patients also diagnosed with gingival overgrowth (p<0.00001). Active CMV replication in saliva and crevicular fluid was significantly associated with viral infection (4/16 patients, p=0.0001, and 3/16, and 3/16, p=0.01). **Conclusions:** Renal transplant patients affected by periodontitis may be at risk of harboring actively HCMV despite receiving prophylactic anti-viral therapy. This study shows that gingival overgrowth and associated periodontal pockets may be sites of HCMV replication. Study elucidated use of saliva and gingival crevicular fluid to detect CMV pp67 mRNA, a marker of active HCMV infection, and provided evidence for a link between CMV associated periodontitis and renal transplant complications.

Abstract# 1300 **Poster Board #-Session: P56-III**

IMPACT OF CALCINEURIN INHIBITORS ON GRAFT FUNCTION OF PATIENTS WITH PROTRACTED DELAYED GRAFT FUNCTION (DGF). Luciene A. Silva,[1] Paula G. Machado,[1] Cláudia R. Felipe,[1] Sung I. Park,[1] Riberto Garcia,[1] Hélio Tedesco-Silva,[1] José O. Medina-Pestana.[1] [1]*Hospital do Rim e Hipertensão–Nephrology Division, Universidade Federal de São Paulo, São Paulo, SP, Brazil.*

It is still controversial the detrimental effect of calcineurin inhibitors on the duration and rate of graft function recovery in patients who develop DGF after cadaveric kidney transplantation. The purpose of this study was to evaluate the use of calcineurin inhibitor in kidney transplant patients at high risk to develop DGF. **METHODS:** 148 recipients of cadaveric kidney allografts received either cyclosporine- (NEO) or tacrolimus- (TAC) based immunosuppressive regimens from the first posttransplant day. Adjunctive immunosuppressive therapy consisted of azathioprine (39%) or mycophenolate mofetil (61%) and prednisone. 19 (13%) patients received concomitant induction therapy with OKT3 (7.4%), ATG (1.4%) or IL-2R mAb (4%). DGF was defined as the need for dialysis during the first posttransplant week. **RESULTS:** Relevant demographic parameters were: donor cause of death (43% CVA), use of vasoactive drugs prior to organ harvesting (96%), mean donor creatinine (1.4 mg/dL), mean time on dialysis (79 months), PRA>10% (28 %), repeat transplant (12%), recipient black ethnicity (49%), mean cold isquemia time of 19.2 hrs. More patients in the TAC group received MMF (88.2 vs. 37.5%, P<0.05). Mean cyclosporine and tacrolimus concentrations were 438 and 13.8, and 270 and 8.9 ng/mL at months 1 and 6, respectively. The incidence of DGF was 57% (NEO= 64% vs. TAC=50%, ns). Of the 84 patients with DGF, 66 (79%) recovered graft function (3 primary nonfunction, 4 vascular thrombosis, 7 graft losses, and 4 deaths before recovery of graft function). Mean time to last dialysis or nadir creatinine were 17,6 ± 14,7 (2 to 87) and 33,5 ± 19,7 days (3 to 110), respectively, with no differences between the two groups. There were no differences in the incidence of biopsy-confirmed acute rejection (NEO= 23% vs. TAC=22%, ns) or any treated rejection (NEO= 43% vs. TAC=32%, p=0.2). At 6 months patient and graft survival were 94% and 81%, respectively (9 deaths and 19 graft losses). Mean creatinine was 1.5 ± 0.4 mg/dL with no difference between the two groups. Neither acute rejection (1.8±0.6 vs. 1.5±0.5 mg/dL, p=0.069) nor DGF (1.6±0.5 vs. 1.5±0.5 mg/dL, p=0.088) had a negative impact on graft function at 6 months. **CONCLUSION:** In this high risk transplant population showing high incidence and protracted DGF, the use of calcineurin inhibitors did not appear to have a significant negative impact on complete recovery of graft function up to 6 months.

Abstract# 1301 **Poster Board #-Session: P57-III**

PERSISTENT ANEMIA: AN UNRECOGNIZED COMPLICATION OF SIROLIMUS/MMF COMBINATION THERAPY. J. Trofe,[1] M. Cardi,[1] M. Clippard,[1] R. Alloway,[1] M. Alonzo,[1] S. Goel,[1] B. Mannion,[1] J. Austin,[1] J. W. Alexander,[1] T. Metze,[1] H. Goodman,[1] R. Munda,[1] S. Dumbauld,[1] M. Hanaway,[1] J. Fidler,[1] J. F. Buell,[1] E. S. Woodle.[1] [1]*Div of Transplantation, University of Cincinnati, OH.*

Profound anemia at time of kidney transplantation (KTX) is less commonly seen since the introduction of erythropoietin (EPO) therapy. After successful KTX, anemia usually corrects within 6-12 weeks. While steroids and calcineurin inhibitors generally do not cause hematologic abnormalities, antiproliferative agents such as mycophenolate mofetil (MMF) and sirolimus (SRL) are well known to cause anemia. **Purpose:** Evaluate the incidence of anemia in SRL/MMF combination therapy compared to SRL or MMF immunosuppressive regimens (IS). **Methods:** Retrospective single center analysis of KTX performed from 1/2000-7/2002. Pts treated for rejection or whom expired within 1 mo were excluded. For analysis, pts were divided into 3 groups: Grp 1=Thymo (90%) FK/**MMF**/Prednisone (or placebo) n=40,Grp 2=Simulect/FK/**SRL**(without MMF or steroids) n=10, Grp 3=Thymo/CYA/**MMF**/**SRL** (without steroids) n=50, and anemia evaluated at 1, 3, 6, and 12 mos. All pts had Median SCrs 1.1-1.3mg/dL at all time points. Anemia was defined as HgB ≤10%, and was managed with iron replacement and/or EPO. Pre and post-KTX factors potentially affecting anemia were evaluated, and Chi Square analyses were performed. **Results:** There were no differences in the following variables among the 3 groups: donor source (LD/CAD), age, race, previous KTX, delayed graft function, SCr, or ACE inhibitor therapy. The incidence of anemia was highest in Grp 3 at all time points evaluated. **Conclusions:** Pts receiving combined SRL/MMF IS have a higher incidence of anemia than when SRL and MMF are not used together. Anemia in SRL/MMF treated pts does not appear to respond to EPO production by the KTX. These observations confirm our clinical impressions that anemia is a major side effect of SRL/MMF combination IS.

Variable	Group 1 (n = 40)	Group 2 (n = 10)	Group 3 (n = 50)	p-value (Groups 1 vs 2)	p-value (Groups 1 and 2 vs 3)
Delayed Graft Function	7 (18%)	1 (10%)	5 (10%)	ns	ns
Overall Incidence of Anemia	18 (45%)	5 (50%)	42 (84%)	ns	< 0.05
Incidence of anemia at 1 month	18 (45%)	4 (40%)	38 (76%)	ns	< 0.05
Incidence of anemia at 3 months	6 (15%)	4 (40%)	21 (42%)	ns	< 0.05
Incidence of anemia at 6 months	1 (3%)	1 (10%)	15 (30%)	ns	< 0.05

Abstract# 1302 Poster Board #-Session: P58-III

DIFFERENTIAL EFFECTS OF CALCINEURIN INHIBITOR (CI) THERAPY ON PANCREATIC β-CELL FUNCTION IN HEPATITIS C (HepC) DIALYSIS PATIENTS AWAITING A RENAL TRANSPLANT. Debbie L. Cohen,[1] Roy D. Bloom,[1] Raymond R. Townsend.[1] [1]*Medicine, University of Pennsylvania, Philadelphia, PA.*

Post-transpant diabetes mellitus (PTDM) is a side effect of immunosupression, particularly when the calcineurin inhibitors (CI) cyclosporine (CYA) and tacrolimus (TAC) are used. We have previously shown an increased incidence of PTDM in HepC+ pts receiving TAC (50%) vs CYA (11%), however the incidence was similar in HepC- pts receiving TAC (10%) vs CYA (9%). This prospective randomized trial was done to determine measures of glucose metabolism (insulin sensitivity - SI, glucose drived glucose uptake - SG and β - cell function) from an intravenous glucose tolerance test (IVGTT) during CI therapy in HepC+ pts on hemodialysis awaiting renal transplant. All subjects had a baseline IVGTT and were then randomized to receive either TAC or CYA for 2 weeks and then a 2nd IVGTT was performed. Dosages of TAC or CYA were adjusted to achieve typical immunosuppression levels. Following a 4 week wasout period, subjects received 2 weeks of the other CI at therapeutic levels and then had a 3rd IVGTT. RESULTS: 1 female (black) subject and 9 male (8 black, 1 white) subjects with a mean age of 43.6±6.6 years were studied. There were no statistically significant changes in SI or SG during TAC or CYA therapy compard with baseline. Pancreatic β-cell function is shown below (expressed as the median value for each treatment in units of mU/mmol). Data were modelled using the software program AKA-glucose. The data were not normally distributed and were further analyzed by the Wilcoxon Rank Sum Testing.

	baseline	CYA	TAC
β-cell function	540	818	272*

*p= 0.004

CONCLUSION: β-cell function was significantly reduced on TAC compared with baseline or CYA therapy. These data are consistent with reduced pancreatic β-cell insulin release in response to glucose as a mechanism of PTDM in TAC treated pts.

Abstract# 1303 Poster Board #-Session: P59-III

NO INCREASED INCIDENCE OF WOUND COMPLICATIONS IN A STEROID-FREE REGIMEN WITH THYMOGLOBULIN INDUCTION AND DELAYED USE OF RAPAMYCIN. R. Brian Stevens,[1] Kecia Christensen,[1] David Mercer,[1] Alejandro Mejia,[1] Lucille E. Wrenshall.[1] [1]*Transplant Division, Department of Surgery, University of Nebraska Medical Center, Omaha, NE.*

Rapamycin is an extremely potent immunosuppressant, increasingly used in steroid-free regimens in combination with FK506. Despite the benefits of rapamycin's use in steroid-free regimens, anecdotal reports of impaired wound healing have dampened enthusiasm for its use. Given these concerns we initiated a rapamycin based steroid-free protocol without the previously reported loading dose (15-mg) and delayed initiation of maintenance rapamycin (5 mg/day) until post-operative day 1-3. We used the same Thymoglobulin (RATG) induction protocol in both a steroid-based (FK506, MMF, prednisone) and steroid-free regimen (FK506, rapamycin). We then compared the incidence of wound and other infectious complications in these two groups. Thirty-six recipients of primary kidney or kidney-pancreas transplants were compared with 26 similar recipients receiving FK506, MMF, and prednisone (age, primary disease, HLA mismatch, etc. were not statistically different). Both groups received induction therapy with Thymoglobulin (total dose 6 mg/kg in four divided doses). The follow up period was 1 – 16 months. There were no statistical differences between the two groups in the incidence of CMV infections, bacterial infections, and leukopenia. Surprisingly, the incidence of wound complications was higher in the triple therapy group (27%) as compared with those receiving FK506 and rapamycin (8%) (p=. 04). There were no statistical differences between the two groups with respect to body mass index or incidence of diabetes. Of note, there were no statistical differences in the incidence of patient death, graft loss, or acute rejection (<5% in both groups). In summary, rapamycin can be safely used in steroid-free regimens without increased risk of wound complications. Thymoglobulin induction permits delayed initiation of rapamycin, reducing potential perturbation of wound healing without an increased risk of cellular rejection.

Reduced Wound Complications With Thymoglobulin Induction And Delayed Initiation Of Rapamycin In A Steroid-Free Regimen

Categories	FK/MMF/P	Rapa/FK	P values
# of patients	N=26	N=36	ND
Kidney Tx	N=19	N=31	ND
Pancreas Tx	N=7	N=5	ND
Deaths	N=1(PTLD)	N=0	0.21
Graft loss	N=1(PNF)	N=1(oxylosis)	0.50
Acute rejection	N=0	N=0	0.50
CMV infections	N=2	N=1	0.30
Bacterial infections	N=0	N=2	0.25
Abscess	N=0	N=1	0.5
Wound disruption	N=7	N=3	0.04

Equal number of LRD and CAD renal transplants in both groups

Abstract# 1304 Poster Board #-Session: P60-III

LONG TERM RENAL ALLOGRAFT SURVIVAL DURING THE LAST DECADE IN SPAIN. Daniel Serón,[1] Manuel Arias,[2] Jose Maria Campistol,[3] Jose Maria Morales.[4] [1]*Nephrology Department, Hospital Universitari de Bellvitge, L'Hospitalet, Barcelona, Spain;* [2]*Nephrology Department, Hospital de Valdecillas, Santander, Santander, Spain;* [3]*Nephrology Department, Hospital Clinic, Barcelona, Barcelona, Spain;* [4]*Nephrology Department, Hospital 12 de Octubre, Madrid, Madrid, Spain.*

The aim is to determine time-dependent modifications in the characteristics of renal transplants patients in Spain during the 90ties and to study risk factors associated with late patient and graft survival. Adult patients transplanted in 1990, 1994 and 1998 with a functioning graft after the first year were eligible to participate. A total of 3365 out of 4688 transplants performed in Spain during the study period, accomplishing the inclusion criteria were recruited. Death with a functioning graft was considered a cause of graft failure. Ten year patient and graft survival were 81 and 68% respectively. Between 1990 and 1998, donor age increased from 32±15 to 43±18 years (p<0.0001), number of HLA mismatches increased from 2.8±1.2 to 3.2±1.2 (p<0.0001) and cold ischemia time decreased from 21±7 to 19 ± 6 hours (p<0.0001). The incidence of delayed graft function remained stable (30 vs 29 %, ns) and the incidence of acute rejection decreased from 39 to 25 % (p< 0.0001). While no patients received mycophenolate, tacrolimus, anti-interleukin 2 antibodies or sirolimus in 1990, these drugs were used in 30.7, 5.0, 2.4 and 1.1 % of patients in 1998. The proportion of patients treated with lipid lowering agents and converting enzyme inhibitors during the first year increased from 6 to 40% (p<0.0001) and from 11 to 23% (p=0.0001), respectively. Patient and graft survival remained stable during the study period. Multivariate analysis showed that the following factors were independently and significantly associated with decreased allograft survival: diabetes as a cause of renal failure, recipient age, acute rejection, last panel reactivity antibodies, serum creatinine at 3 months, an increase of serum creatinine between the 3rd and 12th month, proteinuria at 3 months, an increase of proteinuria between the 3rd and 12th month. The use of lipid lowering agents for any reason during the first year of follow up was associated with an improved survival. Graft survival has remained stable in Spain during the last decade despite an important increase in donor and recipient age and a poorer HLA-matching probably due to a decrease in the prevalence of acute rejection and a more frequent use of use lipid lowering agents that were an independent predictor of improved long term renal allograft survival.

Abstract# 1305 Poster Board #-Session: P61-III

INCIDENCE OF POST TRANSPLANT DIABETES MELLITUS(PTDM) IN HEPATITIS C SEROPOSITIVE(HCV+) AFRICAN AMERICAN KIDNEY RECIPIENTS MAINTAINED ON PROGRAF(FK) AND NEORAL(CSA). Sheng G. Xiao,[1] Daniel Lee,[1] Aparna Kumar,[1] Adrian Lata,[1] Shujing Shang,[1] Michael J. Moritz,[1] Michael Heifets,[2] Mysore S. Anil Kumar.[1] [1]*Surgery/Transplantation, Drexel University College of Medicine, Philadelphia, PA;* [2]*Medicine/ Nephrology, Drexel University College of Medicine, Philadelphia, PA.*

HCV+ kidney transplant recipients of African American group are at a high risk to develop PTDM with calcineurin inhibitor(CI) based immunosuppression as reported from previous studies. The aim of this retrospective study was to find out the incidence of PTDM, patient and graft survival in the in HCV+ African American kidney recipients treated with Prograf and Neoral. Recipients in both the groups were treated with basiliximab/OKT3 induction, FK/CSA, mycophenolate mofetil (MMF) / sirolimus (SRL) and prednisone. Sixty two African American, HCV+ recipients transplanted between 1995 and 2002 were studied. There were 18 patients in FK group and 44 in CSA group. Preoperative history and fasting blood sugar in these patients showed no evidence of diabetes. PTDM was diagnosed with persistent fasting blood sugar ≥ 140mg/dl, or requiring insulin therapy for one month or more. The incidence of PTDM in FK group was 5/18(27%) and in CSA group 3/44(7%) at 1 year after transplantation(p=0.025). IN FK group 1 of 5 recipients with PTDM had been treated with pulse steroid therapy for acute rejection compared to 3 of 3 in CSA group. PTDM in all 7 patients was diagnosed with in 3 months of transplantation.Incidence of acute rejection was 15% in FK and 13% in CSA group(p=ns). Five year actuarial patient survival was 85% and 83% and graft survival was 68% and 69% in FK and CSA groups respectively. This data indicates the actuarial patient and graft survival is comparable between the 2 groups but with a significantly reduced incidence of PTDM in CSA treated HCV+ African American recipients compared to FK. PTDM in Neoral group develops mostly in recipients that were treated for acute rejection by additional doses of steroids while in FK group PTDM may appear even in recipients who were not treated for acute rejection. It is recommended that Neoral should be the calcineurin inhibitor of choice in HCV+ African American kidney recipients to reduce the incidence of PTDM.

Abstract# 1306 **Poster Board #-Session: P62-III**

POST TRANSPLANT DIABETES MELLITUS IN RENAL TRANSPLANTION: A SINGLE CENTER CANADIAN EXPERIENCE 1995-2001. Sita Gourishankar,[1] Gian S. Jhangri,[2] Loreen H. Wales,[3] Marcello Tonelli.[1] [1]*Medicine (Nephrology), University of Alberta, Edmonton, AB, Canada;* [2]*Public Health Sciences, University of Alberta, Edmonton, AB, Canada;* [3]*Nutrition Services, University of Alberta, Edmonton, AB, Canada.*

Post-transplant diabetes mellitus (PTDM) is a serious complication of renal transplantation related to immunosuppression, ethnicity and obesity. We examined the incidence and associated factors of PTDM in our predominantly Caucasian/Southeast Asian (95.2%) renal transplant (tx) population, which consisted of 447 non-diabetics, transplanted between 1995-2001. PTDM was defined as requirement for hypoglycemic medications more than 30 days post-tx. Patients received cyclosporine (CsA) or tacrolimus (TAC), mycophenolate mofetil or azathioprine and prednisone. **RESULTS:** The incidence of PTDM at 6, 12 and 36 months was 5.2%, 5.8% and 7% with an overall incidence of 7.6% (n=33). Mean follow-up time was 49.4 ± 22.1 months and mean time to PTDM onset was 11.6 months. After 1999, 80-87% of our patients were on TAC and 99.6% were on prednisone therapy post-tx. A greater proportion of patients with PTDM were on TAC (46% vs 28%;p=0.04). In univariate analysis, associated factors were ($p<0.05$): age ≥ 45 yrs, induction therapy, rejection, TAC vs. CsA, ATN, day 7 SCr >250 umol/L, cadaver donor. Pretx hemodialysis (p=0.08) and weight at tx (p=0.08) and 6 months post-tx (p=0.052) trended to significance. Dose of steroid, TAC or CsA, and trough levels of TAC and CsA were not associated with PTDM. BMI was not associated with PTDM and only 17% of our population had a BMI ≥ 30. In multivariate analysis, independent factors were ($p<0.05$): age ≥ 45 yrs, rejection and cadaveric donor. Rejection and TAC interacted such that TAC use trended toward significance (p=0.06) with rejection included in the model however excluding rejection resulted in TAC being associated with PTDM (p=0.05). **CONCLUSIONS:** Our 6, 12, and 36 month incidences are slightly lower than in previous reports despite widespread use of calcineurin inhibitor (CNI) and steroid therapy. Previous studies evaluating predictors of PTDM were conducted in populations with high prevalence of African-Americans and obesity. We found that PTDM remained common in a predominantly Caucasian/Southeast Asian population in which high BMI was infrequent. Although body weight was of borderline statistical significance, the majority of risk was conferred by variables relating to clinical outcome (type of CNI, incidence of AR, cadaveric donor). These data suggest that the relative contribution of immunosuppression to the incidence of PTDM may be accentuated in patients without ethnic or anthropometric predisposition.

Abstract# 1307 **Poster Board #-Session: P63-III**

WOUND COMPLICATIONS AFTER KIDNEY TRANSPLANT - HOW MUCH ARE THE NEWER IMMUNOSUPPRESSION DRUGS PLAYING A ROLE? Abhinav Humar,[1] James V. Harmon,[1] Massimo Asolati,[1] Raja Kandaswamy,[1] William D. Payne,[1] Kristin J. Gillingham,[1] Arthur J. Matas.[1] [1]*Surgery, University of Minnesota, Minneapolis, MN.*

Objective: One concern with the newer immunosuppressive drugs is their potential impact on wound healing. Using multivariate analysis, we looked at the impact of various agents on wound complications occurring after transplant. **Methods:** Between Jan 1992 and Sept 2002, 2018 (CAD=987, LD=1031) kidney transplants were performed. The majority were kidney alone (n=1617, 80%); the remainder were simultaneous transplants, with most commonly a pancreas. Of the 2018 recipients, a wound complication developed in 242 (11.9%) . This included either a wound infection (n=169,8.4%) or a wound breakdown (i.e. early dehiscence or late hernia) (n=107,5.3%). Recipients were analyzed by multivariate techniques to determine significant risk factors for a wound complication. Immunosuppression drugs had a significant impact on incidence of wound complications. Use of sirolimus, MMF, and maintenance steroids were all independent risk factors for a wound problem. The type of calcineurin agent (CsA vs. FK) was not a significant variable. Other risk factors were BMI >30 and receiving an extrarenal organ. When wound infections and wound breakdowns were analyzed separately, a similar trend was noted. One notable difference was that recipient age >50 years was an independent risk factor for wound breakdown, but not for wound infection. In a subgroup analysis of recipients in a steroid-avoidance protocol (n=139), the incidence of wound complication by maintainance immunosuppression was the following: CsA/MMF (3.8%), high dose FK/low dose sirolimus (8.7%), Low dose FK/High dose sirolimus (12.5%) (p=0.01). **Conclusions:** Immunosuppression drugs play an important role in the development of wound complications after kidney transplant. Given the significant morbidity associated with these complications, alterations in immunosuppression may be warranted based on the presence or absence of other risk factors for wound complications.

Risk Factor and RR

Risk Factor		RR	p value
Body mass index	≥ 30 vs. < 30	2.56	< 0.001
Rapamycin	yes vs. no	1.90	0.03
MMF	yes vs. no	1.50	0.005
Maintenance steroids	yes vs. no	1.75	0.04
Calcineurin agent	FK vs. CsA	0.90	0.65
Simultaneous extrarenal organ	yes vs. no	1.53	0.07
Reoperation posttransplant	yes vs. no	1.92	0.16
Diabetic status	yes vs. no	1.26	0.18
Recipient age	> 50 vs. 18-50	1.26	0.12
Leak posttransplant	yes vs. no	1.01	0.98

Abstract# 1308 **Poster Board #-Session: P64-III**

PREVENTION OF EARLY BONE MINERAL DENSITY LOSS IN RENAL TRANSPLANT RECIPIENTS UTILIZING CALCIUM AND VITAMIN D THERAPY ALONE. Ion D. Bucaloiu,[1] Craig Wood,[2] James E. Hartle.[3] [1]*General Internal Medicine, Geisinger Medical Center, Danville, PA;* [2]*Department of Clinical Research, Geisinger Medical Center, Danville, PA;* [3]*Department of Nephrology, Geisinger Medical Center, Danville, PA.*

Renal transplantation is associated with early bone loss that increases the subsequent risk of osteoporosis and bone fractures. Published data regarding the prevention of post-renal transplant bone loss is limited. We performed a retrospective analysis on the change in bone mineral density following renal transplantation utilizing a regimen of oral calcium and vitamin D. A baseline DEXA scan was performed during the initial transplant hospitalization on 39 patients undergoing renal transplantation. All patients were treated with steroids, cyclosporine microemulsion and mycophenolate mofetil. Only one patient was subsequently switched to FK 506. Of the 39 patients, 29 patients were begun on 1000 mg of calcium carbonate and 800 IU of vitamin D_3 daily. Dosage reductions were performed if hypercalcemia or hypercalciuria developed. In addition to the above regimen, 5 patients received alendronate 10 mg daily. The remaining 5 patients received no treatment. A follow up DEXA scan was performed at a mean interval of 15 months. Data were analysed retrospectively. Outcome was assessed by the percent change in DEXA absorption/year of follow-up. The groups were compared for: age, gender, baseline PTH, development of hypercalcemia or hypercalciuria, diabetes, creatinine at last follow-up, cumulative prednisone dosage, coumadin therapy, FK506 therapy, incidence of rejection and incidence of clinically evident bone fracture. Patients were excluded if they had been on anticonvulsant therapy. Follow-up DEXA scans showed that no treatment led to a significant decline in bone density from baseline in both the Femur (-4.4%) and Lumbar regions (-8.8%). In contrast treatment with calcium/vitamin D led to no significant change (-0.1% and +1.7% respectively) and addition of alendronate led to a significant increase in the Femur (+6.1%) but not the Lumbar regions (+4.5%). In addition both One-way Anova testing and multple regression analysis showed a significant correlation between treatment group and subsequent DEXA result. Finally there was no evidence of adverse effect on renal function utilizing the treatment regimens with mean creatinines at follow-up of 1.4 (alendronate/calcium/vitamin D), 1.5 (calcium/vitamin D) and 1.8 (no treatment) noted. In conclusion we present data that calcium and vitamin D supplementation alone may impede the typically seen loss in bone mineral density that occurs post-renal transplantation. Furthermore this treatment was accomplished with no impairment in renal function.

Abstract# 1309 **Poster Board #-Session: P65-III**

MANAGEMENT OF ASYMPTOMATIC CHOLELITHIASIS IN PATIENTS AWAITING RENAL TRANSPLANT. Timothy Jackson,[1] Darin Treleaven,[1] Dianne Arlen,[1] Abigail D'Sa,[1] Kim Lambert,[1] Daniel W. Birch.[2] [1]*Medicine, McMaster University, Hamilton, ON, Canada;* [2]*Surgery, McMaster University, Hamilton, ON, Canada.*

Background: There is no consensus in the literature on the most appropriate management of patients with asymptomatic cholelithiasis awaiting renal transplant. The purpose of this study is to delineate the natural history of asymptomatic cholelithiasis in renal transplant patients at our institution and to identify morbidities associated with either pre-transplant cholecystectomy or non-operative management. Methods: We completed a retrospective chart review of all patients awaiting renal transplant at our institution from 1994-2000. All patients underwent routine biliary ultrasonography: cholelithiasis and significant gallbladder abnormalities were identified. Pre-transplant management and both pre and post-transplant outcomes were documented. For patients managed non-operatively, the post-transplant outcomes related to cholelithiasis and gallbladder abnormalities were identified. Results: For the 6 years reviewed, 411 patients awaiting transplant were identified (242 male, 169 female, mean age 45.7 years, etiology of renal failure: 39% glomerulonephritis, 21% diabetes). In this population, 32 patients had cholelithiasis on pre-transplant work-up (7.8%) and 35 had significant gallbladder abnormalities (8.5%: 57% polyps, 34% thick walled and/or contracted, 6% sludge, 3% enlarged common bile duct). Pre-transplant, 12 of 32 patients with cholelithiasis underwent cholecystectomy (32%), with no peri-operative complications. Post transplant, of 19 patients with cholelithiasis none required cholecystectomy (mean follow up 4.8 years, 1 patient was lost to follow-up). Of 35 patients with other gallbladder abnormalities, 2 required cholecystectomy (1 for biliary colic and 1 for questionable malignancy). Conclusions: In this series, no patient developed significant morbidity or mortality related to cholelithiasis or gallbladder abnormalities post-transplant. As in the general population, the magnitude of the risks associated with asymptomatic cholelithiasis does not appear to warrant prophylactic cholecystectomy in patients being considered for renal transplant.

Abstract# 1310 **Poster Board #-Session: P66-III**

SIROLIMUS (SRL)- INDUCED ANGIOEDEMA: REPORT OF TWO CASES. H. Wadei,[1] S. Gruber,[2] J. Garnick,[3] M. West,[2] D. Granger,[2] D. Sillix,[1] A. Haririan.[1] *[1]Department of Medicine; [2]Department of Surgery, Wayne State University School of Medicine; [3]Pharmacy, Harper University Hospital, Detroit, MI.*

SRL is a macrolide that has only recently gained widespread use in organ transplantation, and its full specturm of side-effects is yet to be defined. We therefore describe herein 2 cases of SRL-induced angioedema (AE). **Case 1:** A 53 year old African-American (AA) male with no known drug allergies (NKDA) received a cadaveric renal transplant (CRT) complicated by delayed graft function (DGF). Maintenance immunosuppression (IS) included MMF, a prednisone taper, and SRL (started POD#3; target trough levels 10-15 ng/ml). Beginning day 21, SRL was held for 1 week due to thrombocytopenia. 3 weeks after reinstitution of the drug, he presented with AE (trough level 15 ng/ml). Cessation of SRL, temporary increase in oral steroid dosing, and initiation of H1/H2 blockers led to resolution of the syndrome, with no relapse in the subsequent 9 months. **Case 2:** A 54 year old diabetic male with NKDA received a CRT complicated by DGF. Maintenance IS was as in Case 1. At 3 weeks, the patient was converted from SRL to tacrolimus for thrombocytopenia. 6 weeks later, the patient was switched back to SRL due to uncontrolled diabetes. After 3 doses, he presented with AE. SRL was discontinued, and the AE resolved following therapy as in Case 1. There was no relapse in the following 3 months. **Discussion.** To date, there is no published report of AE complicating SRL use. This syndrome has been linked with a variety of drugs. Given the absence of other AE-associated drugs and the temporal relationship of AE to rechallenge with and cessation of SRL, we feel SRL was the most likely cause. The underlying mechanism is either mast cell activation, which is associated with urticaria, or an increase in vascular permeability by a bradykinin- or complement-mediated mechanism. With the absence of urticaria in both cases, the underlying mechanism was presumably not mast cell-mediated. Indeed, the fact that both patients developed AE shortly after SRL rechallenge suggests an antibody-mediated mechanism, likely affecting the complement pathway. **Conclusion.** AE may occur following reinstitution of SRL after prior exposure. Close monitoring of these cases is warranted.

Abstract# 1311 **Poster Board #-Session: P67-III**

LYMPHEDEMA ASSOCIATED WITH SIROLIMUS IN RENAL TRANSPLANT RECIPIENTS. Walid J. Aboujaoude,[1] Martin L. Milgrom,[2] Laura H. Nayee,[3] Mahendra V. Govani.[1] *[1]Medicine/ Nephrology, Indiana University Hospital, Indianapolis, IN/Marion; [2]Surgery, Indiana University Hospital, Indianapolis, IN/Marion; [3]Pharmacy, Indiana University Hospital, Indianapolis, IN/Marion.*

Introduction: Lymphoceles are common in renal transplant recipients receiving sirolimus. However, recent MEDLINE search revealed no reports of lymphedema associated with the use of sirolimus. **Methods:** We reviewed the charts of 138 adult kidney and kidney-pancreas transplant recipients (performed through 9/30/02), who received sirolimus for more than 6 weeks. The charts of 3 patients with lymphedema were reviewed in detail for demographics, clinical features, diagnostic procedures, management and outcome. Mean follow-up since the first symptoms of lymphedema was 21.67 ± 5.13 (range 16-26) months.

Demographics of all patients

Age	Race	Sex	Cause of ESRD	Donor	Tx date[1]	Siro date[2]	LP
37 y	W	F	IgAN	LD	6/7/00	6/7/00	11 wks
58 y	W	F	GN	CAD	6/9/00	6/10/00	25 wks
63 y	W	F	GN	LD	11/17/99	4/19/01	14 wks

y=years, W=white, F=Female, M=male, IgAN=IgA nephropathy, GN=Glomerulonephritis, HTN=Hypertension, DM=Diabetes Mellitus, LD=Living, CAD=Cadaver, 1=Transplant date, 2=Sirolimus starting date, LP=Latent period between starting sirolimus and first symptoms of lymphedema, wks=weeks

Results: Incidence of lymphedema was 2.2%.

Clinical features, management and outcome of all patients

IR preDx	Clinical feaures	Stop date[1]	Resolution	IR postDx	Recent CR
CysA, siro, pred	Mild edema both legs, Severe edema & redness of LUE and left breast, recurrent lymphangitis, Functioning access LUE	2/20/01	Complete	CysA, MMF, Pred	2.2
Siro, MMF, pred	Mild edema both legs, Severe edema and redness of RUE and right breast, Clotted accesses RUE	6/11/01	Partial, 60-70%	Tacro, MMF, pred	0.8
Siro, MMF, pred	Edema both legs, but severe on left side, allograft right side, No access, Nephrotic syndrome	11/5/01	Partial, 80-90%	MMF, pred	1.5

IR=Immunosuppressive regimen, Dx=Diagnosis, siro=sirolimus, pred=prednisone, tacro=tacrolimus, LUE=Left upper extremity, RUE=Right upper extremity, 1=Sirolimus stop date, CR= Serum creatinine in mg/dl

Diagnostic studies including venograms to rule out venous obstruction were negative. Mammograms and breast biopsies in both patients with breast swelling ruled out malignancy. Sirolimus levels varied within the therapeutic range (5-15 ng/ml) for all 3 patients. On stopping sirolimus, lymphedema improved over weeks to months in all patients. **Conclusions:** Lymphedema is an uncommon but disfiguring complication associated with sirolimus therapy. Further studies are needed to confirm our findings and elucidate the mechanism.

Abstract# 1312 **Poster Board #-Session: P68-III**

MYCOBACTERIAL INFECTION AFTER RENAL TRANSPLANTATION IN A WESTERN POPULATION. Annemie Vandermarliere,[1] An Van Audenhove,[1] Willy E. Peetermans,[2] Yves F. Vanrenterghem,[1] Bart D. Maes.[1] *[1]Department of Nephrology, University Hospital Gasthuisberg, Leuven, Belgium; [2]Department of Internal Medicine, University Hospital Gasthuisberg, Leuven, Belgium.*

Mycobacterial infection is a serious opportunistic infection in renal transplant recipients. The incidence is higher in developing than in developed Western countries. This study is a retrospective review of the records of 2502 renal transplant recipients in Belgium. Fourteen cases of mycobacterial infection (9 Mycobacterium tuberculosis and 5 atypical mycobacterial infection) were diagnosed. The time interval between transplantation and diagnosis was 64 +/- 80 months (mean +/- SD, range 5-188) for Mycobacterium tuberculosis and 92 +/- 75 months (range 14-209) for atypical mycobacterial infection. The localization of Mycobacterium tuberculosis was pulmonary/pleural in 67 % and extrapulmonary in 33 %. The atypical mycobacterial infections were located in skin, tendons and joints. Beside a mild epidemiological exposure in the population, a majority of patients had several risk factors for mycobacterial disease, and factors affecting the net state of immunosuppression (excessive use of steroids, graft dysfunction, chronic viral infections, protein-calorie malnutrition, diabetes mellitus, ...) were very important. On the contrary, the incidence of mycobacterial infection did not increase with the use of more potent immunosuppressive agents (MMF, FK506, sirolimus). The initial antimycobacterial therapy consisted of a combination of isoniazid, rifampicin and ethambutol in all patients. In patients with Mycobacterium tuberculosis infection, a good response to antimycobacterial therapy was obtained. In patients with atypical mycobacterial infection, initial treatment was successful in 4 out of 5 patients, in one patient recurrence was diagnosed. We conclude that the incidence of Mycobacterial infection after renal transplantation has not increased with the use of newer and more potent immunosuppressive therapy. All patients with mycobacterial disease have been treated with higher cumulative doses of corticosteroids compared with the standard transplant patient, and/or had other immunosuppressive conditions correlated with increased for mycobacterial infections. All mycobacterial infections responded well to antimycobacterial treatment without reduction of (target level adjusted) immunosuppressive therapy. Chemoprophylaxis to prevent Mycobacterium tuberculosis disease is still recommended for high risk patients after renal transplantation.

Abstract# 1313 **Poster Board #-Session: P69-III**

INCREASED INCIDENCE OF HYPERCHOLESTEROLEMIA IN RENAL TRANSPLANT RECIPIENTS RECEIVING SIROLIMUS. Steven R. Potter,[1] Sali Aswad,[1] Hamid Shidban,[1] Shirley Mirador,[1] Rafael G. Mendez,[1] Robert Mendez.[1] *[1]National Institute of Transplantation, Los Angeles, CA.*

Introduction: Elevated total cholesterol levels are common in renal transplant recipients and portend an increased risk of cardiovascular events. Multiple immunosuppressive agents have been implicated, however the contribution to dyslipidemia by different agents is poorly elucidated. We retrospectively studied total serum cholesterol levels in 1,315 patients receiving a renal transplant between 1996 and 2001, who were treated with a calcineurin inhibitor (FK or cyclosporine (CYA) and sirolimus (SRL) or mycophenolate mofetil (MMF). **Objectives:** To determine the incidence of hypercholesterolemia in a large series of transplant recipients receiving CYA or FK and SRL or MMF. **Methods:** A retrospective review of 1,415 patients transplanted at a single center between Jan. 1996 - Jan. 2001. There were 340 (24.0%) recipients excluded because of missed blood draws, changes in immunosuppression, or lost to follow-up, leaving 1075 evaluable patients. 645(60%) recipients were male; 724(67%) received cadaveric kidneys and 357(33%) received a living donor kidney. 482(45%) patients were hispanic, 240(22%) white, 136(12.6%) asian, and 102(9.4%) african american. Hypercholesterolemia defined as serum total cholesterol ≥240 mg/dl. Recipients received CYA & MMF (n=68; 4.8%) or FK & SRL (n=106; 7.48%). Recipient age, weight, sex, race, pretransplant prevalence of hypertension or diabetes and renal cold ischemia times equivalent for all groups. Cholesterol measured one day after transplant; 2-4 mos, 4-7 mos, 9-12 mos, 12-24 mos and at end of follow-up period. Patients followed for a mean of 37 mos. **Results:** The use of CYA or FK with SRL was associated with higher likelihood of hypercholesterolemia at all time points after transplantation than the use of CYA or FK with MMF.

TABLE 1

	BASELINE	2-4 MO	4-7 MO	9-12 MO	12-24 MO	END OF F-UP
RAPA & CYCLO (RC)	7.35%	33.53%	24.71%	17.14%	21.14%	24.42%
RAPA & FK (RF)	13.20%	29.11%	19.76%	17.50%	19.76%	22.50%
CELLCEPT & CYCLO (CC)	3.55%	13.51%	12.83%	10.77%	20.00%	17.84%
CELLCEPT & FK (CF)	3.56%	13.89%	14.86%	7.12%	9.96%	10.13%

Conclusions: The use of SRL with FK or CYA was associated with higher serum cholesterol levels than the use of MMF with either calcineurin inhibitor. Patients receiving MMF and FK had lowest serum cholesterol levels at >12 mos. follow-up, with MMF and CYA equivalent to this regimen until 7 mos. post transplant. A trend towards lower cholesterol levels with increasing follow-up was observed in all subgroups from 2 mos. to one year postransplant.

Abstract# 1314 **Poster Board #-Session: P70-III**

QUALITY OF LIFE IN RENAL TRANSPLANTATION; THE EFFECT OF IMMUNOSUPPRESSION AND AGE IN PATIENTS PERCEIVED QOL. Argiris Asderakis,[1] Owain Evans,[1] Ann Marsden,[1] Vasia Kavadas,[1] Adib Khanafer.[1] *[1]Transplant Unit, University Hospital of Wales, Cardiff, United Kingdom.*

Background and aim: Patients with renal transplant demonstrate better Quality of Life (QOL) than patients on dialysis. Aim of this study was to measure the subjective QOL of patients with functioning grafts and associate it with risk factors, including the use of immunosuppressives. **Patients and methods:** 44 renal transplant patients with functioning grafts, at least 3 months post transplant were interviewed with the use of three instruments of measurement of QOL: Kidney Transplant Questionnaire (KTQ), SF36 - a questionnaire for chronic illness, and EORTC health thermometer. The questions in KTQ and SF-36 were grouped in dimensions and mean scores calculated. **Results:** Patients were 4 months to 20 years post transplant (median 27 months). The mean global KTQ score was 5.26 and the mean global SF-36 was 58.27. The worst SF-36 was in general health (mean 48.86). Age >50 was associated with lower QOL in 7 out of 14 parameters studied (Anova test $p<0.05$) in particular **physical condition** and **physical functioning**. Women had a slightly worse KTQ in appearance than men ($p=0.08$). **SF-36 Vitality** was improved 12 months after the transplant($p=0.08$). Testing with Anova, it was found that patients on Tacrolimus had a worse score in SF-36 **physical functioning**, **role limitation due to emotional factors**, **bodily pain** and the **health thermometer**. Since this could have been due to interaction, we used univariate and multivariate analysis. There was interaction between immunosuppressive used and both age and creatinine ($p=0.01$ and $p=0.04$ respectively). The immunosuppressive used remained though, a significant factor after correcting for the effect of age, sex, creatinine, time post transplant and time spent on dialysis ($p=0.026$). We tested the effect of Cyclosporin vs Tacrolimus based on the 'linearly independent pairwise comparisons among the estimated marginal means'. This test showed the difference between the two drugs to be a true one for SF-36 **Physical functioning** ($p=0.016$), for **Health thermometer** ($p=0.1$) and for SF-36 **General health** ($p=0.10$). **Conclusions-Discussion:** Patients post renal transplant perceive their quality of life as good, but they have limitations in their physical functioning and general health. Age>50 years is strong predictor of low QOL score in many dimensions. Tacrolimus seems to limit physical functioning and also the perceived general health. This is not to say that we should not use Tacrolimus, but we should be aware of its limitations in certain patient groups.

Abstract# 1315 **Poster Board #-Session: P71-III**

ROLE OF GLYCOMETABOLIC CONTROL ON RENAL FUNCTION AND BLOOD PRESSURE AFTER PANCREAS/KIDNEY TRANSPLANTATION. Ennio La Rocca,[1] Jelena Bojanin,[1] Paolo Fiorina,[1] Elena Orsenigo,[2] Carlo Socci,[2] Marco Cristallo,[2] Valerio Di Carlo,[2] Paolo Manunta,[3] Chiara Lanzani,[3] Giampaolo Zerbini,[1] Antonio Secchi.[1] *[1]Medicine, San Raffaele Scientific Institute, Milan, Italy; [2]Surgery, San Raffaele Scientific Institute, Milan, Italy; [3]Nephrology, San Raffaele Scientific Institute, Milan, Italy.*

Insulin resistance was identified as risk factor for hypertension and consequently diabetic nephropaty.On the contrary, good glycometabolic control has been shown to exert beneficial effects on diabetic complications, including nephropathy. **Aim of the study:** to evaluate the relationship among glycemic control, insulin resistance, renal function and blood pressure, in pancreas/kidney transplanted patients in good glycemic control. **Patients and methods:** in a cohort of 50 pancreas/kidney transplanted patients was estimated glycated hemoglobin (HbA1C % range 4.6-7.1; nv 3.5-6) and insulin resistance based on fasting plasma glucose, insulin (homeostasis model assessment of insulin resistance [HOMA-IR]). Blood pressure was evaluated by ambulatory 24-hour monitoring, while renal function by creatinine levels (Cr: mg/dl) and creatinine clearance values (Cr/cl: ml/min). Immunosoppressive treatment included CSA, FK 506, MMF and steroids. **Results:** nocturnal blood pressure circadian rhythm was abnormal or inverted in a great number of patients studied. Creatinine, Cr/cl and blood pressure clearly correlated with HbA1c (HbA1c vs Cr: $r = .4$, $p = .01$; HbA1c vs Cr/cl. $r = -.3$, $p < .05$; HbA1c vs PAD/diurnal: $r=.3$, $p= .01$; HbA1c vs PAM/nocturnal: $r=.3$, $p < .03$). No correlation were observed with insulin levels (F IRI sd $=14 \pm 6$ µU/ml) and insulin resistance (HOMA 2.9 ± 12.5). Renal function strictly correlated with blood pressure (Cr vs PAM/diurnal: $r=.3$, $p=.001$; Cr vs PAM/nocturnal: $r=.2$, $p= .01$; Cr/cl vs PAM/nocturnal: $r= -.3$, $p=.009$). **Conclusion:** In pancreas/kidney transplanted patiens, we observed a strict relationship among low HbA1c values, better renal function and low blood pressure, independently from insulin levels and insulin resistance. Results suggest an important effect of glycemic control on renal graft function and consequently on blood pressure.To prolong graft survival, the better glycometabolic control must be achieved after organ transplantation.

Abstract# 1316 **Poster Board #-Session: P72-III**

ECONOMIC REVIEW OF LAPAROSCOPIC VERSUS OPEN LIVING KIDNEY DONOR NEPHRECTOMY. Jill Martin,[1] Joseph Buell,[2] Edward Zavala,[1,3] Jane Benjey,[3] Leslie Trumbull,[3] Barry Marshall,[3] Michael Hanaway,[2] E. Steve Woodle.[2] *[1]College of Pharmacy; [2]Department of Surgery, University of Cincinnati; [3]Transplant Services, University Hospital, Cincinnati, OH.*

Laparoscopic Donor Nephrectomy (LDN) is rapidly becoming a standard technique for living kidney donation. Donors recover quickly and return to their normal activity level sooner, which has been helpful in mitigating some of the disincentives of being a living kidney donor. The institutional economic impact of this technique has not been reported and was evaluated as compared to open donor nephrectomy (ODN). **Methods:** Forty LDNs were compared to 35 ODNs performed between 1/97-10/01 at a single institution. LOS, OR time, and financial data were obtained for the initial surgical admission to discharge and one year post-operatively. Charges were obtained from the institution's activity based cost accounting system (HBOC-Trendstar). **Results:** There was no significant difference between the groups regarding age, race, or weight. As seen in the table below, the LOS was significantly lower for the LDN, irrespective of approach. In contrast, the OR time and charges were significantly higher in the LDN as compared to the ODN, resulting in higher overall charges for the LDN for the initial donor stay. The technically more challenging right kidney donor nephrectomies did not show a significant increase in resource utilization. There were only 3 readmissions, 2 LDN left (total 4 days) and 1 ODN (total 3 days).

	Open Donor (n = 35)	Lap/Left (n = 35)	Lap/Right (n = 5)	Lap/Total (n = 40)
LOS (days)				
Mean	3.83	3.09*	2.40*	3.00*
St. Dev.	±1.12	±1.15	±0.55	±1.11
OR Time (minutes)				
Mean	245.89	318.80*	336.60**	321.03**
St. Dev.	± 61.29	±48.64	±25.54	±46.53
OR Charges (dollars)				
Mean	$4,672.98	$8,577.38**	$8,868.42**	$8,792.42**
St. Dev.	±$1,646.42	±$2,485.25	±$2,800.65	±$2,453.71
Total Charges (dollars)				
Mean	$11,240.63	$18,842.53**	$18,952.59**	$19,342.42**
St. Dev.	±$3,965.79	±$4,509.67	±$4,633.98	±$4,070.93

Compared to Open donor: * = $p < 0.01$ ** = $p < 0.0001$

Conclusion: While LDN has been reported to provide improved patient satisfaction, there is a significant increase in hospital charges compared to ODN. Medicare pays for the increased costs of the LDN through the kidney acquisition cost center; however, only 45% of kidney transplants are paid for by Medicare as the primary payor. Reimbursement from other payors varies. Transplant programs need to have a heightened awareness of the increased costs of LDN.

Abstract# 1317 **Poster Board #-Session: P73-III**

SYSTEMIC HEPARINIZATION IS NOT NECESSARY IN LAPAROSCOPIC DONOR NEPHRECTOMY. L. Thomas Chin, Yolanda Becker, Sean Hedican, Timothy Moon, Stephen Nakada, Jon Odorico, Stuart Knechtle, Anthony D'Alessandro. [1]*Surgery, University of Wisconsin, Madison, WI.*

Systemic heparinization has been used routinely in laparoscopic donor nephrectomy, whereas it is not used in open donor nephrectomy. Critics of the laparoscopic approach have suggested that the requirement for heparin and subsequent protamine administration poses additional risks. After becoming fascile with laparoscopic nephrectomy, we stopped routine heparinization of our donors. In this study, we compare outcomes of laparoscopic donor nephrectomies performed with and without systemic heparin. **Methods:** Donor nephrectomies were performed via a standard flank approach or via a totally laparoscopic approach. In the intial laparoscopic group, 3000 Units of heparin was administered 3 minutes prior to renal artery clamping. After clamping of the renal vessels, 50 mg of protamine was given. No heparin was administered for the open or later laparoscopic groups. In all cases the kidney was flushed for 1 minute on the back table with cold lactated Ringer's solution containing heparin 10 U/ml. Serum creatinine, incidence of rejection, and graft survival were examined retrospectively; the groups were compared using the Wilcoxon rank-sums test and the log rank test respectively. **Results:** 158 open and 136 lap nephrectomies were performed from 1999-2001. 68 of the 136 lap nephrectomies (lapNx) were perfomed without heparin. The no heparin lapNx group included 10 double and 1 triple artery nephrectomy. All lapNx were left sided. There were no adverse effects related to either heparin or protamine administration. The mean warm ischemic time in the lapNx group was 3.4 min. The mean warm ischemic time in the open group was not recorded. There was no difference in postoperative serum creatinine at 1 week, 3, 6, 9 and 12 months post-operatively, acute rejection, nor graft survival between groups. One graft failed secondary to renal artery thrombosis in the open group. No thrombotic complications occurred in either lapNx group. Time zero back table biopsies were available for review from 10 of the 68 no heparin lapNx group as well as 19 subsequent no heparin lapNx biopsies, and no evidence of microthrombi were seen. **Conclusion:** Laparoscopic donor nephrectomy can be performed safely without systemic heparinization even in the presence of multiple arteries.

One year graft survival/rejection

	Graft Survival	Rejection
Open (n = 158)	97%	28%
LapNx Heparin (n = 68)	98%	24%
LapNx No Heparin (n = 68)	97% p=0.48	25% p=0.98

Abstract# 1318 **Poster Board #-Session: P74-III**

LONG-TERM ANALYSIS OF GRAFT FUNCTION IN RIGHT LAPAROSCOPIC DONOR NEPHRECTOMY: A CASE CONTROLLED SERIES. A. P. Chudzinski,[1] R. E. Boardman,[1] M. Gupta,[1] M. J. Hanaway,[1] T. D. Merchen,[1] M. Clippard,[1] E. S. Woodle,[1] J. F. Buell.[1] [1]*Division of Transplantation, University of Cincinnati, Cincinnati, OH.*

Utilization of right laparoscopic donor nephrectomy (LDN) grafts remains controversial. Right LDN is utilized when left LDN is contraindicated due to vascular and/or ureteral anomalies. The purpose of this study was to compare the right vs. left LDN with respect to donor and recipient outcomes. **Methods:** We retrospectively identified 23 right LDN and compared them to a contemporary control group of 23 left LDN that were matched for donor gender, age, weight, and body mass index (BMI). Warm ischemia time (WIT), intra-operative fluid input and output (I/O), operative time (ORT), estimated blood loss (EBL), donor time to oral intake (TPO), donor length of hospital stay (LOS), donor time to drive (TTD), donor time to return to work (TTW), and recipient graft function with respect to urine output (UOP) and serum creatinine (SCr) were analyzed. **Results:** Donor demographics, donor outcomes, and recipient graft function include:

	Left (n=23)	Right (n=23)	P value
Gender (M/F)	13/10	13/10	ns
Age (yr)	40±10	41±8	ns
Weight (kg)	78±14	79±15	ns
BMI (kg/²)	27±3	26±5	ns
WIT (min)	210±40	248±93	0.04
I/O (L)	2.6±0.5/0.9±0.5	2.3±0.6/0.9±0.3	0.07 / ns
ORT (min)	210±40	194±42	ns
EBL (mls)	158±85	139±71	ns
TPO (hrs)	7±2	6±1	0.09
LOS (hrs)	42±10	40±8	ns
TTD (days)	10±6	7±3	0.09
TTW (days)	30±6	24±5	0.06
UOP (L/day) Day 1	3.5±2.5	2.8±1.7	ns
Day 2	4.0±2.8	3.8±2.9	ns
Day 3	2.8±1.3	3.0±4.2	ns
Scr (mg/dL) Day 1	4.0±2.2	4.2±2.1	ns
Day 3	2.1±1.9	2.6±1.9	ns
Day 7	1.6±1.3	2.4±2.0	ns
Day 30	1.3±0.6	1.7±1.0	ns
3 months	1.3±0.5	1.5±0.7	ns
6 months	1.2±0.4	1.6±0.8	ns
1 year	1.4±0.5	1.5±0.8	ns
1 yr graft survival	96%	91%	ns

Conclusion: Right LDN have slightly longer WIT, however, short and long-term graft function was not adversely affected. These observations support the views of equivalent results with right vs. left LDN.

Abstract# 1319 **Poster Board #-Session: P75-III**

LAPAROSCOPIC DONOR NEPHRECTOMY 1997-2002: DONOR AND RECIPIENT OUTCOMES IN MORE THAN 400 CASES AT A SINGLE INSTITUTION. Burak Kocak,[1] Alan Koffron,[1] Paolo Salvalaggio,[1] Talia Baker,[1] Jonathan Fryer,[1] Dixon Kaufman,[1] Michael Abecassis,[1] Frank Stuart,[1] Joseph Leventhal.[1] [1]*Department of Surgery, Northwestern University, Chicago, IL.*

Laparoscopic live donor nephrectomy (LDN) is now a well established, less invasive alternative to open nephrectomy for living kidney donation. We have reviewed our 5-year experience with LDN, assessing the feasibility and safety of LDN, the function of kidneys removed by LDN, and the incidence of urologic complications in LDN recipients. 405 LDN were performed from 10/97 to 12/02 at our center. Operative technique for LDN remained consistent throughout the series (4 laparoscopic ports, periumbilical or left/right lower quadrant extraction incision) except 5 cases, which were done hand-assisted. The left kidney was removed in 98.8% of donors. 90 donors (22%) had a body mass index of > 30. 94 kidneys (23%) had multiple renal arteries, 12 kidneys (3%) had retroaortic/multiple renal veins. The mean warm ischemia time was 2.6±0.5 minutes. The mean length of postoperative hospital stay was 1.7±0.7 days, which decreased to 1.2±0.5 days in the last 100 cases. Complications of LDN included urinary retention(5), wound infection(4), splenic capsular tear, all managed laparoscopically(3), diaphragmatic tear(1), aortic injury(1), renal arterial injury(1), self-contained adrenal hematoma(1), transient carbon dioxide pneumomediastinum(2), chylous ascites(2), port site granuloma with nerve entrapment(1). 5 of the donors required readmission: Ileus(1), chylous ascites(2) managed conservatively, repair of aortic injury and excision of port site granuloma. All patients made complete recoveries. There were 9 open conversions, of which 6 were in the first 100 cases. There have been no postoperative intestinal obstructions in any donors. The preoperative and postoperative day #7 serum creatinine values of the donors were 0.9±0.2 and 1.3±0.3, respectively. There has been only one recipient of LDN kidneys who experienced delayed graft function with ATN requiring dialysis. There were no graft loses due to LDN technique. Kidneys requiring vascular reconstruction experienced excellent allograft outcomes. There have been no short or long-term allograft urologic complications in this series. LDN is a safe and effective procedure. LDN patients have a rapid postoperative recovery and short hospital stay. With careful surgical technique, delayed graft function and urologic complications in recipients can be avoided. Aberrant vascular anatomy and obesity are not contraindications to LDN, but require experience.

Abstract# 1320 **Poster Board #-Session: P76-III**

LAPAROSCOPIC VERSUS OPEN LIVE DONOR NEPHRECTOMY. THE FIRST PROSPECTIVE CLINICAL TRIAL. Nasser Simforoosh,[1] Abbas Basiri,[1] Seyed Amir Mohsen Ziaei,[1] Ali Tabibi,[1] Nasser Shakhs Salim,[1] Fatemeh Pourrezagholi,[1] Seyed Mohamad Mehdi Hosseini Moghadam.[1] [1]*Urology, Shahid Labbafi Nejad Hospital, Tehran, Tehran, Islamic Republic of Iran.*

Purpose: To our knowledge there is no prospective study to compare laparoscopic and open live donor nephrectomy. There for we decided to compare these two techniques in a randomized controlled trial. **Methods and Material:** From July 23, 2001 until May 21, 2002, 80 recipients underwent live renal transplantation. Patients were entered this clinical trial upon inclusion criteria of the study. Donors were selected for laparoscopic (study group) or open donor nephrectomy (Control group) technique in a randomized fashion. 40 patients were operated in both groups. **Results:** Donor age and sex did not differ statistically in the two groups. In the laparoscopic and open nephrectomy groups mean follow-up was 142 and 148 days (P=0.72), mean operative time was 251 and 135 minutes (p=0), mean hospital stay was 2.21 and 2.13 days (p=0.73). Mean warm ischemia time was 6.6 minutes in laparoscopic nephrectomy group (LDN) and was less than 2.5 minutes (p=0) in open nephrectomy group (ODN). There was 2 Conversion to open in LDN group. Mean serum creatinine at day 3 in LDN and ODN group was 1.91 mg/dl and 1.46 (p=0.09). At 3 months mean serum creatinine was 1.32 and 1.37 in LDN and ODN group accordingly (p=0.96). There were no ureteral complication in LDN group and there was one ureteral fistula in ODN group. **Conclusion:** To our knowledge this is the first prospective study that laparoscopic donor nephrectomy can give similar outcome of renal transplantation. However laparoscopic approach for donation has several advantages encouraging donors for kidney donation, in an era that kidney shortage is an important obstacle for kidney transplantation.

Abstract# 1321 Poster Board #-Session: P77-III

RIGHT LAPAROSCOPIC NEPHRECTOMIES (LDNs) - ARE THERE ADDITIONAL RISKS? James V. Harmon,[1] Arthur J. Matas,[1] Massimo Asolati,[1] Abhinav Humar,[1] Rainer W. Gruessner,[1] David E. R. Sutherland,[1] David L. Dunn,[1] Raja Kandaswamy.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Intro: Our policy for open nephrectomy has been to use the right kidney with a single artery where there were multiple left arteries. In LDNs, some centers will use the left kidney with 2 good-sized arteries over a single-artery right kidney, because of the concern about the shorter right renal vein in LDNs posing an added risk of thrombosis. Our center has routinely gone to a single-artery right kidney in this situation. Multiple-artery left kidneys were done if the right side also had equally multiple arteries. We studied whether the outcome of single-artery right LDN vs. double-artery left LDN were comparable. **Methods:** Since Dec. 97, we have done 254 LDNs (253 analyzed). There were 205 left and 48 right LDNs. Of the left, 83% were single and 17% multiple and the right, 85% single, 17% multiple. Donor and recipient demographics and outcomes are shown. The only significantly different variable was an increased donor age in right LDNs (p=0.006). In the tables single left aftery LDN results are shown on the extreme right for comparison.

Donor Variables	Single Right (n=41)	Muliple Left (n=35)	Single Left (n=170)
Mean age in years ± SD	47 ± 9	41 ± 13	41 ± 10
Sex M/F (%)	44/56	51/49	46/54
Race Caucasian/non (%)	92/18	89/11	87/13
LOS ± SD	3.3 ± 2.1	3.8 ± 1.7	3.6 ± 1.3
Δ creatinine ± SD	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.2
Number of arteries 1/2/3 (%)	100/0/0	0/97/3	100/0/0

Causes of graft loss (DWFG) include 1 chronic rejection in the right LDN and 1 recurrence of disease in the multiple artery left LDN. Delayed graft function (DGF) is defined as dialysis in the first week. There were no technical graft losses in either group.

Recipient Variables	Single Right (n=41)	Multiple Left (n=35)	Single Left (n=170)
Mean age in years ± SD	47 ± 12	43 ± 13	43 ± 15
Sex M/F (%)	54/46	73/27	60/40
Race Caucasian/non (%)	93/7	88/12	91/9
Implant site R/L/Intraperitoneal	33/65/2	45/52/3	46/50/4
DGF (%)	3	9	6
Patient survival 1/3 year (%)	95/95	100/100	95/95
Graft survival 1/3 year (%)	95/95	97/97	93/92
Creatinine 3/9 mos	1.6/1.6	1.5/1.6	1.6/1.6

Conclusions: Right nephrectomy poses no additional risk compared to left nephrectomy whether it is single or multiple arteries. Multiple arteries lead to increased warm ishemia time (multiple anastomoses) whereas right kidneys come with a shorter vein. In left multiple-artery cases with a single right artery, choice of side should be left to surgeon's judgment and level of comfort.

Abstract# 1322 Poster Board #-Session: P78-III

COMPARISON OF LAPAROSCOPIC VERSUS OPEN HARVEST TECHNIQUES FOR LIVING DONOR TRANSPLANTATION: INITIAL RESULTS OF THE REGISTRY OF SIMULECT USE IN LIVING TRANSPLANTS (RESULTS). Charles B. Cangro, Anne Wiland, Douglas Slakey, William Bennett, Meredith Aull, Jeffrey Fink, Matthew Weir, the ReSULTs Registry Group. *[1]University of Maryland, Baltimore, MD.*

ReSULTs is a transplant registry documenting the transplant experience and outcome of patients who received a renal transplant from a living donor at one of 29 participating US centers. The registry provides an opportunity to compare the laparoscopic (LAP) to the open (OPEN) technique of renal transplant procurement. There are currently 261 transplant registrants (158 related and 103 unrelated) transplanted between August 2001 and October 2002, and induced with basiliximab. Of these registrants, 145 (55.6%) had their kidney harvested by the LAP and 116 (44.4) by the OPEN technique. The recipients and donors did not differ in demographic characteristics, weight or biologic relationship. Tacrolimus or cyclosporine were the calcineurin inhibitors used in 85 (58.6%) vs 31 (21.4%) of LAP and 77 (53.1%) vs 39 (26.9%) of OPEN, respectively. The mean body weight of the LAP and OPEN donors was 78.1 kg vs 76.8 kg, and mean age was 41.0 yrs vs 40.6 yrs respectively. There were 31(22%) of LAP and 57 (49.1%) of OPEN patients with 6 months or more of follow-up. There was no difference in the incidence of delayed graft function (DGF), or the median cold ischemic time in LAP vs OPEN group (0.9 ± 0.8 hrs vs 0.5 ± 1 hrs, p=0.1). The groups had no difference in serum creatinine at Day 1 (D1), discharge (DC), 3 months (M3) and 6 months (M6) but the incidence of biopsy proven acute rejection (AR) during the first 6 months was slightly higher in the LAP vs OPEN patients (p=.04).

	DGF	Pretx Cr*	Cr D1	Cr DC	Cr M3	Cr M6	AR
LAP	12 (8.3%)	7.1	4.2	1.4	1.3	1.4	10 (6.8%)**
OPEN	10 (8.6%)	7.3	4.2	1.4	1.3	1.2	5 (4.3%)

*median Cr expressed in mg/dl, there were no significant differences, **p=0.04

Conclusion: We conclude that the LAP method of living donor kidney harvest has become the predominant technique in the US. There does not seem to be a tendency to utilize any one procurement procedure based on donor size or age. Short-term results are comparable between the LAP and OPEN techniques, although the incidence of DGF was greater than expected in both groups and there was higher acute rejection rate in the LAP group, the significance of which must be confirmed with longer follow-up in ReSULTs.

Abstract# 1323 Poster Board #-Session: P79-III

LAPAROSCOPIC LIVING DONOR NEPHRECTOMY IN DONORS OVER AGE 65 YEARS. Julie Heimbach,[1] Michael Ishitani,[1] George Chow,[1] Mikel Prieto,[1] Scott Nyberg,[1] Matthew Griffin,[1] Mark Stegall.[1] *[1]Transplant Center, Mayo Clinic, Rochester, MN.*

Background As the US population continues to age, the need renal replacement therapy in the elderly continues to increase. However, the shortage of cadaveric organs and the increasingly long waiting period limits the use of cadaveric renal transplantation in older patients. Living donor renal transplant has better patient and graft survival when compared to cadaveric transplantation. In many cases an organ from an older donor may be the only kidney available for an elderly recipient. However, the outcome and complications of patients undergoing laparoscopic donor nephrectomy over age 65 is not known. **Methods**: We analyzed outcomes for donors undergoing a transabdominal, hand-assisted laparoscopic nephrectomy from January 2000 to September 2002. We assessed donor outcomes including perioperative complications, residual renal function, and hospital stay as well as graft function in the recipient. GFR was measured by iothalamate clearance. **Results**: Of 467 laparoscopic donor nephrectomies performed during the study period, 20 patients (4.3%) were ≥ 65 years old (mean 67.7 range 65 to 78). Prior to nephrectomy, 8 had HTN (definedas stable and controlled with a single hypertensive agent), 5 had BMI ≥ 30, and 5 had prior abdominal surgery. Donor complications included 2 incisional hernias, 1 urinary tract infection and 1 retroperitoneal hematoma managed conservatively. Average hospital stay was 2.1 days (range 1-4). Mean iothalamate clearance pre-op was 87.6 ml/min/SA (range 70-112) and post-op was 53.1 ml/min/SA (range 40-106). At 6 months mean proteinuria was 84.7 mg/protein/day. The prevalence and degree of HTN was unchanged. Recipient mean age was 62.4 years (range 37-81). Recipient creatinine clearance upon discharge from clinic at 6 weeks was 47.6 ml/min/SA. Graft survival is 95% and donor and recipient survival is 100%. **Conclusions**: Laparoscopic donor nephrectomy in carefully selected patients over age 65 is safe and results in satisfactory recipient allograft function. Use of older living donors can broaden the donor pool and may improve access to transplantation for elderly patients with ESRD.

Abstract# 1324 Poster Board #-Session: P80-III

LIVING KIDNEY DONATION: EVALUATION OF THREE SURGICAL TECHNIQUES AND DONOR QUALITY OF LIFE. Grzegorz Nowak,[1] Sun Shibo,[1] Peter Barany,[2] Bo-Goran Ericzon,[1] Henryk Wilczek,[1] Ingela Fehrman-Ekholm.[2] *[1]Department of Transplantation Surgery, Karolinska Institute, Huddinge University Hospital B56, Sweden; [2]Department of Nephrology, Karolinska Institute, Huddinge University Hospital B56, Sweden.*

Background: An important issue in living kidney donor transplantation is whether the donation is safe for the donor. A survey of donors who underwent nephrectomy by conventional flank incision between 1964-1995, showed that 5% of them never fully recovered after donation. The aim of this study was to examine how the introduction of new surgical techniques such as nephrectomy by an anterior incision and laparoscopic nephrectomy, and the introduction of new pain-relieving protocols, has influenced the donor's course after donation. Patients and methods: 144 living kidney donors between 1996-2000 were included in the study. The surgical technique and pain-relieving protocols used were evaluated. In addition, a contemporary donor/recipient kidney function analysis was made. Besides analysing hospital records, a questionnaire was sent to all donors living in Sweden (n=128). Results: 48% of the donors underwent flank incision, 35% anterior incision and 17% laparoscopic nephrectomy. The recorded complication frequency was 28, 15 and 18%, respectively. Patients operated on by the anterior incision and patients who underwent a laparoscopic nephrectomy had a reduced stay in hospital ($p<0.03$) and required less total dose of painkillers ($p<0.005$). The response rate to the questionnaire was 92%. 28% of the donors said that they felt better after donation and 5% felt worse. Before donation, 5% considered the medical care to be unsatisfactorily, and after donation 14% said that they had not been attended to well enough. 9% of the donors claimed that they suffered an economical loss as a consequence of their donation. One donor regretted her donation. There was a difference in kidney function between donors and recipients after transplantation (GFR 70±2 vs. 44±2 ml/min/1.73m², respectively, $P<0.001$). Conclusions: The introduction of new surgical techniques, such as nephrectomy by anterior incision and laparoscopic nephrectomy, reduces hospitalisation time and the use of painkillers after donation. However, 5% of the donors still declare that their condition is worse after than before donation. It is also important that donors are fully compensated for economical loss. The lower GFR in the recipients is probably due to several factors, but mainly due to the influence of immunosuppressive drugs. By the use of never, less toxic and more effective immunosuppressive medication improvements in recipient GFR should be possible.

Abstract# 1325 **Poster Board #-Session: P81-III**

MICROINVASIVE DONOR NEPHRECTOMY: ? FUTURE OUTPATIENT SURGERY. Deepak Mital,[1] Roderick Quiros,[1] John Lee,[1] Stephen C. Jensik.[1] [1]*Transplant Surgery Section, General Surgery, Rush Medical College, Chicago, IL.*

Purpose: Advances in minimally invasive techniques have reduced the morbidity after donor nephrectomy significantly. Is it now possible to do this as a same day procedure ? **Methods:** We have studied the results after 50 Microinvasive Donor Nephrectomy procedures performed at our institution between June 2001 and November 2002. This involves a single 2.5 inch extra-peritoneal incison anterior to the 10th rib, without any rib resection. Pain and morbidity is minimal, allowing immediate resumption of activity and a full diet. Most donors are able to drive and return to normal activities within 5-7 days after the nephrectomy. **Results:** Two of these kidney donors were discharged to home on the day of surgery itself. All the 48 others went home on the day after donor nephrectomy. The donors were between 18- 61 years of age. Their weights ranged from 115 to 225 lbs. Renal allograft function was 100% in the recipients. There were no renal artery or vein injuries or thromboses. Since the peritoneum is not entered, there were no injuries to the spleen or bowel. None of the donors had an ileus or bowel obstruction for the same reason. There were no instances of ureteric necrosis, obstruction, stenosis or leak. This compares quite favorably with other minimally invasive techniques, including standard Laparoscopic and Hand Assisted Laparoscopic methods, wherein most series report a length of stay ranging from 2-4 days. Laparoscopic techniques have major limitations which include the well known intraperitoneal complications of : renal artery / vein and other vascular trauma, splenic capsule tears, bowel perforation, ureteral ischemia and delayed bowel obstruction. Since expensive Laparoscopic instruments are not required ,operative time is short and the length of stay just 1 day, costs are minimal. The learning curve is not steep as in Laparoscopic techniques, and allograft function is immediate. **Conclusions:** Microinvasive Donor Nephrectomy may be done safely for the donor , with early kidney function for the recipient, at the same time allowing expeditious discharge of the donor from the hospital with excellent resource utilization. Now that the dust is settling down from the aggressive marketing of laparoscopic donor nephrectomy, it is obvious that this is associated with unacceptable and avoidable risks to the donor and the kidney. The results with the Microinvasive technique bear the promise of donor nephrectomy becoming an outpatient or overnight stay procedure in the near future.

Abstract# 1326 **Poster Board #-Session: P82-III**

LAPAROSCOPIC HAND-ASSISTED DONOR NEPHRECTOMY FOR RIGHT KIDNEYS. A SINGLE CENTER EXPERIENCE. Mikel Prieto,[1] George K. Chow,[1] Mark D. Stegall.[1] [1]*Transplant Center, Mayo Clinic, Rochester, MN.*

Laparoscopic donor nephrectomy is becoming widely accepted as the preferred method for living kidney donation. Many centers, however, have been reluctant to perform right kidneys laparoscopically and preferentially select left kidneys even in the presence of multiple renal arteries. Frequently, when the right kidney needs to be removed the open nephrectomy option is offered. At our center all donor nephrectomies are performed with the hand-assisted laparoscopic technique. The criteria used for kidney selection is the same as the one used in the past for open donor nephrectomies. This study analyzes the outcomes of those donors who underwent a right nephrectomy. **Methods.** From 1/1/00 to 9/26/02, 441 consecutive laparoscopic donor nephrectomies were performed at our institution. In 92 cases (21%) the right kidney was chosen as the donor kidney for the following reasons: double left renal artery (55%); triple left renal artery (9%); early bifurcation of left renal artery (10%); fibromuscular dysplasia or stenosis on the right renal artery (10%); right kidney smaller than left (5%); and multiple cysts or scarring on the CT angiographic image of the right kidney (9%). The male/female ratio was 42/50. Sixty-six were related and 26 were unrelated donors. **Results.** Ninety-two hand-assisted laparoscopic right donor nephrectomies were performed. The kidney was removed through an infraumbilical incision (midline or Pfannenstiel, based on surgeon/patient preference) in 85 (92%) patients. In 7 (8%) obese patients a paramedian incision was used. Eighty-four patients (91%) had no complications, 3 patients (3%) had a wound infection and one patient required reoperation for bleeding in the immediate postoperative period. Hospital stay was 2.2+/-0.5 days. All kidneys were transplanted successfully. There were no significant differences between left and right nephrectomies when comparing operative time, hospital stay, morbidity and allograft function. **Conclusion.** Hand-assisted laparoscopic donor nephrectomy can be safely performed on right kidneys. Kidney selection in donors undergoing a laparoscopic nephrectomy may be based on the usual vascular anatomical criteria. This approach maximizes the number of donors that are eligible for the procedure and decreases the number of grafts with multiple arteries.

Abstract# 1327 **Poster Board #-Session: P83-III**

RENAL TRANSPLANTATION VIA THE EXTRAPERITONEAL APPROACH IN PEDIATRIC RECIPIENTS WITH LOW-BODY WEIGHT. Kazunari Tanabe,[1] Tadahiko Tokumoto,[1] Yuichiro Yamazaki,[1] Hideki Ishida,[1] Nobuo Ishikawa,[1] Naoshi Miyamoto,[1] Hiroaki Shimmura,[1] Motoshi Hattori,[1] Katsumi Ito,[1] Hiroshi Toma.[1] [1]*Department of Urology, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.*

Introduction: The complex nature of pediatric renal transplantation and the often fragile condition of children with end-stage renal disease create the potential for a number of complications. We have performed renal transplantation via the extraperitoneal approach at our institution even in children who weigh less than 15 kg. We retrospectively evaluated whether renal transplantation via the extraperitoneal approach is safe and acceptable in low-body weight pediatric kidney transplant recipients. Patients and Methods: From February 1983 to December 2001, 1,751 patients underwent renal transplantation at our institution, including 84 boys and 59 girls with a mean age of 10.3 years who comprised the study group (age 1-16). Mean weight was 30 kg at transplantation, and 31 and 112 patients weighed less than 15 kg and 15 or more kg, respectively. All patients were treated with cyclosporine- or tacrolimus-based immunosuppression, including methylprednisolone and azathioprine or mizoribine. Living-related and cadaveric renal transplantation was performed in 130 and 13 patients, respectively. ABO-compatible and ABO-incompatible living renal transplantation was performed in 131 and 12 patients, respectively. The extraperitoneal technique was performed in all recipients, even in low-weight children. Results: During observation, 10 surgical complications (6.7%) developed. There were no gastrointestinal complications. The recipients whose weight was less than 15 kg developed 6 surgical complications (19%), including significant vesicoureteral reflux to the graft, bladder leakage, subcapsular hematoma, renal artery stenosis, retroperitoneal bleeding, and lymphocele in 1 patient each. In contrast, the recipients weighing 15 or more kg developed 4 surgical complications (5.4%) which were significant vesicoureteral reflex in 4 patients. The incidence of surgical complications was less in the recipients who weighed over 15 kg than in those with a low-body weight. Only one graft was lost to a surgical complication (renal artery stenosis). Conclusions: Although the incidence of surgical complications was higher in pediatric recipients of low-body weight than in those weighing over 15 kg, graft loss occurred in only 1 patient. The extraperitoneal technique seemed to be safe, without gastrointestinal complications even in children weighing less than 15 kg.

Abstract# 1328 **Poster Board #-Session: P84-III**

CHANGES IN ENDOTHELIAL FUNCTION BEFORE AND AFTER RENAL TRANSPLANTATION. Huseyin Oflaz,[1] Aydin Turkmen,[2] Rumeyza Kazancioglu,[2] Fehmi Mercanoglu,[1] Banu Bunyak,[2] Memduh Dursun,[3] Kutlay Tutucu,[2] Burak Pamukcu,[1] Mehmet S. Sever.[2] [1]*Cardiology, Istanbul Faculty of Medicine, Istanbul, Turkey;* [2]*Nephrology, Istanbul Faculty of Medicine, Istanbul, Turkey;* [3]*Radiology, Istanbul Faculty of Medicine, Istanbul, Turkey.*

There is an accumulating strong evidence suggesting that endothelial cell dysfunction is an early key event in the development of arteriosclerotic cardiovascular disease observed in chronic renal failure patients. On the other hand, the role of renal transplantation (tx) on endothelial dysfunction is still unclear. The aim of this study was to evaluate the endothelial functions of chronic renal failure patients before tx, while they were on hemodialysis, and after tx by noninvasive brachial arterial ultrasound. Twenty-two (5F, 17M) living related renal tx recipients were included in the study. Endothelial functions of each patient were assessed in 4-6 hours after the last hemodialysis session before tx and 6 and 12 months posttx. At the same time blood biochemistry was obtained for atherosclerotic risk factors. The endothelial functions of the brachial artery were evaluated by using high resolution vascular ultrasound. Endothelium dependent (ED) and independent (EID) vasodilations were assessed by establishing reactive hyperemia and by using sublingual nitroglycerine, respectively. Results are presented as percentage change from baseline values. The mean age of the patients was 33.9±11.6 years and the mean hemodialysis duration was 26.9±26.7 months. All of the patients were transplanted for the first time. A triple immunosuppression consisting of calcineurin inhibitor, mycophenolate mofetil and prednisone was prescribed. The laboratory and brachial artery measurements are presented in Table 1. There was a significant improvement in both ED and EID vasodilation after tx especially at the 12th month. Renal tx provides a better quality of life for chronic renal failure and hemodialysis patients in many aspects. It also provides improvement in endothelial dysfunction by eliminating the uremic environment though not in the early posttx period.

Table 1

	Pretx	Posttx 6 months	Posttx 12 months
Triglyceride, mg/dl	167±33	171±36	168±36
Cholesterol, mg/dl	187±39	183±40	188±36
Hct, %	31.9±4.6*	39.2±5.6	41.6±6.7
Creatinin, mg/dl	9.4±2.4*	1.4±0.4	1.5±0.3
Basal diameter, mm	3.8±0.6	3.8±0.4	3.7±0.3
ED, %increase	5.8±3.8*	8.4±2.4*	12.6±2.4
EID, %increase	9.6±4.4*	16.5±4.5*	18.9±4.1

* $p<0.05$, ED: endothelium dependent vasodilation, EID: endothelium independent vasodilation

Abstract# 1329 **Poster Board #-Session: P85-III**

CHANGES IN MICB EXPRESSION ARE ASSOCIATED WITH CELLULAR STRESS FOLLOWING RENAL TRANSPLANTATION. Isabel Quiroga,[1] Mariolina Salio,[2] Dicken D. H. Koo,[1] Dawn Shepherd,[2] Lucy Cerundolo,[1] Vincenzo Cerundolo,[2] Susan V. Fuggle.[1] *[1]Nuffield Department of Surgery, University of Oxford, Oxford, Oxfordshire, United Kingdom; [2]Tumour Immunology Unit, Niffield Department of Medicine, University of Oxford, Oxford, Oxfordshire, United Kingdom.*

MICA and MICB (MHC class I-related chain A and B) are highly polymorphic genes that encode molecules closely related to MHC class I and are induced on epithelial cells in response to stress. Their precise function is not fully understood, but they are recognised by γδ, CD8+ αβ T cells and NK cells via NKG2D and may play a role in autoimmunity, tumour and virus recognition. Importantly, incompatible donor MIC genes have recently been shown to initiate the formation of antibodies in recipients of organ transplants. The aim of this study was to determine whether there are changes in expression of MICB following renal transplantation and whether changes are associated with graft rejection and function. Paired renal biopsies obtained from living donor (n=10) and cadaveric renal allografts (n=50) before and 7 days post-transplant were stained with a rabbit polyclonal antibody specific for MICB. The level of staining was compared to that of HLA class II, molecules known to be up-regulated on renal tubules during inflammatory responses. Variable levels of tubular expression of MICB were evident in pre-transplant biopsies [high 6/60 (10%), low/negative 13/60 (22%), intermediate 35/60 (58%)]. High levels of expression of MICB were significantly associated with induction of MHC class II on renal tubules in pre-transplant donor biopsies. Following transplantation, MICB was up-regulated on the renal tubules of 17/60 (28%) biopsies. This was significantly associated with the induction of MHC class II antigens (p= 0.04). Acute rejection (AR, diagnosed using the Banff '97 classification) and delayed graft function (DGF, requirement for dialysis in the first week after transplantation) can both be considered objective markers of cellular stress within the transplanted kidney. At day 7, 37/60 transplants were found to have AR and/or DGF. There was a strong association between upregulation of MICB and cellular stress, 15/17 biopsies with up-regulated MICB expression had AR and/or DGF (p=0.003). In summary, this is the first study that demonstrates that MICB expression is induced following renal transplantation. MICB expression is associated with HLA class II induction and with cellular stress in the transplanted kidney.

Abstract# 1330 **Poster Board #-Session: P86-III**

DO THE PRESENCE OF MULTIPLE RENAL ARTERIES (MRA) AND NEED FOR RENAL ARTERIAL RECONSTRUCTION (RAR) WORSEN OUTCOMES AFTER RENAL TRANSPLANTATION? D. P. Foley,[1] J. S. Odorico,[1] L. A. Fernandez,[1] B. D. Shames,[1] L. T. Chin,[1] Y. T. Becker,[1] B. N. Becker,[1] J. D. Pirsch,[1] S. J. Knechtle,[1] A. M. D'Alessandro,[1] M. Kalayoglu,[1] H. W. Sollinger.[1] *[1]Division of Transplantation, University of Wisconsin Medical School, Madison, WI.*

Decision algorithms in living donor (LDRTx) and cadaveric renal transplantation (CRTx) are influenced by the presence of MRA. In this analysis we examined whether the presence of MRA, or the need for RAR influenced outcomes after renal transplantation. Methods: We completed a retrospective review of 2150 renal transplants performed at our institution between Jan, 1994 and Dec, 2001. Data were collected from the transplant database and through review of operative reports. Renal transplants were divided into 5 groups: 1 RA(n= 1750)(control), 2 RA (n=361) and > 2 RA (n=39) for the first analysis and RAR(n= 274) vs no RAR(1 RA + 2 RA without RAR: n= 1876)(control) for the second analysis. Outcomes included patient (pt) and graft survival, transplant renal artery stenosis (TRAS), renal artery thrombosis (RAT), and delayed graft function (DGF-dialysis required before POD #7). Associations were analyzed using the Cox proportional hazards model. Analyses were stratified for transplant type (CRTx vs LRDTx vs SPK) and transplant number (primary vs nonprimary). Results: A significantly higher risk of death was seen in primary transplant recipients with 2 RA (RR=1.51, p=0.03) and > 2 RA (RR=2.29, p=0.03) and those requiring RAR (RR= 2.01, p=0.001). Multivariate analysis demonstrated RAR to have a stronger influence on death (RR= 2.04, p=0.006) than MRA. In contrast, neither 2 RA nor > 2 RA increased the risk of graft failure. However, RAR did moderately increase graft failure risk (RR=1.34, p=0.03). Risk of TRAS(n = 75) was significantly greater in patients with 2 RA (RR= 2.66, p=0.0001) and those with > 2 RA (RR= 3.29, p =0.02) compared to 1 RA controls. RAR also significantly increased the risk of TRAS compared to controls (RR=2.84, p=0.0001). Risk of RAT (n=21) was increased in the > 2 RA group (RR=7.0, p= 0.003) but not in the 2 RA group (RR=0.86, p=0.80). RAR had no significant impact on the development of RAT. Risk of DGF (n= 302) was not significantly increased by the presence of 2 RA or >2 RA, but was modestly increased by the need for RAR (RR=1.42, p=0.02). Conclusions: The presence of MRA significantly increased the risk of death, incidence of TRAS and RAT after renal transplantation. The need for RAR also negatively impacted pt and graft survival and development of TRAS and DGF. Recipient outcomes need to be considered when transplanting kidneys with MRA.

Abstract# 1331 **Poster Board #-Session: P87-III**

OBJECTIVE, REAL-TIME, INTRAOPERATIVE ASSESSMENT OF RENAL PARENCHYMAL PERFUSION USING INFRARED IMAGING. Alexander Gorbach,[1] Donna Simonton,[1] S. John Swanson,[2] Allan D. Kirk.[2] *[1]Diagnostic Radiology, The Clinical Center, National Institutes of Health, Bethesda, MD; [2]Transplant Section, NIDDK, National Institutes of Health, Bethesda, MD.*

It has long been appreciated that prolonged ischemia impairs allograft function, and that delayed graft function correlates with increasing rates of acute and chronic rejection. Although these associations are well recognized, methods for objectively quantifying ischemic renal injury have been less forthcoming. We have hypothesized that the predominant mediators of ischemia-associated injury are those factors that impair parenchymal perfusion. To date there has been no clinically applicable way to directly and objectively assess renal parenchymal (as opposed to large vessel) perfusion. There is also no objective method for relating therapeutic interventions aimed at improving perfusion with their intended effect. Recent developments in infrared (IR) technology have allowed for measurements of temperature with accuracy to the hundredth of a °C. This method has the advantage of imaging entire fields and allowing for real-time assessment of specific areas or aggregate assessment of large areas. We have investigated whether high-resolution intraoperative infrared imaging would be an effective method to quantify parenchymal perfusion and predict subsequent graft function following renal transplantation. Recipients of live donor (n=8) and cadaveric donor (n=5) grafts were evaluated from the time of graft reperfusion using an Infrared Focal Plane Array camera used to image local temperature gradients simultaneously across the entire transplanted kidney by passively detecting IR emission. The camera has a sensitivity of 0.02°C. One hundred images were obtained at 1 sec intervals and digitized. Sequential digital images were taken with the plane of the IR camera's lens positioned parallel to the plane of the graft. Image acquisition continued for 3-8 minutes following reperfusion. As anticipated, the rate of return of renal function varied considerably between living donor and cadaveric cases. Cold ischemic time ranged from 35 minutes to 29 hours and was bimodally distributed. Renal rewarming time (RT) as determined by IR imaging was closely correlated with cold ischemic time ($P<0.001$, $R^2 = 0.81$). It also predicted subsequent return of renal function with RT negatively correlated with the regression slope of creatinine ($p = 0.02$, $R^2 = 0.38$) and the regression slope of BUN ($p = 0.07$, $R^2 = 0.26$). These data demonstrate that IR imaging provides non-invasive whole kidney measurement of reperfusion. This technology may be useful to objectively assess methods to limit reperfusion injury post transplant.

Abstract# 1332 **Poster Board #-Session: P88-III**

MAGNETIC RESONANCE ANGIOGRAPHY FOR PREOPERATIVE EVALUATION OF LIVE KIDNEY DONORS. Amer Rajab,[1] Ronald P. Pelletier,[1] Mitchell L. Henry,[1] Elmahdi A. Elkhammas,[1] Ginny L. Bumgardner,[1] Ronald M. Ferguson.[1] *[1]Surgery/ Transplantation, The Ohio State University Medical Center, Columbus, OH.*

Background: Careful evaluation of the renovascular anatomy in potential living-related kidney donors is essential regarding the presence of accessory renal arteries. Conventional arteriography has been the standard for delineating the renal arterial supply. However, it is an invasive procedure requiring nephrotoxic contrast and radiation. Magnetic resonance angiography (MRA) has emerged as an attractive non-invasive alternative. In addition, MRA has the advantages of evaluating the anatomy of the kidney parenchyma. The present study evaluated the accuracy and usefulness of MRA in potential living kidney donors. A retrospective evaluation of the result of MRA in the last 189 living kidney donors and the finding during donor operation was performed.Results: MRA was correct in identifying the renovascular anatomy in 173 donors (91.5%). In 16 patients (8.5%), MRA was inaccurate. In ten patients, MRA reported less numbers of arteries than what was found during operation. In seven patients, MRA identified renal pathology that influenced our decision regardless of the number of arteries: 1) one kidney was smaller than the other, 2) the kidney had a small stone, 3) the kidney had 1.5 cm renal cyst, and 4) renal arteries had fibromascular disease or stenosis. The misreading of the MRA did not affect the outcome in the recipient. Renal artery stenosis was identified in three patients on MRA and was confirmed during surgery. Three venous anomalies were identified; two retro-aortic left renal veins and one left renal vein draining into the left common iliac vein. The charge for the MRA at our institute is $1,487 comparing to $4,086 for the angiogram. We conclude that MRA is an adequate test for donor evaluation.

Abstract# 1333 Poster Board #-Session: P89-III

PROSPECTIVE COMPARISON OF COMPUTERIZED TOMOGRAPHY ANGIOGRAPHY AND MAGNETIC RESONANCE ANGIOGRAPHY WITH SELECTIVE RENAL ANGIOGRAPHY IN LIVING KIDNEY DONOR ASSESSMENT.

Kenneth Beasley,[1] Christopher Neville,[1] David Bach,[2] Richard Rankin,[2] David Peck,[2] Elaine O'Riordan,[2] Dean Smith,[2] Mary Anne Henry,[1] Jennifer Cross,[1] Andrew House,[3] Vivian McAlister,[1] Anthony Jevnikar,[3] Allison Spouge,[2] Lisa Thain,[2] Patrick Luke.[1] *[1]Surgery, London Health Sciences Centre, London, ON, Canada; [2]Radiology, London Health Sciences Centre, London, ON, Canada; [3]Medicine, London Health Sciences Centre, London, ON, Canada.*

For years, the gold standard in the evaluation of living donor renal vascular anatomy had been selective renal angiography (SRA). Because of the potential morbidity associated with SRA, both Computerized Tomography Angiography (CTA) and Magnetic Resonance Angiography (MRA) were prospectively evaluated in the assessment of renal donors. Fifty one potential living donors were evaluated between July 2000 and October 2002. Two patients were excluded because of intra-abdominal abnormalities diagnosed on CTA (lymphoma, renal mass). All patients had SRA and 44 underwent donor nephrectomy (DN). 54 renal units were prospectively evaluated by MRA and 22 by CTA. Radiologists interpreting CTA and MRA were blinded to the results of the SRA. We used SRA supplemented by findings at DN as our gold standard and defined a positive test as an abnormality found on the evaluated imaging modality. SRA yielded a sensitivity of 83%, specificity of 97%, positive predictive value (PPV) of 83%, and negative predictive value (NPV) of 97% when compared with findings at DN. CTA was found to have 75% sensitivity, 100% specificity, 94% NPV and 100% PPV. CTA was correct in identifying 3 of 4 accessory arteries. MRA was found to have 54% sensitivity, 87% specificity, 88% NPV and 50% PPV. MRA correctly identified only 7 of 11 accessory arteries and incorrectly identify 6 accessory arteries not present on SRA or DN. Additionally, two patients diagnosed with fibromuscular dysplasia by SRA were not identified by MRA. In its current state of evolution, MRA is not capable of replacing SRA in living renal donor imaging. We are continuing to assess the validity and predictive value of CTA in the evaluation of renal donors.

Abstract# 1334 Poster Board #-Session: P90-III

UTILITY OF DUPLEX DOPPLER ULTRASOUND (DUS) FOR THE EVALUATION OF TRANSPLANT RENAL ARTERY STENOSIS (TRAS.

Mahesh Goel,[1] David A. Goldfarb,[1] L. Laperna,[2] S. Whitelaw,[2] S. M. Flechner,[1] C. S. Modlin,[1] I. S. Gill,[1] A. C. Novick.[1] *[1]Urological Institute, Cleveland Clinic Foundation, Cleveland, OH; [2]Department of Vascular Medicine, Cleveland Clinic Foundation, Cleveland, OH.*

Objective: DUS is a useful screening tool for renal artery stenosis in native kidneys but its role for TRAS is less clear. This retrospective study was done to determine the role of DUS in the management of TRAS. Material and Methods: Patients with clinical suspicion of TRAS who underwent DUS between 1/ 1998 - 1/ 2001 were included in the study. Clinical suspicion of TRAS was defined by uncontrolled hypertension, abnormal renal function, or the findings of a transplant renal artery bruit. Patients with acute rejection, lymphocele, hematoma or hydronephrosis were excluded. Patients were divided into two groups on the basis of DUS. Group A (peak systolic velocity <200 cm/sec) with low probability of TRAS (0-59% stenosis); and group B (peak systolic velocity >200 cm/sec) with high probability of TRAS (60-99% stenosis). Results: A total of 51 patients met the entry criteria; Group A (26), Group B (25). Nine patients in Group A underwent further radiological investigation based upon a high clinical suspicion. Eight patients underwent MRA, 6 showed stenosis. Angiography was performed in 5 patients and all showed stenosis. One was TRAS, but 4 were stenosis to the inflow artery adjacent to the transplant artery (ARAS). Three angioplasties were performed; 2 for ARAS, 1 for ARAS and TRAS. Intervention in this group did not improve renal function or blood pressure. In Group B, 19 patients underwent further radiographic evaluation. Six did not due to low clinical suspicion. Twelve underwent MRA and 10 were positive for stenosis. Of the 10 positive MRA, 5 had angiography. The other 5 had insignificant stenosis or stenosis at another site on MRA. There were a total of 13 angiographies performed in group B, 10 showed TRAS, 3 showed ARAS. Ten of these patients underwent angioplasty, 8 for TRAS, and 2 for ARAS. Of the 3 patients who did not undergo any further treatment 2 had less than 30% stenosis, while 1 had a low-pressure gradient across the stenosis. 7/10 Group B patients showed improvement either in BP or renal function after angioplasty. Conclusion: Overall suspicion for TRAS based upon clinical findings remains critically important for diagnosis. A high probability DUS is more likely to identify a stenosis that will result in blood pressure/renal function improvement with treatment. A low probability study does not eliminate the possibility of a stenosis.

Abstract# 1335 Poster Board #-Session: P91-III

IMPACT OF 3-D IMAGING WITH MULTISLICE HELICAL CT FOR EVALUATION OF LIVE RENAL DONORS UNDERGOING LAPAROSCOPIC NEPHRECTOMY.

Dushyant V. Sahani,[2] Jonathan Mercer,[3] Chieh-Min Fan,[2] Matthew G. Nuhn,[1] Nahel Elias,[1] Jennifer McGowan,[2] Peter R. Mueller,[1] Dicken S. C. Ko.[1] *[1]Department of Surgery, Transplantation Unit, Massachusetts General Hospital, Boston, MA; [2]Department of Radiology, Massachusetts General Hospital, Boston, MA; [3]Department of Urology, Massachusetts General Hospital, Boston, MA.*

Purpose: To evaluate the sensitivity and specificity of 3-D multislice helical computed tomography (MSCT) in imaging of vascular, collecting system, and renal parenchyma in patients undergoing live kidney donation. **Materials and Methods:** Preoperative knowledge of vascular anatomy and parenchymal disease is critical in patients undergoing live donor nephrectomy. MSCT represents a substantial improvement in technology that lends itself to the comprehensive evaluation of renal donors. 80 consecutive potential renal donors were referred for 3D CT angiography for vascular mapping underwent multiphasic CT (Light speed QX\i, GE). An unenhanced helical scan was performed followed by a triphasic scanning after injection of 150 ml contrast at a rate of 5-6 ml/sec. Acquisition time for each phase was approximately 22 sec with arterial phase images at 25 sec (1.25-mm), venous phase images at 70 sec (2.5-mm) and excretory phase images at 10 minutes (2.5-mm). 3-D maps of the renal arteries, vein and the ureters generated were reviewed by two readers and the findings were confirmed at surgery. **Results:** There was overall agreement between MSCT arteriography and intraoperative findings in 74 cases (92.5%). Double renal arteries were identified in 6 cases (7.5%). Accessory arteries measuring less ≤ 1.5 mm and early branching single vessels simulating dual arteries were misdiagnosed on initial interpretation. Addition venous tributaries occurred in 8 patients (10%) whereas only one patient was found to have no additional tributary seen intraoperatively. Sensitivity and specificity of CT arteriography for detecting accurate venous anatomy were 90.0 and 98.6%, respectively. Misdiagnoses included early venous bifurcations and supernumerary tributary veins, which were poorly opacified. A single collecting system in all kidneys was identified correctly with CT urography. Overall, the sensitivity and positive predictive value of CT imaging in correctly determining the combined vascular, ureteral, and parenchymal anatomy in the harvested kidney were 90, 100 and 100%, respectively. **Conclusions:** 3-D multislice helical CT is highly accurate and specific for comprehensive evaluation vascular anatomy, collecting system, and renal parenchyma preoperatively in patients who are candidates for live donor nephrectomy. Poor opacification resulted in a lower sensitivity for venous anatomy.

Abstract# 1336 Poster Board #-Session: P92-III

A RETROSPECTIVE STUDY OF THE PREDICTIVE POWER OF SPIRAL CT ANGIOGRAPHY IN DELINEATING RENAL VASCULAR ANATOMY FOR LIVE DONOR NEPHRECTOMY.

Nicholas R. Brook,[1] Gareth R. Lewis,[1] Kevin Mulcahy,[2] Peter S. Veitch,[1] Michael L. Nicholson.[1] *[1]Department of Surgery, University of Leicester, Leicester, United Kingdom; [2]Department of Radiology, Leicester General Hospital, United Kingdom.*

Introduction Laparoscopic donor nephrectomy (LDN) presents unique surgical challenges, particularly operating in the presence of complex venous or arterial anatomy. This outlines the importance of pre-operative imaging prior to LDN. This study aims to determine the accuracy of spiral CT imaging of donor venous anatomy by comparing CT and operative findings, for both laparoscopic and open donor nephrectomy. P*atients and methods* Forty live donors underwent spiral CT angiogram prior to donation. Scans were reported by the same radiologist. The number and diameter of 'predicted' renal arteries, veins and renal vein tributaries were documented. After back table perfusion, the same details were recorded, and were taken as the 'actual' findings. Tributaries of less than 1 mm diameter were not recorded. Right donor nephrectomy was performed in 7 patients. *Results* 47 actual renal arteries were identified at nephrectomy; of these 46 were predicted by CT. Likewise, 42 actual renal veins were found at nephrectomy, 40 of which were predicted. See Table. Spearman's co-efficient correlations of predicted tributary diameter were 0.16, 0.53, and 0.48 for gonadal, adrenal and lumbar veins respectively. There were no cases of damaged renal vascular anatomy in this series. *Conclusions* Assessment of potential renal donors pre-operatively with spiral CT provides accurate prediction of the presence or absence of the gonadal and adrenal vein, but is less accurate for prediction lumbar vein detection. Prediction of renal vein tributary diameter compared unfavourably with measured diameter at nephrectomy.

Predictive power of spiral CT in donor nephrectomy

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value(%)	Accuracy (%)	Kappa value
No renal vein tributaries	82	46	91	27	77	0.44
Gonadal veins	89	54	84	64	79	0.56
Adrenal Veins	79	89	96	53	81	0.55
Lumbar veins	55	84	80	62	68	0.36

Abstract# 1337 **Poster Board #-Session: P93-III**

SPIRAL CT EVALUATION OF LAPAROSCOPIC KIDNEY DONORS. Truman M. Sasaki,[1] Frederick Finelli,[1] Sayed R. Ghasemian,[1] Diana Y. Barhyte.[2] *[1]Transplantation Services, Washington Hospital Center, Washington, DC; [2]Transplant Research Center, MedStar Research Institute, Washington, DC.*

Background: An arteriogram, the criterion standard for evaluation of kidney donors, is invasive, costly, and time consuming. **Methods:** This study reviews the results of spiral CT evaluation in 251 donor patients dating from September 1997, the inception of the laparoscopic nephrectomy program, to May 2002. Those patients evaluated with angiogram (n=6), MRI (n=2), or outside studies (n=20) and open nephrectomy cases (n=20) were excluded from the analysis. There were 120 males and 131 females with an average age of 35.4 years (range = 18 - 71). There were 131 African Americans. **Results:** Bilateral single renal artery and vein were identified in 186 patients; multiple vessels in one or both kidneys in 65 patients. When spiral CT identified a single artery in a donor kidney (n = 209), it was correct in 192 (92%) and incorrect in 17 (8%) of the cases. When two arteries were visualized in a donor kidney (n = 42), the finding was correct in 37 (88%) and incorrect in 5(12%) of the cases. No false positive readings were found. Among the recipients of the donor kidneys, the mean creatinine on post-operative day 7 for one, two, and three artery kidneys was 1.67 (n=199, range=0.7 - 8.4), 1.75 (n = 47, range= 0.6 - 7.6) and 2.0 (n=5, range = 1.0 - 4.8) respectively. When the mean creatinines are compared between those kidneys with two arteries (X=1.6, n=30) identified pre-operatively to those with an additional artery found during surgery (X=2.0, n=17) there is no significant difference. Those with three arteries could not be compared because all five were identified pre-operatively as having two arteries. The incidental findings included renal pelvis, ureteral anomalies (n=120, adrenal mass (n=2), gall stones (n=3), and benign cysts and masses of the uterer and kidneys (n=30). **Conclusions:** We conclude that spiral CT is the least intrusive evaluation but errors in identifying the number of renal arteries pre=operatively affects the conduct of the surgery and may affect renal function. The surgeon should conduct the operation of all donor nephrectomies as if additional renal arteries are present.

Abstract# 1338 **Poster Board #-Session: P94-III**

HELICAL CT FOR PATIENTS VASCULAR EVALUATION PRIOR TO RENAL TRANSPLANTATION. Amado Andrés,[1] Guillermo Cruceyra,[2] Francisco Gragera,[3] Ana Ramos,[3] Yolanda Revilla,[3] Esther Gonzalez,[1] Josè M. Morales,[1] Manuel Praga,[1] Rafael Diaz,[2] Oscar Leiva.[2] *[1]Nephology, Hospital 12 de Octubre, Madrid, Spain; [2]Urology, Hospital 12 de Octubre, Madrid, Spain; [3]Radiology, Hospital 12 de Octubre, Madrid, Spain.*

Objectives: Calcifications on the aorta and iliac arterial system are very common among patients with chronic renal failure, specially in elderly patients and those with arteriosclerosis risk factors.Contrasting with conventional or magnetic resonance angiography, helical CT provides an accurate representation of the vascular calcifications, and permits us analyse the free-calcium areas where the vascular anastomosis is possible. We present our experience using helical CT in the evaluation of candidates to renal transplantation.**Methods:** Between December 1999 and March 2002 we performed 114 vascular studies with helical CT and MIP reformatted images, prior to kidney transplantation. The criteria for these studies were: patients over 60 years, presence of arteriosclerosis risk factors, vascular calcifications in abdominal x-ray study and more than one renal transplantation.**Results:** 33 patients (29%) were excluded for kidney transplantation since generalised vascular calcifications were revealed by the helical CT. 81 candidates (71%) were included. We have essayed renal transplantation in 28 patients, and in 25 (89%) the vascular anastomosis was possible exactly in the area where the helical CT showed free-calcium artery. Only in 3 candidates, with multiple vascular calcifications in the helical CT, the intraoperative findings did not allow to perform the transplantation. **Conclusions:** The extent and thickness of aorta and iliac arterial system calcifications are accurately depicted by helical CT. Its use in the evaluation prior to renal transplantation allows us to: 1.- Reject patients with universal vascular calcifications. 2.- Predict the right area where the vascular anastomosis can be performed.

Abstract# 1339 **Poster Board #-Session: P95-III**

TRIPLE THERAPY TO ATTENUATE ISCHEMIA REPERFUSION. Angello Lin,[1] C. Sekhon, Prabhakar Baliga,[1] Kenneth D. Chavin,[1] P. R. Rajagopalan,[1] Jeffrey Rogers,[1] A. K. Singh, I. Singh. *[1]Surgery, Medical University of South Carolina, Charleston, SC.*

BACKGROUND: Cellular damages from ischemia reperfusion (I/R) and the subsequent inflammatory reactions may contribute to delayed graft function (DGF) in renal transplantation. Previously studies have demonstrated the beneficial effects of administering N-acetyl cysteine (NAC or N, a potent oxygen free radical scavenger), sodium nitroprusside (SNP or S, vosodilator as a nitric oxide donor), and Phosphoramidon (P, an endothelin antagonist) in a 90 minute warm ischemia reperfusion kidney model. We undertook this study to evaluate their effectiveness in a prolonged cold storage model in kidney transplantation. METHODS: Male Mongrel dogs (15-20kg) were divided into treated and untreated groups. The left kidney was removed, flushed with University of Wisconsin (UW) solution, and stored at 4°C for 48 hours. Autotransplants were performed by a single surgeon and right nephrectomy was completed at the same time. Blood samples were drawn at different time intervals post operatively. Dogs were euthanized based on clinical and laboratory criteria. The treatment group received intravenously NAC (100mg/kg), SNP (2.1mg/kg), and P (1mg/kg) prior to the left nephrectomy and prior to reperfusion. All dogs were followed for their serum creatinine (SCr) and BUN as a marker of renal function. RESULTS: All 5 animals in the control group developed ATN of the transplanted kidney and were euthanized by POD#3. They were anuric with poor clinical conditions. 50% of the treated group (3/6) survived till 14 days when they were euthanized per protocol. The difference in SCr and BUN between the two groups were significant as well on POD#3 before the dogs were euthanized. CONCLUSION: The triple therapy used in this experiment were effective in attenuating the injuries caused by ischemia reperfusion in the current cold storage preservation method. Optimizing treatments and understanding its mechanism will make this therapy more clinically applicable.

Abstract# 1340 **Poster Board #-Session: P96-III**

IMPROVEMENT OF PRIMARY ORGAN FUNCTION BY RETROGRADE OXYGEN PERSUFFLATION OF WARM ISCHEMICALLY DAMAGED PORCINE KIDNEYS. Juergen W. Treckmann,[1] Andreas Paul,[1] Stefan Saad,[2] Julia Hoffmann,[3] Christoph E. Broelsch,[1] Manfred Nagelschmidt.[3] *[1]Clinic of General and Transplantation Surgery, University of Essen, Essen, Germany; [2]2nd Department of Surgery, University of Cologne, Cologne, Germany; [3]Biochemical and Experimental Division, 2nd Department of Surgery, University of Cologne, Cologne, Germany.*

The shortage of viable donor organs for transplantation has stimulated efforts to use also organs of non heart beating donors. For transplanted porcine livers we could show a restitution of organ function with retrograde oxygen persufflation(ROP) after warm ischemia over 60 min. In a porcine model of autologous transplantation retrograde oxygen persufflation of warm ischemically predamaged kidneys was studied to find out the maximum time of warm ischemia allowing kidney function to be restituted. These results were compared to machine perfusion. (MP) The kidneys of 37 pigs were exposed to warm ischemia (WI) for 60, 90 or 120 min. Then 16 kidneys were subjected to ROP for 4 hrs at 4°C, 5 kidneys treated by hypothermic machine perfusion (only 60 min WI), and 16 controls received only cold storage in UW-solution. After autologous transplantation survival for 7 days and plasma creatinine were evaluated as main criteria. Only in the group with 60 WI and ROP all animals survived (n=6). They showed primary renal function with maximum plasma creatinine on day 2 (6,8 mg/dl) and nearly normal values an day 7 (2,1 mg/dl). All of the other groups lost animals due to anuria; the survivors passed significantly higher creatinine levels (ANOVA, p<0,05) which did not normalize until day 7.

Group	Treatment	n	Survival Day 7 n	creatinine at end of trial mg/dl
1	60 min WI, ROP	6	6	2,1
2	60 min WI	7	4	2,5
3	90 min WI, ROP	7	5	10,9
4	90 min WI	6	5	7,81
5	120 min WI, ROP	3	1	16,8
6	120 min WI	3	1	14,8
7	60 min WI, MP	5	3	9,82

In this setting kidneys with 60 min of warm ischemia showed optimal organ function following treatment with ROP. However this effect could not be seen in kidneys with a warm ischemic insult of 90 or 120 min. ROP was superior to machine perfusion. 4 out of 6 resp. 1 out of 3 kidneys with 90/120 min of warm ischemia had a primary but reduced organ function even after simple cold storage.

Abstract# 1341 **Poster Board #-Session: P97-III**

INTRAVENOUS APPLICATION OF SELENIUM CAN REDUCE ISCHEMIA-REPERFUSION INJURY AFTER KIDNEY TRANSPLANTATION FROM NON -HEART BEATING DONOR.

Vladislav Treska,[1] Vilem Kuntscher,[1] Daniel Hasman,[1] Jiri Kobr,[2] Jaroslav Racek,[3] Ladislav Trefil,[3] Ondrej Hes.[4] *[1]Surgery, Charles University Hospital, Plzen, Czech Republic; [2]Childrenas Clinic, Charle University Hospital, Plzen, Czech Republic; [3]Institute of Clinic Biochemistry, Charles Univewrsity Hospital, Plzen, Czech Republic; [4]Siklas Pathological Institution, Charles University Hospital, Plzen, Czech Republic.*

Background: The NHBD kidneys sustain substantial warm ischemic damage, which impaires their function. Ischemia reperfusion injury (IRI) is probably one of the main causes of primary afunction or delayed graft function of kidneys procured from NHBD in comparison with kidneys procured from heart beating donors. **Aim of study:** To evaluate the intensity of IRI after kidney transplantation (KTx) from NHBD and the possibility of its influence by intravenous administration of free oxygen radicals scavenger. **Material and Method:** Landrace pigs (N=20) were used for NHBD animal model. The NHBD simulation was performed by clamping the kidney hilum for 30 minutes prior to kidney retrieval. The kidney was than flushed with HTK solution. The animals were divided into two groups. Group I (N=10) received 30 minutes before KTx selenium intravenously. Group II (N=10) served as a control group.IRI was evaluated by malondialdehyde (MDA) levels in the venous blood serving as factors of free oxygen radicals (FOR) production. The body´s defences against FOR was examined by reduced glutathion levels (GSH) in the venous blood and by antioxidative capacity of plasma (AOC). All markers were evaluated during 120 minutes interval after kidney reperfusion. The study conformed "Guiding Principles in the Care and Use of Animals". **Results:** At the beginning of reperfusion the MDA levels were lower and AOC and GSH levels higher in GI compared with GII. These differences remained for the full duration of the experiment (120 minutes). The average MDA levels in animals of GI were significantly lower when compared with MDA levels in GII. The average AOC of plasma and GSH in GI were significantly higher when compared with GII.- Tab.

MDA, GSH and AOC levels after kidney Tx

	MDA (umol/l)	GSH (mmol/l)	AOC (mmol/l)
GI	1.7+- 0.3	1.3 +- 0.1	1.0 +- 0.5
GII	1.9 +- 0.6	1.1+- 0.4	0.9 +- 0.2
ANOVA	p< 0.01	p< 0.0004	p< 0.02
Wilcoxon	p< 0.002	p< 0.0005	p< 0.08
Median test	p< 0.001	p< 0.0001	NS

Conclusion: Intravenous application of selenium just prior KTx from NHBD significantly reduces the IRI intensity and may have an important effect on the graft function after transplantation. The study was supported by the Grant Agency, Charles University, Praque, No 82/2000.

Abstract# 1342 **Poster Board #-Session: P98-III**

HUMAN TRANSPLANTS FROM CADAVER DONORS EXPRESS INCREASED CHEMOKINE GENES DURING ISCHEMIA/ REPERFUSION INJURY WHEN COMPARED TO LIVING DONOR ALLOGRAFTS.

Nader Fahmy,[1] Venkatesh Krishnamurthi,[1,3] David Goldfarb,[1,3] Charles Modlin,[1,3] Andrew Novick,[1,3] Robert Fairchild.[2,3] *[1]Urological Institute, Cleveland Clinic Foundation, Cleveland, OH; [2]Immunology, Cleveland Clinic Foundation, Cleveland, OH; [3]Transplant center, Cleveland Clinic Foundation, Cleveland, OH.*

OBJECTIVES: Ischemia/reperfusion injury is unavoidable with potential serious consequences on the outcome of organ transplantation. The presence of tissue inflammation initiates a chemokine cascade ending by T cell infiltration into the graft. The key factors directing cell infiltration into inflammatory sites remain unclear. Chemokines are cytokines having chemoattractant properties for leukocytes expressing specific receptors. Previous animal studies have shown rapid induction of chemokine gene expression shortly following reperfusion of ischemic organs. Administration of antibodies to these chemokines improved graft function. The goal of the current study was to investigate chemokine gene and receptor expression before and after reperfusion of both cadaver and living donor human kidney allografts. **Methods:** 22 patients undergoing kidney transplantation were included in this study. 16 patients received allografts from living donors while 6 were procured from cadaver donors. A renal biopsy was taken while the kidney was on slush ice for approximately 1.5 hours (ischemia sample). Another biopsy was taken after approximately 1 hour of reperfusion (reperfusion sample). Chemokine gene and receptor expression was tested using quantitative real-time PCR. We have tested the following chemokines gene expression in the biopsies: IP-10, Mig, I-TAC, RANTES, MCP-1, IL8 and IL-15 and the receptors CXCR3, CCR2 and CCR5. **RESULTS:** Examination of the living donor allografts have revealed a higher expression during ischemia than base levels of IP-10, MCP-1, IL-8 and IL-15. Further more, expression of these chemokines was significantly higher during reperfusion. (P<0.0001). Examination of the cadaver donor allografts has shown increased expression of all chemokines except Mig during ischemia when compared to base level expression. In addition, a further significant increase in the expression of I-TAC, MCP-1, IL-8 and IL-15 was observed during reperfusion. Overall expression of chemokines was increased in cadaver allografts when compared to living donors. **Conclusion:** The expression of chemokines and chemokine receptors is increased during reperfusion of cadaver and living donor renal transplants. This raises the possibility that tissue injury and acute rejection may be attenuated by strategies antagonizing chemokines that direct recruitment of leukocytes into ischemic organs following transplantation.

Abstract# 1343 **Poster Board #-Session: P99-III**

URETEROCYSTOPLASTY AND KIDNEY TRANSPLANT-THE BEST TECHNIQUE FOR BLADDER ENLARGEMENT IN PATIENTS WITH ESRD AND BLADDER DYSFUNCTION.

William C. Nahas, Eduardo Mazzucchi, Ioanis M. Antonopoulos, Afonso Piovesan, Luis E. Ianhez, Lilian M. Araujo, Elias Davi-Neto. *[1]Urology- Renal Transplant Unit, Hospital das Clinicas Universty of São Paulo, São Paulo, SP, Brazil.*

INTRODUCTION: Urinary bladder augmentation is well established for the treatment of dysfunctional bladder in renal transplants patients. All segments of the gastrointestinal tract have been used successfully in augmentation cystoplasty. When disposable dilated ureter provide an excellent source of augmentation material with urethelium and muscular backing, free of the eletrolyte and acid base disturbances and mucus production. We present our experience on ureterocistoplasry and kidney transplantation. METHODS: Augmentation cystoplasty using detubularized, reconfigured, disposable megaureter, with or without concomitant nephrectomy was performed in 8 pts (7 male, 1 female) and subsequently to a kidney transplantation. The etiology of bladder dysfunction was posterior urethral valve 3 pts, neurogenic bladder 3 pts, vesicoureteral reflux 3pts. All had poor bladder compliance and vesicoureteral reflux. Two pts with ureterostomy were enlarged with the distal ureter without removing the kidney, and maintained without dialysis,the kidney was removed after a successful transplantation. All other pts were on dialysis and the kidney was removed at the moment of the enlargement with the ureter, one of then by a laparoscopic procedure. The pts were submitted to another urodynamic study two ms later, three of them did not gained capacity nor improved the compliance, and all of them had been previously submitted to a ureteric reimplantation and were treated by a second enlargement with a bowel segment. They received a kidney transplantation from living related donor after a medium of 4.2 months (2 to 7 months) by an extraperitoneal access. RESULTS: The medium follow up was 45.2 months (1 to 83 months), and the medium creatinine level was 1.3 mg/dL (1 to 1,9 mg/dL), none of the grafts were lost. The pts were totally continent and pts with neurogenic bladder drained their bladder by clean intermittent catheterisation (CIC). Asymptomatic bacteriuria was the most common complication and happened more frequently on the pts on CIC. One pt was submitted to an antireflux procedure two years after kidney transplantation with good evolution. CONCLUSIONS: Ureterocystoplasty combines the benefits common to all enterocistoplasties without adding any of their complications or risks.

Abstract# 1344 **Poster Board #-Session: P100-III**

HOW TO DEAL WITH CHILDREN WITH END STAGE RENAL DISEASE AND SEVERE BLADDER DYSFUNCTION.

William C. Nahas, Eduardo Mazzucchi, Marco Arap, Ioannis Antonopoulos, Affonso Piovesan, Lilian Araujo, Elias David-Neto, Luis Ianhez, Sami Arap. *[1]Urology, University of São Paulo, São Paulo, SP, Brazil.*

INTRODUCTION and OBJECTIVES: Aiming the transplant success, the lower urinary tract has to provide a reservoir capable of carrying an adequate volume at low pressure and to allow an efficient method of drainage. The experience with kidney transplantation in the pediatric population with severe lower urinary tract dysfunction is presented. METHODS: In the last 10 years, 185 kidney transplants were performed at the University of São Paulo in patients under 18 years old, 17 patients with lower urinary tract abnormalities were submitted to 19 kidney transplantations. Mean age at transplantation was 13.3 years (3 to 18 years). The etiology of bladder dysfunction was neurogenic bladder (5 pts), posterior urethral valve (5 pts), vesico-ureteral reflux (4 pts), extrophy (1 pt), severe bladder malformation (1 pt) and prune-belly syndrome (1 pt). Al patients were submitted to cystourethrography and urodynamic evaluation. In one patient the graft was implanted into a urinary diversion. In a second one, a Mitrofanoff procedure was created for easier clean intermittent catheterization (CIC) and bladder drainage. One patient was submitted to an auto-augmentation and a dilated non refluxing ureter was located into the iliac fossa for easier CIC. All other patients had their bladder enlarged. Five patients had their bladder enlarged with the ureter. An intestine segment was used in 12 cases. The graft was located extraperitoneally and whenever possible the ureter was implanted into the bladder. RESULTS: Mean follow-up was 55.2 months (3 to 192 months). Bladder capacity and compliance was markedly improved, except in two patients submitted to ureterocystoplasty who needed a second enlargement with an intestinal segment. Ten patients empty their bladder using CIC. Mean creatinine level was 1.66mg/dL (0.8 to 3.1 mg/L). Urinary tract infection was the most frequent complication. Three grafts were lost (two patients received a second graft). The actuarial graft survival was 100, 100, 90% at 12, 36 and 60 months, respectively. CONCLUSIONS: The ureter, when available, is the most attractive source for bladder augmentation. Intestinal segments can properly be used. When the urinary tract cannot be reconstructed, kidney transplantation into a urinary diversion is the sole solution. Enterocystoplasty is a safe and effective method for the treatment of severe lower urinary tract abnormalities even in children candidates to kidney transplantation.

Abstract# 1345 **Poster Board #-Session: P101-III**

THE TIME-DEPENDANT RISK FACTORS INFLUENTING THE LONG-TERM GRAFT SURVIVAL IN LIVING RENAL ALLOGRAFTS: A JAPANESE SINGLE CENTER EXPERIENCE.

Tadahiko Tokumoto,[1] Kazunari Tanabe,[1] Hideki Ishida,[1] Hiroaki Shimmura,[1] Fusako Toda,[1] Takashi Yagisawa,[1] Takashi Akiba,[2] Hiroshi Toma.[1] *[1]Department of Urology, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [2]Division of Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

OBJECTIVES: Most investigations have revealed that the improvement in early graft survival has not resulted in a corresponding improvement in long-term graft survival. The risk factors for long-term graft survival should be clarified. MATERIALS: Between 1983 and 2001, 1594 renal transplantation were performed under cyclosporine-based or tacrolimus-based immunosuppression at our institution. 1375 renal transplant recipients who received kidneys from living donors were enrolled to clarify the time dependency of risk factors for long-term graft survival in this study. The mean age was 32.4 years (range, 1-69 years), with 899 males and 476 females. 979 recipients were ABO-compatible, 255 recipients were minor-mismatch and 141 recipients were ABO-incompatible, respectively. The mean donor age was 52.7 years (range, 19-79 years). The mean allograft weight was 169g. We examined various possible risk factors, including HLA-AB and –DR mismatches, ABO-blood group incompatibility, graft weight, donor age and sex, recipient age and sex, and the presence or absence of acute rejection (AR) by using the time-dependent, nonproportional Cox's hazards model. RESULTS: Acute rejection episode, donor age, HLA-AB 4-antigen mismatches, ABO-incompatible transplantation, smaller kidney weight the patient's body weight (Kw/Bw ratio less than 2.67), and transplantation from an unrelated living donor were risk factors for long-term graft survivals. Multivariate analysis for time-dependent risk factors showed that donor age of more than 60 years was the most important risk factor for long-term graft failure above 5 years after renal transplantation (hazard ratio: 2.04). In contrast, AR, ABO-blood group incompatibility, and nonrelated donors were significant risk factors for short-term graft failure within 5 years after renal transplantation (hazard ratio: 2.93, 1.51, and 1.69, respectively). CONCLUSIONS: AR, ABO incompatibility, and nonrelated donors were significant risk factors for short-term graft failure. Furthermore, donor age of more than 60 years was a most important crucial risk factor affecting long-term graft survival.

Abstract# 1346 **Poster Board #-Session: P102-III**

TREATMENT OF RENAL ALLOGRAFT ARTERY STENOSIS BY PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY: A 33 CASE REPORT STUDY.

Marie-Béatrice Nogier,[1] Nassim Kamar,[1] Pauline Bernadet-Monrozies,[1] Philippe Otal,[2] Lionel Rostaing,[1] Francis Joffre,[2] Dominique Durand.[1] *[1]Department of Nephrology, Dialysis and Transplantation, CHU Toulouse-Rangueil, Toulouse, France; [2]Department of Radiology, CHU Toulouse-Rangueil, Toulouse, France.*

Among 838 cadaveric renal transplantation performed over the last 12 years, 33 severe renal allogaft artery stenosis (> 50% of the lumen) were observed in 30 patients who received calcineurin inhibitors based immunosuppression. They were treated by percutaneous transluminal angioplasty (PTA) associated to a stent implantation in 8 cases. In 87% of the cases the stenosis was found during the first year following the transplantation. 14 were anastomotic, 14 were truncal and 5 were distal. 90% of the patients had hypertension and therefore received at least a double anti-hypertensive therapy. 5 early complications were observed : 2 non-occlusive dissections treated in situ, 1 stent migration requiring surgery and 2 partial allograft infarction. 3 ostial and 1 truncal severe restenosis above 60 % (12%) were observed 100 ± 12 days after PTA. Three of them were successfully treated by a second PTA. Overall, a year following PTA, there were a decrease in mean arterial blood pressure (101.9 ± 13.2 vs 116.2 ± 16.2 mmHg ; p = 0.04), a decrease in serum creatinine level (130 (79—327) vs 153 (70—300) µmol/L ; p = 0.02) and an increase in serum creatinine clairance estimated by the cockroft formula (55 (21—93) vs 47 (24—82) mL/min ; p = 0.007). 4 patients underwent hemodialysis within the second year following PTA but there were no relationship between those two events. In conclusion, PTA and stenting are safe and effective procedures to treat renal allograft artery stenosis, since there is a low rate of immediate complications (15%) and restenosis (12%).

Abstract# 1347 **Poster Board #-Session: P103-III**

THE SIGNIFICANCE OF PATHOLOGIC ASSESSMENT OF CHRONIC RENAL FAILURE IN RENAL ALLOGRAFT RECIPIENTS.

Yong Bok Koh,[1] Yeong-Jin Choi,[2] In Sung Moon,[1] Chul Woo Yang,[3] Yong-Soo Kim,[3] Byung Kee Bang,[3] Byung Kee Kim,[2] Chang Suk Kang.[2] *[1]Surgery, The Catholic University of Korea, Seoul, Korea; [2]Clinical Pathology, The Catholic University of Korea, Seoul, Korea; [3]Medicine, The Catholic University of Korea, Seoul, Korea.*

Recurrence and progression of original disease are now emerging as critical problems after kidney transplantation (KT). Many renal diseases leading to chronic renal failure (CRF) are well known to recur and affect the prognosis of the allograft. The exact etiology of renal diseases is obscure in most recipients and usually presumed by clinical data. To investigate the pathologic etiology of CRF and to evaluate the clinical significance of the pathologic assessment, 73 recipients without pathologic diagnosis for CRF were included. We used native kidneys obtained from the recipients by unilateral nephrectomy at the time of KT. Gross and light microscopic examination with special stains and mmunofluorescent stains were performed. The mean age was 40 (12-63) years, and male to female ratio was 1.6:1. The mean duration of pre-KT dialysis was 467 (5-3480) days. The presumptive diagnosis for CRF was made in 55/73 cases (75%), and unknown in remaining 18/73 (25%). The pathologic diagnosis was specified in 67 cases (92%), and unspecified in the remaining 6 (8%) due to severe renal changes. Fifty-four cases (74%) were GN, 27 IgAN, 12 chronic sclerosing GN, 7 FSGS, 3 MPGN, 3 MsPGN and 2 MGN. Tubulointerstitial diseases (3 reflux nephropathy, 2 chronic pyelonephritis, 1 chronic tubulointerstitial nephritis) and systemic diseases (4 diabetic GN, 1 lupus GN, 1 Wegeners granulomatosis) were diagnosed respectively in six (8%) recipients, and one (1%) was polycystic kidney. The pre-KT diagnosis was changed after pathologic assessment in 9/55 (16%) cases with presumptive diagnosis. Specific pathologic diagnosis was made in 67 cases (92%) at the time of KT, and significantly correlated with the duration of dialysis (p=0.001). Fifty-one post-KT renal biopsies, performed in 44 recipients from 9 to 911 days after KT, revealed recurrence of renal diseases in six transplants (13.6%). Five cases were IgAN and one was MGN. Recurrent IgANs were diagnosed from 14 to 55 days (mean 29 days) after KT, and a recurrent MGN was diagnosed at 14 days after KT. The pathologic etiology could be elucidated in most cases at the time of KT. The recurrence of original disease seems to start very early after KT, therefore the close pathologic follow-up is recommended. In conclusion, the pathologic assessment in renal allograft recipients is necessary for accurate management and improvement of the transplant survival.

Abstract# 1348 **Poster Board #-Session: P104-III**

COMPARISON OF OUTCOMES WITH STRAIGHT, HAND-ASSISTED AND TELEROBOTIC LAPAROSCOPIC DONOR NEPHRECTOMIES.

Martin A. Maraschio, Stuart Wolf, Robert M. Merion, John C. Magee, Darrell C. Campbell, Jr., Randall S. Sung, Jeffrey D. Punch, Juan D. Arenas.

Introduction: we present our experience with the telerobotic laparoscopic donor nephrectomies, and compare the results to the hand-assisted and straight laparoscopic donor nephrectomies Methods: retrospective review of all hand-assisted, straight and robotic laparoscopic donor nephrectomies performed at the University of Michigan between october of 2001 and november of 2002. Results: 95 laparoscopic donor nephrectomies have been performed during that time period: 49 hand-assisted (HA), 24 telerobotic (TR), and 22 straight laparoscopic (SL). There were no differences on the mean age between groups (41, 42 and 39 for HA, TR and SL). The operative time was similar for the three techniques as well: 203 minutes for HA, 225 minutes for SL and 242 for TR (P<0.05 for TR compared to the other two techniques). A 10% (5/49), 17% (4/24), and 18% (4/22) of all the cases were right nephrectomies respectively for HA, TR and SL. Complications cosisted on myoglobinuria (1), renal artery dissection (1), prolongued ileus (1) and postoperative bleeding for SL; Splenic injury (5), with splenectomy in one case, postoperative bleeding (1), lesion to a polar renal artery (1), and lesion to the external iliac artery (1) for HA; Myoglobinuria (2), bowel pseudo-obstruction (1), acute pancreatitis (1), and lesion to the proximal ureter (1) for TR. The conversion rate to open was 17% (4/24), 0% and 5% (1/22) respectively for TR, HA, and SL. The mean hospital stay was 2, 2.2 and 2.9 days for HA, SL and TR respectively. All patients had a full recovery to normal activities within 4 weeks after surgery for the three laparoscopic techniques. The recipient mean postoperative creatinine at day 30 was 1.41, 1.41 and 1.37 for TR, HA and SL respectively (p=NS). Discussion: TR has proven to be a safe procedure when compared to SL and HA. The longer surgical times could be attributed at least in part to the time consumed on preparing the robotic device and on the fact that we are on the ascending part of the laearning curve with this new procedure. With the advening of new robotic devices, simplier to set up and operate, the use of this procedure will extend to many transplant centers.

Abstract# 1349 Poster Board #-Session: P105-III

OUTCOMES OF 'MINI NEPHRECTOMY' IN OBESE PATIENTS.

Surendra Shenoy,[1] Martin Jendrisak,[1] Karen Hardinger,[2] Jeffrey Lowell,[1] Niraj Desai,[1] William Chapman.[1] *[1]Surgery, Washington University School of Medicine, St. Louis, MO; [2]Pharmacy, Barnes Jewish hospital, St. Louis, MO.*

Purpose: To evaluate the outcome of a minimally invasive open nephrectomy procedure in obese live donors. Background: "Mini nephrectomy" is a posterior-transcostal donor nephrectomy technique, developed to decrease the morbidity associated with other nephrectomy techniques. It uses a 6-8 cm incision to complete dissection and extract the graft from the donor. This technique has been used in both normal and obese donors, to retrieve right or left kidney. We review here the outcome in normal and obese donors. Methods: 85 consecutive 'mini nephrectomies' were performed between February 2001 to November 30, 2002 at a University affiliated Hospital. A prospective evaluation of donor demographics, pertinent laboratory data, operative details, post operative complications, length of stay, return to activity and pain medication requirements was undertaken. Results: Table 1 provides comparison of these parameters between obese (BMI >28) and normal (BMI<28) donors.

Table 1

	Total (n=85)	Normal (n=56)	Obese (n=29)	p value
Followup(months±SD)	12±6	12±5	12±7	NS
Age(years)(mean±SD)	39±10	37±9	43±12	0.02
Sex(m/f)	35/50	22/34	13/16	
Relationship(LRD/LURD)	63/22	43/13	20/9	
BMI(g/m²)(mean±SD)	26.3±3.9	23.8±2.3	30.6±2.0	<0.001
EBL(mL)(mean±SD	170±140±	145±97	217±190	0.03
Transfusion requirement	0	0	0	NS
Dissection time(min)(mean±SD	146±51	134±44	161±56	0.03
Incision length(cm)(mean±SD)	6.8±1.1	6.7±1.0	7.1±1.2	0.046
Length of stay(days)(mean±SD)	2.6±0.9	2.5±0.8	2.8±0.6	NS
PCA requirement(hrs)(mean±SD)	27.2±10	27.7±10	26.7±10.7	NS
Conversion	2	1	1	NS
Post op Complications	2	2	0	

Compared to normal donors, a minimal increase in blood loss (none required transfusions) and operative time was noted in obese donors. Both groups had similar perioperative complications, postoperative length of stay and pain medication requirements. All grafts functioned immediately except for one in normal group. Conclusions: These data demonstrate that 'mini nephrectomy' can be safely performed in obese patients. 'Mini nephrectomy' provides benefits of the open retroperitoneal approach and minimally invasive surgery. Unlike other open nephrectomy procedures this can be performed with no increased morbidity or mortality in overweight but otherwise healthy live donors to retrieve right or left kidney.

Abstract# 1350 Poster Board #-Session: P106-III

A DECISION ANALYSIS OF PRE-EMPTIVE AORTO-CORONARY BYPASS IN RENAL TRANSPLANT CANDIDATES.

Aziz Walele,[1] Alexander Logan,[1] Edward Cole,[1] David Naimark.[2] *[1]Division of Nephrology, University Health Network; [2]Sunnybrook & Women's College Health Science Center, University of Toronto, Toronto, ON, Canada.*

BACKGROUND: Prior to kidney transplantation, cardiac aorto-coronary bypass (ACB) is recommended for amenable, critical (>70%) coronary artery disease (CAD). However, asymptomatic sub-critical (50-70%) CAD may also preclude transplantation. The benefit of pre-emptive ACB is uncertain. *Should ACB be offered to the transplant candidate?* The decision must balance up-front ACB risk and uncertain 'waiting-list' time for transplantation that offers a quality of life and survival advantage versus the potential candidate remaining on life-long dialysis. This predicament offers an opportunity evaluate the choice by decision analysis. METHODS: A theoretical dialysis cohort of male and female transplant candidates, age 45 years accede to pre-emptive ACB prior to a living-donor transplant or activation on the cadaver transplant 'waiting-list'. The model of events subsequent to the decision simulates natural history progression as health-states in a Markov-process. The cohort transit health-states in monthly Markov-cycle iterations that simulate a life time-horizon. Simplifying model assumptions maintain the decision balance. The preferred outcome is a strategy with highest 'expected utility'. Base-case parameter estimates are derived from USRDS and published data sources. RESULTS: The theoretical cohort base-case computation shows the highest expected utility for pre-emptive ACB and transplantation than the life-long dialysis option. Living-donor transplantation after ACB and 6 months event-free recuperation shows an incremental gain for males of 5.3 and females 9.7 quality-adjust life-months. The living-donor option is influenced by candidate preference for dialysis or transplantation. The decision is robust to variables including age and ACB risk <18%. Cadaver transplantation after ACB with a 2.5 year event-free 'waiting-list' time shows an incremental gain for males of 0.5 and females 3.0 quality-adjust life-months. The cadaver transplant option is influenced by candidate preference for dialysis or transplantation. In males, a cadaver transplant option is favored if ACB risk <5.6%, candidate age <52 years and 'waiting-list' time <3.5 years. CONCLUSION: From a potential candidate perspective the preferred choice for asymptomatic sub-critical coronary artery disease is 'pre-emptive ACB' prior to living-donor or cadaver kidney transplantation than a decision to remain on life-long dialysis. Consideration should be given to candidate age, sex, 'waiting-list' time and preference for transplantation.

Abstract# 1351 Poster Board #-Session: P107-III

ADVERSE EVENTS ON THE KIDNEY TRANSPLANT WAITING LIST: INCIDENCE AND RISK FACTORS.

Irene Ma,[1] John Gill,[1] Kathryn Tinckam,[1] David Landsberg,[1] Adeera Levin.[1] *[1]Nephrology, St. Paul's Hospita, Vancouver, BC, Canada.*

As waiting times for cadaveric kidney transplantation continue to increase, the task of monitoring the medical fitness of patients on the transplant waiting list (TXWL) has become increasingly more difficult and resource intensive. In order to develop an effective medical surveillance strategy for waitlisted patients, detailed information regarding the incidence of adverse events and identification of patients at increased risk for adverse events on the TXWL is required. Beginning in June, 1998 we prospectively studied 331 waitlisted and 261 newly activated transplant candidates through their TXWL, peri-transplant and post-transplant course. Information regarding death, cardiovascular events and removal from the TXWL was collected for all patients. During the median follow-up of 30 ± 10 months, 255 patients received a transplant and 69 patients had a total of 137 adverse events. Table one show that the majority of events occurred on the TXWL . Only 4/57 of the patients with non-fatal events on the TXWL subsequently received a transplant. In a Cox regression model, that included patient age, diabetes, history of smoking and cardiovascular disease, New York Heart Association (NYHA) functional class, albumin and hemoglobin, older patients (HR=1.04 per year older, p= 0.01), patients with lower hemoglobin (HR= 1.03 per g/l lower, p = 0.02), smokers (HR= 4.42, p <0.001) and patients with NYHA class 2 or higher (HR= 5.78, p <0.001) were at increased risk for adverse events on the TXWL. We conclude that adverse events are common among patients on the transplant waiting list and that many patients have multiple events. Demographic, historical, functional and laboratory information can be used to identify patients at increased risk for adverse events and modification of some of these risk factors (anemia, smoking, NYHA functional class) may decrease the risk for adverse events.

Event	Total number of events	TXWL	Peri-transpant (within 30 days)	Post transplant
Death	57	47	0	10
MI	20	12	3	5
CABG	12	11	1	0
PTCA	13	7	2	4
CVA	16	10	0	6
On hold for cardiac cause	19	19	n/a	n/a

Abstract# 1352 Poster Board #-Session: P108-III

DIFFERENCES IN SENSITIZATION LEVELS COULD CONTRIBUTE TO THE VARIATION IN WAITING TIMES AND SURVIVAL RATES FOR CADAVERIC KIDNEYS IN WHITES, BLACKS, HISPANICS AND ASIANS.

Alan Ting,[1] Wida S. Cherikh,[1] Yulin Cheng,[1] Christopher F. Bryan.[2] *[1]United Network for Organ Sharing, Richmond, VA; [2]Midwest Transplant Network, Westwood, KS.*

Purpose: Waiting times to cadaveric kidney transplantation vary by candidate ethnicity; Whites have the shortest wait times and non-whites have significantly longer times. Blacks have the lowest graft survival rates and Asians the highest. The purpose of this study was to determine whether the sensitization rate, as measured by % panel reactive antibodies (PRA), could be a significant factor in the variation in wait times and graft outcome seen among the different ethnicities. **Methods:** The proportion of unsensitized (<10% PRA), sensitized (10-79%) and highly sensitized (80+%) White, Black, Hispanic and Asian candidates added to the UNOS/OPTN wait list between 1/1/1999 and 12/31/2001 were calculated. The data were stratified by first or repeat transplant and by gender. The same variables were calculated using a snapshot of the wait list on 12/31/01. The difference between the addition and snapshot data gives the accumulation rate on the wait list. **Results:** There were 25,256 Whites (51%) additions to the wait list, 15,105 Blacks (31%), 6,742 Hispanics (14%) and 2,091 Asians (4%). A snapshot of the wait list on 12/31/01 showed 16,241 Whites (42%), 14,579 Blacks (38%), 5,667 Hispanics (15%) and 1,901 Asians (5%). (1) Percentage (%) of Sensitized (S) and Highly Sensitized (HS) Candidates Added to the Wait List.

	First Transplant		Repeat Transplant	
Ethnicity	S	HS	S	HS
White	13.3	4.2	30.0	24.3
Black	17.9	6.8	35.9	32.9
Hispanic	14.1	4.9	32.2	26.5
Asian	11.4	3.9	22.6	18.4

Blacks had the highest percentage of sensitized and highly sensitized candidates added to the list for a first and a repeat transplant and Asians the lowest. The percentage of sensitized and highly sensitized females was greater than males in all analyses (data not shown). (2) Accumulation Rate on the Wait List. For highly sensitized candidates Blacks had the highest accumulation rate (3.2% for first and 15.5% for repeat transplants), and Asians the lowest (2.0% and 9.9%). For sensitized candidates, Asians had the highest accumulation rate (3.1% and 5.7%). The data for the other ethnicities had no consistent patterns. **Conclusions:** Sensitization levels are highest in Blacks followed by Hispanics, Whites and Asians, and could be a contributing factor in the long wait times experienced by Blacks, but not by Asians. Sensitization levels could also correlate with graft outcome since Asians who have the best outcomes are the least sensitized; whereas Blacks who have the worst outcome are the most sensitized.

Abstract# 1353 Poster Board #-Session: P109-III

IS PREEMPTIVE RENAL TRANSPLANTATION PREFERRED? Nasser Simforoosh,[1] Fatemeh Pourrezagholi,[1] Abbas Basiri,[1] Behzad Einollahi,[1] Ahmad Firouzan,[1] Maryam Moghadam,[1] Mohsen Nafar,[1] Soudabeh Farhangi,[1] Mohamad Hosein Nourbala,[2] Behzad Hajarizadeh,[2] Vahid Pourfarziani,[2] Mahboob Lesanpezeshki,[2] Mohamadreza Khatami.[2] *[1]Urology, Shahid Labbafi Nejad Hospital, Urology Nephrology Research Center, Tehran, Tehran, Islamic Republic of Iran; [2]Urology, Bhghiatolah Hospital, Bhghiatolah University of Medical Sciences, Tehran, Tehran, Islamic Republic of Iran.*

BACKGROUND: To compare the results of preemptive kidney transplantations with patients who were on chronic dialysis before transplantation, this historical cohort study was conducted. **METHODS & MATERIALS:** From 1984 to 2002, 3010 living kidney transplantations were performed in our two centers. Since 1992, 127 preemptive kidney transplantations (study group) were performed which was compared with 186 patients who were on chronic dialysis before transplantation (control group) and underwent transplantation at the same time. All patients in control group have been under dialysis more than 6 months. Survival analysis was performed applying Kaplan-Meier method and compared in two groups applying Log Rank method. **RESULTS:** The difference between two groups were not significant as far as recipient age, sex, etiology of CRF, donor age and sex, donor–recipient relationship and immunosuppressive protocols, were concerned. Difference between study and control group was not significant when rejection episodes were studied while the need for ALG therapy in study group was less and the response to steroid therapy was also better in this group comparing to control group (P=0.002). One year, 2 years and 3 years patient survival were similar in study group which was 96.4 %, 96.4%, 96.4% accordingly comparing to control group which was 97.81%, 96.23% and 96.23%. One year, 2 years and 3 years graft survival was better in study group (preemptive transplantation group) which was 93.7%, 85.4%, 84.8% accordingly comparing to control group (patients on chronic dialysis before transplantation) which were 85.4%, 79.5% and 79.5%. The differences between groups were significant for first and second year (P=0.03 and 0.02 respectively) while in third year was not significant (P=0.06), but the graft survival was still better in study group for third year follow up. **CONCLUSION:** Graft survival in preemptive kidney transplantation was better when compared to patients who were on chronic dialysis before transplantation. This also eliminates the need for AV fistula formation in patients with chronic renal failure with its possible known adverse effects. Therefore, we recommend preemptive kidney transplantation as a better choice for patients with chronic renal failure when it is possible.

Abstract# 1354 Poster Board #-Session: P110-III

MANAGING THE WAITING LIST: UTILITY OF A RENAL TRANSPLANT RE-EVALUATION CLINIC. Jeanine A. DeLucia,[1] Fadi G. Lakkis,[1] Amy L. Friedman,[1] Marc I. Lorber,[1] Richard N. Formica.[1] *[1]Departments of Surgery & Medicine, Yale University, New Haven, CT.*

Introduction: The increased waiting time on the renal transplant cadaveric list and increasing comorbidities of patients who are listed raise the concern that some of these patients may not be medically appropriate for transplantation once a kidney becomes available. To address this problem we initiated a renal transplant re-evaluation clinic to manage our waiting list that includes approximately 650 patients. Results: Between February and October of 2002, we re-evaluated 53 patients who were selected based on their numeric ranking within their respective blood group and not based on age or comorbidities. The mean age of these patients was 52 years and the median waiting time was 4 years (range 2 - 7 years). Seventeen of 53 patients were made temporarily unavailable (TU), three patients who were already TU were kept TU, and one TU patient was reactivated. The major reasons for TU status were cardiovascular comorbidities (34%), lack of updating of routine medical information (17%), psychosocial issues (17%), infection (14%), diagnosed or suspected malignancy (10%), neurological comorbidities (3%), and surgical issues (3%). Thirty-three patients were cleared for transplantation without the need for significant further evaluation. Conclusions: (1) Thirty-two percent of patients who were presumed to be ready for transplantation had to be temporarily inactivated because of major comorbidities requiring further evaluation. (2) Lack of updated medical information was the second most common reason for delaying transplantation. These findings are most likely a reflection of the increasing size of the waiting list, increasing waiting time, and worsening comorbidities of patients listed. These findings emphasize the need for a re-evaluation process at transplant centers handling large number of patients on the list and underscore the importance of up-to-date communication with dialysis centers, referring nephrologists, and patients to alleviate delays in transplantation.

Abstract# 1355 Poster Board #-Session: P111-III

LOW UTILIZATION OF PRETRANSPLANT BILATERAL NATIVE KIDNEY NEPHRECTOMY (PRE-BNX) IN THE US. Bertram L. Kasiske,[1,2,3] Jon J. Snyder.[1] *[1]U. S. Renal Data System, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]Department of Medicine, Hennepin County Medical Center, Minneapolis, MN; [3]Department of Medicine, University of Minnesota, Minneapolis, MN.*

Although indications for PRE-BNX include cancer, infection, uncontrolled hypertension and symptomatic polycystic kidney disease, the frequency of PRE-BNX is unknown. We examined PRE-BNX among first, kidney-only transplants in 1994-2000 using United States Renal Data System patients who had Medicare as their primary insurance payer. Medicare claims (1991-2000) were searched to identify PRE-BX. Out of 35,105 patients, only 722 (2.1%) had PRE-BNX. The proportion of patients with PRE-BNX did not change over the 7-year study period. We used logistic regression to characterize patients most likely to have PRE-BNX. Significant (P<0.05), independent correlates included: age 0-17 y (odds ratio = 6.25; 95% CI = 6.66-8.76, reference 18-44 y), age 45-59 y (1.72, 1.33-2.22), age ≥60 y (1.43, 1.11-1.84); Hispanic (1.32, 1.02-1.70); cystic kidney disease (26.4; 17.8-39.2, reference diabetic nephropathy), hypertensive nephrosclerosis (3.21, 2.10-4.92), glomerulonephritis (2.94, 1.95-4.45), and other causes (6.13; 4.14-9.09); cadaveric donor (0.48; 0.36-0.65, reference live-unrelated donor); up to 1 y of pre-transplant dialysis (3.66, 1.76-7.62, reference pre-emptive transplant), 1-2 y (5.54, 2.68-11.4), 2-3 y (7.20, 3.47-14.9), ≥3 y (10.2 , 4.96-20.9); lean body mass index (BMI) <18.5 kg/m² (1.57, 1.21-2.04, reference 18.5-25.0 kg/m²), obese BMI>30 kg/m² (0.79, 0.62-0.99). In a Cox proportional hazards analysis, PRE-BNX was not independently associated with graft survival, death-censored graft survival, or mortality, after taking multiple patient and donor characteristics into account. We conclude that PRE-BNX is uncommon among the Medicare insured. Patients most likely to have PRE-BNX are either older or younger than age 18-45 y, more likely to have cystic kidney disease or hypertensive nephrosclerosis, and more likely to have a lower BMI than patients who did not undergo PRE-BNX. There were no independent associations between PRE-BNX and posttransplant outcomes. However, the small number of patients with PRE-BNX and the possibility that they are at increased risk from unmeasured co-morbidities should be considered in the interpretation of these results.

Abstract# 1356 Poster Board #-Session: P112-III

EFFECT OF UNIVERSAL LEUKOREDUCTION OF RBC TRANSFUSIONS ON ALLOSENSITIZATION IN POTENTIAL RENAL TRANSPLANT RECIPIENTS. Martin Karpinski,[1] David Rush,[1] John Jeffery,[1] Denise Pochinco,[1] Iga Dembinski,[1] Peter Nickerson.[1] *[1]Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: Allosensitization in individuals awaiting renal transplantation prolongs time on the waiting list, and is associated with inferior patient and graft outcomes. Despite the routine use of EPO, >30% of potential transplant candidates in the UNOS registry have a history of having received a transfusion. One approach to minimize allosensitization rates has been to leukoreduce RBC units prior to transfusion (i.e. reduce WBC count to $<5.6 \times 10^6$ / unit) and Canadian Blood Services instituted a universal leukoreduction policy in 1999. While this practice has been effective at decreasing allosensitization for leukemia/lymphoma patients, there is no data with regards to its efficacy in potential renal transplant candidates. **Method**: Sera pre- and post-transfusion were retrospectively tested for anti-HLA antibodies in 96 potential renal transplant candidates. Samples were screened by both the AHG-CDC and FlowPRA techniques. Sixty-four individuals were transfused prior to universal leukoreduction and 32 were transfused after the implementation of universal leukoreduction. Transfusion associated allosensitization was defined as either the detection of de novo anti-HLA antibodies post-transfusion or an increase in %PRA>30%. **Results**: The rate of allosensitization in the pre-leukoreduction era was no different than the rate in the post-leukoreduction era (16/64 (25%) vs. 10/32 (31%), p=NS). AHG-CDC failed to detect 35% of individuals who became allosensitized as a result of a transfusion, as determined by FlowPRA (AHG-CDC +ve=17/96 vs. FlowPRA +ve=26/96, p<0.05). When individuals were stratified into those at high risk of allosensitization (prior pregnancy, transfusion, or transplant) vs. low risk there was a trend for decreased rates of allosensitization in the low risk group with leukoreduced blood (High Risk: 59% vs. 41% (leukoreduced vs non-leukoreduced), Low Risk: 0% vs 11% (leukoreduced vs. non-leukoreduced) however this did not reach statistical significance. **Discussion**: While universal leukoreduction has reduced allosensitization in leukemia/lymphoma patients this study suggests that this is not the case for potential renal transplant candidates. We speculate that this is due to the fact that the alloimmune response is more intact in potential renal transplant candidates. Therefore, transfusion (even with standard leukoreduced RBC's) should continue to be avoided if feasible in patients awaiting transplantation.

Abstract# 1357 Poster Board #-Session: P113-III

OFFERING A CLINICAL RISK EVALUATION SCORE TO PATIENTS UNDER EVALUATION FOR A KIDNEY TRANSPLANT.

Sarbjit V. Jassal,[1] Douglas E. Schaubel,[2] Stanley S. A. Fenton.[1] *[1]Medicine, University Health Network, Toronto, ON, Canada; [2]Department of Biostatistics, University of Michigan, Ann Arbor, MI.*

Despite increasing knowledge about the predictors of transplant survival, clinicians are unable to offer patients a individualised graft and patient survival probability. Our objective was to develop a series of predictive tables which could offer a survival probability adjusted for comorbidity profile, age, gender and donor source. Methods: Using prospectively collected administrative data from the Canadian Organ Replacement Registry,1988 to 1998, we derived a model for 1-, 3- and 5 year patient survival and graft survival. The model covariates included age, race, gender, treatment period, primary renal disease cause, donor source, months on dialysis and comorbidities (cardiovascular disease, peripheral vascular disease, malignancy, lung disease and/or other serious illnesses). The model was used to predict 1-, 3- and 5 year patient and graft survival rates for 3 clinical scenarios. Patient A, was a 55 yr. old Caucasian male, with renal disease secondary to diabetes, who had a previous history of myocardial infarction and peripheral vascular disease. Patient B was a 45yr. old Caucasian female, with renal disease due to polycystic kidney disease (PKD) with no known comorbid conditions and Patient C was a 65 yr. old male also with PKD and no comorbid conditions Results: A representative sample of the results are shown below (table 1) Conclusions: These prediction tables allow clinicians to discuss the feasibility and predicted outcome of transplantation with patients undergoing clinical evaluation.

Estimated Patient Survival for Patients A -C as given by the Prediction Tables

Cadaveric Donor; 24 month wait time	1 yr Pt Surv	3 yr Pt Surv	5 yr Pt Surv
Patient A	92%	85%	75%
Patient B	98%	96%	93%
Patient C	93%	87%	79%
Living Donor; 12 month wait time			
Patient A	95%	91%	85%
Patient B	99%	98%	96%
Patient C	96%	93%	87%

Abstract# 1358 Poster Board #-Session: P114-III

CONSEQUENCES OF ELIMINATING HLA-B IN CADAVERIC KIDNEY ALLOCATION TO INCREASE MINORITY TRANSPLANTATION: IS THIS THE RIGHT WAY?

Daniel C. Brennan,[1] Mark A. Schnitzler.[2] *[1]Internal Medicine, Washington University School of Medicine, St. Louis, MO; [2]Health Administration Program.*

Introduction: The rate of cadaveric renal transplantation among wait listed African Americans is less than that in Caucasian Americans. There are numerous factors behind this outcome. One, is the fact that the distribution of HLA antigens in wait listed African Americans differs from the distribution in the donor pool. It has been argued that elimination of HLA-B matching from the allocation schema could shift 236 cadaveric transplants annually from Caucasian to African Americans with an estimated 2% increase in the overall rate of graft failure (Roberts et al. Abstract #32, ATC 2002). Subsequently, HLA-B has been removed from cadaveric kidney allocation. The patient survival and economic consequences of this change are unknown. Methods: All data were drawn from the USRDS database. A Markov model was constructed to assess the long term expected outcomes and costs of transplantation including and excluding HLA-B matching in the allocation schema. Additional models were constructed to assess the benefits and consequences of shifting organs from Caucasian to African Americans comparing transplantation to waiting on dialysis. Probabilities of all outcomes were calculated including outcomes while awaiting transplant on dialysis, graft function, graft loss with death, graft loss with return to dialysis, and death. Costs, calculated from the perspective of Medicare, along with quality adjusted life year (QALY) benefits, were estimated according to each associated outcome and discounted to present value. Results: Shifting 236 organs from Caucasian to African Americans increases total QALYs by 395 years and decreases total lifetime costs by $11 million in African Americans. However, this shift reduces total QALYs by 591 years and increases total lifetime costs by $10 million in Caucasian Americans. Further, eliminating HLA-B matching causes an overall 2% reduction in graft survival which reduces total QALYs by 415 years and increases total lifetime costs by $16 million. Conclusion: To gain a total of 395 QALYs and save $11 million in 236 African American patients, we must cause a loss of a total of 1,006 QALYs and $26 million in all other patients. Therefore, we find the elimination of HLA-B from the allocation schema to be a very expensive method of redistributing a small number of organs in favor of African Americans. The transplant community must decide if there are better ways to meet this goal.

Abstract# 1359 Poster Board #-Session: P115-III

PRIORITY FOR CHILDREN IN CADAVER KIDNEY SHARING – THE STRATEGY ADOPTED IN SAO PAULO.

Paulo C. Koch Nogueira,[1] Alexandre S. R. Amaral,[1] Paula G. P. Machado,[2] Reginaldo Boni,[2] Luiz A. Pereira,[3] José O. M. Pestana.[2] *[1]Pediatrics, Faculdade de Ciencias Medicas de Santos, Santos, SP, Brazil; [2]Nephrology, UNIFESP - Escola Paulista de Medicina, Sao Paulo, SP, Brazil; [3]Central de Transplante - Capital, Secretaria de Estado da Saúde, Sao Paulo, SP, Brazil.*

OBJECTIVE - The idea of giving priority to children in cadaver kidney allocation is well accepted. The aim of this study is to evaluate the efficacy of the child priority policy adopted in Sao Paulo, Brazil. METHODS – Ecologic study of the data collected by the Government Transplant Organization in Sao Paulo involving all patients who were included on the waiting list from 13/08/98 until 31/12/01. Proportions were compared using the Chi-square test, and survival curves (Kaplan-Meier method) were constructed from the database using cadaver donor kidney transplants as the dependent variable and the pediatric age (<18 years) as the independent variable; patients who died or underwent a living donor transplant were censored. RESULTS – In the initial 2.6 years of data collection, the policy of priority for children was to direct cadaver kidneys obtained from children < 12 years to child receptors. In the last 0.8 years the policy has been broadened to include cadaver donors < 18 years. Throughout the study period 9,370 patients with end stage renal disease fulfilled the clinical criteria to be enrolled in the waiting list, comprising 9,039 adults (mean age=48.6±14.1 years, 3,760 female) and 331 children (mean age=11.9±5.1 years, 163 female). Over this period there were 1,993 deaths (32/331 children and 1,961/9,039 adults, Chi-square=27.6 p<0.0001), 1,032 living donor transplants (69/331 children and 963/9,039 adults Chi-square=33.9 p<0.0001) and 556 cadaver donor transplants (112/331 children and 444/,9039 adults, Chi-square=478.6 p<0.0001). Three years after being enrolled on the list 26.3% of the children, and 76.7% of the adults were still waiting (Log Rank test=542.53 p<0.00001). Mean waiting time for the whole group (adults+children) was 3.5 years (95%CI=3.5-3.6). For the adult group it was 3.6 years (95%CI=3.6-3.6), while for children it was 2.3 years (95%CI=2.1-2.5). CONCLUSIONS – The adopted policy has been effective to further kidney cadaver transplants in children. This benefit lead to only a slight increase in the waiting time for adults, since the mean waiting time for the adult group is only 0.1 year longer than that observed for the whole group.

Abstract# 1360 Poster Board #-Session: P116-III

IS THE UNOS POINT ALLOCATION FOR HIGHLY SENSITIZED PATIENTS JUSTIFIABLE IN THE ABSENCE OF A STANDARDIZED PRA METHOD AT THE NATIONAL LEVEL?

Gopal Krishnan,[1] Leroy R. Thacker.[2] *[1]Kidney Transplant, Our Lady of Lourdes Medical Center, Camden, NJ; [2]South-Eastern Organ Procurement Foundation, Richmond, VA.*

In our earlier communication (Proc of 10th International Congress of Immunology, New Delhi, India, 1998), we addressed the issue of UNOS point allocation system in the USA for highly sensitized patients on the waiting list, since the PRA test results varied from laboratory to laboratory depending upon the method employed. Also, most laboratories perform T-PRA determination only. Based on the results of our retrospective analysis of the SEOPF database regarding the impact of HLA-DR mismatching on renal allograft survival in retransplants, we recently proposed that B-cell PRA and crossmatches should be routinely performed by histocompatibility laboratories (Transplantation, 2002). The purpose of this investigation was to retrospectively analyze the SEOPF database in order to find out whether any correlation between various T-PRA methods and renal allograft survival exists. The results of the following T-PRA methods were available in the SEOPF database: NIH/Extended (n = 5,439), Wash/Extended (n = 12,968), Anti-globulin (n = 14,724), Flow (n = 2,843) and ELISA (n = 2,393). For secondary analysis, % PRA was broken down into PRA>80%,(n = 2,148) and PRA 15-80% (n = 6,029). A statistically significant difference in renal allograft survival was noticed between the five PRA methods for all recipients (Log-Rank $\chi^2 = 216.1804$, 4 d.f., $p < .0001$; Wilcoxon $\chi^2 = 251.4347$, 4 d.f., $p < .0001$) and in both of the sub-groups of PRA > 80% and PRA 15-80% (Log-Rank $\chi^2 = 34.2649$, 4 d.f., $p < .0001$; Wilcoxon $\chi^2 = 33.9272$, 4 d.f., $p < .0001$ and Log-Rank. $\chi^2 = 59.4393$, 4 d.f., $p < .0001$; Wilcoxon $\chi^2 = 73.4302$, 4 d.f., $p < .0001$ respectively). A post-hoc test of the hypothesis of no difference between PRA testing method and renal allograft survival after adjusting for covariates that are known or suspected to affect graft survival was rejected (Wald $\chi^2 = 134.1068$, 4 d.f., $p < .0001$). Therefore, if an equitable distribution of organs is to be assured to transplant recipients, the UNOS point allocation system should be based upon a standardized method for PRA determination that could be uniformly performed by all laboratories.

Abstract# 1361 **Poster Board #-Session: P117-III**

RETRANSPLANT WITHOUT INTERVAL DIALYSIS: A BETTER OPTION? Massimo Asolati,[1] Arthur Matas.[1] [1]*Surgery, University of Minnesota, Minneapolis, MN.*

Preemptive primary transplantation is associated with better patient and graft survival than transplantation after initiation of dialysis. And for those starting dialysis, post-transplant outcome is incrementally worse for each additional year of pre-transplant dialysis (Kidney Int 58(3):1311-7, 2000). We asked whether recipients undergoing a second transplant also benefitted by transplantation without interval dialysis (i.e., by a preemptive transplant). Between 1984 and 2002, 441 patients with primary graft failure underwent retransplantation. Of these 339 had graft survival >6 months and were included in this analysis. There were 196 males and 143 females; the mean age was 35.3. Of the 2nd tx, there were 125 LD and 213 CAD. Overall, peak PRA was 49% in the dialysis group, 17% in the preemptive; PRA at transplant was 33% in the dialysis group and 11% in the preemptive group. Outcome is shown in the tables.

LD	Pre Tx Dialysis (n=71)			Preemptive (n=54)			
Curr. PRA	9%			0.6.%			
Peak PRA	27%			6%			
	1yr	3yrs	5yrs	1yr	3yrs	5yrs	p
Patient survival	93%	91%	83%	94%	94%	90%	0.3
Graft survival	86%	81%	69%	94%	94%	85%	0.03
D/C Graft surv.	81%	88%	82%	100%	100%	93%	0.03

For LD, preemptive 2nd tx was associated with significantly better graft survival, and better death-censored graft (D-C) survival (table). Graft loss to chronic rejection (CR) occurred in 5% of the preemptive recips (17% losses) vs. 18% dialyzed recips (28% losses). Death with function (DWF) occurred in 24% preemptive recips vs 28% dialyzed.

CAD	Pre Tx Dialysis (n=152)			Preemptive (n=61)			
Curr. PRA	48%			21%			
Peak PRA	59%			23%			
	1yr	3yrs	5yrs	1yr	3yrs	5yrs	p
Patient survival	96%	83%	85%	95%	88%	85%	0.3
Graft survival	90%	81%	70%	34%	82%	73%	0.3
D/C Graft surv.	93%	85%	80%	98%	93%	87%	0.15

For CAD, there was no significant difference in graft survival between preemptive vs dialyzed recips. Graft loss to CR occured in 21% preemptive (38% losses) and 18% dialyzed (33% losses) recips. DWF occurred in 26% preemptive vs 32% dialyzed. **Conclusions:** We found significantly better outcome for preemptive 2nd LD transplants (but not CAD). Larger numbers are needed to determine if the benefit of preemptive LD 2nd transplants is due to decreased sensitization, continuation of immunosuppression from 1st thru 2nd tx, or other factors.

Abstract# 1362 **Poster Board #-Session: P118-III**

LONG-TERM FOLLOW UP OF KIDNEY TRANSPLANT DONORS IS POOR AND INCONSISTENT: A PRELIMINARY REPORT. Ruben Velez,[1] Jennifer Fischer,[1] Tom Parker,[1] Larry Melton,[1] Kim Rice,[1] Karl Brinker,[1] Yousri M. Barri.[1] [1]*Dallas Nephrology Associates, Dallas Transplant Institute, Dallas, TX.*

The current practice of transplant centers is to follow living donors once following donation. There is no available funding for long-term follow up of donors. The burden is usually placed on the donors to follow up with their primary care physicians. In this study we have sent a questionnaire to 220 living donors during the previous 7 years at our institution. The questionnaire included specific question about the presence or absence of hypertension or proteinuria and the level of serum creatinine if known and if they have any other medical problems. At our institution we pass on long term follow up to primary care physicians after a single visit 3-6 month post donation. Sixty-one patients responded to the initial mail out. Thirty-three were females and 28 were males, the mean age was 47 ± 9 years and the mean duration since donation was 20 ± 13 months. Of 61 donors 49 (80%) did not have a follow up serum creatinine since donation, and 28 (46%) did not know if they developed hypertension, and 19 (31%) were not sure if they have developed proteinuria. Of the respondents 8% developed hypertension and 3% had proteinuria. Forty (66%) of donors had no proteinuria, 28 (46%) had no hypertension and 12 (20%) were aware that they have a normal serum creatinine. In conclusion: This preliminary report revealed that the majority of living donors do not follow serum creatinine. Furthermore, a large proportion of donors were unaware of their BP level or the presence of proteinuria. From this limited this study long-term follow-up of living donors has been inconsistent and poor. Adequate funding is needed to cover long-term follow up of living donors preferably by a nephrologist/transplant team.

Abstract# 1363 **Poster Board #-Session: P119-III**

A 12-MONTH ECONOMIC ANALYSIS OF IMMUNOSUPPRESSIVE STRATEGIES CONTAINING FTY720 AND NEORAL VS. MYCOPHENOLATE AND NEORAL FOR RENAL TRANSPLANTATION. C. Marra,[1,3] R. Balshaw,[2,3] A. Kilburg,[4] Z. Kalo,[4] C. David,[4] D. Hricik,[5] P. Keown.[1,3] [1]*Univ of British Columbia, Vancouver, BC, Canada;* [2]*Simon Fraser Univ, Burnaby, BC, Canada;* [3]*Syreon Corp, Vancouver, BC, Canada;* [4]*Novartis Pharma, Basle, Switzerland;* [5]*Case Western Reserve Univ, Cleveland, OH.*

Background: FTY720 is a novel lymphocyte homing agent with a unique mechanism of action that acts synergistically with cyclosporine (CsA). A series of phase 2 renal transplant (RTx) studies have shown that FTY720 reduces the incidence of acute rejection and that FTY720 with reduced dose CsA shows potential clinical benefit over conventional immunosuppressive (IS) strategies. Thus, an economic evaluation was performed to determine the economic attractiveness of FTY720 versus Mycophenolate Mofetil (MMF) in the first 12 months post RTx. Methods: 6-month results from a multicenter, randomized, partially blinded study involving 258 de novo kidney transplant patients were used as the primary clinical data source. In this trial, patients were randomized to one of four arms: 1) FTY720 5 mg + reduced dose CsA (n=72); 2) FTY720 2.5mg + full dose CsA (n=76); 3) FTY720 2.5 mg+ reduced dose CsA (n=72); and 4) MMF+ full dose CsA (n=38). Only the first two FTY720 regimens were included in the economic analysis as the third was clinically inferior. The decision model incorporated the costs and probabilities of initial hospitalization, acute rejection, IS therapy, adverse events, and graft maintenance/failure and dialysis for each strategy. The cost of FTY720 was not included as it has not been determined. Resources were valued in 2001 U.S. dollars. Results: The incidence of biopsy proven acute rejection (BPAR) at 6 months was lower in both FTY720 arms when compared to the MMF arm (13.9%, 13.2%, vs. 18.4%). In addition, the composite endpoint of BPAR, death, graft loss or premature discontinuation was significantly lower in the FTY720 5mg + reduced dose CsA compared to the MMF arm(p=0.048). In economic terms, the two FTY720 arms had lower, total direct 12 month medical costs ($69,639 and $76,545) compared to the MMF arm ($85,582). Both FTY720 arms showed reduced costs of graft loss, dialysis, and maintenance IS. Sensitivity analyses indicated robustness to plausible changes in clinical and cost variables. Conclusions: The model provides a robust description of outcomes and costs post renal transplantation. Combination immunosuppressive regimen based on FTY720 appear to be dominant therapies, simultaneously reducing the incidence of BPAR and the cost of treatment in the first year post kidney transplant for a wide range of potential costs for this novel agent.

Abstract# 1364 **Poster Board #-Session: P120-III**

LONG TERM FOLLOW-UP OF PEDIATRIC EN BLOC RENAL TRANSPLANTATION. Kenneth A. Beasley,[1] Felipe Balbontin,[2] Anthony Cook,[1] Michael Bloch,[1] Vivian C. McAlister,[1] Joseph Lawen,[2] Patrick P. Luke.[1] [1]*Surgery, London Health Sciences Centre, London, ON, Canada;* [2]*Surgery, Dalhousie, Halifax, NS, Canada.*

We reviewed the long-term outcomes of pediatric en bloc renal transplantation at two Canadian centers in the cyclosporine era. Between June 1984 and July 2002, 16 patients received pediatric en bloc renal transplants at Dalhousie University and the University of Western Ontario. Mean recipient age and weight were 45 ± 17 years (15 - 74) and 72.2 ± 14.4 kg (48 - 91), respectively. En bloc kidneys were procured from donors aged 2.1 ± 0.8 years (0.7-4.0) weighing 14.3 ± 2.0 kg (12-17). All patients received calcineurin inhibitor-based therapy along with corticosteroids, and 15 patients received additional antiproliferative agents. Only three patients received antibody-based induction therapy. After a mean follow-up of 3.7 years (0.4 - 15), seven rejection episodes occurred (1 Banff Ia/ 3 Banff 1b/ 2 Banff II/ 1 accelerated antibody-mediated).The rejection episodes were successfully treated with steroids (4), anti-lymphocyte-based therapy (2), and intravenous immunoglobulin along with plasmapheresis (1). Mean serum creatinine at 3 months, 1 and 3 years was 138.8 ± 54.5 µmol/l , 118.6 ± 38.1µmol/l , and 95.1 ± 24.4 µmol/l, respectively. The mean serum creatinine of 5 patients with at least 5-year follow-up was 96.8 ± 12.3 µmol/l. Three-year graft and patient survivals were 94% and 94%. Two deaths with functioning grafts occurred at one and seven years post-transplant secondary to cardiac and infectious etiologies. None of the grafts were lost independent of death. En bloc transplantation has excellent short and long term results. Improving graft function after 3 years represented by falling serum creatinine suggests that these kidneys have excellent renal reserve and growth potential.

CMV, Hepatitis, Miscellaneous Infections and Renal Transplant Complications

Abstract# 1365 Poster Board #-Session: P121-III

CLINICAL COURSE OF LAMIVUDINE-RESISTANT HBV INFECTION IN RENAL ALLOGRAFT RECIPIENTS. Tak-Mao Chan,[1] Pok-Siu Yip,[1] Colin S. O. Tang,[1] Guo-Xiang Fang,[1] Kar-Neng Lai,[1] Stephen K. N. Ho.[1] *[1]Dept of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong.*

We have reported that pre-emptive lamivudine therapy based on HBV DNA level markedly reduced liver-related mortality in HBsAg+ renal transplant recipients (RTR). However, the progressive emergence of lamivudine-resistant HBV variants presents a major concern, and yet the natural history of lamivudine-resistant HBV infection in immunosuppressed subjects remains obscure. Since Jan 1996 we have treated 29 of 53 HBsAg+ RTR pre-emptively with lamivudine based on their increasing HBV DNA levels. All the patients had maintenance immunosuppression with low-dose prednisolone and cyclosporin or tacrolimus. All the patients had effective suppression of HBV DNA after treatment. This is a prospective cohort study to examine the clinical course of patients who have developed lamivudine-resistant HBV variants. 14 patients (48.3%) developed lamivudine-resistance with resurgence of HBV DNA during treatment, whereas treatment was successfully withdrawn (i.e. without relapse) after 24.6±11.9 mon in 9 patients (31.0%). The duration of lamivudine therapy and the post-transplant follow-up for the 14 patients were 56.7±12.5 and 97.4±44.9 mon respectively. Lamivudine-resistance emerged after 16.9±7.0 mon (range 10-35) of treatment, with 78.6% within the first 18 mon. Patients with lamivudine-resistant variants showed similar age, gender, baseline eAg status, and post-lamivudine eAg sero-conversion (25.0 vs 33.3%, P=1.00) compared to those who did not develop resistance. Liver biochemistry deteriorated in 11 (78.6%) patients after the emergence of lamivudine-resistance, which was transient in 4 (36.4%) but became chronic in 6 (54.5%) patients. The remaining patient developed severe exacerbation, but responded to the addition of famciclovir. Peak HBV DNA after the emergence of resistance was lower ($1.26\pm1.09x10^9$ vs $6.26\pm12.23x10^9$ copies/ml, P=0.011), while peak ALT was higher (196±117 vs 77±47 iu/l, P=0.005), compared to the respective pre-treatment levels. None of the patients with lamivudine-resistant HBV had liver-related mortality or hepatocellular carcinoma, while the latter affected 2 patients in the other group. We conclude that half of lamivudine-treated HBsAg+ RTR are complicated by drug resistance. Although the subsequent peak HBV DNA is lower than the pre-treatment level, exacerbation of hepatitis is common, and half of these patients develop chronic active hepatitis. Thus, lamivudine-resistant HBV variants will present an escalating clinical problem in immunosuppressed RTR, before the advent of effective therapy.

Abstract# 1366 Poster Board #-Session: P122-III

HEPATITIS B VIRUS CHARACTERIZATION AND COINFECTION WITH HEPATITIS C, HEPATITIS D, HEPATITIS G AND TT VIRUSES IN RENAL TRANSPLANT PATIENTS. David J. B. Machado,[1] Joao Renato R. Pinho,[2] Marcilio F. Lemos,[2] Regina C. Moreira,[2] Adriana P. Campri,[2] Alessandra E. Nascimento,[2] Flair J. Carrilho,[1] Luiz E. Ianhez.[1] *[1]Nephrology, University of Sao Paulo, Sao Paulo, SP, Brazil; [2]Virology, Instituto Adolfo Lutz, Sao Paulo, SP, Brazil.*

Hepatitis B has an aggressive course under immunosuppression and it can be worse in patients confected with hepatitis C or D. To evaluate the occurrence of hepatitis C (HCV), hepatitis D (HDV), hepatitis TT (TTV) and hepatitis G (HGV) viruses in patients with hepatitis B infection elected for treatment and to establish hepatitis B virus genotypes, subtypes, pre-core, basal core promoter and polymerase variants in 22 renal transplant patients in Brazil. Hepatitis B viral replication was determined by serology (HBeAg) and by Amplicor HBV Monitor, Roche. Gene sequencing assessed surface genotypes, subtype, pre-core, basal core promoter and HBV polymerase variants. Hepatitis D infection was determined by serology (Diasorin, Italy) and TTV/ HGV by "in house" polymerase chain reaction (PCR). Hepatitis C was evaluated either by serology and PCR. Alanine aminotransferase levels were elevated in 13 patients (59,0%) and HBeAg was positive in 18 patients (81%). HBV DNA level was $1.12 \times 10^9 \pm 2.0 \times 10^9$ copies/ml. Genotype D was found in 17 (77%) and genotype A in 5 patients (23%). All patients with genotype D were ayw3 and all patients with genotype A had adw2 subtype. Pre-core M1, pre-core M2, pre-core G1899A, basal core promoter and YMDD variants occurred in four, one, one, six and zero patients, respectively. HBeAg negative patients had pre-core M1 (2 patients- 50%), pre-core M2 (1 patient –25%) or basal core promoter (1 patient – 25%) mutation. Anti-HCV positivitiy was found in 1 patient but PCR was negative, and PCR was positive in an Anti-HCV negative patient. HDV co infection was null. TTV and HGV infection were demonstrated in 11 patients (50%) and in 9 patients (41%), respectively. TTV/HGV/HBV co infection occurred in 4 patients (18%). There was no correlation between co infection occurrence and ALT levels, viral load level or liver grade at biopsy. HBeAg negative patients had lower ALT levels (p<0,01). In conclusion hepatitis B patients frequently have transfusion transmitted viruses co infection. As viruses co infection may modify response to therapy or determine ALT flares after HBV suppression, it should be assessed in future trials. Pre-core variants are the major pre-core phenotype determinants. YMDD variants coexistence cannot be find with wild type prior treatment with nucleoside analogues.

Abstract# 1367 Poster Board #-Session: P123-III

LONG-TERM IMPACT OF HEPATITIS B AND HEPATITIS C INFECTION IN RENAL ALLOGRAFT RECIPIENTS. Ping Zhang, Jianghua Chen, Jianyong Wu. *[1]The Nephrology Center,The First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, Zhejiang, China.*

Objective To study the Long-term impact of hepatitis B and hepatitis C infection in renal allograft recipients. **Methods** 983 recipients were retrospectively studied and divided into HBV group, HCV group and hepatitis-negative group. Life-table survival estimates were used to evaluate the impact of hepatitis B or hepatitis C on renal allograft recipients. The rate of acute rejection (AR) and chronic allograft nephropathy (CAN) and the causes of death were analyzed too. **Results** 108 recipients were HBV positive (10.99%), 31 were HCV positive (3.15%) and 844 were hepatitis-negative(85.86%). Patient survival by life-table analysis was lower in HBV group with high mortality of liver failure (14.8%, P<0.001). There was no significantly difference of survival in six years between HCV group and hepatitis-negative group. The morbidity of CAN was 12.96% in HBV group (p<0.01), 25.81% in HCV group (p<0.01) and 5.6% in hepatitis-negative group. There were no difference of the rate of AR and the mortality of non-liver failure among three groups. **Conclusions** Renal allograft recipients who are hepatitis B have an increased risk of death after transplantation compared with hepatitis negative recipients and the major hazard is liver failure. The recipients who are hepatitis C in six years do not have an increased risk of death after transplantation compared with hepatitis negative recipients. Both have high morbidity of CAN.

Abstract# 1368 Poster Board #-Session: P124-III

THE IMPACT OF HEPATITIS B AND C VIRUS ON RENAL TRANSPLANT PATIENT AND GRAFT SURVIVAL. Elizete Keitel,[1] Roberta Pozza,[1] Auri F. Santos,[1] Antonio N. Bittar,[1] Rosana M. Bruno,[1] Daniela Seeling,[1] Joao Carlos Goldani,[1] Valter D. Garcia.[1] *[1]Nephrology, Santa Casa Hospital, Porto Alegre, Rio Grande do Sul, Brazil.*

Aim: to evaluate the long term patient and graft survival of renal transplants recipients with positive serology for B and /or C hepatitis virus compared to recipients with negative serology. Methods: retrospective analysis of 452 renal transplants, with positive serology (group H; n=151) or not (group NH; n=301) for B and / or C hepatitis virus, performed from August 1991 to July 1997. The patients were followed by at least 5 years. The patient and graft survival were done by Kaplan-Meier method. Results: Out of 452 patient, 151 (33.4%) had positive serology for B and /or C hepatitis virus; (35 (7.7%) positive for B virus, 103 (22.8%) for C virus, e 13 (2.9%) for B and C. The mean age was in group H: 37±15 years and group NH: 32 ±14 years (p=0.001). The proportion of male was 60.3% in group H and 55.5% in group NH (p=0.36)). The first transplants were 88.7% in GH and 92% in group NH (p=0.50). The proportion of cadaver donor was 55.7% in group H and 49.2% in group NH (p=0.23). The actuarial patient survival at 5 and 10 years was 83% and 67% *vs* 93% and 86%for group H and group NH, respectively (p<0,001). The actuarial graft survival at 5 and 10 years was 63% and 44% *vs* 68% and 59% for group H and group NH, respectively (P=0,02). Graft loss caused by death occurred in 44.2% (34/77) in group H and in 21.4% (24/112) in group NH (p=0.001). The causes of death were similar in both groups. Conclusion: the presence of antibodies for hepatitis C and antigen for hepatitis B is associated to a long term lower graft and patient survival. The significance of these results should be compared with patient survival with hepatitis that remained in the waiting list.

Abstract# 1369 Poster Board #-Session: P125-III

POSTRANSPLANT MEMBRANOPROLIFERATIVE VERSUS MEMBRANOUS GLOMERULONEPHRITIS: OUTCOMES AND PROGNOSTIC FACTORS. Milagros Ortiz,[1] Esther González,[1] Nuria Esforzado,[2] Beatriz Domínguez-Gil,[1] Juan C. Herrero,[1] Agustín Gómez de la Cámara,[3] Enrique Morales,[1] Federico Oppenheimer,[2] Amado Andrés,[1] Jose L. Rodicio,[1] Jose M. Campistol,[2] Jose M. Morales.[1] *[1]Nephrology Department, 12 de Octubre Hospital, Madrid, Spain; [2]Renal Transplant Unit, Clinic Hospital, Barcelona, Spain; [3]Epidemiology Department, 12 de Octubre Hospital, Madrid, Spain.*

Postransplant glomerulonephritis (PTGN) are considered as risk factors for graft loss. However, there is no enough information comparing clinical course and prognosis among different types, mainly among those hepatitis C virus (HCV) related PTGN. In the present study, we analyze and compare clinical course, survival and prognostic factors after renal transplantation in a a group of patients with PTGN [membranoproliferative (MPGN) and membranous (MGN)]. A total of 38 cases of PTGN from two transplant units in Spain were included in the study. Twenty one (55.2%) showed a MGN (18 of them with positive ELISA-2 anti-HCV antibodies) and 17 (45%) had been diagnosed of MPGN (13 of them with positive ELISA-2 anti-HCV antibodies). A comparison between both groups was done in terms of graft and patient survival. A multivariate analysis was carried out in order to identificate those factors related to graft loss. Graft survival in patients with MPGN was 100%, 47% and 35% at 1, 3 and 5 years, respectively. Same values for MGN were 100%, 71% and 52% (p NS). Patient survival was 100%, 95% and 90% versus 100%, 90% and 77% at 1,3 and 5 years, respectively in both groups. No differences in survival rates were noticed

independently of HCV status. Age, gender, acute rejection, antirejection therapy, baseline immunosuppression, hypersensitized status, HCV serology, HCV RNA, severity of liver disease, clinical presentation and specific therapy for glomerular disease were similar in both groups. In the multivariate analysis (logistic regression), only remission of the nephrotic syndrome [OR 12.51 (1.54-101.75)] and normal renal function at diagnosis [OR 17.5 (1.33-230.43)] were associated with a favourable outcome.]In conclusion, no differences were found between patients with posttransplant MGN and MPGN in terms of graft and patient survival. Our results also suggest that HCV positive serology is not a factor conditioning a worse outcome in these PTGN. Only the remission of the nephrotic syndrome and the presence of a normal renal function at diagnosis had a prognostic value, both conditioning a better outcome of the kidney graft.

Abstract# 1370 Poster Board #-Session: P126-III

IMPROVED DETECTION OF HCV INFETION AND HCV GENOTYPES IN HEMODIALYSIS PATIENTS USING HCV TMA AND TMA-LIPA ASSAYS. Nasreen S. Khan,[1] Sali Aswad,[1] Hamid Shidban,[1] Rafael Mendez,[1] Robert Mendez,[1] Lorraine Comanor.[2] *[1]National Institute of Transplantation Laboratory, National Institute of Transplantation, Los Angeles, CA; [2]Clinical Research Consultant, Palo Alto, CA.*

Introduction: compared to the general population with 0.3-1.5% prevalence of HCV infection, the prevalence among hemodialysis (HD) patients ranges from 10-40%. Early detection of HCV is needed to decrease transmission in HD centers, and to refer patients for their liver disease evaluation prior to kidney transplantation. Active HCV infection can be difficult to detect as serological and biochemical tests may fail to indicate infection. Serum HCV RNA detection by reverse transcriptase PCR may not have sufficient sensitivity, and may be affected by heparin which is used during dialysis. **Objective**:We evaluated whether HCV TMA assay based on transcription mediated amplification (Bayer Diagnostics, CA)could identify HCV infection in a greater number of HD patients than RT-PCR. **Methods:** A total of 2321 patients were screened during Jan.2001-Jan.2002 using HCV EIA 2.0 (Abbott. Laboratories, IL). EIA(+) samples were tested by RT-PCR after RNA extraction(automated NucliSens RNA extraction, bioMerieux, Inc, NC), using primers for 5' untranslated region of HCV RNA. TMA was performed directly on serum samples. cDNA produced during TMA was amplified to determine HCV genotype of discordant samples by a novel TMA-LiPA protocol. **Results:** HCV RNA testing by both PCR and TMA was performed on a subset of 80 EIA(+), and 100 EIA (-)HD patients (table.1)**EIA(+) group:**HCV RNA was detected in 53.8% and not detected in 30% of samples by both assays. Of the 13 discordant samples HCV RNA was detected in 14.7% by TMA but not by PCR, and in 2.2% samples by PCR only. **EIA(-)group**: HCV RNA was detected in 2% of the samples and was not detected in 95% of the samples by both methods. In the remaining 3% samples, HCV RNA was detected by TMA only. **HCV genotyping:** The fact that an HCV genotype was determined by TMA-LiPA assay for 11EIA(+), and 2 EIA(-) samples confirms that samples harbored HCV RNA (table.2). Furthermore, 2 EIA(-)HD patients tested positive by EIA 3.0 provides evidence of HCV infection in these patients. **Conclusisons:** we recommend implementing HCV testing algorithm with currently most sensitive and specific tests available for improved detection and characterization of active HCV infection in HD patients.

HCV RNA detection by PCR and TMA in EIA (+) and EIA(-) patients

# of samples	HCV EIA 2.0	HCV RT-PCR	HCV TMA
43	+	+	+
11	+	-	+
2	+	+	-
24	+	-	-
2	-	+	+
3	-	-	+
95	-	-	-

Resolution of TMA(+)PCR(-)Discordant results

# of samples	HCV genotype
EIA(+)	
5/11	1a
1/11	1b
1/11	1
2/11	3a
2/11	nt
EIA(-)	
2/3	1b
1/3	nt

Abstract# 1371 Poster Board #-Session: P127-III

OUTCOME OF HEPATITIS C VIRUS (HCV)-INFECTED PATIENTS ON THE KIDNEY TRANSPLANT WAITING LIST. Roy D. Bloom,[1] Gabriel Sayer,[1] Peter Abt,[1] Abraham Shaked,[1] Jeffrey Berns,[1] Rajender Reddy.[1] *[1]Departments of Medicine and Surgery, University of Pennsylvania, Philadelphia, PA.*

Though HCV infection is associated with post-transplant morbidity and mortality, the course of HCV-infected patients on the kidney transplant waitlist is poorly characterized. Our program began prospective screening of all kidney and kidney-pancreas transplant candidates for HCV in 1992, initially by serological testing and more recently by RT-PCR for HCV RNA as well. As of 6/30/02, HCV had been identified in 290 patients by at least one of these tests. Thus far, 135 of the 290 patients have been transplanted and 17 others have listed elsewhere. The purpose of this study was to examine outcomes of the residual 138 HCV+ patients who were not transplanted. Seventy nine (57%) of these patients are currently listed for transplantation. The remaining 59 (43%) patients have been removed from the list; 44 of them have died, either before or after delisting. The mean time to death has been 61 ± 10 (SEM) months (range 9-301) since starting renal replacement therapy and 38 ± 4 months (range 1.63-88) since evaluation. Indications for delisting have been death (n=21), as well as non-compliance, advanced liver disease or other comorbidities (n=20, 8 and 10 respectively). An unadjusted analysis demonstrated that, compared to waitlist survivors, those who died were less likely to be African-American (50% vs 67%, P=0.055), prior transplant recipients (4.5% vs 13.8%, P=0.08) or immunized against hepatitis B surface Ag [HbsAb+] (36% vs 52%, P=0.03). The duration of renal replacement therapy prior to evaluation was shorter in patients who died than survived (26 ± 11 months vs 42 ± 9 months, P=0.047). Patients who died were older at evaluation (50 ± 2 yrs vs 46 ± 1 yrs, P=0.04) and had a higher frequency of diabetes (61% vs 35%, P=0.003) than waitlist survivors. There were no differences in gender, transient ALT elevations, requirement for, or modality of dialysis, alcohol or intravenous drug use between patients who died or survived on the list. A multivariate logistic regression model was fit with the above covariates that had unadjusted relationships (P<0.1) with death on the waitlist. By this analysis, only diabetes remained associated with waitlist mortality (OR 3.4, CI 1.4-8.4, P=0.008).**Conclusion: 1.** There is a high rate of mortality among HCV+ patients awaiting kidney transplantation **2.** The risk of death in these patients is strongly associated with the presence of diabetes, a recognized complication of HCV infection. Frequent pre-transplant reevaluation of such individuals is clearly warranted.

Abstract# 1372 Poster Board #-Session: P128-III

LONG TERM OUTCOME OF HEPATITIS C VIRUS (HCV)-INFECTED KIDNEY RECIPIENTS WITHOUT PRE-TRANSPLANT DIABETES. Roy D. Bloom,[1] Gabriel Sayer,[1] Kevin Mange,[1] Alden Doyle,[1] Simin Goral,[1] Robert A. Grossman,[1] Ali Naji,[1] James Markmann,[1] Marty Sellers,[1] Rajender Reddy.[1] *[1]Medicine and Surgery, University of Pennsylvania, Philadelphia, PA.*

HCV is commonly associated with pre- (pre-DM) and posttransplant diabetes mellitus (PTDM) and is a major cause of morbidity and mortality in kidney transplant recipients. Very little data exists regarding chronic survival of nondiabetic patients who receive renal allografts. The purpose of this study was to examine the long-term outcome of 97 HCV+ kidney transplant recipients, not known to have DM pretransplant, who were identified and prospectively followed during the past decade in our transplant program. HCV was discovered before and after transplantation in 83 (67%) and 14 (33%) patients respectively. Mean (± SEM) posttransplant follow up has been 77 ± 5 months. Both CyA (n=65) and tacrolimus (n=32) have been used as primary immunosuppressive agents. 31% patients have developed PTDM, a mean of 13 ± 3 months after transplantation. Twenty nine (30%) patients have died, including 11 patients with functioning kidney transplants. An unadjusted analysis was performed to compare characteristics of patients who died to survivors. Sixty one percent patients who died, returned to dialysis before death, compared to 34% graft failure rate among survivors (P=0.015). Compared to survivors, patients who died had higher rates of acute rejection (69% vs 34%, P=0.006) and attendant use of methylprednisone (69% vs 41%, P=0.02) and antilymphocyte therapy (52% vs 23%, P=0.02). HCV was diagnosed posttransplant in 24% patients who died as opposed to 10% of survivors (P=0.05). There were no significant differences in age, demographics, duration of ESRD, clinically apparent liver disease or infectious complications between the two groups. Posttransplant follow up to the end point of death or current survival has been likewise similar. PTDM occurred in 32% of patients who survived and 27% who died (P=ns). Cox regression was performed on the above covariates that had unadjusted relationships with mortality. Only posttransplant diagnosis of HCV remained associated with mortality (OR 4.0, CI 1-11, P=0.02) **Conclusion: 1.** Late (posttransplant) diagnosis of HCV is associated with an increased risk of mortality in long-term HCV-infected kidney recipients without pre-DM, although clinically apparent liver disease is not increased. **2.** PTDM, a common complication of HCV infection, does not impact on patient outcome within the first decade after transplantation.

Abstract# 1373 Poster Board #-Session: P129-III

RIBAVIRIN PHARMACOKINETICS IS DEPENDENT OF RENAL FUNCTION. Nassim Kamar,[1] Efthymios Manolis,[2] Thierry Lafont,[2] Lionel Rostaing,[1] Etienne Chatelut.[2] *[1]Nephorology, Dialysis and Transplantation, CHU Toulouse-Rangueil, Toulouse, France; [2]Institut Claudius Regaud, Toulouse, France.*

Ribavirin is approved for the treatment of chronic hepatitis C virus (HCV) infection. However, no recommendation exists for dosing patients with impaired renal function. We performed a pharmacokinetic study in 21 HCV positive renal or liver transplant patients (from 27 to 73 years old, M/F: 15/6) treated with ribavirin (1000 mg/d) and α -interferon-2b. The mean creatinine clearance (ClCr) calculated by the Cockcroft-Gault equation was 57 mL/min (17—89). Twelve blood samples were obtained during a 96-h period after the first single administration of 1000 mg. After the first PK/PD profile the patients received ribavirin at 1000 mg/day. A blood sample was taken monthly just before and 2 hours after the oral administration. Plasma ribavirin concentrations were determined by HPLC. 429 plasma concentrations were analyzed by a population pharmacokinetic method using the NONMEM program according to a two-compartment model with first-order absorption, proportional and a combined (i.e., additive plus proportional) model for inter-individual and residual variability, respectively. The mean observed ribavirin apparent clearance (CL/F) was 9.1 L/hr (with an inter-individual variability of 39%), which was lower than the mean values observed in patients with normal renal function that were enrolled in the developmental clinical trials of ribavirin (i.e., 17.9 L/h for females, and 21.5 L/h for males). The influences of the following covariates on CL/F were examined: age, sex, body weight (BW), serum creatinine (Scr), ClCr, hemoglobin, and graft status. CL/F was highly correlated with ClCr ($r = 0.63$, $p<0.01$). The final regression formula was CL/F (L/h) = 32.5(± 3.5) x BW x (1 – 0.0094(± 0.0012) x AGE) x (1 – 0.42(± 0.08) x SEX) / Scr, where sex = 0 for males, and 1 for females; Scr in µmol/l. Gender had a larger influence on CL/F than that corresponding to the Cockcroft-Gault equation (i.e., 15%). This study confirms the need for individual adaptive dosing of ribavirin in patients with renal dysfunction. The equation could support design-specific clinical trials for these patients.

Abstract# 1374 Poster Board #-Session: P130-III

TREATMENT OF HEPATITIS C ASSOCIATED COMPLICATIONS IN RENAL TRANSPLANTATION. Annette Bruchfeld,[1] Annika Wernersson,[2] Lars Stahle.[3] *[1]Renal Medicine; [2]Pathology; [3]Clinical Pharmacology, Huddinge Univ Hospital, Karolinska Institute, Sweden.*

Background:HCV is prevalent in renal transplantation(RTx). Studies show impaired survival in the long-term. HCV nephritis/vasculitis can affect graft survival. Fibrosing cholestatic hepatitis(FCH) has been reported. Therapy in RTx is controversial since interferon(INF) can induce acute rejection. Cases/methods : 2 diabetic patients with renal/pancreas Tx (2 patients)15 and 16 years previously, 1 patient with FSGS with RTx 3 years previously, all HCV-PCR positive. Pat 1 with GFR 25ml/min presented with malaise, weight loss, jaundice,rising creatinine and a liver biopsy consistent with FCH. Pat 2 presented with GFR 10 ml/min,loss of insulin production,weight loss, fever,low-grade CRP,leukopenia,anemia and trombocytopenia. HCV-related vasculitis with cryo, RF, low complements was diagnosed. Pat 3 with GFR 30 ml/min presented with malaise, weight loss, rising liver enzymes and diabetes.Liver biopsy showed toxicity/fibrosis. MMF(MPA/MPAG) was monitored with HPLC assessing AUC. Ribavirin-HPLC measured through levels aiming at 10-15 µmol/L. Outcome: Pat 1 on CyA/cortocosteroids(CS), was given INF 1,5 MU daily aiming at 3 MU three times weekly(t.i.w). CyA was withdrawn,MMF 500mg x 2 was added all resulting in rapid improvement with complete reversal of pathological liver tests and improving renal function. Antiviral therapy was 6 months with sustained viral clearance. Infectious episodes however complicated therapy. Pat 2 on azathioprin(AZA)/CS, was given INF 1,5 MU daily aiming at 3 MU t.i.w eventually switched to pegylated INF 50 µg weekly. AZA was switched to MMF 500 mg x 2. Vasculitic manifestations cleared, insulin production was regained but not renal function. HCV viral load diminished significantly but did not clear. INF was stopped after 9 months on dialysis with no signs of vasculitis. Pat 3 was on FK/AZA/CS. AZA was discontinued.Ribavirin(RIBA) 400 mg daily with RIBA monitoring lead to improved liver function and stabilization of diabetes.RIBA induced anemia was treated with EPO. Liver biopsy 15 months later showed progression with cirrhotic lesions. INF was added to RIBA. 6 months therapy achieved low viral load, normalization of pathological liver tests except γ-GT, but therapy was discontinued due to dehydration and infection.The patient eventually died of liver failure. Conclusion: INF/RIBA or INF/MMF has not been described for HCV in RTx. 3 cases with severe disease manifestations benefited from therapy with no rejections .Combination therapy might possibly lower the rejection risk and make antiviral therapy for HCV more accessible in RTx.

Abstract# 1375 Poster Board #-Session: P131-III

IMPAIRED RENAL ALLOGRAFT BUT NOT PATIENT SURVIVAL IN PATIENTS WITH ANTIBODIES TO HEPATITIS C VIRUS. Louise Giblin,[1] Michael R. Clarkson,[1] Joseph J. Walshe,[1] John Donohue,[1] Patrick O'Kelly,[1] Mary Keoghan,[1] David Hickey,[1] Peter J. Conlon.[1] *[1]Nephrology and Renal Transplantation, Beaumont Hospital, Dublin, Dublin, Ireland.*

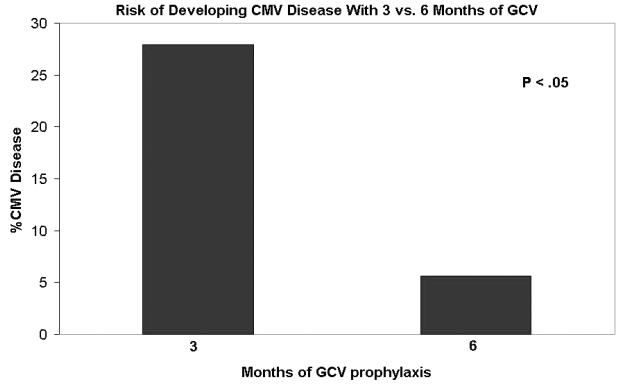
Introduction: The long-term impact of hepatitis C virus (HCV) infection in renal transplant patients remains controversial. The incidence of hepatitis C in the Irish end-stage renal disease (ESRD) population is low by international standards. No data exists regarding the outcome of renal transplantation in this sub-group of Irish ESRD patients. *Methods*: We retrospectively analyzed the outcome of renal transplant patient's known to be hepatitis C antibody positive and compared them to the outcome of the general transplant population. Primary data was obtained from the Irish Renal Transplant Registry. 34 patients received a total of 57 grafts of which four were from living-related donors. The majority of transplants were performed prior to the introduction of prospective testing for hepatitis C in 1991. All patients were treated with standard immunosuppression (steroids, azathioprine, and /or calcineurin inhibition). *Results*: The graft survival rate was significantly less in the HCV positive cases: 70.3, 62.1 and 46.38% at 1, 5 and 10 years respectively, compared with 84.8, 77.4 and 69.0% in HCV negative patients (P<0.0001). Chronic allograft nephropathy was the commonest cause of graft loss. The patient survival rate was similar in both groups: 94.0, 90.7, and 83.5% in the HCV positive patients at 1, 5, and 10 years, compared with 95.2, 91.5 and 83.5% in the HCV negative cases respectively (P = NS). The commonest cause of death in this patient group was cardiovascular complications. *Conclusion*: In the Irish renal transplant population the presence of hepatitis C antibodies, before or after transplantation, is associated with a worse long-term graft, but not patient survival.

Abstract# 1376 Poster Board #-Session: P132-III

LOWER RISK OF DEVELOPING CMV DISEASE IN HIGH RISK PATIENTS WITH 6 MONTHS OF PROPHYLACTIC GANCICLOVIR. Alden Doyle,[1] Simin Goral,[1] Bob Grossman,[1] Roy Bloom.[1] *[1]Renal Electrolyte Hypertension, University of Pennsylvania, Philadelphia, PA.*

Background: Despite oral Ganciclovir prophylaxis, CMV disease continues to be a problem in high risk renal transplant recipients. **Methods:** A retrospective analysis of 104 high risk CRT and LDT patients (CMV positive donor/CMV negative recipient) to compare the tolerability and efficacy of oral ganciclovir for 6 months (n=18) with the standard 3 month course (n=86). **Results:** There were no significant differences in cadaver kidney use, recipient race, gender, age, transplant history, renal diagnosis, or preexisting diabetes between groups. There was a statically significant reduction in the rate of CMV disease in the group treated for 6 months (figure 1). The use of any cytolytic therapy was slightly higher in the 6 months group (93% vs. 74%). The use of Tac, MMF, and Rapa were not significantly different in the two groups. CMV disease manifested as abdominal pain (22% vs. 21%), fever (39% vs. 38%), failure to thrive (9% vs. 8%), or leukopenia (25% vs. 4%), all cases were successfully treated after diagnosis. No patients in either group were unable to complete the course of ganciclovir therapy. **Conclusion:** In high risk renal transplant recipients, six months of oral ganciclovir prophylaxis is associated with a lower rate of CMV disease than three months.

Figure 1



Abstract# 1377 Poster Board #-Session: P133-III

CYTOMEGALOVIRUS DISEASE IN THYMOGLOBULIN TREATED TRANSPLANT RECIPIENTS DESPITE GANCICLOVIR OR VALGANCICLOVIR PROPHYLAXIS. Enver Akalin,[1,2] Vinita Sehgal,[1,2] Scott Ames,[2] Sabera Hossain,[2] Lisa Daly,[2] Barbara Murphy,[1,2] Jonathan S. Bromberg.[2] *[1]Renal Division; [2]Recanati/Miller Transplant Institute, Mount Sinai School of Medicine, New York, NY.*

Cytomegalovirus (CMV) is the most common opportunistic infection following solid organ transplantation. The clinical patterns and predictors of CMV disease in kidney and/or pancreas transplant patients on ganciclovir or valganciclovir prophylaxis were studied. We conducted a retrospective analysis of 129 transplant recipients (112 kidney, 5 combined kidney/liver, 4 combined kidney/pancreas, and 8 pancreas after kidney), who received transplantation between January 1st, 2001 and March 31st, 2002 at our center. CMV disease was diagnosed by positive blood hybrid-capture RNA-DNA hybridization test, or tissue histopathology. CMV seronegative recipients (R) who received organ from CMV seronegative donors (D) without Thymoglobulin induction had acylovir prophylaxis. All other patients received 3 month CMV prophylaxis with oral ganciclovir or valganciclovir. Median follow-up after transplantation was 12 months (range, 6 to18 months). 14 patients received CMV prophylaxis with acyclovir, 68 ganciclovir, and 47 valganciclovir. The overall incidence of CMV disease at 1-year posttransplant was 14% (4% tissue-invasive, 10% non-invasive). Only one patient had CMV disease during 3 month CMV prophylactic period. The remaining 17 patients had diagnosis of CMV after the completion of CMV prophylaxis, a median of 8 weeks later (range, 2-28 weeks). Univariate analysis indicated that induction treatment with Thymoglobulin and CMV antibody status were the strongest predictors for the development of CMV disease. 15 of 60 patients (25%) receiving Thymoglobulin developed CMV disease, representing 83% of the CMV patients. This rate was significantly higher when compared with patients that received basiliximab (7 patients, none had CMV disease) or no induction treatment (only 3 of 62 patients (4.8%) had CMV disease) (p=0.002). D+/R- patients developed higher rates of CMV disease (8 of 17 patients (47%)), when compared to D+or-/R+(9 of 84 patients (10.7%)) or D-/R- group (1 of 28 patients (3.6%)) (p=0.0002). CMV incidence was not statistically different between patients treated with ganciclovir or valganciclovir (15% vs 17%, respectively). CMV disease continues to be prevalant in organ transplant recipients, who received Thymoglobulin induction treatment or seronegative recipients of seropositive donors, despite the introduction of valganciclovir. These patients may require longer prophylaxis, or other treatment options to decrease the incidence of CMV disease.

Abstract# 1378 Poster Board #-Session: P134-III

TRANSIENT CMV ANTIGENEMIA IN INTERMEDIATE RISK (R+/D+OR-) KIDNEY TRANSPLANT RECIPIENTS WITHOUT ANTIVIRAL PROPHYLAXIS IS A COMMON EVENT AND IS NOT ASSOCIATED WITH AN ADVERSE SHORT-TERM GRAFT OUTCOME NOR OVERT CMV CLINICAL DISEASE. Ramon Vanegas-Carrero,[1] Juan G. Sierra-Madero,[2] Teresa Munoz,[2] Luis E. Morales-Buenrostro,[1] Luis Gonzalez-Michaca,[1] Ricardo Correa-Rotter,[1] Josefina Alberu.[3] *[1]Nephrology, Inst. Nal. Ciencias Medicas Y Nutricion S.Z., Mexico, City, Mexico; [2]Infectious Disease Dept, Inst. Nal. Ciencias Medicas Y Nutricion S.Z., Mexico, City, Mexico; [3]Transplantation, Inst. Nal. Ciencias Medicas Y Nutricion S.Z., Mexico, City, Mexico.*

Pretransplant CMV seropositive kidney transplant recipients (KTR) carry a low risk for CMV disease [unless they receive antilymphocyte therapy (ALT)], however, administration of antiviral prophylaxis is a common management strategy with the theoretical objective of avoiding the indirect effects of CMV. This prospective study evaluated the incidence of CMV infection/disease in this group of patients and its impact on short-term graft function, in the absence of CMV prophylaxis. **Methods**: 33 adult KTR IgG/CMV seropositive before transplant were included. All received triple drug immunosuppressive therapy without ALT induction. Clinical and laboratory information were closely monitored during the follow up (f-u) period. Acute rejection (AR) episodes were biopsy confirmed. CMV *pp65* blood antigenemia assay was performed biweekly for the first 3 months posttransplant and then monthly up to 6 months. If positive (one antigen positive cell/150x10^3), the antigenemia assay was performed weekly. No CMV prophylaxis was given. **Results.** At a mean of 138±64 days posttranplant f-u, 24 (73%) KTR remained antigenemia negative, and 9 (27%) developed a positive result in the assay; only one of the latter developed mild symptoms suggestive of CMV disease. The time elapsed between the transplant and the first positive antigenemia was 17± 6 days; the number of positive cells/assay fluctuated from 1 to 1900, and the mean duration of antigenemia before its spontaneous disappearance was 31±52 days. The mean SCr was 1.35±1.08 and 1.4±0.51 mg/dl before the first positive antigenemia, and at the last f-u visit, respectively (p=0.37). For those who remained antigenemia negative during f-u, the SCr was 1.16±0.6 and 1.16±0.37 mg/dl at one month and last visit, respectively. The overall incidence of AR was 18.2%, with no difference between antigenemia + and negative patients. Patients treated for AR with methylprednisolone had a 50% incidence of CMV antigenemia which appeared after rejection therapy. **Conclusions.** Transient CMV antigenemia with no clinical consequences either in the short-term graft function or in CMV disease, is a frequent event in KTR with intermediate risk. Even though this is a small sample, the results obtained question the current widely used strategy of universal prophylaxis for all KTR.

Abstract# 1379 Poster Board #-Session: P135-III

VALGANCICLOVIR PROPHYLAXIS FOR CYTOMEGALOVIRUS DISEASE IN KIDNEY AND SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANT RECIPIENTS. Gaetano Ciancio,[1] George Burke,[1] Leibovici Zvi,[1] David Roth,[2] Warren Kupin,[2] Anne Rosen,[1] Adela Mattiazzi,[2] Joshua Miller.[2] *[1]Surgery, University of Miami, Miami, FL; [2]Medicine, University of Miami, Miami, FL.*

Introduction and Objective: Concerns have been raised about the possibility of increased of cytomegalovirus (CMV) disease as result of the use of potent immunosuppressive drugs like Daclizumab, tacrolimus, mycophenolate mofetil, cyclosporine and rapamycin, which are used routinely in our transplant program. We are assessing prospectively the efficacy and safety of oral valganciclovir as prophylaxis for CMV disease in patients with highly potent immunosuppression. **Methods:** 163 consecutive patients undergoing renal (Kid) or simultaneous pancreas-kidney (SPK) transplantation starting July 2001 were enrolled. Valganciclovir is administered orally 900 mg once daily for 3 months. Dosage is adjusted according to serum creatinine. Blood was sampled before transplantation from the donors and recipients for CMV serology and nested polymerase chain reaction (PCR) for CMV DNA, and after transplantation from the recipients only at monthly intervals until 6 months. Patients are observed for the development of any CMV-like illness during follow-up. Daclizumab (1 mg/kg) was given as induction therapy on the day of surgery, and every other week for a total of 5 doses. **Results:** A total 163 recipient-donor pairs have been enrolled so far. Immunosuppression was tacrolimus-mycophenolate mofetil based in 105 patients (Kid= 92, SPK= 13), cyclosporine-rapamycin in 12 (Kid= 12), and tacrolimus-rapamycin based in 31 (Kid= 16, SPK= 15). The CMV serologic status was D+/R+ in 66, D+/R- in 29, D-/R- in 21 and D-/R+ in 47 pairs. Among the 163 patients one developed CMV-Hepatitis and no side effects by oral valganciclovir has been seen so far. **Conclusions:** Valganciclovir is safe and effective as prophylaxis for cytomegalovirus disease in kidney and simultaneous pancreas-kidney transplant recipients who have received highly potent immunosuppression.

Abstract# 1380 Poster Board #-Session: P136-III

A SURVEY ON THE CLINICAL MANAGEMENT OF CMV INFECTION RISK IN KIDNEY TRANSPLANT RECIPIENTS IN FRANCE. Anne Crochard,[1] Christophe Legendre,[2] Yvon Lebranchu,[3] Isabelle Durand-Zaleski,[4] Claire Pouteil-Noble,[5] Stephen Beard.[6] *[1]Laboratoire GlaxoSmithKline, Marly le Roi, France; [2]Service de Néphrologie, Hôpital Saint-Louis, Paris, France; [3]Service de Néphrologie, Hôpital Bretonneau, Tours, France; [4]Service de Santé Publique, Hôpital Henri Mondor, Créteil, France; [5]Service de Néphrologie, Hôpital Lyon-Sud, Pierre Bénite, France; [6]RTI Health Solutions, Manchester, United Kingdom.*

Introduction : Prophylaxis practices of CMV infection after kidney transplantation are heterogeneous. The aim of our study was to obtain information about current clinical practices of prevention of CMV in France. Methods: A postal survey questionnaire was sent to the 35 adult renal transplantation centers throughout France related to year 2000 data. Informations collected were: number of renal transplantations, proportion of patients in CMV risk groups (donor/recipient CMV serostatus and the prevention strategy against CMV infection according to the CMV risk group. It also focused on the numbers of CMV infections and diseases observed and their current treatments. Results: Overall 31 centers completed the survey (89%). The centers performed 1641 adult transplantations during the year 2000. The average number of renal transplantations per center was 53 per year, ranging from 20 to 140. Around 20% of patients were considered at high risk of CMV infection (D+R-), 59% were R+ and 21% were at low risk (D-R-). However, the distribution of these risk groups varied widely across centers.The most commonly adopted strategy in the D+R- group was prophylaxis, alone in 58% of cases and combined with a pre-emptive strategy in 31%. In this group oral valaciclovir alone was chosen in 51% of patients receiving prophylaxis treatment whereas ganciclovir alone was used in 37%. In the group of R+ patients, treatment strategies appeared to be the same irrespective of the donor CMV status and the most frequently used strategy was pre-emptive therapy alone (42%). In this group oral valaciclovir alone was chosen in 55% of patients receiving prophylaxis treatment whereas ganciclovir alone was used in 41%. With regard to D-R- patients, no prophylaxis was done and the most common approach was pre-emptive treatment in 63% of cases. The numbers of cases of CMV infections per year were estimated at 164 CMV syndromes and 52 CMV tissue invasive diseases predominantly in D+R- patients. Whatever the severity of the disease, IV ganciclovir was the most frequently formulation drug used. Conclusion: There is an important heterogeneity across centers regarding clinical practices of CMV prevention and treatment of CMV diseases following renal transplantation in France. Because 21% of high risk patients (D+/R-) still develop CMV diseases, CMV infection remains a challenge for the transplantation community.

Abstract# 1381 Poster Board #-Session: P137-III

CYTOMEGALOVIRUS (CMV) PROPHYLAXIS WITH VALGANCICLOVIR (VAL) BASED ON DONOR/RECIPIENT (D/R) SEROSTATUS IN AFRICAN-AMERICAN (AA) RENAL ALLOGRAFT RECIPIENTS (RAR). J. Garnick,[1] A. Haririan,[2] D. Sillix,[2] M. West,[3] D. Granger,[3] S. Penumalee,[2] K. Morawski,[3] S. Gruber.[3] *[1]Pharmacy, Harper University Hospital, Detroit, MI; [2]Nephrology/ Medicine, Wayne State University School of Medicine; [3]Transplant Surgery, Wayne State University School of Medicine, Detroit, MI.*

Use of intravenous (IV) or oral ganciclovir (GCV) for CMV prophylaxis in RAR is well established, with reported infection rates of 10-15% overall and 25-35% in the D+/R- group. In contrast, there is a paucity of data examining the efficacy of the prodrug VAL in RAR, particularly in the AA population. AAs are potentially at higher risk for developing CMV infection due to greater use of antilymphocyte induction and higher doses of mycophenolate mofetil (MMF). Therefore, we describe our experience using VAL in 49 adult AA RAR [14 living-donor (LD); 13 retransplants; recipient age = 45.5 ± 10.8; mean time ESRD = 6 years] transplanted from 10/31/01 to 9/3/02 and followed for 8.2 ± 3.5 (range 3-13) months (mo). VAL was dosed once daily for 90 days according to D/R serostatus: high risk [D+/R-] received 900 mg (n = 7); moderate risk [D+/R+, D-/R+] received 450 mg (n = 38); and low risk [D-/R-] received no prophylaxis (n = 4), with adjustment for renal function. Thymoglobulin® (5-11 days; n = 22) or basiliximab (BSX) (n = 27) was used for induction depending on patient risk factors. Initial maintenance immunosuppression consisted of MMF 1 g BID, prednisone taper, and either tacrolimus (TAC) (n = 23) or sirolimus (n = 26), depending on the absence or presence of delayed/slow graft function, respectively. Target trough levels were 10-15 ng/ml for both agents. Overall, only two patients (4%) developed symptomatic CMV infection (pp65 antigenemia), and there were no cases of tissue invasive disease. Both patients were primary, LD RARs in the D+/R- group (2/7, 29%) and received BSX and TAC. Case 1 occurred at 6 mo posttransplant. Case 2 occurred at 4 mo in conjunction with enterococcal bacteremia. CMV infection resolved in both cases following sequential IV GCV and VAL therapy without relapse. Case 2 developed significant leukopenia following combination VAL and linezolid treatment, which resolved upon discontinuation of the latter. This patient subsequently developed grade IA acute rejection (AR), resolving completely with steroids. Only 2 other patients developed AR, both with noncompliance (overall AR = 6%). We conclude that VAL dosed according to D/R serostatus provides very effective CMV prophylaxis in the high-risk AA RAR. Given the absence of CMV infection in the moderate-risk group, the daily VAL dose may be reducible to 450 mg in the D+/R- group without sacrificing efficacy.

Abstract# 1382 Poster Board #-Session: P138-III

PROCALCITONIN—A VALID SCREENING MARKER IN EARLY DIAGNOSIS OF HCMV INFECTION IN RENAL ALLOGRAFT RECIPIENTS. Bin Liu,[1] Changsheng Ming,[1] Fanjun Zeng,[1] Zhengbin Lin,[1] Weijie Zhang,[1] Zhisui Chen,[1] Shi Chen.[1] *[1]Institute of Organ Transplantation, Tongji Hospital, Wuhan, Hubei, China.*

Objectives To investigate the role of the new inflammation marker procalcitonin (PCT) in early differential diagnosis of human cytomegalovirus (HCMV) infection in renal allograft recipients. **Materials and Methods** Thirty-six renal transplant recipients (30 males, 6 females, whose mean age were 34 years, ranging from 21 to 63 years old) with fever were enrolled. The duration of postoperation time were 1 month to 46 months. Twenty recipients were diagnosed as having bacterial respiratory tract infections by detection of peripheral blood leukocyte (PBL) and pathogen culture in body fluid in combination with clinical symptoms and chest x-ray test, Twelve were diagnosed as HCMV infection by using ELISA to test anti-HCMV IgG, IgM from serum samples and immunohistochemistry method to test HCMV antigen expression from urine. The other four were diagnosed as superinfection with both bacterium and HCMV. Besides, plasma samples were analyzed using a semi-quantity BRAHMS PCT-Q test in all patients and results were observed in an hour. Therapeutic protocols included adjustment of immunosuppressive regimes, oxidation therapy and use of antibiotics or ganciclovir, etc. **Results** Anti-HCMV IgM titres were elevated significantly in 9/16 patients with the positive rate being 56.3%, yet HCMV antigen was detected in 16/16 patients with the positive rate being 100%. PCT values kept normal (<0.5ng/ml) in 12/12 patients with simple HCMV infection, but increased moderately or significantly (>10ng/ml) in the other patients with bacterial infections or superinfection. PCT levels decreased to normal range gradually in 20 cases in which infections were controlled effectively, while 4 with continuous elevated PCT levels died finally due to respiratory failure and MSOF. **Conclusions** HCMV antigen detection is a sensitive and specific method for HCMV infection but not a quick indicator since it may take 3 days to obtain results. PCT has been proven to be a reliable and fast diagnostic parameter for systemic bacterium, fungus and parasite infections. In those renal transplant recipients with sepsis who remain normal ranges of plasma PCT level, HCMV infection may be considered firstly. Thus PCT is a valid screening marker for early diagnosis of HCMV infection.

Abstract# 1383 Poster Board #-Session: P139-III

FLUCONAZOLE IS EFFECTIVE IN DECREASING THE INCIDENCE OF COCCIDIOMYCOSIS IN KIDNEY TRANSPLANTS IN AN ENDEMIC AREA. Vimal Vermani,[1] Janis E. Blair,[2] Joy L. Logan.[1] *[1]Department of Medicine, University of Arizona, Tucson, AZ; [2]Division of Infectious Diseases, Department of Internal Medicine, Mayo Clinic Hospital, Phoenix, AZ.*

Background: Coccidioidomycosis (cocci) is a fungal infection endemic to the southwestern USA. In immunocompetent persons, coccidioidal infection ranges from clinically inapparent to symptomatic pulmonary infection, rarely with dissemination. Symptomatic cocci occurs more frequently and has a higher likelihood of dissemination, morbidity and mortality in immunocompromised persons such as transplant recipients. Prior to the use of antifungal prophylaxis, Cohen et al reported 18 coccidioidomycosis (cocci) infections in 260 renal transplant patients (6.9%) in Arizona, during the 1970's. **Aim:** To determine the incidence of coccidioidal infection among kidney transplant recipients using fluconazole as prophylaxis. **Methods:** We retrospectively reviewed the records of 199 patients undergoing renal transplantation at our centers since 1998. Recipients with a prior history of cocci or positive cocci serology received lifelong prophylaxis with daily fluconazole. All other patients were considered at low risk for coccidioidal infection and received prophylaxis protocols adopted by our centers. **Results:** Eleven patients with a history of cocci infection and 5 with positive serologies prior to transplantation were initially identified to receive lifelong prophylaxis; 14 of these continued prophylaxis indefinitely. None of these 14 patients on lifelong fluconazole had recurrent cocci. The other two patients only took fluconazole for six months. The remaining 183 patients received fluconazole for either one month (n=91), one to three months (n=8), or three to six months (n=83). One patient did not receive any prophylaxis. Four patients developed clinical cocci an average of 10 months (4 months to 2 years) after transplantation. These four had not been identified as high risk patients and received fluconazole prophylaxis. Two of these 4 were still receiving prophylaxis at the onset of their clinical cocci infection. The infection was controlled in three patients and the fourth died of disseminated disease. The overall incidence of cocci infection in these patients was 2.0% **Conclusions:** Fluconazole effectively decreased the incidence of cocci from 6.9% to 2.0% and prevented reactivation of prior infection in kidney transplant recipients, although comparison with historical controls is confounded by differences in immunosuppression. While the best dose and duration of fluconazole therapy remains uncertain, these data suggest that prophylaxis is warranted in endemic areas.

Abstract# 1384 Poster Board #-Session: P140-III

PROBLEMS OF POST TRANSPLANT TUBERCULOSIS IN A DEVELOPING COUNTRY. Anwar Naqvi,[1] S. Adibul Hasan Rizvi,[1] Zafar Hussain,[1] Naqi Zafar,[1] Ejaz Ahmed,[1] Sajid Sultan.[1] *[1]Transplantation, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Sindh, Pakistan.*

Purpose: To study the High incidence of post transplant tuberculosis (PTTB) and the problems in diagnosis and treatment. Methods: Between 1986-2000, 958 patients received a intrafamilial transplant. Of these 142 (14.8%) developed PTTB. Initially diagnosis was based on clinical, radiological, smear and histological criteria. Later diagnosis was based mainly on culture, DNA based PCR methods and histology. Immunosuppression was by triple drug regimen in majority. Anti TB therapy was Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PZA) and Ethambutol (ETH) for 2 months and RIF, INH, ETH or RIF, INH, PZA for 7 months in 98 patients and RIF, INH, ETH for 2 months and RIF, INH for 7 months in 44 patients. At initiation of ATT CyA dose was increased by 75%. Abbreviated AUC was undertaken on day 10 for CyA adjustment. Last 76 transplants have been randomized into two groups, one receiving INH prophylaxis and the other not. Statistical analysis included Kaplan Meier survival estimates and Student's t test. Results: The time of onset on PTTB was 0-6 month in 30%, 7-12 months in 22% and beyond 12 months in 48%. Mean age was 30 years (Range 10-50). The site of TB was pulmonary in 54%, extra pulmonary in 29%, disseminated in 3% and remained unknown in 14% (largely in the initial cases). Initially clinical/radiology constituted diagnostic basis in 52%, culture/smear 35% and histology 10%. In the latter part of the study culture/PCR constituted 51%, clinical/radiology 20% and histology 33%. The common problem with ATT therapy was interaction of Rifampicin with CyA. Pre ATT CyA Co was 260 ng/ml and mean dose 328 mg/day while on ATT Co was 185ng/ml and dose increased to 512mg/day. The cost of drug in TB group for 9 months was $5000 vs $2000 in non TB group ($p<0.01$). Mortality rate was 29%. Of the 41 deaths, 18 died with functioning graft. The major cause of death was infection (Non TB) in 28%, fulminant TB in 20% and dialytic non compliance 14%. Overall patient survival at 1 and 5 year was 87% and 70% respectively. Of the 38 patients on INH prophylaxis none developed TB as compared to 3 in non INH group. Conclusion: The high incidence of PTTB remains a challenge for diagnosis and treatment. PCR method has helped in early diagnosis. Cost of therapy remains high due to necessary use of Rifampicin as TB is resistant to other drugs. INH prophylaxis has encouraging initial results and needs further assessment.

Abstract# 1385 Poster Board #-Session: P141-III

RISK FACTORS AND ADVERSE EFFECTS OF URINARY TRACT INFECTION IN LIVE RELATED RENAL ALLOGRAFT RECIPIENTS. Zafar Hussain,[1] S. Adibul Hsan Rizvi,[1] Anwar Naqvi,[1] Naqi Zafar,[1] Ejaz Ahmed,[1] Sajid Sultan.[1] *[1]Transplantation, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Sindh, Pakistan.*

Purpose: To evaluate the impact of persistent or recurrent urinary tract infection especially with resistant organism on live related renal allograft function and survival. Methods: One hundred renal transplantation performed in 1997 and followed up for 1 year were analysed for urinary tract infection. All patients were on Co-trimoxazole for prophylaxis against pneumocystic carini infection. Immunosuppression was by triple drug regimen. Results: There were 75 males and 25 females. Forty six patients developed at least one episode of infection with majority of the first episode occurring in the first 2 months after transplantation. In thirty-two, infection was persistent or recurrent. E. coli and pseudomonas accounted for 88% episodes and almost all were resistant to Co-trimoxazole. Incidence was significantly higher in females compared to males (76% vs 36%, p = 0.001). Patients with infection had a higher prevalence of anatomically abnormal tract (28%) compared to those without infection (8%) (p = 0.001). Ureteric stent was present in 71% of infected patients but only in 40% of non-infected patient (p = 0.002). Infection was also higher in those who experienced rejection compared to rejection free patients, (72 % vs 40%, p = 0.001). At 1 year post transplant serum creatinine exceeding 2mg/dl was seen in 36.9% of infected patients and 16% of infection free patients. Five grafts were lost in infected patients and one in non-infected group. Conclusion: Co-trimoxazole has not been effective for prophylaxis and against urinary tract infection due to emergence of resistant strains of uropathogen. Female sex, placement of stents, anatomical abnormalities and rejection emerged as significant factors leading to urinary tract infection. Graft function was poor in the infection group after 1 year of transplantation. Strategies to prevent and aggressively treat urinary tract infection needs reassessment.

Abstract# 1386 Poster Board #-Session: P142-III

***MYCOPLASMA HOMINIS* SEPTIC ARTHRITIS IN A PEDIATRIC RENAL TRANSPLANT RECIPIENT.** Ayesa Mian, Alan Farney, Susan Mendley. *[1]U Maryland, Baltimore, MD.*

Septic arthritis (SA) usually occurs in young children, often from Staphylococcus. With chronic immunosuppression, SA may be subtle, caused by atypical or fastidious organisms. This15 yr old African-American female without previous joint disease developed *Mycoplasma hominis* SA in her right hip two months after cadaveric renal transplant (Tx). Initial immunosuppression was basiliximab, prednisone, tacrolimus (target 12-15 ng/ml) and mycophenolate (1 g BID). Early post-Tx course included acute vascular rejection, Banff IIA, treated with 10 days of thymoglobulin (Cr from 6 to 1.8 mg/dl), as well as hypoalbuminemia, anasarca, and failure of wound closure, but no infection. Six weeks post-Tx, leukopenia occurred (WBC 2600 /mm³) with right leg pain and hip flexor weakness without trauma. She was afebrile. Radiographs of hips and knees were normal. Over three weeks, pain localized to the lateral aspect of the thigh/hip with worsening leg weakness and a new limp. Labs: stable Cr 1.6 mg/dl; WBC 4700/mm³, 83% PMNs, 8% bands; ESR 69 mm/hr; and CRP <0.5 mg/dl. Narrowing of the hip joint space was then seen on radiograph; MRI showed moderate joint effusion. Aspiration yielded 10 ml of turbid fluid, with WBC 79,000/mm³ (98% PMN) but no organisms, crystals, or acid fast bacilli were seen. The joint was incised and drained with empiric therapy of Vancomycin and Ceftriaxone; immunosuppression was reduced. Synovial fluid cultures grew Mycoplasma after 5 days; blood and urine cultures were sterile. Antibiotics were changed to doxycycline and levofloxacin. Later identification showed *M. hominis*, sensitive to doxycycline (MIC<0.03 mcg/ml) and levofloxacin (MIC<0.5 mcg/ml). After eight weeks of antibiotic therapy, hip function improved and gait normalized. Sixteen months later there has been no relapse of *M. hominis* infection. Radiographs show joint space narrowing from previous cartilaginous destruction. This case is instructive in that the presentation of SA was subtle and indolent, without fever or leukocytosis. While *M. hominis* is reported in adult Tx patients, often in infected wounds, it has not been described in pediatric recipients. A high index of suspicion for atypical organisms is required to detect *M. hominis*. The fairly intense early immunosuppression may have contributed to the susceptibility to this organism. Optimal therapy for *M. hominis* SA is not well established and relapses occur. However, our patient has remained free of ongoing infection with stable graft function (Cr 1.4 mg/dl) on moderate immunosuppression with prednisone, tacrolimus and mycophenolate.

Abstract# 1387 Poster Board #-Session: P143-III

OUTCOMES OF LAPAROSCOPIC DONOR NEPHECTOMY AND LIVING DONOR RENAL TRANSPLANTATION IN THE MORBIDLY OBESE. Julie K. Heimbach,[1] Sandra Taler,[1] Michael Ishitani,[1] George Chow,[1] Mark D. Stegall,[1] Mikel Prieto.[1] *[1]Division of Transplantation Surgery, Mayo Clinic and Foundation, Rochester, MN.*

Background: Over the past two decades, the prevalence of obesity (BMI ≥ 30 kg/m²) and in particular morbid obesity (BMI>35 with an associated comorbidity) has increased markedly. Diabetes mellitus is a common comorbidity and the most common cause of end stage renal disease. While the survival benefits of living donor renal transplantation are well demonstrated, the outcomes of living donor kidney transplantation involving either morbidly obese donors or recipients is not known. **Methods**: We examined outcomes for patients undergoing laparoscopic donor nephrectomy and living donor transplantation from January 2000 through September 2002. We assessed donor outcomes including operative time, peri-operative complications, hospital stay and residual renal function, and recipient outcomes including graft function and peri-operative complications. **Results**: Of 467 consecutive laparoscopic renal donors, there were 39 patients with a BMI≥ 35 (mean 37.8, range 35 to 50). The mean operative time was similar for the morbidly obese versus non-morbidly obese donors (198.2 ± 41.2 minutes vs. 181.9 ± 39.9 minutes, p=.42.). Wound complications were more frequent in the morbidly obese group (8% vs.3% , p≤0.01) though the overall complication rates (including conversions to open nephrectomy) were similar. Average hospital stay was also similar in both groups (2.1 vs. 2.4 days). There were no deaths or serious complications in either group. Residual renal function in the morbidly obese donor measured by iothalamate clearance was 74 ml/min/SA (range 59-111). Of 505 consecutive living donor renal transplant recipients, 47 had a BMI ≥ 35 (mean 38.6, range 35-50). Morbidly obese recipients had a similar incidence of wound infections (5.3% and 1.3%, p=0.1) and lymphoceles (4.2% and 1.2%, p=0.2). Overall graft survival was lower in the obese group compared to the non-morbidly obese group (89.4% vs 96.3% p=0.03). Recipient survival was 100% in the morbidly obese group and 98.1% in the non-obese group. **Conclusions**: With careful patient selection, morbid obesity does not pose undue risk to either donor or recipient. Given the severe and increasing shortage of cadaver organs and the superior outcome for live donor recipients, acceptance of morbidly obese donors appears to be justified. While morbidly obese recipients are at slightly higher risk for graft failure, denying access to transplant based on morbid obesity alone does not appear to be justified.

Abstract# 1388 Poster Board #-Session: P144-III

CADAVERIC RENAL TRANSPLANTS WITHOUT DELAYED GRAFT FUNCTION HAVE OUTCOMES EQUIVALENT TO LIVING DONOR RENAL TRANSPLANTS: A SIX-YEAR EXPERIENCE FROM TWO TRANSPLANT CENTERS. Angelo N. Arnold,[1] Lorita M. Rebellato.[2] *[1]Transplant Immunology and Renal Transplant Program, Sentara Norfolk General Hospital, Norfolk, VA; [2]Departments of Pathology and Surgery, East Carolina School of Medicine, Greenville, NC.*

Patients with end-stage renal disease can have three transplant options; a living-related donor (LRD), a living-unrelated donor (LURD), or a cadaver donor (CAD) transplant (TX). Some patients struggle with the decision to accept this gift from a LD and many patients do not have a LD option. An important part, of the decision process, is accurate information concerning the potential for a good outcome with different donor types. We report our combined experience for 610 renal TXs, with and without delayed graft function (DGF), defined as any dialysis in the first week after operation. All TXs were performed from 1995 through 2001 at Sentara Norfolk General Hospital, Norfolk, VA or East Carolina University School of Medicine, Greenville, NC. The analysis included LD TXs with immediate function (IF) and excluded peri-operative technical failures or deaths, and incomplete records (N=36). The recipients were 46% Female, 55% African American (AA), 89% first transplant, and 8% pediatric. The average recipient age was 43 years (range 2-77 years). Immunosuppression was primarily cyclosporine, mycophenolate mofetil, and prednisone. Induction with an antilymphocyte cytolytic agent or IL2 receptor antibody was given to 66% of the patients for high immunological risk and/or calcineurin inhibitor sparing. The 610 TXs were divided into CADIF (N=200), LRDIF (N=250), LURDIF (N=59) and CADDGF (N=101) groups. We report that the CADIF group had a similar TX outcome as the LRD and LURD groups. The CADIF group was more sensitized than the LRDIF and LURDIF groups (p<0.001) and had more HLA mismatches than the LRDIF group (p<0.002). In contrast to the CADIF group, the CADDGF group did poorly.

Impact of Donor Source and Function on TX Outcome

	CADIF	LRDIF	LURDIF	CADDGF
Actuarial PS 5 Years	96.6%	92.2%	93.3%	74.5%
Actuarial GS 5 Years	70.8%	75.9%	66.4%	43.7%
Actuarial GS 5 Years AA Recipients	63.5%	66.3%	60.3%	37.7%
Serum Creatinine at Year 3 (mean±SEM)	1.7±0.1	1.5±0.1	1.7±0.1	2.1±0.2
Peak T cell PRA (mean±SEM)	20±2%	7±1%	3±1%	30±4%
HLA-ABDR Mismatch (mean±SEM)	2.7±0.1	2.3±0.1	4.2±0.1	3.0±0.2

Conclusion: CAD TXs without DGF have PS, GS and renal function, as measured by serum creatinine at year three, that is equivalent to LD TXs. Recipient and donor factors that can reliably predict DGF should be identified. This information can be used to make an informed decision about transplantation. Preventing DGF will improve long-term outcome.

Abstract# 1389 Poster Board #-Session: P145-III

FENOLDOPAM FOR THE PREVENTION OF DELAYED GRAFT FUNCTION IN RENAL TRANSPLANTATION. Nicolas A. Muruve,[1] Joseph Tobias,[2] Andi Arnautovic,[1] Indira Arnautovic.[1] *[1]Surgery / Urology, University of Missouri, Columbia, MO; [2]Anesthesia, University of Missouri, Columbia, MO.*

INTRODUCTION: Cadaveric donors are the main source of kidneys for renal transplantation. In order to shorten wait time, methods to expand the donor pool include using older donors, non heart-beating donors and dual kidney transplants have been sought. These organs can be associated with an increased risk of delayed graft function (DGF) which can shorten graft survival and increase hospital costs. We designed a pilot study to determine if fenoldopam, a selective renal vasodilator that is an agonist for D1-like dopamine receptors, can reduce the rate of DGF in cadaveric renal transplantation.METHODS: 20 consecutive recipients of cadaveric renal transplants were selected to receive fenoldopam. This was compared with the 20 most recent transplants performed at our institution prior to this study. Fenoldopam was administered at an initial rate of 0.2 mcg/kg/min and continued for 48 hours or stopped when the patient was transferred out of the ICU. DGF was defined as dialysis in the post-op period or a serum creatinine that did not drop from pre-op values after surgery. Donor age, cause of death, HLA mismatch and cold ischemia times were recorded. Side effects from fenoldopam and dose used were noted. DGF rates in the study group were then compared with that of the historical control group. 1 tailed t-test was used for statistical analysis.RESULTS: To date a total of 16 patients have enrolled in the study. No statistically significant difference was found between control and study patients respectively in recipient age (mean 52 vs. 46) p=0.11, donor age (34.1 vs. 34.3) p=0.64, and cold ischemia time (1238 min vs.1063 min) p=0.2. Antigen mismatch also was similar between control and study patients (3.6 vs 3.3). There were 7 females in both the control and study groups. Donor cause of death was also similar with 4 of 16 (25%) donors dying from CVA in the study group versus 5 of 16 (31%) donors in the control group. Fenoldopam did appear to improve early graft function with a total of 9 of 16 (56%) control patients experiencing DGF versus 4 of 16 (25%) in the study group. Mild hypotension with fenoldopam was observed that responded to dose reduction. Dose ranged from 0.05 mcg/kg/min to 0.5 mg/kg/min.CONCLUSIONS: Our preliminary data suggests that fenoldopam use in cadaveric renal transplantation reduces DGF rates. Complications appear minimal as all patients tolerated the drug. We anticipate continued accrual to this study.

Abstract# 1390 Poster Board #-Session: P146-III

IMMUNOSUPPRESSION DOES NOT REQUIRE MODIFICATION IN PATIENTS WITH DELAYED RENAL ALLOGRAFT FUNCTION. Barry J. Browne,[1] Cynthia Op't Holt,[2] Osemwegie E. Emovon.[3] *[1]Surgery, Baystate Medical Center, Springfield, MA; [2]Surgery, Mayo Clinic, Jacksonville, Jacksonville, FL; [3]Medicine, Medical University of South Carolina, Charleston, SC.*

Delayed graft function (DGF) is commonly believed to adversely impact both short- and long-term renal allograft outcome. Because immunosuppressive therapy is commonly altered after DGF is identified, retrospective analyses are difficult to interpret. We therefore prospectively sought to examine the natural history of DGF in a controlled patient population under identical immunosuppressive protocols. Methods: Adult patients undergoing cadaveric renal transplantation were treated with sequential triple drug immunotherapy. High-dose steroids were administered in the operating room and rapidly tapered to 20mg prednisone by POD #6. Cyclosporine (CsA) microemulsion was begun on POD #1, and dosed asymmetrically at 12hr intervals to reach a daytime Cav of 650 ng/ml (utilizing 2hr and 6hr levels) while PM doses were adjusted to an AM trough of 300ng/ml. Mycophenolate(1000 mg q12hr) was added on POD #3 in most patients and discontinued after 3 months. No induction agents were used. All patients were followed for at least 1 year. Results: 60 consecutive patients received 64 allografts (4 double grafts). 17 patients required dialysis and were considered to have DGF. Eight of these patients received marginal organs turned down by at least one other center. Cold ischemia time was significantly longer in patients with DGF (24hr vs 19hr, p<0.01). All patients were treated as planned and there were no major protocol violations. One patient had primary nonfunction and was excluded from analysis. CsA trough and Cav values were similar between groups. Eight patients required biopsy during the first month for failure to improve but acute rejection was only identified in one patient. No patient required dialysis after POD #14. Mean serum creatinine levels (mg/ml) were:

*p<0.05	Week 1*	Month 1*	Month 3	Month 6	Year 1
DGF(n=17)	10 ± 1.3	2.0 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1
No DGF	2.1 ± 0.2	1.5 ± 0.1	1.5 ±0.1	1.5 ± 0.1	1.5 ± 0.1

Although serum creatinine levels fell more slowly in patients with DGF, there was no significant difference by 3 months and the creatinine clearance was not significantly different between groups after 1 year (71cc/min in DGF vs 61cc/min, p=0.13). Our data demonstrate that alterations in routine immunosuppressive strategies may not be necessary to achieve equivalent outcomes in patients with DGF.

Abstract# 1391 Poster Board #-Session: P147-III

A COMPARATIVE SIDE EFFECT PROFILE AMONG RENAL TRANSPLANT RECIPIENTS TREATED WITH SIROLIMUS OR MYCOPHENOLATE MOFETIL PLUS A CALCINEURIN INHIBITOR AND CORTICOSTEROIDS AT A SINGLE TRANSPLANT CENTER. Marc I. Lorber,[1] Richard N. Formica,[2] Kathleen M. Lorber,[1] Margaret J. Bia,[2] Douglas J. Smith,[2] Giacomo P. Basadonna,[1] Fadi Lakkis,[2] Amy L. Friedman.[1] *[1]Sect of Organ Transplantation; Dept of Surgery, Yale University School of Medicine, New Haven, CT; [2]Section of Nephrol; Dept of Internal Medicine, Yale University School of Medicine, New Haven, CT.*

Sirolimus immunosuppression is efficacious, and recent results suggested associated anti- mesenchymal effects may favorably impact graft arteriosclerosis. Some have suggested the SRL safety profile may be inferior vs. MMF, and this analysis evaluated whether side effects among 121 renal transplant recipients receiving SRL with reduced dose CNI (n=62) or MMF with conventional CNI (n=59) were different. Trough SRL concentrations were 10-15 ng/ml. Among SRL treated patients, CsA target troughs were 75-100 during the initial 3 months and 50 ng/ml thereafter; target troughs for patients receiving Tcl were 5 ng/ml. MMF, 1 gram twice daily was adjusted according to patient tolerability. Among MMF treated patients, CsA troughs were 150-200 ng/ml during the initial 3months and 100-150 ng/ml thereafter; target Tcl troughs were 5-10 ng/ml. There was no graft loss or patient death, and serum creatinine was similar (SRL, 1.6±0.7; MMF 1.5±0.5). The incidence of acute rejection was below 10% in both MMF (8.5%) and SRL (9.7%) treated recipients (p=ns). The side effect profiles have been summarized:

Value	SRL (n=62)				MMF (n=59)				P value
FOLLOW UP	3 mo	6 mo	12 mo	18 mo	3mo	6mo	12mo	18mo	
Hematocrit	39 ± 5	36 ± 5	38 ± 7	40 ± 6	37 ± 4	39 ± 5	41 ± 4	42 ± 6	ns @ 18 mo
Cholesterol (mg/dl)	221 ± 48	225±49	226 ± 37	237±21	198 ± 41	193±35	185±41	196 ± 31	p= 0.004@18mo
Triglycerides	270±229	255±170	309±221	302±216	175±96	184±126	152±83	220±142	ns @ 18 mo
Blood Pressure	131/77	135/76	131/76	130/80	131/76	132/81	132/79	127/78	ns @ 18 mo
Testosterone (males)	3.0 ng/ml				3.1ng/ml				ns

Additionally, lymphoceles, wound complications, and CMV rates were similar among all patients. These results provide strong support that SRL combined with low dose CNI and steroids is safe and efficacious. Cholesterol elevation was modest, and other potentially relevant side effects noted in pre- clinical and early clinical SRL experiences such as effects on reproductive hormones in males were similar to MMF treated patients. These excellent outcomes support the need for long- term trials to establish whether SRL based regimens will improve graft function.

Abstract# 1392 Poster Board #-Session: P148-III

RELATIONSHIP BETWEEN PRA, ANTIBODY TITER AND ALLOIMMUNIZATION IN HIGHLY SENSITIZED ESRD PATIENTS. Smita Vaidya,[1] David Partlow,[1] Kristine K. Gugliuzza,[2] John A. Daller.[2] *[1]Pathology, University of Texas Medical Branch, Galveston, TX; [2]Surgery, University of Texas Medical Branch, Galveston, TX.*

This study was designed to answer three specific questions: (1) what are the anti-HLA antibody titers of high PRA patients? (2). What type of allo immunization is responsible for high titer anti-HLA antibodies? (3). How do various ethnic populations respond to allo immunization? **Methods**. Anti-IgG titers of 65 high PRA (range 50-100%, median 84%) patients were determined by serially diluting their peak reactive sera from 1:1 up to 1:1024 dilutions and screening these dilutions by antiglobulin method against a panel of 30-40 HLA typed T lymphocytes. Immunological histories of transfusions (TF), prior transplant rejection (TX) and prior pregnancies (Preg) of these 65 high PRA patients were compared with those of 80 low PRA patients (range 0-10%, median 3%). Out of total145 (65 high PRA and 80 low PRA) patients, 57 were Blacks (B), 48 were Whites (W), 36 were Hispanics (H) and 4 were Asians (A). Fisher Exact test was used for the statistical analysis of the data. P value less than 0.05 was considered statistically significant. **Results.** Antibody titers of patients with PRA between 80 to 100% were generally greater than 1:1024 (range 1:256 –>1:1024, median >1:1024). Antibody titers of the patients with PRA between 79- 50% were 1:64 (range 1:32-1:256, median 1:64). In general, neither TF(p=0.8) nor Preg (p=0.9) sensitized primary transplant recipients. However, TX, followed by TF was highly immunogenic (p=0.00016). Similarly motherhood, followed by either TX (p=0.00005) or TF (0.0001) was highly immunogenic. Acute rejection was highly likely to induce strong anti-HLA response than chronic rejection ($p=8.9x10^{-13}$). Among various ethnic groups, B were highly likely to receive TF following rejection than either W or H (p=0.002 and 0.003 respectively), consequently, B had high titer anti-HLA antibodies (>1:1024) than either W or H (p=0.002 and 0.003 respectively). **Summary.** (1) Patients with PRA greater than 80% have extremely high titer HLA antibodies. These titers decline with decrease in PRA. (2). TF and Preg by themselves may not be immunogenic in primary transplant patients; however, they are highly immunogenic in regraft recipients. (3). B are more likely to receive TF following rejection, consequently they are highly likely to develop strong anti-HLA response. **Conclusion**. Blood transfusion should be avoided whenever possible in all patients who are immunized by either previous transplants or pregnancies.

Abstract# 1393 Poster Board #-Session: P149-III

CYTOMEGALOVIRUS (CMV) GENOTYPE AND RESPONSE TO THERAPY IN SOLID ORGAN TRANSPLANT RECIPIENTS WITH CMV DISEASE. Atul Humar,[1] Deepali Kumar,[1] Christian Gilbert,[2] Guy Boivin.[2] *[1]Multi-Organ Transplantation, University of Toronto, Toronto, ON, Canada; [2]Research Center in Infectious Diseases, Centre Hospitalier Universitaire de Quebec, Quebec City, QC, Canada.*

Background: CMV can be classified into four glycoprotein B (gB) genotypes based on sequence variation in the UL 55 gene. There may be an association between CMV gB genotype and clinical outcome including response to therapy although the latter has not been well assessed in previous studies. We looked at the effect of CMV gB genotype on response to therapy in solid organ transplant patients treated for CMV disease. Methods: The CMV gB genotype was determined directly from pre-therapy leukocyte samples using PCR amplification of a region of UL55, followed by restriction analysis based on HinF I and Rsa I digestion in 50 organ transplant patients with CMV disease. CMV viral loads were determined at regular intervals after starting therapy. Genotype results were correlated with response to ganciclovir therapy, including viral load half-life, time to viral clearance, and disease recurrence. Results: The distribution of CMV gB genotypes was: gB1 19/50 (38%); gB2 9/50 (18%); gB3 12/50 (24%); gB4, 2/50 (4%) and 8 patients (16%) had mixed infection. Median days to viral clearance was not significantly different among genotypes although patients with mixed infection had a trend towards delayed viral clearance (28.4 days vs. 19.5 days; p=0.17). Viral load half-life calculated by plotting decays curves after start of therapy was not significantly different among the 4 genotypes. Again, patients with mixed infection had a trend towards prolonged viral half-life (T1/2=8.4 days vs. 3.9 days; p=0.18). CMV gB genotype did not affect the rate of disease recurrence or the occurrence of tissue invasive disease vs. CMV viral syndrome (p=NS for all comparisons). Conclusions: The gB genotype causing CMV disease does not seem to significantly influence response to therapy including time to viral clearance, viral load half-life and rate of disease recurrence, although patients with mixed infections may have a slower response to therapy.

Abstract# 1394 Poster Board #-Session: P150-III

EFFICACY OF LOW-DOSE VALGANCICLOVIR FOR THE PREVENTION OF CMV AFTER ORGAN TRANSPLANTATION. Mark S. Kelley,[1] Marsha R. Honaker,[1] M. Francesca Egidi,[2] M. Hosein Shokouh-Amiri,[2] Amy L. Christenson,[1] A. Osama Gaber.[2] *[1]Pharmacy Division of Transplantation, University of Tennessee Health Science Center, Memphis, TN; [2]Department of Surgery, Division of Transplantation, University of Tennessee Health Science Center, Memphis, TN.*

With ganciclovir, we have had a significant decline in the incidence of CMV infection and disease despite the concurrent emergence of more potent immunosuppressants. Recently, valganciclovir, the valyl ester of ganciclovir, has become available. Due to its enhanced pharmacokinetics, valganciclovir has considerable promise in the prophylaxis against CMV and offers convenient once-daily dosing. We report our recent experience with valganciclovir 450mg once daily as well as a historical cohort that received ganciclovir 1 gram three times daily (both adjusted for renal function). All patients transplanted at our center from 8/1/01 to 9/30/02 that received either valganciclovir or ganciclovir as CMV prophylaxis were included in the analysis.

Demographic Characteristics

	Cytovene Group (n=62)	Valcyte Group (n=68)	ρ
Organ type - # (%)			
Kidney	49 (79)	47 (69)	NS
Pancreas	10 (16)	12 (18)	NS
Liver	3 (5)	9 (13)	NS
CMV Serology - # (%)			
Negative/Negative	7 (11)	4 (6)	NS
Negative/Positive	17 (28)	19 (28)	NS
Positive/Negative	5 (8)	16 (23)	0.017
Positive/Positive	33 (53)	29 (43)	NS
Induction - # (%)			
Anti-Thymocyte	50 (81)	54 (80)	NS
IL-2 Antagonist	2 (3)	3 (4)	NS
No Induction	10 (16)	11 (16)	NS
Maintenance Immunosuppression - # (%)			
FK/MMF/Prednisone	32 (52)	40 (59)	NS
FK/Rapamycin/Prednisone	16 (26)	13 (19)	NS
Rapamycin/MMF/Prednisone	14 (23)	15 (22)	NS

Patient Outcomes

	Cytovene Group (n=62)	Valcyte Group (n=68)	ρ
Mean follow-up (months)	10.7 ± 3.7	4.6 ± 3.6	0.000
Patient Survival - # (%)			
Kidney	46 (94)	45 (96)	NS
Pancreas	10 (100)	12 (100)	NS
Liver	2 (67)	6 (67)	NS
Graft Survival - # (%)			
Kidney	45 (92)	43 (91)	NS
Pancreas	8 (80)	10 (83)	NS
Liver	2 (67)	6 (67)	NS
Incidence of AR - # (%)			
Kidney	6 (12)	6 (13)	NS
Pancreas	1 (10)	0 (0)	NS
Liver	0 (0)	1 (11)	NS
CMV Infection/Disease - # (%)	0 (0)	1 (1)	NS

The only significant difference in toxicity was a higher white blood cell count at one month in the ganciclovir cohort (7.1 ± 2.8 vs 6.1 ± 2.3; ρ=0.034). There were no patients who discontinued therapy due to adverse effects in either group. These data suggest that both ganciclovir and valganciclovir offer significant efficacy in the prevention of CMV infection and disease post-transplant. Furthermore, both agents were well tolerated. Larger prospective, randomized trials will be needed to confirm our findings and validate the use of this promising new agent in the prevention of CMV infection and disease in transplant recipients.

Abstract# 1395 Poster Board #-Session: P151-III

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) AMONG EBV-SEROPOSITIVE COMPARED WITH EBV-SERONEGATIVE PEDIATRIC TRANSPLANT RECIPIENTS. Upton Allen,[1] Gabrielle Farkas,[1] Diane Hébert,[1] Sheila Weitzman,[1] Raymond Tellier,[2] Bo Ngan,[2] Annie Fecteau,[3] David Grant,[3] Lori West,[1] Samia Wasfy.[1] *[1]Pediatrics, Hospital for Sick Children, Toronto, ON, Canada; [2]Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada; [3]Surgery, Hospital for Sick Children, Toronto, ON, Canada.*

Introduction: Post-transplant Lymphoproliferative Disorder (PTLD) due to the Epstein-Barr Virus (EBV) is a major concern after pediatric transplantation. While patients at greatest risk are EBV-seronegative recipients who receive EBV-seropositive organs, PTLD may also occur in EBV-seropositive recipients. Knowledge of the factors that are associated with PTLD in the latter group may assist with early diagnosis, prevention and treatment. **Methods**: We compared the clinical, epidemiologic and laboratory characteristics of PTLD among EBV-seropositive versus EBV-seronegative pediatric transplant recipients. Patients were identified through a PTLD Registry, in which cases of PTLD were prospectively assembled. This study targeted biopsy-proven PTLD cases over a period of four years (1997-2000, inclusive). **Results**: Twenty-two cases of PTLD were diagnosed during the study period. PTLD cases occurred at a median of 22.8 months post-transplantation (range 1-131). The transplanted organs were livers (59.1%), hearts (22.7%), kidneys (13.6%) and lungs (4.6%). The median age of children with PTLD was 26.2 months (range 6.1-194). Among children with PTLD, the ratio of EBV-seropositive recipients to EBV-seronegative recipients was approximately 1:4. The mean ages at the time of diagnosis of PTLD were 114.3 (sd ± 58.6) versus 38.3 ± 48.1 months (P = 0.02), among EBV-seropositive and EBV-seronegative recipients, respectively. In addition, the time to the development of PTLD was shorter in EBV-seropositive recipients (means 17 ± 22.9 compared with 46.5 ± 39.1 months; P = 0.04). The 2-year survival rate was 50% (3/6) for EBV-seropositive recipients who developed PTLD during the study period. The corresponding rate for EBV-seronegative recipients was 100% (16/16, P = 0.01). There were no significant differences in the pathologic features of PTLD lesions among EBV-seropositive versus seronegative recipients. **Conclusion:** Approximately 25% of PTLD cases occurred among EBV-seropositive recipients. These children were older with a worse outcome compared with their seronegative counterparts. In an effort to guide possible preventive strategies, additional studies are planned to further characterize the factors that may be predictive of an increased risk of PTLD among EBV-seropositive pediatric transplant recipients.

Abstract# 1396 Poster Board #-Session: P152-III

PHARMACODYNAMICS OF SAFETY AND EFFICACY FOR GANCICLOVIR AFTER ITS ORAL ADMINISTRATION AND FROM ITS PRO-DRUG, VALGANCICLOVIR, IN SOLID ORGAN TRANSPLANT RECIPIENTS. H. Wiltshire,[1] C. Paya,[2] M. D. Pescovitz,[3] A. Humar,[4] E. Dominguez,[5] K. Washburn,[6] E. Blumberg,[7] B. Alexander,[8] R. Freeman,[9] N. Heaton,[10] R. Tansley,[1] Valganciclovir Solid Organ Transplant Study Group. *[1]Roche Products Ltd, Welwyn Garden City, Herts, United Kingdom; [2]Mayo Clinic, Rochester; [3]Department of Surgery, Indiana University; [4]Toronto General Hospital, ON, Canada; [5]University of Nebraska Medical Center; [6]University of Texas Health Science Center, San Antonio; [7]Hospital of the University of Pennsylvania; [8]Duke University Medical Center; [9]New England Medical Center; [10]King's College, London, United Kingdom.*

Background: A Phase III study was conducted to compare the safety, efficacy and PK of ganciclovir (GCV) after oral administration of its capsule formulation and its pro-drug valganciclovir (VGCV), for prophylaxis of CMV-disease in liver, heart and kidney transplant recipients. As part of this study, we investigated the relationship between systemic exposure to GCV and anti-CMV efficacy and hematological safety. Methods: The PK of GCV was analysed using a population approach with data from 248 of the 372 patients treated. Viral load and haematological measurements were taken from all subjects at regular intervals during and after the 100 days' treatment. The correlation between individual exposure to GCV during the prophylaxis phase with the incidence of CMV viremia during and after treatment, CMV disease in the 6-month post-transplant period and hematological toxicity was assessed. Results: The development of viremia during prophylaxis, and for the month following, was appreciably greater if exposure {AUC(0-24h)} to GCV was < 35 µg.h/mL (median value of all subjects = 40 µg.h/mL). On Day 100, 13% of patients had a measurable viral load (> 400 copies/mL) if the average AUC was 25 - 35 µg.h/mL (55 subjects) but only 3% at 35 - 45 µg.h/mL (n=67). There was no relationship between AUC and anemia and only a weak tendency to increased neutropenia (< 1000 cells/µL) and leukopenia (< 3500 cells/µL). For example, 42 % of patients with average AUC of 25 - 35 µg.h/mL had an episode of leukopenia compared to 49% at > 50 mg.h/mL. Those patients who received GCV experienced, on average, 1.7-fold lower exposure than those on VGCV with only 27%, compared to 76%, having AUCs > 35 mg.h/mL. These results are consistent with the later incidence of viral breakthrough observed in the VGCV arm of the study after cessation of therapy. Conclusions: The greater systemic exposure to GCV delivered by VGCV, compared to oral GCV, was associated with delayed development of viremia. However, there was only a weak association between AUC and hematological toxicity.

Abstract# 1397 Poster Board #-Session: P153-III

HEART AND LIVER TRANSPLANT RECIPIENTS ARE AT A LOW RISK FOR POLYOMA VIRUS BK VIREMIA AND NEPHROPATHY. Dechu Puliyanda,[1] Nurmamet Amet,[1] Archana Dhawan,[1] Lara Hilo,[1] Raju K. Radha,[1] Suphamai Bunnapradist,[1] Lawrence S. C. Czer,[1] Paul Martin,[1] Stanley C. Jordan,[1] Mieko Toyoda.[1] *[1]Divisions of Kidney, Cardiac and Liver Transplantation, Cedars Sinai Medical Center, Los Angeles, CA.*

Background: BK nephropathy is thought to result from reactivation of virus in the transplanted kidney in an immunocompromised host. Our previous data shows that the plasma rather than urine BK PCR is more likely to correlate with active disease. The incidence of BK viremia in solid organ transplants other than the kidney has not been systematically studied. Here we performed a retrospective evaluation of BK PCR analysis in heart and liver transplant recipients, and compared them with kidney allograft recipients. **Methods**: Archived plasma samples obtained from 51 heart (Jan '94 to April 2002), 45 liver (Jan '97 to April 2002) and 162 renal (Jan '99 to August 2002) transplant recipients who received transplantation at our center were submitted for BK-PCR. Plasma samples between 6 and 18 months post-transplant were used for the analysis based on the previously reported median time for BK viremia and BK nephropathy in renal allograft recipients. Immunosuppressive regimen at 3 months post transplantation in renal transplant recipients consisted of CSA (150-200ng/ml) or Tacrolimus (6-8pg/ml), Cellcept and Prednisone; in liver transplant patients Tacrolimus (5-6pg/ml); and in the heart transplant recipients CSA (100-200ng/ml) or Tacrolimus (8-10pg/ml) with azathioprine or Cellcept. **Results**: 8 of the 162 renal transplant recipients had BK viremia (10 - 10^7 copies/500ng DNA) with nephropathy. BK viremia was not found in any cardiac allograft recipients and 44/45 liver transplant recipients were also negative. Low copy numbers (<5) were detected in 1 liver transplant recipient whose serum creatinine was stable (0.9- 1.0mg/dl). The prevalence of BK viremia in kidney transplant recipients wa s 4.9% vs 0% in the heart and 2.2% in liver transplant recipients with 95% confidence intervals of 2.15-9.49%, 0-6.9% and 0-11.7% respectively. **Conclusion**: Based on our single center experience we believe that recipients of isolated heart or liver allografts are at very low risk for development of BK viremia and nephropathy. These data also strongly suggest that BK nephropathy evolves from the transplanted kidney. Genotypic variation between the latent virus in the allograft and native kidney,renal injury during procurement and higher immunosuppression are the likely predisposing factors for greater risk of BK nephropathy in the renal allograft recipients.

Abstract# 1398 Poster Board #-Session: P154-III

EBV MONITORING BY QUALITATIVE AND QUANTITATIVE PCR: A TWO-TIER STRATEGY FOR THE IDENTIFICATION OF LYTIC VIRAL EXPANSION INTO PTLD. Robert E. Cirocco,[1] Rodriguez Riena,[1] Eloundou Irvana,[1] Fola Amole,[1] Sharon Babishkin,[1] Tomaki Kato,[1] Paoulo Rosconni,[1] Naveen Mital,[1] Violet Esquenazi,[1] George W. Burke,[1] Andreas Tzakis,[1] Joshua Miller.[1] *[1]Department of Surgery, Division of Transplantation, University of Miami and the Veterans Affairs Administration Hospital, Miami, FL.*

Transplant patients under immunosuppressive therapy are at risk for initial infection and/or reactivation from a latent herpes virus, the Epstein Barr Virus (EBV). This serious complication is highly associated with an often-fatal condition, Post Transplant Proliferative Disease (PTLD). Initial identification and monitoring levels of EBV in these patients is vital in antiviral and/or immunosuppressive drug dose tailoring. The object of this study was to evaluate the two-tier approach of EBV monitoring by: 1) screening our transplant recipients by a qualitative PCR for EBV to identify detectable levels of EBV and 2) a quantitative assay to determining the EBV levels in the identified "positive" patients by Real-Time PCR. One part of this study involved quantitation of two compartments in each sample, the whole blood (cells and plasma) to identify expansion in the B cells and plasma alone to identify an active lytic infection. We studied patients who were tested by both qualitative and quantitative assays from 1/02 to 10/02. Of the fifty-seven positive qualitative EBV PCR patients tested, fifty-three patients had detectable levels in the quantitative assay. There was a 92.9% correlation of positive patients between the qualitative and quantitative EBV PCR assays. If we analyze patients with greater than two positive qualitative PCR tests, i.e. consistently positive in the qualitative assay,a 98.2% correlation was observed between the both assays. In the quantitative assay we analyzed 2683 samples from 340 patients from 1/00 to 11/02. Two hundred ninety three samples from 63 patients exhibited (significant) levels >2,000 copies/ml in the blood only. There was no morbidity. Sixty-six samples from 25 patients exhibited >2,000 copies/ml in both blood and plasma. Of these 25 patients, 15 demonstrated biopsy proven PTLD. In conclusion: 1) the use of qualitative EBV PCR identifies transplant recipients that have detectable viral genomes in the blood. 2) The quantitation by real-Time PCR of EBV levels in the blood and plasma allows the clinician insight for the adjustment of therapy. 3) EBV levels in the plasma have a high degree of correlation to the development of PTLD. And 4) Using the qualitative EBV PCR for screening and quantitative EBV PCR for determining changing levels, enables the physician the most efficient method for EBV monitoring.

Abstract# 1399 Poster Board #-Session: P155-III

DROTRECOGIN ALFA (ACTIVATED) (XIGRIS™) IN ABDOMINAL ORGAN TRANSPLANT RECIPIENTS WITH SEVERE SEPSIS. G. Mark Baillie,[1,2] Jennifer H. Birsner,[1] David J. Taber,[1,2] Jeffrey Rogers,[1] Angello Lin,[1] Osemwegie Emovon,[1] Fuad Afzal,[1] Prabhakar K. Baliga,[1] P. R. Rajagopalan,[1] Kenneth D. Chavin.[1] *[1]Division of Transplant Surgery, Medical University of South Carolina, Charleston, SC; [2]Department of Pharmacy Services, Medical University of South Carolina, Charleston, SC.*

Sepsis is a leading cause of death in abdominal organ transplant recipients. Drotrecogin alfa (activated), recombinant human activated protein C (rhAPC), is indicated for the reduction of mortality in patients with severe sepsis who have a high risk of death. Although the PROWESS study demonstrated significantly lower mortality in patients with severe sepsis who received rhAPC than in those who received placebo, organ transplant recipients were specifically excluded from the trial. In addition, an increased risk of bleeding associated with rhAPC therapy may preclude its use in some patients. We report our series of abdominal organ transplant recipients with severe sepsis who were treated with rhAPC. METHODS: We reviewed all abdominal organ transplant recipients at our hospital between 12/01 and 08/02 with sepsis treated with rhAPC. All patients met institutional criteria for rhAPC use. rhAPC was infused at 24mcg/kg/hr for 96 hrs. Infusions were temporarily interrupted for invasive procedures. Data collection included demographics, APACHE II score at initiation of therapy, etiology of sepsis, mortality, vasopressor requirements, and bleeding complications. RESULTS: Six patients received rhAPC. All patients completed the 96hr infusion. No severe bleeding complications requiring discontinuation of therapy were observed in any patient. Vasopressor requirements were reduced in all patients after initiation of rhAPC. Even patients who expired had initial hemodynamic improvement with rhAPC. Patient survival at 6 months post-rhAPC therapy was 33% (2 of 6 patients).

Table 1: Patient Information

			Patient Survival			# of Vasopressors Required	
Patient Demographics	APACHE II Score	Sepsis Source	96 hr	28 Day	90 Day	rhAPC Start	rhAPC End
59yo WM Oltx	37	pneumonia	Alive	Exp	Exp	2	1
55yo WM Oltx	18	pneumonia	Alive	Alive	Alive	3	1
33yo WM pancreas	15	CMV/GVHD	Alive	Exp	Exp	1	1
42yo WM SPK	16	Perf Viscus	Alive	Alive	Alive	2	0
72yo WF Oltx	28	Perf Viscus	Alive	Alive	Alive	2	1
60 yo WF Oltx	32	Pneumonia	Alive	Exp	Exp	2	1

CONCLUSION: Although the safety and efficacy of rhAPC for the treatment of severe sepsis in abdominal organ transplant population has yet to be established in large, multi-center trials, our results suggest the risk of increased bleeding with rhAPC may not preclude its successful use in abdominal organ transplant recipients.

Abstract# 1400 Poster Board #-Session: P156-III

COMPARATIVE EFFICACY AND SAFETY OF LOW DOSE VALGANCICLOVIR VS. ORAL GANCICLOVIR FOR THE PREVENTION CYTOMEGALOVIRUS DISEASE IN RENAL ALLOGRAFT RECIPIENTS. Daniele K. Gelone,[1] Diane Cibrik,[1] Sarah Vogler,[1] Alan Leichtman,[1] Kathleen Lake.[1,2] *[1]Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Surgery, University of Michigan, Ann Arbor, MI.*

Valganciclovir HCl (VGCV) is the L-valyl ester of ganciclovir (GCV), which is rapidly converted to GCV after oral administration. VGCV was developed to improve bioavailability and enhance compliance through a once daily dosage regimen (900mg/d). Preliminary pharmacokinetic data suggested that similar AUCs are achieved with VGCV 450mg/d as compared to GCV 1.0g/tid. **Aims:** The purpose of this review was to evaluate the efficacy and safety of low dose VGCV (450mg/d) for CMV prophylaxis (CMVpx) vs. oral GCV(1000mg/tid) CMVpx. **Methods:** Between the years 2000-2002, 290 adult kidney transplant recipients were retrospectively reviewed for CMVpx regimen. In 2002, our program changed CMVpx from GCV to VGCV. Transplant recipients at high risk (D-R+) or intermediate risk (D+R+ or D+R-) for CMV received either oral GCV (1000mgTID) or VGCV (450mg/d) adjusted for renal function on post-op day 1 through 90. Hgb, absolute neutrophil count(ANC) and platelets (PLT) were reviewed at 1, 3, and 6 months post-op. CMVpx was initiated for any rejection episode treated with antibody. **Results:** 141 GCV and 76 VGCV recipients were identified. Based on the serologic status of the donor (D) and recipient (R), 40 of the GCV cohort and 23 of the VGCV cohort were at high risk for CMV(p=0.76). Induction use was greater in the VGCV (44.7%) arm compared to the GCV arm (29.1%) (p=0.021). Mean follow-up for the GCV and VGCV patients were 11.7±0.75 & 5.14±2.15 months, respectively (p<0.05). The overall incidence of CMVD confirmed by antigenemia was 5% with GCV prophylaxis compared to 3.9% with VGCV prophylaxis(p=1.0). Subgroup analysis by CMV sero-status revealed 4.3% of the R-D+ VGCV patients acquired CMVD compared to 12.9% of R-D+ GCV patients (p=0.402). R+D+ VGCV (5.9%) & GCV (3.6%) recipients experienced similar rates of CMVD (p=0.63). The mean time to CMVD was 4.9 months. No R+D- patients developed CMVD. CMVD occurred after the 90 day prophylaxis period in all patients except for 1 in each arm who developed CMVD during the CMVpx. Both agents were well-tolerated. Zero patients exhibited abnormalities in ANC (<500/mm3) or PLT count (<50,000/mm3) up to 6 months post-op. 4 patients in the GCV arm experienced anemia. (Hgb <8.0g/dL). **Conclusions:** Compared to GCV (1000mg/TID), low dose (450mg/d) VGCV appears equally safe & efficacious in the prevention of CMVD in renal allograft recipients while providing patients a decreased pill load and convenient once daily regimen.

Abstract# 1401 Poster Board #-Session: P157-III

PREOPERATIVE DETERMINATION OF CARDIAC TROPONIN T PREDICTS ONE-YEAR MORTALITY IN RENAL TRANSPLANT CANDIDATES. Britta Weidtmann,[1] Johannes Rixe,[1] Margit Muller-Bardorff,[1] Gert Richardt,[2] Martin W. Strik,[3] Lutz Fricke.[3] *[1]Medizinische Klinik II, Universitaetsklinikum Luebeck, Luebeck, Germany; [2]Herzzentrum, Segeberger Kliniken, Bad Segeberg, Germany; [3]Interdisziplinaeres Transplantationszentrum, Unversitaetsklinikum Luebeck, Luebeck, Germany.*

Cardiovascular disease is the primary cause of morbidity and mortality in patients with end-stage renal disease (ESRD) and renal transplant recipients (RTR's). Cardiac troponin T (cTnT), a structural protein of the thin filament, is a highly specific and sensitive marker for myocardial cell injury and its role for risk stratification in patients with acute coronary syndromes (ACS) is well established. The value of cTnT determinations in patients with ESRD has been questioned, but although the exact pathophysiologic mechanism of the troponin release remains still unclear, the detection ≥ 0,1 µg/L results in an up to 7-fold increased mortality. The purpose of this study was to determine the presence and prognostic implications of elevated cTnT in RTR's before and after renal transplantation. 160 RTR's were prospectively enrolled and serum cTnT was collected preoperatively, daily postoperatively until discharge from the transplantation unit and 12 month after renal transplantation. The one-year follow up is completed up to now in 68 patients (51,5 ±12,7 yrs., m/f:1,3/1) and already at this subgroup highly significant results are obvious. In comparison to pretransplant cTnT levels one year after renal transplantation with improved renal function there was a significant reduction of the cTnT values (median 0,013 µg/L, 25./75. percentile 0,0/0,046 µg/L versus median 0,0 µg/L, 25. and 75. percentile 0,0 µg/L [p=0,005]). The overall mortality from cardiac and non-cardiac causes was 10,3% (n=7); the preoperative cTnT values of the RTR's who died were significant higher (median 0,059 µg/L, 25./75. percentile 0,039/0,222 µg/L versus median 0,0 µg/L, 25./75. percentile 0,0/0,035 µg/L [p=0,002]). Furthermore, the RTR's were subgrouped according to their preoperative cTnT-levels (A:0-0,029 µg/L; B:0,03-0,099 µg/L; C:≥0,1 µg/L); with rising cTnT levels the incidence of the combined end point (death and ACS within 12 month) increased significantly (A: 4,8%; B: 15,8%; C: 57,1% [p=0,002]). Thus, the preoperative determination of cardiac troponin T allows the detection of a high risk collective for adverse cardiac events and death from all causes among the patients after renal transplantation and should become part of pretransplant evaluation.

Abstract# 1402 Poster Board #-Session: P158-III

FACTORS OF URETERAL NECROSIS IN RENAL TRANSPLANTATION. Georges Karam,[1] F. Maillet, S. Parant, J. M. Nguyen, J. P. Soulillou, M. Giral. *[1]Urology, CHU Hotel-Dieu, Nantes, France.*

The main cause of urinary fistula in renal transplant is the ischemia of the distal ureter, which has lost its arterial supply. However, ischemia alone could not explain the late occurrence of many fistulas beyond the first month. **The objective** of this retrospective study was to establish, on a large cohort of kidney graft patients, which parameters could be involved in the occurrence of ureteral necrosis. **Patients and Methods:** Between January 1990 and December 2001, 1629 renal transplantations have been performed in our center. All data were computerized in a cross-audited and validated data bank (DIVAT). Studied parameters were: donors age (≤55 vs.>55 years), gender, cause of death and serum creatinin before procurement; recipients age (≤55 vs .>55years), gender, initial disease (nephropathy vs. uropathy), anti-PRA antibodies (≤25% vs. > 25% and >80%) and retransplantation (1 vs. ≥ 2), cold ischemia time, delayed graft function, HLA incompatibilities, induction and maintenance immunosuppression, right or left kidney, number of arteries, site of transplantation and the presence or not of a double J stent. Parameters of the follow-up were the number and timing of acute rejection episodes, CMV infection (viremia, PCR), acute pyelonephritis and ureteral necrosis. Ureteral histological analysis was performed in 25 cases (necrosis, leukocyte infiltration and CMV or BKV inclusions). Uni and multivariate statistical tests have been used with an alpha risk at 5%. **Results:** Fifty-six cases of ureteral necrosis (3.4% IC: 2.6-4.2%) have been observed. The average time for diagnosis was 49±39 days (1-156 days). Univariate and Cox model have shown that ureteral necrosis was significantly and independently correlated with donor age (p=0,006) and the occurrence of a CMV infection during the follow-up (p=0,003). Ureteral histological studies have shown CMV and BKV inclusions respectively in 4 and 6 cases, arterial thrombosis, arterial dissection and venous thrombosis respectively in 4, 1 and 16 cases. Ten out of 16 were recent and 6 were former thrombosis with signs of repermeabilisation. No histological acute rejection was observed. Patient and graft survival rate were respectively 87% and 66% for the first group and 86% and 58% for the second group (p=NS). **Conclusion:** Ureteral necrosis is not only related to the section of the ureter and of its vessels. Donor's age and cytomegalovirus infection were significantly associated with its occurrence. Analysis of the chronology of CMV infections compared to the ureteral necrosis is under process.

Abstract# 1403 Poster Board #-Session: P159-III

A PROSPECTIVE, RANDOMIZED CONTROLLED TRIAL COMPARING ORAL GANCICLOVIR WITH WEEKLY-MONITORED CMV-ANTIGENEMIA IN RENAL TRANSPLANT PATIENTS WITH A HIGH-RISK FOR CMV INFECTION. Margaret Queiroga,[1] Maria Cristina R. Castro,[1] Lilian M. P. Araujo,[1] Cristiane F. Alves,[1] Erica Kakehashi,[1] Claudio Panutti,[2] Luci VilasBoas,[2] William C. Nahas,[1] Luiz E. Ianhez.[1] *[1]Divisions of Urology and Nephrology - Hospital das Clinicas, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil; [2]Instituto de Medicina Tropical, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil.*

Severe CMV infection is very common in patients receiving anti-lymphocyte therapy. A 90-day prophylaxis with oral Ganciclovir (GCV) is very effective but expensive. Weekly-monitored CMV antigenemia ($CMV_{antig.}$) and treatment of early infection (pre-emptive therapy) seems also safe and possibly cheaper. We conducted a randomized controlled trial comparing these 2 approaches for patients receiving ATG/OKT3. Out of the 114 pts transplanted during 1-year period, 34 received ATG (n=29) or OKT3 (n=5) and were randomized (1:3) either to oral GCV (2.25grs/d/90d - group A, n=9) or to weekly-monitored CMV_{antig} (group B, n=25) and followed for 180 days. 3 pts were excluded due to early losses. Groups were similar regarding age, gender, number of retransplants, race or immunosuppression. None of the patients in group A developed CMV infection or died during the follow-up period. 1 pt lost his graft. In group B, there were 3 graft losses (p=NS) and 5 deaths (NS) (4 infectious and 1 cardiovascular). Two of these five deaths occurred in pts who developed CMV infection, although with negative $CMV_{antig.}$ at the time of death. In group B, 19 pts (83%) developed CMV infection diagnosed by a median antigenemia of 8 (3–47) cels/3x10^5 leukocytes, 34±13 days after Tx. They were treated with GCV and all of them became antigenemia negative after a mean of 12±6 days. At the end of the follow-up period, only one patient, in group B, had a recurrence of CMV infection that was successfully treated with IV GCV. No CMV invasive disease occurred in any patient. The costs of prophylaxis vs pre-emptive therapy were analyzed. The hospitalization time was similar for both groups (42±16 vs 43±20 days, p=NS). In group B, 104 $CMV_{antig.}$ were performed. In group A, the mean cost (only oral GCV) was U$2.344/pt while in group B (antigenemia + GCV) it was U$2.335/pt (p=NS). These data indicate that either the prophylatic use of oral GCV or the frequent monitoring of antigenemia can be adopted in this CMV high-risk group. However, the high incidence of CMV infection (82%), in the antigenemia group, may compromise the long-term course of the transplant. Considering the similar costs of both methods, the prophylaxis with oral GCV seems to be the ideal approach for this CMV high-risk cohort.

Abstract# 1404 **Poster Board #-Session: P160-III**

POLYOMA VIRUS IN PEDIATRIC SOLID ORGAN TRANSPLANT RECIPIENTS EVALUATION USING PCR TECHNIQUES. Jean Herman,[1] Mark Van Ranst,[2] Robert Snoeck,[2] Rita Van Damme-Lombaerts.[1] *[1]Pediatric Transplantation, University Hospital Gasthuisberg, Leuven, Belgium; [2]Laboratory of Virology, Rega Institute KUL, Leuven, Belgium.*

Polyoma virus (PV) infection and related interstitial nephritis is being increasingly recognized as an important cause of allograft dysfunction in renal transplants. Two strains are associated with disease in humans: BK virus (BKV) and JC virus (JCV). We monitored transplant recipients for polyoma in urine and blood using a quantitative PCR assay with the aim of evaluating the incidence and clinical relevance of polyoma infection. We assessed the efficacy of antiviral treatment in 1 patient with a biopsy proven polyoma nephritis. Patients and Methods: Pediatric renal transplants (n=44) recipients were evaluated for the presence of BKV and JCV in blood and in urine using a quantitative PCR technique specific for each virus. Recipients of liver (n=9) and of heart (lung) transplants (n=2) were also screened. Results: None of the liver and heart transplants were positive for BKV or JCV. BK viruria was observed in 12 out of the 44 renal recipients (27%), while BK viremia was concomitantly demonstrated in 4 (9 %). JC viruria was found in 6 renal recipients (14%). None of the patients with JC viruria or isolated BK viruria presented renal dysfunction. Among the 4 patients with BKV in urine and blood, one was negative at transplantation, presented important viruria at 6 weeks post Tx and developed concomitant BK viremia following treatment of a biopsy-proven acute vascular rejection at 12 weeks. A biopsy, a few weeks later, revealed polyoma nephritis. Reduction of immunosuppression (IS) lead to stabilization of the slightly reduced renal function. A second child presented at 14 months post Tx with deterioration of renal function and very high load of polyoma virus in urine and blood; biopsy revealed polyoma nephritis. Reduction of IS was followed by a biopsy proven acute rejection. The child was treated with cidofovir (1 mg/kg/dose,q 2 weekly, with probenecid) and methylprednisolone. The graft function, though reduced, stabilized, the viral load dropped in blood but remained elevated in urine. The 2 other patients did not show any signs of graft dysfunction though one of them had a persistent very high load of BKV in blood and urine up to 22 months post Tx. Conclusion: PV was detected in renal but not in liver or heart transplants. Polyoma viruria without viremia seems inoffensive. Polyoma nephritis occured in 2 out of the 4 children with BKV viremia and viruria. Reduction of IS may lead to rejection whose treatment in turn lead to reactivation of the virus. Cidofovir may represent a useful treatment.

Abstract# 1405 **Poster Board #-Session: P161-III**

DIABETES MELLITUS AND DISLIPEMIA ARE INDEPENDENT RISK FACTORS FOR DEVELOPPING RENAL ARTERY STENOSIS (TRAS) AFTER KIDNEY TRANSPLANTATION (RT). Josep Mª Puig, Anna Oliveras, Mª Josep Soler, Susana Vazquez, Marisa Mir, Josep Lloveras. *[1]Nephrology Department, Hospital del Mar, Barcelona, Spain.*

Graft disfunction secondary to vascular complications such as transplant renal artery stenosis (TRAS) following RT may affect graft survival and patient morbidity. Our aim is to ascertain the possible risk factors for developping TRAS. In a cohort of 503 RT performed between 1979 and 2002, 34 patients were diagnosed of TRAS by arteriography (incidence: 6,7%). Univariate analyses were performed for several donor and recipient variables (x^2 test). Significant variables were then analyzed by a multivariate Cox proportional hazards model. Results (A = TRAS vs. B = non TRAS) Other analyzed variables such as: age, gender, cigarette smoking, lipid profile and HT post RT, stroke, peripheral vascular disease, acute rejection episodes, cytomegalovirus infection, renal function, 24h-proteinuria, plasma fibrinogen, serum potassium, hematocrit, donor age, donor HT, donor type (cadaveric or living-related), acute tubular necrosis, cold ischaemia time, anastomotic type and number of arteries were not significative risk factors for TRAS. We conclude that: 1) diabetes mellitus and dislipemia prior to RT are independent risk factors for renal artery stenosis in renal transplanted patients. 2) Hypertension and ischaemic cardiac disease are also predictive risk factors in the univariate analyses, but not in the multivariate model.

	Univariate analyses			Multivariate analyses		
Diseases prior RT	A	B	p	OR	95%CI	p
Hypertension	82.9%	65,2%	0.003			
Diabetes Mellitus	22,9%	3.7%	< 0.001	4,5	1,2-17.1	0.028
Isch. cardiopathy	11.4%	3.1%	0.0037			
Dislipemia	78.8%	6.1%	< 0.001	50.4	19.8-128.7	< 0.001

Abstract# 1406 **Poster Board #-Session: P162-III**

RELATIONSHIP OF HEPARIN TO BLEEDING AND THROMBOSIS WHEN PREVENTING RENAL TRANSPLANT GRAFT THROMBOSIS. Andrew S. Mathis,[1,2] Nisha Dave,[1] Gary S. Friedman.[3] *[1]Pharmacy Practice, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ; [2]Pharmacy, Saint Barnabas Medical Center, Livingston, NJ; [3]International Regenerative Medicine, West Orange, NJ.*

PURPOSE: Patients with hypercoagulable states (HCS) are at greater risk for post-transplant renal allograft thrombosis. Therapeutic doses of heparin reduce the risk of renal artery/vein thrombosis in these patients, but are associated with a high bleeding risk (60%). Little is known about why this bleeding risk occurs. This study documented heparin anticoagulation in renal transplant recipients to evaluate factors associated with bleeding risk, and determine the relationship between heparin administration and optimal partial thromboplastin time (PTT) ratio (PTT times control). METHODS: Medical records of 28 consecutive adult renal transplant recipients from 1998 to 2002 who received unfractionated heparin (UFH) for prevention of renal thrombosis, starting within 24 hours of the procedure, were retrospectively evaluated. Patient medical history, demographic and UFH dosing characteristics were collected, and bleeding and thrombosis were documented. RESULTS: Eighteen patients (64.3%) had clinically important bleeding, with 9 patients requiring surgery for major bleeding. Mean initial UHF rate and mean duration of UFH administration was 6.77 U/hr and 9.33 days. Patients with and without bleeding were similar in demographic characteristics, mean PTT and maximum INR. Bleeding occurred at a mean PTT ratio of 2.5±1, compared with an overall mean (all PTT ratios) of 1.54±0.33 in bleeders, and 1.49±0.35 in non-bleeders (p=0.001). There was a trend toward higher maximum PTT (p=0.052) and longer length of stay (p=0.12) in patients with bleeding. An increase in PTT ratio from 1.5-1.9 to 2-2.4 to 3-3.4 increased bleeding from 4.2% to 12.8% to 14.7%, respectively. Of the 18 patients who bled, 15 received surgical antibiotic prophylaxis with cefotetan. Cefotetan appeared more likely to promote bleeding (75%) compared to other surgical prophylaxis (37.5%, p=0.091). Three thrombotic episodes occurred (2 renal and 1 pulmonary), all at PTT ratios <1.5 (mean 1.07); PTT ratio ≥1.5 resulted in 100% freedom from thrombosis. CONCLUSIONS: Heparin anticoagulation in renal transplant patients with HCS reduces the risk of thrombosis but an unacceptable risk of bleeding is confirmed. Higher PTTs and cefotetan prophylaxis may be bleeding risk factors. Optimal PTT ratio appears to be 1.5-1.9 to prevent thrombosis and bleeding.

Abstract# 1407 **Poster Board #-Session: P163-III**

MYCOBACTERIAL INFECTIONS OCCURRING AFTER KIDNEY TRANSPLANT. Tun Jie,[1] Deepali Kumar,[2] Atul Humar,[2] James V. Harmon,[1] Massimo Asolati,[1] Arthur J. Matas,[1] Kristen J. Gillingham,[1] Abhinav Humar.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN; [2]Medicine, University of Toronto, Toronto, ON, Canada.*

Background: Kidney transplant recipients have several risk factors that may predispose them to mycobacterial infections. We looked at such infections occurring after kidney or kidney/pancreas transplant to determine incidence, risk factors, and outcomes. **Results:** Of 3921 transplants performed between 1984-2002, 18 (0.45%) recipients were identified that developed mycobacterial infection at some time posttx. Of these, 10 were male and 8 female, 11 had a cadaver transplant, and 7 a living donor. Mean age at transplant for this group was 37.5 years. Racial background was as follows: Caucasian (n=12), African American (n=2), Native Indian (n=2), Hispanic (n=1), and Middle Eastern (n=1). The majority had a kidney alone (n=13). But 5 recipients had simultaneous transplant of a second organ: pancreas (n=3), islets (n=1), and liver (n=1). None of the 23 recipients had a documented mycobacterial infection pretransplant; 1 recipient did have a positive Mantoux test at the time of transplant and then developed pulmonary TB 4 months posttransplant. In the remaining 17 patients, pretransplant Mantoux testing was either negative (n=10) or unavailable (n=7). Mean time to development of infection was 3.3 years (range=2 weeks to 12 years). The most common site of infection was respiratory (n=6). Other sites included musculoskeletal (n=3), extremity wound (n=3), skin (n=2), gyn (n=1), and other (n=2). Only 3 of the infections were with mycobacterial tuberculi; the others were with avium (n=1), scrofulaceicum (n=1), chelonei (n=2) or some other non-tuberculus acid fast bacilli. Risk factors were present in may of the recipients, depending on the site of infection. This included occupational exposure, chronic non-healing wounds, accidental sof tissue injury, or previous TB exposure. A prior episode of acute rejection was significantly more common in those with vs. without infection (at 12 months posttransplant, 60.9% vs. 31.5%, p=0.001). With mean followup of 12.5 years since transplant and 9.2 years since infection, 13 of the recipients are alive and well. The most common causes of death in the remaining 5 were cardiovascular (n=3), followed by sepsis (n=2). Patient and graft survival rates were comparable to recipients with no infection. **Conclusions:** Infection with mycobacterium is uncommon after KTx; the majority are with a non-tuberculus type of organism. Most cases will have some identifiable risk factor. Increased level of immunosuppression likely also plays a role.

Abstract# 1408 Poster Board #-Session: P164-III

IMPACT OF MULTIPLE KIDNEY GRAFT ARTERIES ON RENAL FUNCTION AND PROTEINURIA ONE YEAR FOLLOWING TRANSPLANTATION. Matthias Buchler,[1] Elodie Fabre,[2] Jean M. Halimi,[1] Azmi Al Najjar,[1] Jean M. Boutin,[2] Hubert Nivet,[1] Yves Lanson,[2] Yvon Lebranchu.[1] *[1]Nephrology-Clinical Immunology, CHU Bretonneau, Tours, France; [2]Urology, CHU Bretonneau, Tours, France.*

Introduction: Multiple renal arteries (MRA) are common and do not contraindicate renal transplantation. The influence of MRA on immediate complications such as arterial thrombosis, bleeding and/or urologic fistulae have been reported but the effects of MRA on renal function are less well known. We performed a one year retrospective analysis of renal function and 24 hour proteinuria, in all renal recipients grafted in our center between 1996 and 2000. **Patients and methods:** A total of 262 patients who received a kidney graft in the study period (259 cadaveric and 3 living related donors) were divided into two groups: Patients receiving a graft with MRA (n=72, 27.5%; group A) and those with one artery (n=190, 72.5%; group B). There was no significant difference between groups in terms of sex, cold and warm ischemia times, presence of panel reactive antibodies and/or immunosuppression. Donor and recipient ages were significantly lower in group A than in group B (39± 15.4 vs 45± 15.2 yrs, $p<0.05$ and 40.4± 13.5 vs 46.0± 16.4 yrs, $p<0.05$ respectively). The need for at least one dialysis in the first week post-transplantation was comparable in group A and B (25% vs 21%, p=0.50). **Results:** There was no significant difference in serum creatinine levels (126± 45.8 vs 120± 49.9 micromol, p=0.47), systolic (138 ± 16 vs 140 ± 18 mmHg, p=0.80) or diastolic blood pressure (80 ± 12 vs 81 ± 11 mmHg, p=0.75) at one year. Anti-hypertensive drugs were given in 94.4% of patients in group A and 87.9% in group B (p=0.17). Nevertheless proteinuria was more frequent (61% vs 48%) and significantly higher in patients with MRA compared to patients without MRA (0.24± 0.85g/24h vs 0.1± 1.5g/24h respectively, $p<0.03$). Patient (98.6% vs 99.0%) and graft survival (91.7% vs 95.8%) at one year was not significantly different between groups. **Conclusion:** MRA is frequent and observed in nearly 30% of kidney transplants. Kidney with MRA can be transplanted because survival and graft function was not worse at one year but the higher proteinuria observed might influence long term graft survival. Longer follow-up is required to answer this question.

LIVER: LRD

Abstract# 1409 Poster Board #-Session: P165-III

PREDICTORS OF LIVING LIVER DONATION: FAITH, EDUCATION AND CLOSE RELATIONSHIP TO THE RECIPIENT. Kristel K. Hunt,[1] Anshu Chandra,[1] Kelly Smith,[1] Dianne LaPointe-Rudow,[1] Jean C. Emond,[1] Robert S. Brown, Jr..[1] *[1]Center for Liver Disease and Transplantation, New York Presbyterian Hospital, NY, NY.*

After completing the evaluation process, as many as a third of potential living donors (LD) change their mind. Our goal was to analyze donor characteristics that may predict withdrawal. **Methods:** Between 7/98 and 10/02, 237 individuals passed preliminary screening for LD. 161 patients subsequently completed full psychosocial and medical evaluations. Data regarding the potential donor's relationship to the recipient, their age, sex, ethnicity, education, employment and religious practices was abstracted from the evaluation charts. We analyzed select variables with Pearson's correlation coefficient; significant variables were then introduced into a LR model. Individuals ineligible for medical or recipient reasons were not included in the model predicting withdrawal. Statistical significance was defined as $\alpha=0.05$. **Results**: 44 evaluations were for pediatric and 193 for adult recipients; there were as many men as women. There were more Hispanics (41 vs. 29%, p=0.02) than in the general transplant recipient population. The mean age of the potential donors was 37(+/-10.5) years. 161 were 1° relatives (31% parents, 45% children and 24% siblings) of the recipients, 22 were spouses of adult recipients, 20 were other relatives, 18 friends and 11 other non-relatives. 91% of donors for children were their parents. 94 (58%) of the candidates proceeded to donation; 55 (37%) changed their mind, 44 (30%) were ineligible on medical or psychiatric grounds and 25 (17%) for recipient reasons. 5 (3%) may have been pressured to donate and were therefore excluded. After controlling for all other variables, age, sex and race were not predictive of donation. Compared to 1° relatives, spouses of adult recipients were 5.3 times (p=0.02) more likely and other relatives and friends 2.5 and 5 times (p= 0.06 and 0.09) less likely to go through the process. OR for donation was 26 ($p<0.0001$) for those describing themselves as religious and 3.4 (p=0.06) for those actively practicing their faith. Having attended some college had an OR of 5.0 (p=0.02) and graduating from college, 7.1 (p=0.002). OR for those with a graduate degree was 2.7 (p=NS). Employment was not predictive of donation. **Conclusions:** Faith, higher education levels and close relationship to the recipient predicted successful donation at our center. It may be prudent to employ early psychosocial evaluation and education prior to medical evaluation in certain donor candidates. Studies of cost-effectiveness of various evaluation methods are needed.

Abstract# 1410 Poster Board #-Session: P166-III

KINETICS OF REGENERATION OF LIVER VOLUME AND OF LIVER FUNCTION AFTER RIGHT HEMIHEPATECTOMY IN LIVING LIVER DONORS. Silvio Nadalin,[1] Massimo Malagó,[1] Giuliano Testa,[1] Mechtild Beste,[2] Christoph Jochum,[2] Volker Penndorf,[2] Thobias Schroeder,[3] Guido Gerken,[2] Christoph E. Broelsch.[1] *[1]General and Transplantation Surgery, University of Essen, Essen, Germany; [2]Internal Medicine and Gastroeneterology, University of Essen, Essen, Germany; [3]Radiology, University of Essen, Essen, Germany.*

Aim: To study the kinetics of regeneration of liver volume and of quantitative liver function after right hemihepatectomy (segment 5-8) in living liver donors. **Material and Methods:** From January 2000 up to July 2002 we performed a right hemihepatectomy (segment 5-8) in 27 healthy adult living liver donors (age : 33.5±11 years). Preoperative, at 10th postoperative day (POD 10), at 3rd , 6th and 12th postop. month (POM 3, POM6 and POM 12) we measured the following parameters: 1-Liver volume by use of MRI 1.5 Tesla (Magneton Sonata, Siemens , Erlangen, Germany) through ultrafast T1-w-GRE sequences in axial acquisition, 1 cm thick. 2- Galactose Excretion Capacity (GEC) test as expression of cytosolic liver function (mg/kg BW/min) and reported in relation to liver volume (GEC/ml liver volume). **Results:** Within the first 10 post. days we observed a mean value of 94% regeneration of liver volume calculated in relation to the postoperative residual volume without significant variation in the next months. The initial liver volume was never reached. The kinetics of functional regeneration (GEC/ml liver volume) referred to the initial preop. values showed a 25% decrease at POD 10, an increase up to 125% at POM 6 and a return to initial values at POM12 although only 80% of original liver volume was reached. **Conclusion:**Liver volume and cytosolic liver function after right hemihepatectomy in living liver donors, have different kinetics of regeneration especially within the first 10 postoperative days.

Abstract# 1411 Poster Board #-Session: P167-III

PREVALANCE AND SIGNIFICANCE OF VARIATIONS IN THE INTRAHEPATIC SEGMENTS OF PORTAL VEINS: EXPERIENCE IN 100 LIVING DONORS BASED ON PREOPERATIVE HELICAL COMPUTERIZED TOMOGRAPHY EVALUATION. Sebnem Örgüç, Ugur Gürgan, Deniz Nart, Cigdem Arikan, Sema Aydogdu, Zeki Karasu, Murat Zeytunlu, Murat Kilic, Yaman Tokat, Yildiray Yuzer. *[1]Organ Transplantation and Research Center, Ege University Medical Faculty, Izmir, Turkey.*

Objective: To review the prevalence of surgically significant portal venous anatomic variations encountered in 100 living liver donors, to correlate them with surgical findings and to determine the role helical Computerized Tomography (CT) on the selection of candidates and surgical management. Methods: We examined 100 consecutive living donor candidates with helical CT using 5-10mm slices, reconstructed at 3mm intervals. 3-D renderings of the portal vein and its branches were created. The influence of CT data on surgical planning is discussed. Results: Normal portal venous anatomy was found in 88 cases. Twelve cases had variations of the portal vein. Of these 7 were trifurcation of portal vein into left main, right anterior and right posterior branches. The other 5 had a the right anterior portal branch originating from left main portal branch or vise versa depending on the route of intermediate segment. Left lateral segment transplantation (S 2,3) was performed on 25 pediatric cases, five had a portal variation which did not have any influence on the surgical management. In 62 adult recipients right lobe liver transplantation (S 5,6,7,8) was done. In seven cases double portal veins, namely right anterior-posterior sectoral portal branches, were either combined on the bench table or anastomosed separately. One donor candidate was refused due to multiple vascular variations. The other 12 candidates were excluded for various reasons other than vascular abnormalities. Conclusions. Helical CT delineates the normal anatomy and demonstrates variations in the portal system of liver. Clinically relevant information regarding liver volume, fatty infiltration of parenchyma, vascular anatomy and coincidental upper abdominal lesions can be assessed using the data of a single examination. Isolated portal vein anomaly is not an indication for refusal of a living liver donor candidate. However preoperative knowledge of portal variations alters surgical management.

Abstract# 1412 **Poster Board #-Session: P168-III**

CAN LIVING DONOR LIVER TRANSPLANTATION PROMOTE TOLERANCE? Pam M. Kimball,[1] Robert Fisher.[1] [1]*Surgery, Medical College of Virginia, Richmond, VA.*

A unique feature of living donor liver transplantation (LDLT) is the period of explosive liver regeneration after surgery. Although undefined, the regenerating liver must release a plethora of growth hormones and cytokines. Whether these systemic changes impact recipient response to alloantigens is unknown. We evaluated post-LDLT recipient in vitro responses to donor specific and mitogenic challenge. Proliferation, cytokine secretion and apoptosis were measured. Patients were poorly to moderately DR matched with their donors: 3 patients were 2 DR mismatched and 2 patients were 1 DR mismatched. UNOS score was l (n=1), 2b (n=2) and 3 (n=3). All patients received mycophenolate mofetil, Prograf and prednisone. PBMC were collected on POD 13 ± 4 and challenged in donor specific mixed lymphocyte culture (dMLC) and with the mitogen PHA. Recipients were proliferatively hyporesponsive to dMLC challenge but showed a robust response to PHA. Proliferative response to dMLC was minimal (SI of 1.1 ± 0.3). In contrast, proliferative response to PHA was strong (SI of 48 ± 17 , p=.01). Secreted cytokine profiles showed minimal cytokine elaboration in response to dMLC but a TH1 profile in response to PHA. Secreted TNF levels were lower in dMLC vs PHA (64 ± 31 vs 4092 ± 1621 pg/ml, p=.03). IL10 levels were also lower in dMLC vs PHA cultures (1.2 ± 8 vs 142 ± 72 pg/ml, p=.05). In contrast, IL2 levels were equivalent (34 ± 6 vs 55 ± 25 pg/ml, p=ns). Lastly, the level of apoptosis was measured by elisa. Indices of apoptosis were low and equivalent in PBMC challenged with dMLC and PHA (686 ± 134 vs 470 ± 71 units, p=ns). Patients were clinically followed for l year post LDLT. All patients (100%) were rejection-free. Two patients died within 30 days of LDLT from non-immunologic causes (cardiac arrest and aspergillus infection). The remaining patients had stable graft function at one year. CONCLUSION. This small pilot study suggests that recipients of LDLT may enjoy a period of donor specific hyporesponsiveness after transplantation which is not mediated by apoptosis. Whether this period of 'tolerance' is a consistant feature of LDLT and will permit reduction of immunosuppression is a question worthy of comprehensive investigation.

Abstract# 1413 **Poster Board #-Session: P169-III**

UTILITY OF LIVER BIOPSY AND COMPUTED TOMOGRAPHY IN EVALUATION OF CANDIDATES FOR LIVING DONOR LIVER TRANSPLANTATION. Rafik M. Ghobrial,[1] Constantino Fondevila,[1] Piyaporn Limanond,[1] Steven S. Raman,[1] Charles Lassman,[1] James Sayre,[1] Sammy Saab,[1] David S. K. Lu,[1] Ronald W. Busuttil.[1] [1]*The Dumont-UCLA Transplant Center, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Objective: To determine the clinical utility of unenhanced computed tomography (CT) in the assessment of macrovesicular steatosis in potential donors for living related liver transplantation. **Patients and methods**: Of 50 potential living donors, we analyzed 42 candidates who underwent CT within 4 weeks of core liver biopsy. An experienced liver pathologist, blinded to both CT and surgical findings, retrospectively reviewed all the biopsy specimens and determined the degree of macrovesicular steatosis as graded on a percentage scale. A radiologist blinded to the histological grading calculated a mean hepatic density (MHD) in each donor liver and a mean splenic density (MSD). A liver density index (LDI) was derived and defined as the difference between MHD and MSD. The body mass index (BMI; kg/m2) was determined for each patient. Using linear regression analysis, the histological percentage of macrovesicular steatosis was correlated with both the LDI and BMI respectively. **Results**: Overall, LDI correctly predicted the degree of macrovesicular steatosis in 38 of 42 cases (90.5%). Four of 4 livers with LDI below10 Hounsfield (HU) correlated with >30% macrovesicular steatosis. Nine if 11 livers with LDI between 10 and 5 HU correctly predicted a range of 6-30% steatosis (relative contraindication). In 2 of 11 cases, the LDI overestimated the degree of hepatic steatosis. An LDI above 5 HU correctly predicted 25 of 27 livers with 0-5% steatosis. In 2 of 27 cases, parenchymal hemosiderin deposition elevated the LDI into normal range despite mild histological steatosis. Using a linear regression analysis, the degree of histological macrovesicular correlated well with CT LDI (r= 0.92) and marginally with BMI (r= 0.45). Of the 27 potential donors with normal livers on CT and acceptable LDI levels, 4 (14.8%) were deemed poor donor candidates because the core biopsy revealed subtle findings of hepatic necrosis and nonspecific hepatitis. **Conclusions**: Although unenhanced CT quantifies the degree of macrovesicular steatosis relatively well, it may preclude a liver biopsy only in patients with low LDI. In the pre-operative evaluation of living related donors with normal LDI, a core biopsy may still be necessary to detect those with both fatty liver and coexistant hemosiderin deposition or radiologically occult diffuse liver diseases.

Abstract# 1414 **Poster Board #-Session: P170-III**

INDICATION FOR HEPATIC VENOUS BRANCHES RECONSTRUCTION IN RIGHT-LOBE LIVING RELATED LIVER TRANSPLANTATION - ANATOMICAL AND VOLUMETRIC STUDY USING 3 DIMENSIONAL COMPUTED TOMOGRAPHY. Toshiro Ogata,[1] Koji Okuda,[1] Hideo Matsuo,[1] Kei Fujiki,[1] Hiroto Ishikawa,[1] Masashi Yasunaga,[1] Nobuharu Uchida,[1] Hikonobu Horiuchi,[1] Shigeki Hikida,[1] Hisafumi Kinoshita,[1] Kazuo Shirozu,[1] Shigeaki Aoyagi,[1] Hiroyoshi Mizote.[2] [1]*Department of Surgery, Kurume University School of Medicine, Kurume-City, Fukuoka, Japan;* [2]*Department of Pediatric Surgery, Kurume University School of Medicine, Kurume-City, Fukuoka, Japan.*

Purpose In the right-lobe liver graft without the middle hepatic vein (MHV), reconstruction of hepatic venous branches, such as inferior right hepatic vein (IRHV), middle right hepatic vein (MRHV), hepatic vein of segment 8 (V8) and hepatic vein of segment 5 (V5), is important to preserve maximum graft liver function. In this paper, the anatomical and volumetric study of the hepatic venous branches determined by 3 dimensional computed tomography (3D-CT) were investigated to establish a indication for reconstruction in living related liver transplantation (LRLT). **Materials and methods** Seventy-five patients in adult were examined with 3D-CT, injecting contrast materials intravenously. Anatomical variation and volumetry of the distribution area of the superior hepatic vein (SRHV), MRHV, IRHV, V5 and V8 were assessed by computer analyzed 3D-CT imaging. The distribution volume rates of hepatic venous branches in right lobe were calculated. **Results** *Anatomical variation* : In 12 cases, SRHV was short and IRHV, MRHV, large V5 were distributed to the posterior segment compensatingly. IRHV was detected in 41 cases, MRHV in 9 and large V5 in 16. In cases with short SRHV, the compensatory branches considered to be reconstructed. In 21 cases, MHV ramified two main branches near the orifice to VCI, in which one of the branches distributed to the anterior segment and another to the medial segment. In these cases, the right-lobe graft can be harvested with only the branch distributed to the anterior segment without circulatory disturbance of the remnant donor liver, and the branch can be reconstructed easily in graft implantation. *Volumetric study* : The average volume rates of SRHV, V5, MRHV, IRHV in right lobe was 63% (16~92%), 28% (19~43%), 8.5% (1.4%~4.5%), 19% (7.2~60%). The average distribution rates of V5, MRHV, and IRHV more than 5 mm and less than 5 mm in diameters were 28% and 14%, respectively. About 23% of these branches less than 5 mm in diameters distributed more than 20% of right lobe, and they were considered to be reconstructed. **Conclusion** Preoperative 3D-CT was useful as indication of hepatic venous branches reconstruction in LRLT considering ramification pattern and distribution of hepatic venous branches.

Abstract# 1415 **Poster Board #-Session: P171-III**

MEDICAL EVALUATION OF 170 POTENTIAL RIGHT LOBE LIVER DONORS. Elizabeth A. Pomfret,[1] James J. Pomposelli,[1] Fredric D. Gordon,[1] Mary Ann Simpson,[1] David L. Burns,[1] Edward Kreske,[1] Michele L. Sheehy,[1] Urmilla Khettry,[1] W. David Lewis,[1] Roger L. Jenkins.[1] [1]*Liver Transplantation and Hepatobiliary Surgery, Lahey Clinic Medical Center, Burlington, MA.*

Background: The evaluation of potential live liver donors (LD) is an important component of successful live donor adult liver tranplantation (LDALT) utilizing right lobe grafts. Careful medical and psychosocial evaluation of all potential donors is critical in diagnosing occult pathology thereby reducing the incidence of poor outcome for both donors and recipients. **Methods:** 170 potential right lobe LD between 18 and 55 years old (mean 36.9±9.6), sharing either a genetic or significant "emotional" relationship with the recipient were evaluated between 12/98 and 12/02. Patient demographics, preoperative medical data and surgical outcome were collected prospectively and recorded in a comprehensive database. Preoperative liver biopsy was performed selectively in patients with mildly elevated liver enzyme levels, BMI>28, evidence of steatosis by ultrasound or helical CT, or positive hepatitis B serological studies (HBsAb and/or HBcAb positive and HBsAg negative). **Results:** 95 men and 75 women with compatible blood type were evaluated and 56 (32.9%) underwent LD right hepatectomy and another 5 (2.9%) are currently awaiting surgery. 86 potential donors (50.6%) were rejected for either donor (n=43) or recipient (n=43) reasons and 23 (13.5%) elected not to participate after initial screening.

Reasons for Donor Rejection (N=43)

Reason	Frequency	Percent
Inadequate LV	8	18.6
Abnormal LFT	3	7.0
Unsafe anatomy	6	14.0
Steatosis	8	18.6
Medical	16	37.2
Psychosocial	2	4.7

LV=liver volume, LFT=liver function tests

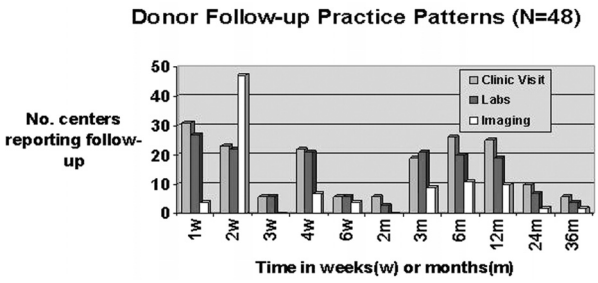
Liver biospy was performed in 23 potential LD (13.5%) with 58% being abnormal (macrosteatosis, non-alcoholic steatohepatitis (NASH), chronic portal inflammation, primary biliary cirrhosis (PBC) and hepatitis C infection (HCV)). Newly diagnosed conditions that prohibited donation were identified in 29 of the 147 potential LD (19.7%) who decided to proceed with the evaluation process. These included: macrosteatosis, hemochromatosis, PBC, NASH, HCV, polycystic liver and kidney

disease, renal cell carcinoma, multiple splanchnic arterial aneurysms, abnormal cardiac stress test and psychiatric disorder. **Conclusions:** Only 33% of potential donors actually go on to right lobe donation. One in 5 donors were diagnosed with a new medical or psychiatric condition that precluded donation. Of these 66% were due to liver related pathology. Many of these conditions were diagnosed noninvasively, therefore selective liver biopsy is advocated.

Abstract# 1416 Poster Board #-Session: P172-III

PRACTICE PATTERNS FOR LONG-TERM FOLLOW-UP OF ADULT-TO-ADULT RIGHT LOBECTOMY DONORS AT U.S. TRANSPLANTATION CENTERS. Kimberly L. Beavers,[1] Joseph Cassara,[1] Mark Russo,[1] Roshan Shrestha.[1] [1]*Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Protocols used by transplant centers to care for donors following right hepatectomy for living donor liver transplantation (LDLT) are not well described in the medical literature. Our goal was to describe practice patterns for the long-term follow-up of adult-to-adult right lobectomy donors at U.S. transplantation centers. Methods: All adult liver transplantation centers listed with UNOS were surveyed between May and November 2002. A transplant coordinator from each center completed a telephone questionnaire designed to define donor follow-up patterns. Data analysis was performed using STATA software, version 7 (College Stations, TX). Results: Of 97 adult liver transplantation centers, 90 (92.8%) completed the survey. 96% of participants were transplant coordinators, 2% hepatologists, and 2% surgeons. 51 (56.7%) programs have performed LDLT to adult recipients. Over 1044 right lobectomies have been performed. 38% of active programs have performed fewer than 10 donation right lobectomies (range 1-101). 35 (68.3%) programs have a formal follow-up protocol, and an additional 13 centers reported usual patterns. The protocols range from no formal follow-up, to visits every few weeks in the early postoperative period followed by evaluation every 6 months (Table). Evaluation beyond 12 months is typically on an as-needed basis. Personal psychosocial support services following donation are unusual and include: regular phone calls from the coordinator (5), quality of life instruments (3), scheduled follow-up with the psychologist (1) or a satisfaction survey (1). Several centers provide group newsletters, combined donor-recipient support groups, recognitions parties, and certificates. Conclusions: There is significant variability in how transplant centers are following donors over time. Formal psychosocial support following donation is rare.



Abstract# 1417 Poster Board #-Session: P173-III

RIGHT HEPATIC LOBECTOMY FOR AN UNDERLYING DISEASE PROCESS VS. FOR DONATION IN LIVING DONOR LIVER TRANSPLANT. Donavon Hess,[1] Timothy D. Sielaff,[1] Brooke Glessing,[1] William D. Payne,[1] Abhinav Humar.[1] [1]*Surgery, University of Minnesota, Minneapolis, MN.*

Background: Right hepatic lobectomy (RHL) from a healthy liver donor has been considered to carry similar risk as right hepatic lobectomy from patients with an underlying disease process (such as tumor), although objective data are lacking. Our aim was to compare the outcomes of patients undergoing RHL for liver donation vs. those undergoing RHL for disease. **Results:** A retrospective review of 20 liver donors and 22 patients with liver disease who underwent RHL was performed. Patients with disease were older and had more major comorbidities, including cancer in 21/22, than living donors. Surgical procedure was essentially similar in the 2 groups, consisting of a standard RHL with transection of the liver just to the right of the middle hepatic vein. There were no significant differences in intraoperative estimated blood loss (EBL) or transfusions for the 2 groups. Postoperatively, patients undergoing RHL for disease had a shorter length of hospital stay (patients were discharged after return of bowel function and oral medication pain control) compared to liver donors. No analyzed study factor correlated with the increased hospital stay. Complications were more frequent in those with underlying disease. Complications in patients with underlying disease included bile leak x1, atrial fibrillation x 3, and wound infection x 2. Only 1 donor developed a complication— a central line infection. There were no differences in the rise or return to normal of postoperative lab values including liver function tests, albumin, or INR (p=ns).

Hepatic Lobectomy: Disease vs. Donation

Study Factor	Disease	Donor	p value
Age (years)	57.7 ± 2.9	34.5 ± 1.9	< 0.01*
# male/female	7/15	10/10	0.23
# comorbid illnesses	2.0 ± 0.1	0.1 ± 0.1	< 0.01*
EBL (mL)	514 ± 71	398 ± 59	0.26
Units blood transfused	0.5 ± 0.3	0.1 ± 0.1	0.33
Length of stay (days)	6.3 ± 0.3	7.8 ± 0.3	< 0.01*
Major complications	6	1	< 0.05*

Conclusion: Complications were more frequent in patients undergoing RHL for disease but, despite more advanced age and associated medical comorbidities, these patients had a shorter hospital stay compared to living donors. This appears to be a result of slower return of bowel function and more difficulty with pain control in the living donors.

Abstract# 1418 Poster Board #-Session: P174-III

GRAFT WEIGHT TO RECIPIENT WEIGHT (GW/RW) RATIO: A POOR PREDICTOR OF OUTCOME AFTER PARTIAL LIVER TRANSPLANTS IN ADULT RECIPIENTS. Abhinav Humar,[1] Khalid Khwaja,[1] Brooke R. Glessing,[1] Elizabeth Larson,[1] Rainer Gruessner,[1] John R. Lake,[1] Angelika C. Gruessner,[1] Timothy D. Sielaff,[1] Willam D. Payne.[1] [1]*Surgery, University of Minnesota, Minneapolis, MN.*

Background: GW/RW ratio is widely used in pediatric living donor transplants to determine adequacy of graft size. Studies have shown that a low value (GW/RW<1.0%) is correlated with poor outcomes. We sought to determine if this was also true for adult living donor transplants (LDT) and cadaver split liver transplants (SLTs) performed for 2 adult recipients. **Results:** Between 1999-2001 we performed 40 partial liver transplants: 20 adult SLTs from 10 cadaver donors and 20 adult LDTs using the right lobe. All cadaver splits were performed in the midplane of the liver, generating a right and left lobe, which were both transplanted into adult-sized recipients. All adult LDTs were performed using the right lobe, with preservation of the middle hepatic vein with the donor. Recipients were analyzed in 2 groups: GW/RW <0.9% (n=15, mean=0.75%, range=0.65-0.85%) and GW/RW ≥0.9% (n=25, mean=1.21%, range=0.94-1.64%). Recipient characteristics of the 2 groups including MELD scores and UNOS status were similar. 80% of recipients in the ≥0.9% groups had received the right lobe vs. 67% in the <0.9% group. 56% of recipients in the ≥0.9% group had received a LDT, vs. only 40% of the recipients in the <0.9% group. GW/RW ratio was a poor predictor of early graft function. Serum INR, bilirubin, and ALT were not significantly different between the 2 groups at 1 day, 1 week, and 1 month posttransplant (Table). With mean follow up of 19 months, 13 of 15 (87%) recipients in the <0.9% group are alive with functioning grafts (2 early deaths due to hepatic artery thrombosis). In the ≥0.9% group, 22 of 25 (88%, p=ns) recipients are alive and well (1 early death from primary non-function, 2 late deaths from suicide and tumor recurrence). Only 1 recipient had primary non-function posttransplant (a cadaver SLT left lobe recipient with high pretransplant MELD score and GW/RW=1.48%). **Conclusions:** GW/RW ratio using 0.9% as a cut-off value is a poor predictor of outcome after partial liver transplants in adult recipients. Other formulas, perhaps using body suface area and MELD scores, or a different cut-off value for GW/RW ratio may be better predictors.

Recovery of liver function by GW/RW ratio

	GW/RW < 0.9%	GW/RW ≥ 0.9%	p value
Bilirubin (mg/dL):			
Pretransplant	4.8	2.2	ns
1 day	6.8	7.0	ns
1 week	3.4	3.8	ns
1 month	0.9	1.1	ns
INR			
Pretransplant	1.4	1.2	ns
1 day	1.5	1.5	ns
1 week	1.3	1.3	ns
1 month	1.2	1.2	ns

Abstract# 1419 **Poster Board #-Session: P175-III**

DONOR QUALITY OF LIFE AFTER LIVE DONOR ADULT LIVER TRANSPLANTATION (LDALT). Eric Richman,[1] Alyson M. Nixon,[1] Elizabeth A. Pomfret,[1] Mary Ann Simpson,[1] James J. Pomposelli,[1] Fredric D. Gordon,[1] W. David Lewis,[1] Roger L. Jenkins.[1] *[1]Liver Transplantation and Hepatobiliary Surgery, Lahey Clinic Medical Center, Burlington, MA.*

Background: The disparity between the number of adults requiring liver transplantation and the supply of available cadaveric organs has resulted in increased utilization of live liver donors. Extensive screening procedures have been instituted to insure the physical and psychological well-being of potential and actual living liver donors. We report the results of a prospective Quality of Life survey administered to all LDALT donors prior to surgery, and at 1 week, 1, 3 and 6 months, and 1 year postoperatively. **Methods:** Two survey instruments were used at each time point; the standard SF36 (V2) instrument and a customized survey developed with input from members of our donor evaluation team. To date, we have entered a total of 22 live donors. All have completed surveys through month 3; 17 have completed surveys through month 6; and 9 have completed all planned data points. **Results:** Pain and discomfort related to surgery were noted at 1 week and 1 month, with resolution in all cases by month 3. At all time points, 21 donors reported themselves as still willing to donate; this included 2 donors whose recipients died and 2 donors who experienced postoperative complications. One donor expressed ambivalence regarding donation indicating unwillingness to donate on surveys at 1 week and 1 and 6 months, and willingness to donate on the 3 month and 1 year surveys. This was our youngest donor at age 18. No donor reported "outside" pressure to donate, but 2 reported "self" pressure on the 1 week survey. All donors reported improvement in their relationship with the recipient and no change or improved relationships with their spouse or significant other. Most donors (18/22) expressed concern for out-of-pocket costs related to donations with emphasis on lost wages and transportation/lodging costs in the immediate postoperative perios. Three donors reported clinical depression with resolution in 2 and continuing treatment in 1. One additional patient experienced a transient reactive depression following the death of the recipient. **Conclusion:** Our results indicate that LDALT donors find the overall experience to be positive with regard to their personal relationships to recipient and family members. They also emphasize the need to adequately address financial issues associated with LDALT donation and suggest that special attention be paid to young donors to insure that they fully understand the expected postoperative events associated with donation.

Abstract# 1420 **Poster Board #-Session: P176-III**

IMPROVED OUTCOME OF LIVING-DONOR RIGHT LOBE LIVER TRANSPLANTATION WITH REVASCULARIZATION OF THE MIDDLE-HEPATIC VEIN. M. S. Cattral,[1] P. D. Greig,[1] C. Vollmer,[1] I. McGilvray,[1] M. Walsh,[1] A. Wei,[1] M. Molinari,[1] D. R. Grant. *[1]Multiorgan Transplant Program, Toronto General Hospital, University Health Network, Toronto, ON, Canada.*

Living-donor liver transplantation of right lobe grafts (RL-LDLT) has become an important option for adults with liver failure. The technical aspects of RL-LDLT are evolving. Management of the venous outflow from segments V and VIII of the graft, and the value of maintaining flow from these segments via the middle hepatic vein (MHV) is controversial. In this study, we compare the outcome of recipients that received liver grafts with or without revascularization of the MHV. Between Apr 00 and Nov 02, 49 RL-LDLTs were performed in 29 males and 20 females with a mean age of 51 ± 14 yrs. In all patients, the right hepatic vein (RHV) was anastomosed to the native RHV, which was enlarged by extending the orifice into the IVC. The MHV was revascularized in 21 patients either directly to the native MHV (17 patients) or with an interposition graft (4). A 3rd venous anastomosis between a segment VI or VII vein and the vena cava was performed in 7 of these patients and a 4th venous anastomosis were performed in 1. Among the 28 patients in whom the MHV was not reconstructed, a second venous anastomosis was performed in 9 patients (segment VIII, 4 patients; segment VI, 5); and 2 in this group had a 3rd anastomosis. The 2 groups were similar with respect to etiology of liver disease, graft/recipient weight ratios, cold and warm ischemic times, and blood product utilization during surgery. Without MHV revascularization congestion of segments of V and VIII was evident in most patients; splenectomy was performed in 1 patient with severe congestion to reduce portal vein inflow. MHV revascularization resulted in uniform graft perfusion and no congestion; hemodynamic measurements in 1 patient revealed that 56% of the total venous outflow was via the MHV. Serum liver tests and prothrombin time normalized more rapidly in patients with MHV drainage. Actuarial patient survival at 6 months was 92% and 77% among patients with and without MHV drainage, respectively (p=ns). Death in 2 patients without MHV drainage was directly related to graft failure from congestion. These data suggest that MHV revascularization may be a critical determinant of graft and recipient outcome. Maintaining flow through the MHV is likely to be of particular value when graft size is marginal or in recipients with severe portal hypertension.

Abstract# 1421 **Poster Board #-Session: P177-III**

LIVING DONOR LIVER TRANSPLANT WITH RENOPORTAL ANASTOMOSIS FOR SPONTANEOUS SPLENORENAL SHUNT. Shigeru Marubashi,[1] Kunihito Kotoh,[1] Kazuhiko Hashimoto,[1] Masaru Kubota,[1] Shogo Kobayashi,[1] Shinji Yamamoto,[1] Hiroaki Nagano,[1] Keizo Dono,[1] Shoji Nakamori,[1] Koji Umeshita,[1] Masato Sakon,[1] Morito Monden.[1] *[1]Surgery and Clinical Oncology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Spontaneous splenorenal shunt (SSS) is not rare in end stage liver disease. Portal vein is often thrombosed and liver transplant cannot be indicated due to the difficulty of portal reconstruction in SSS patients. In this study, we investigated the feasibility and outcome of living donor liver transplant with renoportal anastomosis for portal reconstruction. **Patients and methods** Among thirty-nine patients who underwent living donor liver transplants in our institute from March 1999 to December 2002, two adult patients had spontaneous splenorenal shunt prior to the transplants.*Case 1* 29-year-old female was admitted due to end stage liver disease secondary to Primary screlosing cholangitis (PSC). Portal vein flow was not detected, and markedly developed splenorenal shunt was observed. Living donor liver transplant was performed using right lobe graft from her husband. Left internal jugular vein of the recipient was used for a graft. Under extracorporeal venovenous bypass, left renal vein was clamped and ligated, then left renal vein was anastomosed to jugular vein graft in end-to-end fashion. Vein graft was then anastomosed to right portal vein of the liver graft. Her postoperative course was uneventful and liver regenerated normally. Portal hemodynamics and her liver and renal functions are normal 29 months after transplant. *Case 2* 61-year-old male was admitted due to end stage liver disease of unknown cause with small hepatocelullar carcinomas. Portal vein flow was not detected, and markedly developed splenorenal shunt was observed. Living donor liver transplant was performed using the same technique as in the case 1, except a catheter was placed into one of veins of splenorenal shunt and portal vein flow and pressure were studied postoperatively with Doppler ultrasonography and electric manometer. Portal vein flow was maintained steadily after the transplant and portal pressure was decreased from 40 mmHg on Day1 to 20 mmHg on Day 5. His postoperative course was uneventful, and his liver and renal functions are normal 4 months after transplant. **Summary** Living donor liver transplant with renoportal anastomosis using internal jugular vein graft is a safe and feasible option for ESLD with spontaneous splenorenal shunt.

Abstract# 1422 **Poster Board #-Session: P178-III**

DO WE NEED TO RECONSTRUCT THE MIDDLE HEPATIC VEIN IN ADULT TO ADULT LIVING DONOR LIVER TRANSPLANTATION? John P. Roberts,[1] Nancy L. Ascher,[1] Igal Kam,[2] Tom Bak,[2] Chris Freise,[1] Mike Wachs.[2] *[1]Department of Surgery, University of California San Francisco, San Francisco, CA; [2]Department of Surgery, University of Colorado Health Science Center, Denver, CO.*

Reconstruction of the middle hepatic vein (MHV) has been advocated in adult to adult living donor transplantation to preserve graft outflow. Many groups report a high percentage of MHV reconstructions during graft implantation. We examined outcome following adult to adult living donor transplantation at two centers to determine the necessity for MHV reconstruction. 118 adult to adult living donor transplants were performed in two centers. 6 patients have died (actual patient survival of 95%). 16 patients required re-transplantation (actual graft survival 84%). MHV reconstruction was performed only rarely and only to the recipient MHV without using interposition grafts. Inferior right hepatic veins (IRHV) were re-implanted when they were greater than 5-10 mm. 3 MHVs (3%) were reconstructed and 15 IRHVs. Of the 3 MHVs reconstructed, 1 thrombosed, and one had reversal of flow on the post-operative ultrasound, but both grafts had excellent long term function. Conclusions: Excellent patient and graft survival can be obtained with rare MHV reconstructions. Decompression of the segments with obstructed veins probably occurs by retrograde portal venous flow in those segments.

Abstract# 1423 Poster Board #-Session: P179-III

PERMANENT PORTACAVAL SHUNTING TO AVOID HYPERPERFUSION OF THE ALLOGRAFT AFTER A RIGHT LOBE LIVING RELATED DONOR LIVER TRANSPLANTATION. Murat Kilic, Gokhan Unsal, Murat Zeytunlu, Yildiray Yuzer, Yaman Tokat. *[1]Organ Transplantation and Research Center, Ege University Medical Faculty, Izmir, Turkey.*

Background: Hyperperfusion and swelling of the allograft is a relatively new entity encountered particularly after partial liver transplantation. Various forms of portacaval diversion may be employed to overcome this problem. Herein we report our experience with a case requiring a permanent portacaval shunt to overcome hyperperfusion of the transplanted allograft. Material and Method: A 39 years old male patient who had cirrhosis secondary to chronic hepatitis B infection underwent a right lobe living donor liver transplantation from his 36 years old wife. The patients weighed 62 kg and the weight of the right lobe was 685 grams resulting in a graft to recipient body weight ratio of 1.1 %. The cirrhotic liver was removed by preserving the native vena cava without a temporary portacaval shunt. The right hepatic vein of the donor liver was anastomosed to the right hepatic vein cuff of the recipient and two accessory veins draining segment 5 and 8 were anastomosed separately to the vena cava using recipient's umbilical vein as a conduit. The recipient's portal vein was found to be partially thrombosed and had cavernous transformation. The thrombus was cleaned without difficulty and the enlarged portal vein trunk was anastomosed to the donor right portal vein in an end-to-end fashion. Rapidly after reperfusion the liver started to swell resulting in small capsular tears and bleeding from the cut surface. The hepatic veins were checked to be patent and the portal vein was clamped. A side-to-side portacaval shunt between the native portal vein and vena cava was performed using a 12 mm polytetrafluoroethylene graft. Upon releasing the clamp the liver did not suffer from hyperperfusion and the rest of the procedure was carried uneventfully. Result: The postoperative course was uneventful and the patients was discharged home on day 26. The patient is well and alive with an 8 month follow-up and the shunt is still patent on ultrasound examination. His liver function tests are within normal limits without any sign of hypo or hyperperfusion of the liver. Conclusion: Hyperperfusion of the transplanted liver is a serious problem affecting partial liver allografts and permanent portacaval shunting may be good alternative to overcome this problem.

Abstract# 1424 Poster Board #-Session: P180-III

CALCULATION OF GRAFT LIVER SIZE BASED ON BODY SURFACE AREA (BSA) IS AS RELIABLE AS RADIOLOGIC GRAFT ESTIMATION FOR LIVING DONOR LIVER TRANSPLANTATION. Talia B. Baker,[1] Paolo R. Salvalaggio,[1] Alan J. Koffron,[1] Jonathan P. Fryer,[1] Andres Blei,[1] Michael M. Abecassis.[1] *[1]Dept. of Surgery and Internal Medicine, Division of Transplantation and Hepatology, Northwestern University, Chicago, IL.*

Introduction: Living donors represent an evolving and crucial aspect of liver transplantation. An accurate prediction of adequate functional liver mass is a fundamental component of donor selection. Radiologic volume estimation is viewed by most centers as the gold standard in estimating liver volume (LV) of living donors. **Aim:** To compare a simple and reliable method based on body surface area (BSA) to radiologic volume estimation for both right lobe(RL) and left lateral segment (LLS) living donors. **Methods:** Between 1997 and the present, 70 living donor liver transplants (RL, n= 27; LLS, n= 43) have been performed at our institution. Complete records were available for 54 (RL, n=23; LLS, n=31). LV was calculated based on the formula of Heineman et al, {Liver Transpl Surg 1999 5(5):366} : LV = 1072.8 x BSA-345.7. Actual graft volume (AGV) was weighed at the time of procurement. Radiologic graft volume(radiologic-GV) by MRI or CT were obtained for all donors. Based on LV and AGV, we calculated graft volume (calculated-GV). Accuracy of our calculations were tested by comparing the ratio between radiologic-GV and calculated-GV to Actual-GV. **Results:** Using our analysis we predicted that calculated-GV for RLs would be 47+/-6.6% x LV, and for LLS would be16+/-4.7% x LV. AGV, radiologic-GV, and calculated-GV for both RL and LLS are displayed in the table below. Comparing radiologic-GV and calculated-GV for RLs demonstrated predictive ratios of 1.29+/-0.2 and 1.002+/-0.14 for each method respectively($p<0.05$). For LLS, the predictive ratios were 0.95+/-0.3 and 1.10+/-0.4, respectively($p<0.05$). **Conclusions** Although radiologic-GV is a useful tool for predicting functional liver mass for living donor transplants, we propose that calculation of liver mass based on BSA is as reliable and less cumbersome than radiologic measurement. Therefore, we suggest that radiologic measurement of living donor grafts (both RL and LLS) is redundant and thus obsolete.

Graft Volume Predictions for Living Donor Transplants

	AGV(gm)	radiologic-GV(gm)	calculated-GV(gm)
RL	819.7+/-142.3	1058+/-230	809.5+/-114.7
LLS	282.2+/-105.3	278.7+/-138	284.7+/-62

RL calculated-GV= 47+/-6.6% x LV; LLS calculated-GV =16+/-4.7% xLV

Abstract# 1425 Poster Board #-Session: P181-III

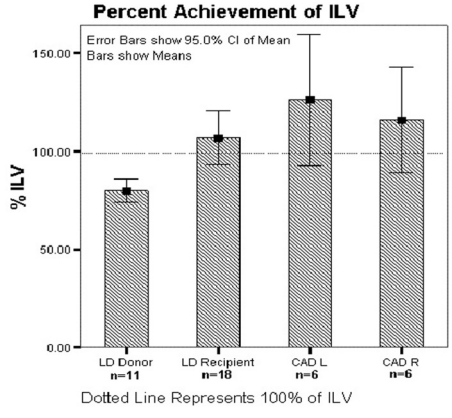
INSULIN SENSITIVITY AND β-CELL FUNCTION IN LIVING-DONOR LIVER TRANSPLANTATION. Martin Stockmann,[1] Sabine Nolting,[1] Thomas Konrad,[2] Diana Hünerbein,[1] Thomas Steinmüller,[1] Peter Neuhaus.[1] *[1]Dep. of Translplantation Surgery, Charité - Campus Virchow Klinikum, Berlin, Germany; [2]Institute of Metabolism Research, Frankfurt, Germany.*

INTRODUCTION: Alterations of glucose metabolism after liver transplantation or liver resection are not well understood. Aim of this study was analysis of insulin sensitivity and β-cell function in living-donor liver transplantation of the right hepatic lobe using a minimal modeling technique of glucose disappearance. **METHODS:** We investigated 13 donors and recipients before and 10 days after right lobectomy or liver transplantation of the right lobe. Insulin sensitivity (SI) was assessed by a computer-assisted minimal modeling technique devised by Bergman et al. using a frequently sampled intravenous glucose tolerance test with 300 mg/kg BW glucose 50% (Konrad et al., 1999). β-cell responsiveness (first and second phase of pancreaticβ-cell secretion) was determined by a c-peptide modeling analysis (SAAM II software). In addition a bioelectrical impedance analysis was done. **RESULTS:** Liver donors showed a SI of $4.01 \pm 0.90 \times 10^{-4}$ min^{-1} x µU/ml before operation which decreased after right lobectomy by 40% to SI $2.42 \pm 0.38 \times 10^{-4}$ min^{-1} x µU/ml, β-cell function was unchanged. Compared to controls recipients were insulin resistant before transplantation (SI $1{,}73 \pm 0{,}36 \times 10^{-4}$ min^{-1} x µU/ml, $p<0.05$). β-cell function was not significantly different. Ten days after transplantation SI increased by 48%, β-cell function was not significantly different. Bioelectrical impedance analysis showed no significant differences. **DISCUSSION:** Right lobectomy in healthy liver donors resulted in markedly impaired insulin sensitivity of the peripheral tissues with unchanged β-cell response 10 days after the operation. Living-donor liver transplantation of the right lobe improved disturbed insulin sensitivity already 10 days after the operation despite high immunosuppression with glucocorticoids and tacrolimus. Thus, liver function itself seems to play a more pronounced role in glucose metabolism than know until now.

Abstract# 1426 Poster Board #-Session: P182-III

LIVER REGENERATION AFTER LIVING DONOR AND CADAVER SPLIT LIVER TRANSPLANTS (SLTs): DONORS VS. RECIPIENTS. Kambiz Kosari,[1] Kevin M. Williams,[2] Maria L. Rodrigues-Gomes,[2] Galia Rosen-Tirosh,[2] John R. Lake,[1] William D. Payne,[1] Timothy D. Sielaff,[1] Abhinav Humar.[1] *[1]Surgery, University of MN, Minneapolis, MN; [2]Radiology, University of MN, Minneapolis, MN.*

Background: There is significant interest in patterns of liver regeneration after partial liver transplants. We looked at liver regeneration, as measured by CT volumetric measurements to determine if there were significant differences between living donors (LD), their recipients, and recipients of cadaver SLTs. **Methods:** We studied liver volumes in 42 adult patients who had either received a partial liver transplant (right lobe LDLT, n=18; right lobe cadaver SLT, n=6; left lobe cadaver SLT, n=6) or who had donated their right lobe for LDLT (n=12). Liver volume was measured at 3 months postop using CT scan, and compared to the patient's ideal liver volume (ILV), calculated using the equation ILV=-794.41 + 1,267.28 x Body Surface Area (BSA) (m2). **Results:** LD vs. cadaver donors were older ($p<0.01$) and smaller in size ($p=0.06$). Comparing recipients who received a cadaver vs LD transplant, the former had higher levels of INR, bilirubin, and ALT during the 1st posttx week. By 1 month posttx, this difference was not apparent. Donors of right lobe grafts, had similar lab values to those seen with their recipients. With regards to measured liver volume at 3 months postop, LD liver donors achieved 79.8% of their ILV; this was less than that seen in cadaver R lobe (116.0%; $p=0.006$), cadaver L lobe (126.3%; $p=0.001$), and LD R lobe (106.9 %; $p=0.006$) recipients (figure). As a function of change from immediate postop liver volume, living donors had a 82.9% increase in liver volume, vs. 124.5% for LD recipients, 132.7% for CAD R lobe recipients, and 167.6% for CAD L lobe recipients. **Conclusion:** Liver regeneration, as measured by CT volume, seems to be greatest in cadaver SLTs. In LD transplants, recipients seem to have greater liver growth vs. the donors. One possible explanation is greater stimulus for liver growth in patients with preexisting liver disease.



Abstract# 1427 Poster Board #-Session: P183-III

IMPACT OF HLA MATCHING ON HEPATITIS C RECURRENCE FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION. Ian C. Carmody,[1] Rafik M. Ghobrial,[1] David Gjertsen,[2] Elaine F. Reed,[2] Douglas G. Farmer,[1] Constantino Fondevila,[1] Yue-Ming Huang,[1] Lena Tang,[1] Ronald W. Busuttil.[1] *[1]Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA; [2]Pathology and Laboratory Medicine, Geffen School of Medicine at UCLA, Los Angeles, CA.*

Introduction: Hepatitis C (HCV) is the most common indication for orthotopic liver transplantation (OLT) in North America. Previously we have shown more rapid and severe HCV recurrence following adult living donor OLT. The aim of this study is to assess the impact of HLA matching on the time to recurrence of HCV after OLT. **Methods:**Out of 510 patients we identified 227 who underwent primary cadaveric OLT for HCV and had complete HLA matching data between 1990 and 2000 at out center. HLA data for A, B and DR loci were obtained from both UNOS and the our tissue typing laboratory. Recurrent HCV was diagnosed by histological evidence of recurrence in the presence of biochemical graft dysfunction. **Results:** Median follow-up time was 30 months. There were no transplants performed with either 0 or 1 HLA donor recipient mismatches (MM). In combined Class 1 and 2 HLA mismatches recurrence rates were 26.8 and 25.8 recurrences per 100 person-years for 2 and 3 MM respectively. In contrast, with 4/5 or 6 MM recurrence rates were reduced to 21.8 and 15.2 respectively. When only Class 1 (A and B) MM were considered recurrence rates dropped from 34.6 with 1 MM to 17.1 for 4 MM respectively. Similar trends were found in HLA A with 1 and 2 MM, there were 25.3 and 18.4 recurrences per 100 person-years and HLA B with 1 and 2 MM, 24.9 and 19.5 recurrences per 100 person-years.With regard to HLA Class 2 (DR only) mismatching with 1 and 2 MM there were 23.5 and 19.4 recurrences per 100 person-years. **Conclusions:**Our study shows a clear trend towards reduced HCV recurrence rates with higher Class 1 or 2 HLA mismatches. HLA mismatching therefore appears to confer protection against HCV recurrence. Our finding may have significant impact on adult living-related donor transplantation for HCV.

Abstract# 1428 Poster Board #-Session: P184-III

PEG INTERFERON α-2b (PEG) AND RIBAVIRIN IS EFFECTIVE AND SAFE AFTER LIVER TRANSPLANTATION (LT). Fredric D. Gordon,[1] Denise Morin,[1] Andrew Keaveny,[1] Eric Goldberg,[1] Stephen C. Fabry,[1] Elizabeth A. Pomfret,[1] James J. Pomposelli,[1] W. David Lewis,[1] Mary Ann Simpson,[1] Roger L. Jenkins,[1] Urmila Khettry.[1] *[1]Hepatobiliary and Liver Transplantation, Lahey Clinic Medical Center, Burlington, MA.*

Background: Recurrent hepatitis C (HCV) is an increasing cause of graft loss in patients undergoing LT. Treatment of HCV after LT is often possible, as pre-transplant contraindications may no longer exist. Our group has shown that standard interferon (IFN) and ribavirin therapy can result in a 29% sustained response (SR) rate. The aim of this study is to determine if treatment with PEG and ribavirin is safe and effective in eradicating HCV after LT. **Methods**: 12 patients with recurrent HCV documented by viremia and liver biopsy after LT were identified. The characteristics of the group included: genotype 1 in 10 subjects and genotype 3a in 2 subjects. Grade 3-4 inflammation was seen in 3/12 and stage 3-4 fibrosis in 4/12. The median pre-treatment viral load was 4,100,000 copies/cc (range 28,000-13,000,000). The median time to initiation of treatment was 44 months (range 11-144 months) after LT. The primary immunosuppressive agent was tacrolimus in 9/12 and cyclosporine in 3/12. All patients were prescribed PEG 1.5 mcg/kg/wk. Ribavirin was begun at 200 mg bid and increased weekly until a maximum of 400mg bid was achieved. Treatment is planned for 12 months with a 6 month follow-up period. Therapy was stopped if the patient became intolerant of side effects or irreversible cytopenias developed. **Results**: All patients have had at least 6 months of therapy and/or follow-up. 6 (50%) were HCV RNA undetectable at 6 months of therapy. 4 patients have completed 12 months of therapy and are HCV RNA undetectable (two genotype 1 and two genotype 3a). Two patients discontinued treatment within one month because of erythropoietin-resistant anemia (n=1) and hepatic decompensation resulting in death (n=1). 7 patients required erythropoietin support and 2 patients also required G-CSF. Other side effects not requiring dose reduction included headache (n=3), fatigue (n=6), diarrhea (n=2), anorexia (n=2), and depression (n=4). No episodes of rejection were seen. **Conclusions**: 1) Undetectable HCV RNA levels can be achieved in LT recipients treated with PEG/ribavirin. 2) PEG/ribavirin therapy can be used safely in LT recipients, however, side effects are frequent and colony stimulating factors are needed in more than 50% of patients. 3) End of treatment and SR rates are anticipated. 4) A current large randomized multicenter trial will further address sustained virological and histologic response rates.

Abstract# 1429 Poster Board #-Session: P185-III

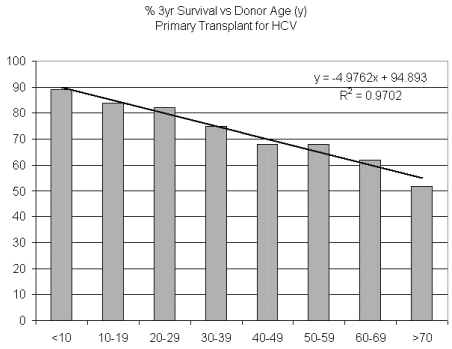
VIRTUAL ABSENCE OF CYTOMEGALOVIRUS DISEASE IN LIVER TRANSPLANT RECIPIENTS RECEIVING SIROLIMUS WITH 3-DAY CORTICOSTEROID TAPER AS PRIMARY IMMUNOSUPPRESSION. Ariana Wallack,[1] Nancy Stolpman,[2] Tracy Steinberg,[3] Marcelo Kugelmas,[1] Thomas Bak,[3] Igal Kam,[3] Gregory T. Everson,[1] James F. Trotter.[1] *[1]Division of Gastroenterology/Hepatology, University of Colorado Health Sciences Center, Denver, CO; [2]Department of Pharmacy, University of Colorado Health Sciences Center, Denver, CO; [3]Division of Transplant Surgery, University of Colorado Health Sciences Center, Denver, CO.*

Background: Cytomegalovirus (CMV) disease is the most common infection following liver transplantation, occurring in approximately 20 % of recipients. CMV disease is associated with a higher cost and a higher incidence of subsequent infections after transplantation. We noted an extremely low incidence of CMV disease in our cohort of liver transplant recipients receiving sirolimus as part of a primary immunosuppressive protocol and report our experience here. **Methods:** All 150 patients transplanted between January 2000 and October 2001 with sirolimus and tacrolimus or cyclosporine A and 3-day corticosteroid taper were analyzed. All patients had follow-up for at least one year after transplantation. Comparisons were made with 191 historical controls transplanted between 1997 and 1999 who received tacrolimus or cyclosporine A and 14-day corticosteroid taper. The incidence of CMV disease (defined as: positive tissue culture or positive immmunohistochemical stain of tissue or positive culture of the blood or CMV DNA > 3,000 associated with clinical symptoms (fever, malaise, cytopenia)) were recorded for each patient for 365 days after transplantation. Risk factors for acquisition of CMV were analyzed including: "CMV mismatches" (donor CMV IgG positive, recipient CMV IgG negative), OKT3 use and calcineurin-inhibitor blood levels. **Results**: The incidence of CMV disease in patients receiving sirolimus was 3/150 (2 %) which was 88 % lower than historical controls 33/191 (17 %), $p < 0.001$. The proportion of "CMV mismatches" in the sirolimus group (23/150 or 15.3 %) was not different than the control group (26/191 or 13.6 %), p = ns. OKT3 was administered to a significantly lower proportion of sirolimus patients (15.3 %) vs. the control group (37 %), $p < 0.05$. Overall mean tacrolimus and cyclosporine A levels were significantly lower in the sirolimus patients by 36 % and 28 %, respectively, $p < 0.05$. **Conclusions:** 1) CMV disease is virtually absent in liver transplant recipients administered a prednisone-free, sirolimus immunosuppressive regimen. 2) Possible explanations for this finding include the absence of prednisone, significantly lower blood levels of calcineurin-inhibitors and reduced administration of OKT3..

Abstract# 1430 Poster Board #-Session: P186-III

THE IMPACT OF DONOR AGE ON PATIENT AND GRAFT SURVIVAL AFTER PRIMARY LIVER TRANSPLANT FOR HEPATITIS C. Sasan Roayaie,[1] Aisha Sarkar,[1] Sukru H. Emre,[1] Thomas M. Fishbein,[1] Charles M. Miller,[1] Myron E. Schwartz.[1] *[1]Recanati-Miller Transplantation Institute, Mount Sinai Medical Center, New York, NY.*

Purpose: To determine the impact of donor age on patient and graft survival after transplant for hepatitis C (HCV). **Methods:** Patients undergoing their first liver transplant between 6/89 and 5/00 were analyzed. Recipient and donor demographics and preoperative labs as well as era of transplant, postoperative immunosuppression, episodes of rejection, use of OKT3 and presence of hepatocellular carcinoma were analyzed. HCV patients were compared to non-HCV patients. **Results:** Of the 1324 patients transplanted during this period, 480 were HCV positive. Median follow-up was 79.6 ± 34 months. Donor age, and recipient PT, BUN and creatinine were the only variables that significantly correlated with patient survival on univariate analysis for HCV patients. Donor age and recipient PT were the only significant variables on multivariate analysis. There was a significant inverse linear relationship between donor age and 3-yr patient survival for HCV patients (fig 1). Donor age was the only variable associated with graft survival for HCV patients. There was a trend toward lower patient (p=0.08) and graft (p=0.075) survival when comparing HCV (45 & 36% @ 10yr) to non-HCV patients (57 & 50% @ 10yr). Donor age was not a significant predictor of patient or graft survival for non-HCV patients. Mean donor age rose from 35.8 yr in the '90-92 era to 48.4 in '96-98. Patient and graft survival were significantly lower for HCV than non-HCV patients in the '96-98 era. **Conclusions:** Donor age is an independent predictor of patient and graft survival after primary transplant for HCV but not for other indications. Donor age has increased over time with a concomitant decrease in patient and graft survivals for HCV patients. Modification of the liver allocation schema to preferentially direct younger donor organs to recipients with HCV would result in higher graft and patient survival for the entire population of liver recipients.



Abstract# 1431 Poster Board #-Session: P187-III

POSTTRANSPLANT HEPATITIS C RECURRENCE IS INFLUENCED BY NON-VIRAL TRANSPLANT RELATED FACTORS: TEN-YEAR ANALYSIS IN OVER 300 PATIENTS. Rafik M. Ghobrial,[1] Yue-Ming Huang,[1] Douglas G. Farmer,[1] Ian C. Carmody,[1] Jeffrey Gornbein,[1] Randy Steadman,[1] Sammy Saab,[1] Leonard Goldstein,[1] Hassan Yersig,[1] F. Duraszo,[1] S. Han,[1] Lena Tang,[1] Ronald W. Busuttil.[1] *[1]Surgery, Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: End stage liver disease (ESLD) caused by Hepatitis C virus (HCV) infection is the leading cause for OLT. Unfortunately, HCV recurrence following orthotopic liver transplantation (OLT) may lead to graft failure and death. Although viral related factors predisposing to HCV recurrence have been widely investigated, the effects of non-viral transplant related variables that may influence posttransplant recurrence are not yet defined. **Methods:** Retrospective review of patients who underwent primary OLT for HCV over a ten year period at our center. HCV recurrence was estimated by graft biopsy. Time to recurrence was considered as primary endpoint. Four donor, 5 recipient and 2 operative variables that may affect outcome were analyzed. Univariate comparison utilized log-rank methods. Cox proportional hazard regression model was employed for multivariate analyses. **Results:** Recurrence-free survival for the 307 pts in this study was 69% and 37% at 1 and 5 years, respectively. Of donor factors, donor hospital stay >5 days (relative risk, RR 2.1; P 0.0002) and progressive increase of donor age (RR 1.37 with ages between 20-39 versus RR 2.2 with 50-59 years, (P 0.04) were univariately associated with shorter time and increased risk of HCV recurrence posttransplantation. Other risk factors, by univariate analysis, included recipient AST levels >81 U/L (RR 1.9, P <0.001), warm ischemia time > 45 min (RR 2.7, P<0.001), and cold ischemia time > 10 hours posttransplantation. Increased donor/recipient HLA matching exhibited a trend for early HCV recurrence. Multivariate examination demonstrated that progressive increase in donor age, donor length of hospital stay > 5 days (RR 3.7, P <0.001), warm ischemia time > 45 min (RR 2.6, P <0.001) and urgent recipient status (RR 1.6. P 0.02) were significant independent predictors of early HCV recurrence following OLT. **Conclusions:** Peri-transplant donor and recipient factors impact posttransplant recurrence-free survival following OLT for HCV. Our findings predict an increased rate of HCV recurrence in the current era of transplantation that dictates liberal criteria of cadaveric organ use and selection of critically ill patients for transplantation.

Abstract# 1432 Poster Board #-Session: P188-III

INFECTIOUS COMPLICATIONS IN ADULT TO ADULT LIVING RELATED LIVER TRANSPLANTATION. Deepali Kumar,[1] Mark Cattral,[1] Paul Greig,[1] David Grant,[1] Atul Humar.[1] *[1]Multi-organ Transplant, University of Toronto, Toronto, ON, Canada.*

Background: Adult-to-adult right lobe liver transplantation has become more common due to the relative shortage of cadaveric donors. Infectious complications post-living related (LRD) liver transplantation have not been systematically reviewed and may differ from cadaveric transplants. We conducted a retrospective study comparing infectious complications in adult LRD liver transplant recipients vs. cadaveric transplant recipients. Methods: A chart review was conducted of all LRD transplants performed at our institution. Patients were compared to those who had undergone standard orthotopic liver transplantation. Only microbiologically documented infections with a compatible clinical syndrome were included in the analysis. These included bacterial infections and opportunistic infections such as CMV disease and invasive fungal infections. Results: Data was reviewed for 43 LRD transplants and compared with a cohort of 200 cadaveric transplant recipients. Baseline characteristics including age, gender, and induction immunosuppression were not significantly different between LRD and cadaveric transplants. The rate of opportunistic infections was comparable or slightly lower in LRD vs. cadaveric transplants and included CMV disease (4.7% vs. 16.0% respectively, p=0.05) and invasive fungal infections (4.7% vs. 8.5% respectively, p=NS). Bacteremia occurred at a similar rate in the two groups (25.6% for LRD vs. 19.5% for cadaveric, p=NS) at a mean time of 46.3 vs. 56.7 (p=NS) days post-transplant. However, there was an overall greater rate of microbiologically confirmed bacterial infections in LRD transplants (37.2% vs. 21.5%, p=0.03). Of these, biliary tract complications were significantly greater in LRD transplants (34.9% vs. 9.5%, p<0.01). Specifically, cholangitis occurred in 7.0% vs. 1.0% (p=0.04) of LRD and cadaveric recipients respectively and post-operative bile leak occurred in 27.9% vs. 8.5%, (p<0.01). A significant portion of bile leaks in the LRD patients were secondary to cut-surface bile leaks (5/12; 42%). Conclusions: Overall rates of infection are similar in LRD vs. cadaveric liver transplants. However, biliary tract complications such as bile leaks and cholangitis were more common in LRD recipients. Cut-surface bile leaks are a more common problem in LRD transplants.

Abstract# 1433 Poster Board #-Session: P189-III

NON-RESPONDERS OF INTERFERON/RIBAVIRIN TREATMENT FOR RECURRENT HEPATITIS C FOLLOWING LIVER TRANSPLANTATION. Gregory A. Smallwood,[1] Laurel Davis,[2] Enrique Martinez,[3] Andrei C. Stieber,[2] Thomas G. Heffron.[2] *[1]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [2]Department of Surgery, Emory University School of Medicine, Atlanta, GA; [3]Department of Medicine, Emory University School of Medicine, Atlanta, GA.*

Background: Treatment of recurrent hepatitis C (HCV) following liver transplant currently includes ∝ -interferon with ribavirin. **Objective:** The aim of this study is to evaluate non-responder protocols for patients failing treatment for recurrent hepatitis C following liver transplantation. **Methods:** Follwing liver transplantation, 67 patients, all serum RNA positive for hepatitis C with histological evidence of recurrence underwent treatment with ∝ -interferon and ribavirin. For patients that failed treatment, patients were begun on either amantadine with interferon/ribavirin or pegulated interferon with ribavirin. **Results:** Of the initial 67 patients, there was a viral clearance in only 14.9 % (10/67). 79.1% (53/67) of the patients had a biochemical response by month 3 with a decrease in ALT [161 (± 91) u/L vs. 71.2 (± 130) u/L; p = 0.003], total bilirubin [1.6 (± 0.9) ng/dl vs. 1.2 (± 0.5) mg; p = 0.05], viral load (2.5 X 10^6 vs. 0.9 X 10^6, p = 0.02) and AST [146 (± 500) u/L vs 68 (± 103); p = 0.002). Patients were withdrawn from treatment [30/67 (44.7 %) due to adverse events associated with bone marrow or hemoglobin suppression. Non-responders were bgun on amantadine (n = 12) with interferon/ribavirin or pegulated interferon/ribavirin (n = 15). In the amantadine group, 3 (25%) had to discontinue due to slurred speech, dizziness, and increased depression. No biochemical response was seen with amantadine [ALT (42.8 (± 6.5) u/L vs. 51.6 (± 11.7) u/L; p = ns), AST (51 (± 11.7) vs. 49.4 (± 7.1) U/l, p = NS), and T. bilirubin (1.1 (± 0.1) mg/dl vs. 1.3 (± 0.1) mg/dl; p = NS]. Although no patients cleared the virus, one log drop was seen (1.6 X 10^6 vs. 0.9 X 10^6; p = 0.4). The pegulated group had 4 (26.7%) patients drop out with with one hospitalized for diarrhea and dehydration. 30% (n = 5) had dose reductions due to adverse events. 3 (20%) patients had complete viral clearance during treatment with 2 (13.3%) patients maintaining a sustained response. Pegulated biochemistries were reduced [ALT (78 (± 62) u/L vs. 60 (± 54) u/L), AST (70 (± 23) u/L vs. 59 (57) u/L), and T. bili (1.4 (± 1.1) vs. 1.2 (± 05) u/L]. Survival during treatment was decreased if the patient was African-American (p = 0.01) or had a prednisone dose of > 15mg/day (p = 0.03) at week 6 following liver transplant. **Conclusions**: Pegulated interferon with ribavirin appears to be superior to amantadine with interferon/ribavirin when used in non-responders for hepatitis C viral clearance.

Abstract# 1434 Poster Board #-Session: P190-III

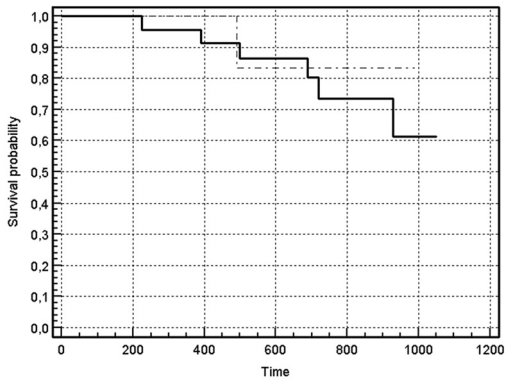
VALGANCICLOVIR TREATMENT OPTION FOR REUCRRENT, RESISTANT HEPAITITS B FOLLOWING LIVER TRANSPLANTATION. Gregory A. Smallwood,[1] Enrique Martinez,[2] Andrei C. Stieber,[3] Thomas G. Heffron.[3] *[1]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [2]Department of Medicine, Emory University School of Medicine, Atlanta, GA; [3]Department of Surgery, Emory University School of Medicine, Atlanta, GA.*

Background: Valganciclovir is a prodrug of the antiviral agent, ganciclovir. Ganciclovir has previously been shown to have antiviral activity against the hepatitis B virus but is currently not used due to the low oral bioavailability and toxicities. **Objective:** The aim of this review is to evaluate the use of valganciclovir for lamivudine resistant, recurrent hepatitis B following liver transplantation. **Methods:** All hepatitis B surface antigen positive patients received, after liver transplant, hepatitis B immune globulin (HBIG) and titrated to maintain hepatitis B surface antibody (HBSAnti) levels above 500 i.u./ml along with lamivudine. With recurrence of the hepatitis B surface antigen, patients were started on valganciclovir 900mg orally, daily. Viral loads were monitored and followed during treatment. **Results:** Over the last 10 years, 42 patients have been transplanted for chronic complications of hepatitis B. At time of transplant, 21 (50%) were actively replicating as demonstrated by the presence of hepatitis E antigen or HBV DNA. With the use of high dose immunoglobulin protocol, only 6 (14.3 %) had recurrence of HBV surface antigen at a mean time to recurrence being 501 (± 281) days. Patients (n = 3) that were actively replicating at time of surgery had similar time to recurrence compared to non-replicators (n = 3) [391(± 175) days vs. 525 (± 301) days, p = NS]. Of the 6 patients with recurrence, 1 recurred prior to the advent of lamivudine and did not survive. Three patients have been maintained on lamivudine alone without resistance developing (1368 ± 402 days). The last two patients (each replicators) developed recurrence that was resistant to lamivudine and was begun on valganciclovir. The first patient over three months had a reduction in HBV PCR from 1,910 pg/ml to 103 pg/ml. With the second patient, initial results indicated a reduction in HBV PCR from 560 pg/ml to 230 pg/ml but due to neutropenia development, dose of valganciclovir was held and HBV increased to 780 pg/ml. With the resumption of dosing, HBV PCR resumed a decline. **Conclusions**: Valganciclovir has antiviral activity to the hepatitis B virus and may be of benefit in lamivudine resistant HBV following liver transplantation. With the recent approval of adefovir for lamivudine resistant HBV, valganciclovir may be useful in combination with adefovir for resistant HBV. Further studies will be required to determine the usefulness of valganciclovir in treatment of post-transplant hepatitis B recurrence.

Abstract# 1435 Poster Board #-Session: P191-III

HEPATITIS "C" RECURRENCE (HCV) DOES NOT WORSENS GENERAL OUTCOME IN ADULT LIVING DONOR LIVER TRANSPLANTATION (ALDLT). Hans Van Vlierberghe,[1] Roberto Troisi,[2] Salvatore Ricciardi,[2] Isabelle Colle,[1] Marleen Praet,[3] Pasquale Conoscitore,[4] Uwe J. Hesse,[2] Bernard de Hemptinne.[2] *[1]Hepato-Gastroenterology; [2]General, Hepato-Biliary and Liver Tx Surgery; [3]Pathology, Ghent University Hospital Medical School, Ghent, Belgium; [4]Gastroenterology, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy.*

Introduction. ALDLT is an established treatment option for selected patients with end-stage liver disease. Preliminary data demonstrated that HCV recurs earlier and is more severe in ALDLT patients in comparison to cadaveric liver transplantation (CLT). In this study we report on the one-year follow-up of our cohort of HCV patients receiving ALDLT or CLT. **Methods.** Between 10/1999 and 9/2002, 26 patients with end-stage HCV-related liver cirrhosis (6 female/20 male) (mean age of 60 ± 7 year) received CLT whereas 17 patients (5 female/12 male) (mean age of 56 ± 4 year) underwent ALDLT. Diagnosis of HCV recurrence wad made on increased transaminases, detectable serum HCV-RNA level and histological findings on biopsy. Biopsies were performed when clinically indicated. Bilirubin concentration, PTT and ALT activity were compared between the two groups at different time interval: 4,12,24,36 and 48 weeks following transplantation. **Results.** HCV recurrence was diagnosed in 10/26 CLT patients vs. 6/17 ALDLT (p = 0.1). Time until recurrence was longer in patients receiving an ALDLT (158 ± 114 days vs. 227 ± 154 days, p = 0.4). Of the biochemical parameters, only bilirubin concentration at week 4 was significantly different between ALDLT and CLT patients (3.1 ± 4.3 vs. 1.26 ± 0.83 mg/dl, p = 0.04), reflecting the presence of a less hepatic mass in LDLT patients. Thirteen CLT patients and 9 ALDLT patients received a liver biopsy (p = 0.9). Timing of liver biopsy, grade of activity and fibrosis and overall survival were similar in both groups (p = 0.6).



Conclusion. At a FU period of 1-year, there is no difference in outcome between end-stage HCV patients receiving an ALDLT or CLT. Considering that long-term outcome does not differ, ALDLT is a good treatment option for patients with HCV-related liver cirrhosis.

Abstract# 1436 Poster Board #-Session: P192-III

FINANCIAL AND CLINICAL IMPACT OF VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE) IN LIVER TRANSPLANT PATIENTS: A MATCHED CONTROLLED STUDY. M. Gearhart,[1] J. Martin,[1] E. Zavala,[1] D. Wetzel,[1] T. Merchen,[1] M. Gupta,[1] J. Morelli,[1] F. Weber,[1] J. Aranda-Michel,[1] M. Bass,[1] S. Rudich,[1] M. J. Hanaway,[1] E. S. Woodle,[1] J. F. Buell.[1] *[1]Department of Transplantation, The University of Cincinnati, Cincinnati, OH.*

Liver transplant recipients are at risk for multi-drug resistant infections due to immunosuppresion and broad-spectrum antibiotic use before and after transplantation. This study evaluates the clinical and financial impact of VRE on transplant recipients compared to a matched control. **Methods:** Liver transplant recipients with VRE from '95-'02 were identified and compared to matched (age, gender, UNOS status, liver disease and transplant date) controls. Demographics, co-infections, antibiotic use, length of stay, abdominal surgeries, biliary complications, survival and financies were compared to matched controls. **Results:** A total of 19 pts developed 28 VRE infections. 38 non-VRE patients served as matched controls. The four most common sites of VRE infection were blood (35%), peritoneal fluid (35%), bile (20%), and urine (12%). Median time from transplant to infection was 48 days (4-348). There were no significant differences in demographics. The VRE group had a higher incidence of prior antibiotic use with vancomycin, altrovafloxacin, and gentamicin. (95% vs. 34%;p<0.05). The VRE group experienced more abdominal surgery (20/19 vs. 3/38; p=.029), biliary complications (9/19 vs. 9/38; p=.018) and a longer length of stay (42.5 vs. 21.7 days; p=.005). ICU stay showed a trend to increased length of stay in the VRE group. Survival in the VRE group and non-VRE group was 52% vs. 82% respectively (p= .048). Six of the 19 VRE patients were treated with linazolid for 8 infections, 4/6 patients survived. Eight patients were treated with quinpristin/dalphopristin for 9 infections, 2/8 survived. Trends for increased financial impact were seen in hospital cost, OR cost, pharmacy cost, blood bank costs, and miscellaneous costs for the VRE group. Laboratory cost was the only area was there was statistical significance was found ($6500 vs. 1,750;p= .02). **Conclusion**: Patients with VRE infections have a poorer clinical outcome compared to patients without VRE infections. Whether the VRE infection is the cause of the increased complications or is simply a marker for poor outcomes is yet to be determined. VRE pts were noted to utilize more financial and hospital resources. A trend towards improved survival was noted with linezolid compared to quinpristin/dalphopristin in a small number of pts, but further trials are necessary to determine if a real difference exists.

Abstract# 1437 Poster Board #-Session: P193-III

LIVER RETRANSPLANTATION IN HCV-INFECTED PATIENTS: AN 8-YEARS, SINGLE CENTER EXPERIENCE. Jose R. Nery,[1] Caio Nery,[1] Guy Neff,[1] Marzia Montalbano,[1] K. Safdar,[1] Silon Brito,[1] Seigo Nishida,[1] Tomoaki Kato,[1] David Levi,[1] Juan Madariaga,[1] Pablo Bejarano,[1] Phillip Ruiz,[1] Eugene Schiff,[1] Andreas Tzakis.[1] *[1]University of Miami, Miami, FL.*

Aims: HCV-related ESLD is the dominant indication for LTx. We reviewed our group experience with LRetx , to determine if the results in patients primarily transplanted for HCV-related ESLD should justify or contra-indicate LRetx in this setting. **Patients and Methods:** From June/94-May/02, 1114 adults underwent 1249 LTx. HCV-cirrhosis was the primary indication in 491 patients (44%). 125 patients were retransplanted once (116); twice (8) or three times (n=1); 65 had been primarily transplanted for HCV cirrhosis (Group I) and 60 for other types of ESLD (Group II). The causes for LRetx were classified as primary non-function(PNF), technical(TEC), and chronic dysfunction(CHR), the latter defined as graft failure occurring after the 1st post-transplant month, in the absence of vascular and biliary complications. **Results:** The rates of LRetx among patients with HCV disease and other causes of ESLD were 13% and 10%, respectively (p=0.05). Patient and graft survival rates were 49% / 45% and 67% /63% in Groups I and II, respectively (p=0.04 / 0.04). The rates of PNF and TEC were not different between Groups I and II, however the incidence of CHR was higher in Group I (32% vs 17%, p=0.04). LRetx for CHR resulted in 73% and 36% graft loss in Groups I and II, respectively (p=0.04). **Conclusion:** HCV is related to increased incidence of graft destructive chronic disease, and has a negative impact on the results of liver retransplantation. In view of these results and the scarce donor resources, further studies are required to support retransplantation in HCV-infected patients.

Abstract# 1438 Poster Board #-Session: P194-III

TREATMENT OF HEPATITIS C FOLLOWING LIVER TRANSPLANTATION. Michael J. Osgood,[1] Marcelo Kugelmas,[1] Tracy Steinberg,[2] Thomas Bak,[2] Michael Wachs,[2] Igal Kam,[2] Gregory T. Everson,[1] James F. Trotter.[1] *[1]Division of Gastroenterology/Hepatology, University of Colorado, Denver, CO; [2]Division of Transplant Surgery, University of Colorado, Denver, CO.*

Background: Hepatitis C virus (HCV) is the most common indication for liver transplantation. Previous studies report limited efficacy of anti-viral therapy after transplantation. We report the experience at our center in the treatment of HCV in our post-transplant cohort. **Methods:** Records of all patients receiving transplants for HCV at our center from 1996 until June 2002 were reviewed. Patients who received a course of treatment for HCV were categorized as viral responders or non-responders based on polymerase chain reaction detection of HCV RNA at end-of-treatment (EOT) and for sustained viral response (SVR) if six months or more had elapsed since EOT. Courses of therapy were followed until October 2002. A full course of treatment was defined as follows: genotype 1, 12 months of treatment or 6 months of treatment with < 2 log decrease in HCV-RNA; genotype non-1, 6 months of treatment. **Results:** One hundred sixty six patients were identified. Demographics were as follows: mean age 49.4 years; 71% male, 29% female; 74% Caucasian, 21 % Hispanic, 4 % Asian, 1 % African-American, and 1 % Native American; HCV genotype was available for 87 patients, of whom 84% were genotype 1, 7% genotype 2 and 9% genotype 3. Thirty five of these 166 patients (21%) were treated with interferon/ribavirin, pegylated interferon/ribavirin or consensus interferon/ribavirin therapy. Overall mean aspartate aminotransferase, alanine aminotransferase, and total bilirubin at start of treatment were 273 IU/l, 239 IU/l, and 4.1 mg/dl, respectively. Thirteen patients completed full treatment, 9 were discontinued prior to completion of treatment, 7 were ongoing at the time of review and 6 were treated elsewhere. Of the 13 completed courses, EOT viral response was seen in 2 patients; of these 2 patients 1 relapsed and 1 (8 %) had SVR. Of the 9 courses discontinued early, EOT viral response was seen in 4 patients and SVR occurred in 2. Fifteen of 35 patients (43%) experienced one or more of the following adverse events: rejection (8/35), psoriasis (2/35), shingles (2/35), hepatitis (2/35), sepsis (1/35), bronchitis (1/35), and pneumonia (1/35). Seven patients stopped therapy due to an adverse event. Six patients required erythropoetin and 7 required G-CSF. Overall, 3/35 (9 %) of patients had SVR. **Conclusions:** 1) Post-transplant anti-viral therapy for HCV is relatively unsuccessful. 2) Adverse effects of therapy limited treatment in many patients.

Abstract# 1439 Poster Board #-Session: P195-III

HEPATITIS C RNA DROP OF TWO LOGS AT 12 WEEKS MAY PREDICT RESPONSE TO TREATMENT OF POST-TRANSPLANT HCV. Nazir Rahim,[1] Katherine Suggett,[2] Christoph Troppmann,[2] Colette Chambers,[2] John McVicar,[2] Lorenzo Rossaro.[2] *[1]Department of Internal Medicine, University of California, Davis Medical Center, Sacramento, CA; [2]Section, Transplant Medicine, University of California, Davis Medical Center, Sacramento, CA.*

Background: Studies in the non-transplant setting have suggested that achievement of ≥ 2 log decrease in hepatitis C virus (HCV) RNA levels at 12 weeks with combination interferon (IFN) and ribavirin (RBV) favorably predicts sustained viral response (SVR). Patients without ≥ 2-log drop do not experience SVR, and it is recommended they stop therapy (12 week stopping rule). Reported SVR rates using combination IFN and RBV for recurrent HCV after liver transplantation (LT) range from 10%-20%. Aim: To determine if ≥ 2 log drop in HCV-RNA at 12 weeks predicts undetectable HCV-RNA at 24 weeks in patients with recurrent HCV after LT treated with escalating doses of combination IFN and RBV. Methods: We prospectively analyzed data from ten patients (90% male, mean age 48 years, 60% genotype 1, primary immunosuppression with tacrolimus and prednisone) with biopsy proven recurrent HCV after LT. IFN dosing started at 1 MIU tiw, increased by 0.5 MIU biweekly to a maximum dose of 3 MIU tiw. RBV dosing started at 200 mg bid, increased by 200 mg biweekly to ≥10 mg/kg with intention to treat for 48 weeks. Results: To date, 9 of 10 patients completed 24 weeks of therapy. One patient dropped out of study prior to 24 weeks of therapy due to hepatic decompensation. All patients achieved ≥ 80% of target doses at 12 weeks. All patients had a decrease in HCV-RNA during treatment (Baseline: 2.6±2.1, 12 weeks: 0.22±0.34, 24 weeks: 0.12±0.22 MIU, p<0.05). Three patients (all genotype 3) had ≥2-log decrease in HCV-RNA at 12 weeks. The same three patients were the only ones who had negative qualitative HCV-RNA at 24 weeks. Those who failed to achieve ≥2 log decrease in HCV-RNA at 12 weeks also failed to demonstrate viral clearance at 24 weeks (p<0.05). Conclusion: Although we did not use pegylated IFN or have SVR results, our data suggest that the 12-week stopping rule may also apply to patients treated with standard IFN and RBV for HCV recurrence after LT.

Abstract# 1440 Poster Board #-Session: P196-III

THROMBOELASTOGRAPH MONITORING CHANGES IN BLOOD COAGULATION OF CIRRHOTIC PATIENTS UNDERGOING MOLECULAR ADSORBENT RECIRCULATING SYSTEM. Cataldo Doria,[1,2] Victor L. Scott,[1,2] Lucio Mandala',[2] Giuseppe Caruana,[2] Salvatore Gruttadauria,[2] Mario Magnone,[2] Carlo Scotti-Foglieni,[2] Ignazio R. Marino.[1,2] *[1]Surgery, Thomas E Starzl Transplantation Institute, Pittsburgh, PA; [2]Surgery, Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Palermo, Italy.*

Coagulopathy is one of the main features of liver cirrhosis. It is multifactorial in origin and often time causes life threatening complication. We, herein describe the effect of molecular adsorbent recirculating system (MARS), a cell-free dialysis technique, on the blood coagulation of cirrhotic patients. From September 2000 to July 2002, 9 patients, were treated with the following indications: 6 (66.6%) acute-on-chronic liver failure (AoCHF), 3 (33.3%) untreatable pruritus. We studied the effect of MARS on platelets count, prothrombin time (PT), international standardized ratio (INR), thromboelastography (TEG), coagulation factors II, V, VII, VIII, IX, X, XI, XII, XIII, von Willebrand (vWF), Lupus Anticoagulant (LAC), Protein C (PC), Protein S (PS), Antitrombin III (AT3), Plasminogen (PLG), α 2 Antiplasmine (ALFA 2), D-Dimer (DD), Fibrine monomers (FM), Complement (C), and C_1Inactivator (C_1INV), before and after MARS. Five males (55.5%) and 4 females (44.4%), age 47–70 (median 56), underwent 12 courses (4 to 7 session each) of MARS treatment. We found statistically significance difference in the platelets count, prothrombin time; reaction time (R) konstant time (K), alfa angle, maximal amplitude (MA); factor VIII, vWF, and DD when measured before and after MARS.

	Statistically significant variables	
Pre-MARS coagulation test	Bleeders mean ± SD	Non-bleeders mean ± SD
Platelet count	36.4 ± 11.8	58.1 ± 29.2
Reaction time (TEG)	854.6 ± 164.4	735 ± 202.3
Konstant time (TEG)	2958.3 ± 2799.9	1288.5 ± 2308.8
Alfa angle (TEG)	11.8 ± 10	34 ± 11
Maximum amplitude (TEG)	26.6 ± 34	44.4 ± 13.6
Factor VIII	93.36 ± 37.3	107.6 ± 55.2
von Willebrand factor	179.06 ± 10.1	184.2 ± 33
D-Dimer	535.76 ± 194.9	631.1 ± 346.8

Table 1

However, no difference were identified for the other variables studied. It appears that MARS induces coagulopathy mainly through a platelet mediated mechanism, which could be mechanically destroyed during the blood passage through the MARS filters or in alternative a cytokine-mediate mechanism activated by MARS can be postulated as responsible for the coagulopathy.

Abstract# 1441 **Poster Board #-Session: P197-III**

THE EFECT OF ANTI-FUNGAL PROPHYLAXIS ON OUTCOMES AFTER ADULT LIVING DONOR LIVER TRANSPLANT (LDLT). David D. Douglas, Janis Blair, Pratima Sharma, Vijayan Balan, Hugo Vargas, Thomas Byrne, Jorge Rakela, Wyn Harrison, Adyr Moss, David C. Mulligan. *[1]Division(s) of Transplant Medicine and Surgery, Mayo Clinic Hospital, Phoenix, AZ.*

Invasive fungal infections occur with a reported incidence between 4-42% after cadaveric liver transplantation and have been associated with a high mortality rate. They are very few reports on the incidence of fungal infection after adult living donor liver transplant and no previous reports on the effect of anti-fungal prophylaxis. From June 1, 1999 through December 1, 2002 our program performed 130 adult liver transplants (107 1st cadaveric transplants, 7 re-transplants in 6 patients, and 16 living donor transplants (LDLT). A total of 15 invasive fungal infections were identified: 11 Candida, 2 Coccidioides, 1 Aspergillus, and 1 Scedosporium. Fungal infection rates were 6.5% (7/107) for 1st cadaveric transplants, 50% (3/6) for re-transplants (p = 0.009 compared to 1st cadaverics), and 31% (5/16) for LDLT (p = 0.009 compared to 1st cadaverics). Fungal related mortality rates were 29% (2/7) for the 1st cadaveric transplants, 66% (2/3) for the re-transplants, and 60% (3/5) for the LDLTs (p = NS in all groups). Because of a concern about increased fungal infections in our first ten LDLT, we instituted anti-fungal prophylaxis with low dose amphotericin B 10 mg IV q day for 7 days followed by fluconazole 200 mg po q day for 21 days in all susequent LDLTs. We then analyzed the differences between these two groups (see table 1). The incidence of hepatic artery thrombosis and biliary complications (leak or stricture) were similar in these two groups. One patient from the non-prophylaxis group was excluded from analysis when calculating the fungal infection rate because he was on fluconazole at the time of transplant due to positive Coccidioides serologies.

Effects of Anti-Fungal Prophylaxis in LDLT

	NO Prophylaxis (LDLT 1-10)	Prophylaxis (LDLT 11-16)	p value
Hepatic Artery Thrombosis	20% (2/10)	17% (1/6)	NS
Biliary Complications	60% (6/10)	50% (3/6)	NS
Fungal Infection Rate	56% (5/9)	0% (0/6)	0.04
Fungal Related Mortality	60% (3/5)	0% (0/0)	NS
Overall Mortality	30% (3/10)	0% (0/6)	NS

Conclusions: 1) There is an increased incidence of invasive fungal infections in LDLT compared to 1st cadaveric liver transplants. 2) Fungal infections is associated with a high mortality rate in all groups. 3) Antifungal prophylaxis is effective in significantly lowering the fungal infection rate following LDLT and seems warranted in this group of patients.

Abstract# 1442 **Poster Board #-Session: P198-III**

IS SEVERE MORBID OBESITY A CONTRAINDICATION TO LIVER TRANSPLANTATION? Jonathan S. Fisher,[1] Claudio Tombazzi,[2] A. Osama Gaber,[1] Santiago R. Vera,[1] Nosratollah Nezakatgoo,[1] A. Bashar Abdulkarim,[1] M. Hosein Shokouh-Amiri.[1] *[1]Section of Transplantation, University of Tennessee, Memphis, TN; [2]Section of Gastroenterology, University of Tennessee, Memphis, TN.*

The incidence of morbid obesity has increased to more than a third of the population in the U.S. That shift is reflected in the population awaiting liver transplantation. In fact, a greater number of patients with severe morbid obesity (BMI >40 kg/m²) are now being referred for liver transplants. Previous reports have suggested increased early postoperative morbidity and mortality for obese patients undergoing liver transplantation. **Purpose:** To evaluate a single center experience transplanting livers into patients with severe morbid obesity (BMI >40 kg/m²). **Method:** A retrospective chart review of first transplant, cadaveric liver recipients at a single center from 1998 to 2002. There were 10 patients identified with BMI >40. This group was compared to a concurrent cohort of 67 nonobese patients (BMI<30) with similar demographics with respect to age, race, etiology of liver disease, MELD score, preoperative creatinine clearance and incidence of diabetes mellitus. Outcomes measured included length of stay, need for re-exploration, one-year patient and graft survival, incidence of rejection, development of new onset diabetes post-transplant, and serologic markers of organ function at one year post-transplant. **Results:** Length of stay in intensive care unit and hospital, need for reoperation, incidence of rejection, and graft and patient survival at one year were no worse for patients with severe morbid obesity.

BMI	Hospital Stay	ICU Stay	Incidence of Reoperation	Incidence of Rejection	Graft Survival	Patient Survival	PTDM
kg/m2	days	days	% at 1 year	% at 1 year	% at 1 year	% at 1 year	% at 1 year
>40 (n=10)	10.8+/-4.0	3.1+/-1.2	20	30	100	90	60
<30 (n=67)	14.1+/-10.8	3.5+/-2.9	18	25	94	91	31

No statistical difference (p value > 0.1) between groups with BMI <30 and >40 for all outcome variables.

There was a trend toward increased incidence of post-transplant diabetes mellitus in the group with BMI >40 (60% vs. 31%). Serum albumin (3.9+/-0.5 mg/dl v. 3.9 +/-0.4 mg/dl) and alanine transaminase (68+/-66 IU/l v. 70+/-94 IU/l) as markers of hepatic function and serum creatinine (1.4+/-0.3 mg/dl v. 1.4+/-0.5 mg/dl) as a marker of renal function were not statistically different between BMI>40 and BMI<30, respectively. **Conclusions:** One-year results of liver transplantation are not adversely affected by severe morbid obesity. Therefore, morbid obesity should not be considered a contraindication for liver transplantation.

Abstract# 1443 **Poster Board #-Session: P199-III**

ULTRA SHORT-TERM USE OF HBIG FOLLOWING OLT IN HBV-CIRRHOSIS. Gerd Otto,[1] Maria Hoppe-Lotichius,[1] Mathias Wunsch,[1] Christian Moench,[1] Stefan Kanzler,[2] Ansgar W. Lohse.[2] *[1]Transplantation and Hepatobiliary Surgery, Johannes Gutenberg University, Mainz, Germany; [2]1st Medical Department, Johannes Gutenberg University, Mainz, Germany.*

Hepatitis B hyperimmune serum (HBig) is usually given to prevent HBV reinfection following liver transplantation (LTx). HBig is expensive and particularly in combination with lamivudine the duration of its administration is under debate. Due to irregular administration of HBig the anti HBs antibody level dropped to nontherapeutic levels in the majority of our patients with HBV cirrhosis. We report the features of reinfection. **Patients and methods:** Twenty six patients transplanted between 9/97 and 11/02 for HBV cirrhosis were enrolled in this retrospective study. In addition to HBV infection 8 patients had a HDV superinfection. Lamivudine was given in 13 patients before LTx (2x400 mg/day). Three patients had a YMDD mutant when transplanted. Following LTx lamivudine was infinitely given in all patients. HBIg was used intraoperatively (10,000 U) and during the first postoperative week (2,000 U per day). Thereafter we aimed at a target level over 100 U/ml. **Results:** The HBV DNA was 541±2126 pg/nl (mean ± SD) before LTx. The median follow up has been 926 days (16 to 1792 days). One- and 3-year survival are 91 and 86 %, respectively. No patient was lost to follow up but due to reasons HBig administration was prematurely discontinued. It dropped below the therapeutic level by day 92 following LTx in 50 % and by day 167 in 80 % of the patients. Only 11 % had a therapeutic level exceeding 1 year. Reinfection occurred in 2 patients transplanted with YMDD mutants (40 and 230 days after LTx) and in one patient with HDV superinfection (1260 days after LTx). All other patients remained free of recurrence despite unmeasurable HBig titers. **Conclusion:** The unvoluntary short-term prophylaxis with HBig did not lead to an unacceptable high recurrence rate. Recurrence occurred only in two patients with YMDD mutants and, surprisingly, in a patient with delta superinfection. The duration of HBig adminitration may probably be limited to the perioperative period.

Abstract# 1444 **Poster Board #-Session: P200-III**

DOES HISTOLOGY HELP PREDICT AGGRESSIVE RECURRENT HCV AFTER TRANSPLANT. Charmaine A. Stewart, Michael Crawford, Anne Burke, Emma Furth, Kim Olthoff, Abraham Shaked. *[1]University of Pennsylvania, Philadelphia, PA.*

Hepatitis C (HCV) recurs in approximately 95% of patients who have undergone liver transplantation.Of these 10% develop a rapidly progressive course post liver tranplant (LT). The factors that lead to aggressive hepatitis C postLT are ill defined. The purpose of this study is to determine whether a histological lesion, collagenization of the space of Disse, is an index lesion that predicts aggressive disease. Aggressive HCV is defined, in this study, as the development of fibrosing cholestatic hepatitis (FCH), bridging fibrosis or cirrhosis, based on liver biopsy obtained at 3 months or thereafter. Methods: This is a cohort study of patients who underwent LT for HCV at the University of Pennsylvania transplant center between 1993 and 2001 and who had at least 1 liver biopsy 3 months or more post liver transplant. The subjects were divided into group1 if collagenization of the space of Disse was found and group 2 if this lesion was absent on the first liver biopsy obtained 3 months or more after LT. The subjects were followed prospectively to determine if they were at higher risk to develop aggressive HCV. In addition, correlations were made with preoperative, intraoperative and postoperative variables to determine if there were other factors that predicted development of aggressive HCV. The preoperative variables that were examined were, bilirubin, INR and creatinine; intraoperative variable was cold ischemic time; and postoperative variables were transaminases, gamma glutamine transferase (GGT), alkaline phosphatase and bilirubin obntained at 90 days and 1 year post transplant. Results: Of the 137 subjects who were transplanted for HCV, 91 subjects had undergone at least 1 liver biopsy. 18 (94% men) had collagenization of the space of Disse and were assigned to group 1, the remaining 73 (75% men) were in group 2. The median age (range) for groups 1 and 2 were, 49 yr (34-60) and 48 yr (34-68), respectively; the sex distribution was 94% and 75% males in groups 1 and 2, respectively. Preoperative laboratory studies revealed no significant difference was found in INR or creatinine in either group, p value 0.566 and 0.18, respectively. However, bilirubin was higher in group 1 than in group 2, median 2.95 vs 2.65 (p=0.579). There was no difference found in cold ischemic time between groups (413 mins vs 433 min.for groups 1 and 2, respectively). Neither was there correlation between collagenization of the space of Disse and postoperative laboratory studies, namely bilirubin, transaminases or GGT in predicting aggressive liver disease(p-values >0.05). After a median follow up time of 1111 days, 3 patients from group 1 progressed to bridging fibrosis. Whereas, none of the subjects from group 2 developed cirrhosis, except one subject who during the follow-up period acquired collagenization of the space of Disse and then developed cirrhosis. One subject from group 1 who had collagenization of the space of Disse then developed FCH. Overall 3 patients progressed to cirrhosis, one of whom was in group 2. The relative risk of developing aggressive HCV in group 1 was 1.7 compared with group 2, p=0.22. Conclusion: Collagenization of the space of Disse does not predict aggressive HCV.

Abstract# 1445 Poster Board #-Session: P201-III

CYTOMEGALOVIRUS INCREASES THE RATE OF GRAFT LOSS AFTER LIVER TRANSPLANTATION. Karen L. Hardinger, Mark A. Schnitzler, Daniel C. Brennan, Niraj Desai, Jeffrey Lowell, Surendra Shenoy, William Chapman. *[1]Transplantation, Barnes-Jewish Hospital at Washington University, St. Louis, MO.*

Cytomegalovirus (CMV) is recognized as a significant cause of morbidity after liver transplantation. The CMV donor seropositive/ recipient seronegative group is most commonly associated with CMV disease and poor outcomes. The purpose of this study was to review our experience with CMV and access the impact of long-term outcomes. **Methods.** A retrospective analysis was performed of all adult liver transplant recipients at a single transplant center between January 1996 and December 2000 (n=235). The analysis included patients who survived at least 90 days after transplantation (n=224). The regimens used for CMV prophylaxis varied at our institution, most patients (78%) received acyclovir for viral prophylaxis. The use of ganciclovir varied, especially in CMV recipient seropositive patients (10%). CMV infection (defined as positive culture or PCR) was treated with at least 21 days of intravenous ganciclovir. **Results.** There were a total of 26 (11.1%) patients who developed CMV infection during the first 90 days after transplantation. The overall rate for CMV was greatest in the CMV donor seropositive/ recipient seropositive group, 18.0%. The rate for CMV was 7.1% in the CMV donor seropositive/ recipient seronegative group, 5.0% in the CMV donor seronegative/ recipient seropositive group, and 0% CMV donor seronegative / recipient seronegative group (P=0.017). Six patients (two donor negative/recipient positive, three donor positive/recipient positive, one donor positive/recipient negative) expired at a mean of 11 months after the diagnosis of CMV infection. At one year after transplant patient and graft survival was 94.4% in patients without history of CMV and 80.4% in patients with history of CMV. Patient and graft survival one year post transplant (P=0.008) and at two years post-transplant (P=0.03) was lower in patients with CMV when compared to patients without CMV. **Conclusions.** Attention to prophylactic strategies is important even in the CMV recipient seropositive groups. Cytomegalovirus is associated with a significantly higher rate of death and graft failure, even in "low-risk patients". Cytomegalovirus has important detrimental effects on long-term outcomes after liver transplantation supporting consideration of more aggressive prophylactic strategies, even in recipient seropositive patients.

Abstract# 1446 Poster Board #-Session: P202-III

FIRST POST-TRANSPLANT DAY ALANINE AMINOTRANSFERASE SERUM CONCENTRATION PREDICTS EARLY HEPATITIS C RECURRENCE AFTER LIVER TRANSPLANTATION. Massimo Rossi,[1] Stefano Ginanni Corradini,[2] Manuela Merli,[2] Rosanna De Marco,[2] Maria Teresa Siciliano,[2] Paola Tanzilli,[2] Gilnardo Novelli,[1] Giovanni Casciaro,[1] Massimo Iappelli,[1] Francesco Nudo,[1] Andrea Onetti Muda,[3] Stefano Natalizi,[3] Giancarlo Ferretti,[4] Pasquale Berloco,[1] Adolfo Francesco Attili.[2] *[1]Dipartimento di Chirurgia Generale "Paride Stefanini"Azienda Policlinico Umberto I, Rome, Rome, Italy; [2]Div. Gastroenterologia Dip. Medicina Clinica, Azienda Policlinico Umberto I, Rome, Rome, Italy; [3]Dip.Medicina Sperimentale e Patologia, Azienda Policlinico Umberto I, Rome, Rome, Italy; [4]Dip.Malattie Infettive e Tropicali, Azienda Policlinico Umberto I, Rome, Rome, Italy.*

Background & Aims: In living donor liver transplantation hepatectomy could lead to a more severe hepatocellular injury followed by rapid hepatocellular regeneration favouring viral replication and accelerating subsequent HCV disease recurrence. We investigated whether also in the case of cadaveric liver transplantation (LT) the degree of peri-operative hepatocellular injury is related with the timing of HCV disease recurrence. ***Methods:*** Eleven primary cadaveric orthotopic whole adult LT performed in HCV-RNA positive patients were included in the study during 02/2001-7/2002 at a single institution. Patients were studied for blood chemistry for three days after LT. HCV disease recurrence was diagnosed by liver biopsy. Statistical analysis was performed by ANOVA ***Results:*** One patient died for primary non function and was excluded from the study. The remaining 10 patients were retrospectively assigned to two groups according to the subsequent occurrence or absence of HCV disease recurrence (DR). Group A comprised 5 patients with early (within 5 months from transplantation) DR, occurring after a mean post-LT period of 3.3 months (range 1.5-5.0). Group B comprised 5 patients without evidence of HCV disease recurrence (mean follow-up 9.9 months; range 5.0-18.3). Group A as compared with group B was characterized by: a) a higher serum post-LT Alanine Aminotransferase (ALT) concentration at day 1 (1396.8±303.8 vs 338.4±33.4 IU/L; $p<0.01$) and at day 2 (1461.8±394.1 vs 368.4±59.4 IU/L; $p<0.03$); b) a higher serum post-LT total bilirubin at day 3 (6.1±0.7 vs 3.1±1.0 mg/dl; $p<0.05$). No intergroup differences were found with regard to recipient and donor age, recipient MELD score, and cold ischemia time. ***Conclusions:*** High ALT serum concentration at day 1 post-LT is a significant predictor of subsequent early hepatitis C recurrence, suggesting that early hepatocellular injury favours viral replication. If these results are confirmed in a larger number of patients, it will be possible, immediately after LT, to identify patients with higher risk for early viral recurrence and treat them in a timely manner.

Abstract# 1447 Poster Board #-Session: P203-III

ULTRASOUND AND HISTOLOGICAL CHANGES OF LIVER AFTER INTRAPORTAL ISLET TRANSPLANTATION. Paola Maffi,[1] Federico Bertuzzi,[1] Enzo Angeli,[2] Carlo Paties,[3] Carlo Fedeli,[1] Rita Nano,[1] Valerio Di Carlo,[4] Alessandro Del Maschio,[2] Antonio Secchi.[1] *[1]Department of Medicine, Scientific Institute San Raffaele, Milan, Italy; [2]Department of Radiology, Scientific Institute San Raffaele, MIlan, Italy; [3]Department of Pathology, Scientific Institute San Raffaele, Milan, Italy; [4]Department of Surgery, Scientific Institue San Raffaele, Milan, Italy.*

Few histological studies reported features of transplanted islets in human,taken from needle biopsy of the liver.No histological modification of the hepatic tissue was described in any of these studies. Furthermore no data are available on liver imaging after the islet trransplantation. The aim of our investigation was to study the impact on liver structure of intrahepatic islet transplantation.31 diabetic patients,who underwent islet after kidney transplant,were evaluated with sonogram of liver and color doppler of the portal vein,before islet transplant,immediately after the infusion and every 6 months,through a 8 years follow-up.All patiens received fresh islets and were immunosuppresed with ATG,steroids,cyclosporin and azatioprine or micofenolate mofetil.A percutaneous needle biopsy was performed when liver echotexture modifications were observed.Serum liver enzyme (AST,ALT,GGT),fasting C-Peptide (F-C-Pep),glycaeted haemoglobin (HbA1c) and Exogenous Insulin Requirement (EIR) were regularly collected.Micro and macro focal hyperechogenicity and focal steatosis,without any sign of peripheral portal thrombosis,were observed in 7 cases 6 months after transplantation.They lasted from 1 to 7 years in 5 cases, while they disappeared in 2 cases after 6 months and 8 years respectively.In the 5 cases of persistent focal steatosis a needle biopsy was taken from the focal areas of hyperechogenicity, under sonography guide, while in 1 case it was taken after the regression of the imaging changes.The histological examination demonstrated: mild to moderate, mainly macrovesicular steatosis,focally and random distributed,unrelated to the acinar zones,and focal glycogen accumulation in the nucleus;portal fibrosis and inflammation were absent or not significant.The biopsy of the case with regression of sonogram alterations showed normal features.No laterations of liver enzyme were observed.F-C-Pep ≥1 ng/ml was coexistent with hyperechogenicity, with a tendency to disappear when focalsteatosis was less evident.The mean HbA1c% was 8.2±0.8; the EIR was < 50% of the pre-transplant dose. In conclusion:the imaging and histological changes of teh liver after islet transplantation could be considered a local reaction to islet engraftment and/or the expression of an over-stimulus of insulin secretion, determining the storage of fat and glycogen in the liver, as already shown in the animal model.

Abstract# 1448 Poster Board #-Session: P204-III

INCREASED INCIDENCE OF ALLOGRAFT PANCREATITIS IN PATIENTS TREATED WITH SIROLIMUS COMPARED TO MYCOPHENOLATE MOFETIL. Dorothea R. Dreyer,[1] John F. Valente,[1] Christopher T. Siegel,[1] Thomas C. Knauss,[2] Kenneth A. Bodziak,[2] David S. Seaman,[1] Donald E. Hricik,[2] James A. Schulak.[1] *[1]Department of Surgery, University Hospitals of Cleveland, Cleveland, OH; [2]Department of Medicine, University Hospitals of Cleveland, Cleveland, OH.*

We reviewed the incidence of pancreatitis (PCT) with sirolimus (SRL) therapy in 39 pancreas transplants performed from 1/1/2000-11/30/2002 at the University Hospitals of Cleveland. All 22 simultaneous kidney pancreas (SPK) and 17 pancreas after kidney (PAK) transplants received tacrolimus (0.1 mg/kg/day), prednisone and basiliximab (94.8%) or thymoglobulin (5.2%). We defined 2 groups based on initial therapy: Group 1 (N=21) received mycophenolate mofetil (MMF, 1gm bid), Group 2 (N=18) received SRL (15 mg load, then 5 mg/day, adjusted to maintain levels of 10-20 ng/mL). There was no difference in history of native PCT (0%), tacrolimus levels, or use of drugs known to cause PCT. Data were comparable for age (41.1±8.8 vs. 40.5±7.3 years), antigen mismatch (3.9±2.2 vs. 4.0±2.1), panel reactive antibody (5±17 vs. 15±33%), cold ischemia time (CIT, 11.5±4.1 vs. 13.7±4.8 hours), and immediate graft function (95.2 vs. 94.4%) for groups 1 and 2 respectively. Trends towards higher one year graft survival (95.2 vs. 77.8%), male gender (71% vs. 58.8%) and SPKs (76.2% vs. 33.3%) were noted in Group 1. Allograft PCT, defined as a two fold increase in amylase and lipase, was observed in five Group 1 patients (23.8%), with 3 rejections, 1 necrotizing, and 1 idiopathic case (both following conversion to SRL) vs. nine group 2 patients (50%, p=0.024 independent samples t-test) with 2 rejections, 3 necrotizing and 4 idiopathic cases. Five open allograft explorations failed to confirm immunologic rejection. In group 2, 6 received empiric rejection therapy, temporally improving PCT in two. All 9 cases of necrotizing PCT(4) or unexplained PCT(5) occurred following exposure to SRL. Logistic regression analysis found initial SRL use, not transplant type (SPK vs. PAK), to correlate with PCT (p=0.0974). In a separate analysis, CIT, degree of antigen mismatch and triglyceride levels pre-PCT did not correlate. Early trends in Group 2 included higher fasting glucose (101±29 vs. 90±34 mg/dL), higher C peptide levels (3.8±1.6 vs. 3.0±1.8), higher triglyceride levels (320±149 vs. 152±118), a greater return to insulin (27.7% vs. 14.2%), and lower 2 year graft survival (66.6 vs. 95.2%) compared to Group 1. SRL was associated with a greater risk of necrotizing and unexplained PCT following pancreas transplant. Triglyceride levels were not elevated sufficiently to explain the incidence of PCT with SRL.

Abstract# 1449 Poster Board #-Session: P205-III

SINGLE HIGH DOSE FRESENIUS-ATG OR FIVE DOSES DACLIZUMAB EQUALLY REDUCE THE FREQUENCY OF ACUTE REJECTION EPISODES IN SPK RECIPIENTS. Johan W. de Fijter,[1] Paul J. van der Boog,[1] Jaap J. Homan van der Heide,[2] Marko J. Mallat,[1] Ilias N. Doxiadis,[1] Andre G. Baranski,[1] Rutger J. Ploeg,[2] Jan Ringers,[1] Leendert C. Paul.[1] *[1]Leiden University Medical Center, Leiden, Netherlands; [2]University Hospital Groningen, Groningen, Netherlands.*

Simultaneous pancreas kidney transplantation (SPK) is associated with a higher incidence of acute rejection episodes (ARE). After renal transplantation daclizumab (DAC) or single high dose anti thymocyte globulin (ATG-Fresenius) has been shown to reduce ARE when given in combination with cyclosporine and steroids. We assessed the safety and efficacy of DAC or ATG prophylaxis in combination with triple therapy in SPK recipients. Methods: A total of 67 type 1 diabetes mellitus patients, ages 18-50, scheduled to undergo primary SPK were analysed. Organs were allocated without prospective matching for HLA antigens. Forty patients were prospectively randomised to receive either prophylactic therapy with DAC i.v. 1 mg/kg on the day of surgery and every other week for a total of five doses or with a single high dose of ATG (9 mg/kg) before reperfusion. A cohort without prophylactic antibodies was used for retrospective comparison. All patients received CsA at 8 mg/kg/day, adjusted to defined trough levels, MMF 2 g/day orally and prednisone, gradually tapered to 10 mg at 12 weeks. A biopsy was performed for all suspected cases of kidney or pancreas rejection, unless contraindicated. Results: Baseline and transplant characteristics were comparable among groups. Mean recipient age was 40±7 yrs and 60% were male. Pre-emptive transplantation in 30% of patients, mean donor age 32±12 yrs, mean CIT 13±3 hrs (pancreas) and 14±4 hrs (kidney). Both ATG and DAC therapy resulted in a 50% ($p<0.0005$) reduction in incidence of ARE at 6 months. Time to rejection was significantly shorter for ATG (12±2 vs 40±48 days;$p<0.005$), with comparable proportions of steroid-resistant ARE with or without induction therapy. No significant differences between the groups for clinical outcome or functional parameters were found. One-year patient, kidney and pancreas graft suvival was 96, 97 and 94%. At 6 and 12 months HbA1c levels were 5.3±0.7 and 5.0±0.6% and calculated GFR 54±11 and 56±10 ml/min respectively. Conclusions: A single high dose Fresenius-ATG or five gifts of DAC as prophylactic treatment were well tolerated and equally effective in lowering the incidence of ARE in SPK recipients. Up to 3 years posttransplant no long-term adverse sequelae of the immunoprophylaxis was observed but also no additional benefit of decreased acute rejection episodes in graft survival or function.

Abstract# 1450 Poster Board #-Session: P206-III

PERITONEAL INFECTION AFTER SIMULTANEOUS PANCREAS KIDNEY (SPK) TRANSPLANTATION. Wolfgang Steurer,[1] Jacques Malaise,[1] Thierry Berney,[1] Richard Nakache,[1] Wolf Otto Bechstein,[1] EUROSPK Study Group. *[1]Eurospk Central Office, Brussels, Belgium.*

PATIENTS AND METHODS: Two hundred and five SPK transplant recipients from 10 centers in Europe and 1 in Israel were included in this open, prospective, randomized, parallel-group study. Following induction with antithymocyte globulin, patients were given either tacrolimus (Tacro) or ciclosporine-microemulsion (Ciclo) along with mycophenolate mofetil and steroids. Data were analyzed regarding post transplant infections. Among them, 136 patients were on hemodialysis (group HD), 28 were not yet on hemodialysis (preemptive SPK) (group ND) and 41 were or had been on peritoneal dialysis (group PD) prior to transplantation. Peritoneal Infection (PI) was defined as the combination of intra-abdominal germ identification with leucocytosis and fever, requiring start or change in antibiotherapy and / or surgical or percutaneous drainage. RESULTS: PI occurred in 29/205 patients (14.1%): 10 patients in PD group (24%) versus 14 in HD group (10.3%) ($p = 0.0208$) and 5 in ND group (17.8%). There is no difference in the occurrence of PI between Ciclo and Tacro groups. Another risk factor for peritonitis is pancreas exocrine enteric drainage with 16% of peritonitis versus 3% in bladder drained pancreas ($p=0.0353$). There are more peritonitis amongst patients who did not receive any prophylactic antibiotics during the post operative period (26.9% vs 11.3% $p = 0.0312$). Nevertheless, for those who received antibiotics, there are still more peritonitis if they were on PD before transplant than in HD : 23.5% vs 7.7% ($p = 0.0101$). The rate of abdominal infection was 30.8% in patients who required a relaparotomy after transplantation vs 11.7% in patients without early re-intervention ($p = 0.0093$). In the multivariate analysis, significant risk factors were: relaparotomy (RR:2.97, $p=0.0221$); peritoneal dialysis (RR:2.84, $p=0.0128$); donor BMI > 25 kg/m² (RR:3.57, $p=0.0029$). When peritonitis occurs, pancreas loss is encountered 2 times more often (27.6% versus 13.1%) ($p=0.0432$) and death 10 times more (10.3% versus 1.1%) ($p=0.0029$) . CONCLUSION: peritonitis occurred more frequently in patients under PD before SPK transplantation. Post operative prophylactic antibiotherapy decreases the risk for peritonitis.

Abstract# 1451 Poster Board #-Session: P207-III

DOES SURGICAL TECHNIQUE INFLUENCE OUTCOMES AFTER SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION? R. J. Stratta,[1] R. R. Alloway,[2] A. Lo,[3] E. E. Hodge.[4] *[1]Department of General Surgery, Wake Forest University, Winston-Salem, NC; [2]Department of Internal Medicine, University of Cincinnati, Cincinnati, OH; [3]Department of Pharmacy, University of Tennessee, Memphis, TN; [4]Roche Research Laboratories, Nutley, NJ.*

Background: Since 1995, many centers have switched from bladder to enteric drainage of the exocrine secretions in simultaneous kidney-pancreas transplantation (SKPT). Enteric exocrine drainage may be performed with either systemic (systemic-enteric [S-E]) or portal (portal-enteric [P-E]) venous delivery of insulin. Controversy exists regarding the optimal surgical technique. **Methods:** From March 1999 to May 2001, a total of 297 SKPT patients were enrolled into a prospective, multicenter, randomized, open-label, comparative trial of two Daclizumab dosing strategies versus no antibody induction in combination with Tacrolimus, MMF, and steroids in SKPT recipients. Surgical techniques were center specific. **Results:** A total of 171 patients (58%) underwent SKPT with S-E drainage, 96 (32%) with P-E drainage, and 30 (10%) with systemic-bladder (S-B) drainage. The two groups randomized to Daclizumab induction were similar with regard to surgical technique (64% S-E, 25% P-E, 11% S-B drainage). However, the third group randomized to no antibody induction had a higher proportion of cases (53%) performed with P-E drainage. ($P < 0.05$). All patients received similar maintenance immunosuppression. Demographic characteristics were comparable according to surgical technique (mean age 39 years, 60% male, 12% African-American). Pancreas cold ischemia times were highest with S-E and S-B drainage (15.0 hours and 14.9 hours, respectively), and lowest with P-E drainage (13.5 hours, P = NS). Six-month outcomes are shown in the table below:

	S-E (N=171)	P-E (N=96)	S-B (N=30)	P-Value
Patient Survival	166 (97%)	95 (99%)	29 (97%)	NS
Graft Survival:				
Kidney	161 (94%)	94 (98%)	28 (93%)	NS
Pancreas	149 (87%)	88 (92%)	26 (87%)	NS
Delayed Graft Function:				
Kidney	14 (8%)	5 (5%)	5 (17%)	NS
Pancreas Thrombosis	13 (7.6%)	5 (5.2%)	3 (10%)	NS
Acute Rejection	32 (19%)	25 (26%)	9 (30%)	NS
Oral Hypoglycemics	11 (6%)	5 (5%)	0	NS
Hgb A1C (%)	5.6	5.7	5.4	NS
Infection	84 (49%)	56 (58%)	19 (63%)	NS
Patients Readmitted	105 (61%)	61 (63.5%)	19 (63%)	NS

Excellent survival rates were achieved with all three surgical techniques. P-E drainage was neither associated with a higher risk of pancreas thrombosis nor associated with a lower incidence of acute rejection. Enteric drainage was not associated with a higher risk of infection. Pancreas graft function and readmissions were similar regardless of surgical technique. **Conclusion:** The six-month results of this multicenter study suggest no significant differences in outcomes in SKPT recipients according to surgical technique.

Abstract# 1452 Poster Board #-Session: P208-III

PERI AND POST OPERATIVE RESOURCE UTILIZATION FOR ISLET TRANSPLANTATION: THE INITIAL TRANSPLANTATION. E. Y. Zavala,[1,4] M. Hanaway,[2] V. R. Peddi,[3] H. R. Rilo,[2] J. E. Martin,[4] B. Marshall,[1] R. Alloway,[3] J. Buell,[2] J. B. Matthews,[2] E. S. Woodle.[2] *[1]Transplant Services, The University Hospital; [2]Dept. of Surgery; [3]Dept. of Medicine; [4]College of Pharmacy, University of Cincinnati, Cinicinnati, OH.*

An FDA approved human islet transplant program was established in July of 2001. We analyzed the economic outcomes for the first islet transplant procedure in our first six islet transplant recipients. **Methods:** A well defined clinical protocol was established for islet transplantation based on FDA requirements. All islet transplant candidates had type 1 diabetes with minimal co-morbidities and hypoglycemic unawareness . Hospital resource utilization was analyzed for the islet transplant procedure and three months post-discharge followup using LastWord, the hospital-based charge capture system. **Results:**Six initial islet transplants were performed in six patients. The clinical outcoms were: one case insulin free and no hypoglycemic awareness; three cases of 50% reduction in insulin dose required and some improvement in hypoglycemic awareness; two cases of 25% reduction in insulin dose required with slight-to-no improvement in hypoglycemic unawareness. The mean charges per transplant were $88,439 ± $31,771 inclusive of three months followup care. The mean length of stay (LOS) was 3.17 days ± 2.40 days. One partial portal vein thrombosis caused an extended LOS of eight days and this case also had a five day readmission in the three month followup period for a rectal ulcer.

Abstract #1452 Figure

Patient	Transplant#	LOS (days)	Readmit LOS	Total Transplant Charges and 3 mos. Followup
1	1	2	0	$59,237
2*	1	2	0	$49,586
3	1	2	0	$99,905
4	1	8	5	$137,420
5	1	2	0	$84,307
6	1	3	0	$100,179
		Mean = 3.17		Mean = $88,439
		S.D = ±2.40		S.D = ± $31,774

*2 months of post-transplant followup

Conclusion: Our initial series of performing the first islet transplant in six patients shows mean charges at three months of $88,439 that is approximately $20,800 less in charges compared to $109,325 for solitary pancreas transplantation estimated from the Milliman and Robertson modeled charges. These charges do not include charges for the second transplant.

Heart Transplantation II

Abstract# 1453 Poster Board #-Session: P209-III

NONINVASIVE ASSESSMENT OF REJECTION IN HEART TRANSPLANT PATIENTS:THE ROLE OF DONOR SPECIFIC ANTIBODIES AND CELL MEDIATED IMMUNITY. Mayra M. Lopez-Cepero,[1] Joel Fernandez,[2] Mark Weston.[2] *[1]Transplant Immunology Laboratory, LifeLink Foundation, Tampa, FL; [2]LifeLink Transplant Institute, Tampa, FL.*

Cardiac allograft rejection is currently assessed using biopsy-guided histologic classification systems. The presence of anti-HLA antibodies (Ab) in patients is well recognize as a risk factor for allograft failure and their assessment is routinely done pre-transplant (TPX). Monitoring of such Ab post-TPX, however, is not commonly performed although a number of studies have shown an association between their appearance and rejection episodes. Twenty nine heart transplant recipients were tested for anti-HLA Ab prior to TPX and at the time of endomyocardial biopsy (EB) post-TPX. The presence and specificity of IgG anti HLA Class I and II was determined by Flow Cytometry using purified HLA antigen coated beads (One Lambda). Group A (N=4) 3 patients had no anti-HLA Ab prior to TPX and developed donor specific Ab in a period of 3 wks to 3 months post-TPX. Five to 8 weeks after donor specific anti-HLA Ab was detected a severe rejection episode ocurred confirmed by EB (grade 2-3A). The remaining patient in this category had donor specific anti HLA Ab prior to TPX and showed increased levels of donor specific Ab plus de novo donor anti-HLA Ab prior to confirmed rejection. Group B (N=4) had no detectable anti-HLA Ab however had severe rejection episodes 3-10 weeks after TPX as detected by EB (grade 2-3A). Group C (N=21) patients had no Ab prior to TPX, have not developed anti-HLA Ab post-TPX with no rejection episodes post TPX. Patients developing anti-HLA Ab early after TPX have demonstrated an increased risk for acute rejectionand poorer graft survival. Patients in Group B had no detectable anti HLA Ab pre and post TPX. These patients may benefit from cell mediated assays that monitor donor specific reactivity. The In vitro CMI assay (Cylex) detects cell mediated immunity (CMI) on CD4+ T lymphocytes in immunesuppressed patients by measuring the ATP concentration from CD4+ T lymphocytes following stimulation with a mitogen, PHA. We postulated that the use of the In vitro CMI could help us to predict such rejection episodes in the absence of detectable donor specific Ab. None of the patients in Group B have developed further rejection eopisodes. However, we have been able to detect increased ATP levels prior to a rejection episode in patients with anti-HLA IgG. The use of donor specific anti-HLA Ab and CMI assays, together with the patients clinical picture will provide a very useful tool in the early detection of rejection episodes.

Abstract# 1454 Poster Board #-Session: P210-III

A RANDOMIZED TRIAL SWITCHING FROM AZATHIOPRINE TO MYCOPHENOLATE MOFETIL IN CARDIAC TRANSPLANT PATIENTS WITH NEWLY DIAGNOSED ANGIOGRAPHIC TRANSPLANT CORONARY ARTERY DISEASE. Jon A. Kobashigawa,[1] Yuzuru Takano,[1] Jonathan M. Tobis,[1] Gregory A. Cogert,[1] Maria Espejo Vassilakis,[1] Jaime D. Moriguchi,[1] Hillel Laks.[2] *[1]Cardiology, The David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Cardiothoracic Surgery, The David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Once transplant coronary artery disease (TCAD) has been diagnosed in heart transplant patients, there is no effective therapy. The use of mycophenolate mofetil (MMF), has been suggested to slow the development of TCAD in animal models. **Methods**: We randomized 30 heart transplant patients with newly diagnosed angiographic TCAD to remain on azathioprine (AZA), n=13, or switch to MMF, n=17, both in conjunction with cyclosporine (CSA) ± prednisone. Angiography was performed one year later in all randomized patients. Analysis by quantitative coronary angiography (QCA) was performed measuring three designated sites in each artery at baseline and one year later for each group. Computerized software (Echoplaque 2.0.x Indec) was utilized for specific QCA measurements which provided a mean coronary diameter score for each patient. Further assessment included development of a >50% progression of disease or a new lesion greater than 50% stenosis. **Results:** There were no differences between the AZA and MMF groups in the change of mean vessel diameter from baseline to one year (AZA = -0.02 ± 0.29 mm vs. MMF = -0.02 ± 0.26 mm, p=0.98). There was a new >50% lesion noted in 38% of the AZA patients as compared to 47% of the MMF pts (p=0.63). There were no differences in ejection fraction, new wall motion abnormality, or cardiac output between both groups. QCA assessment of each separate artery (left anterior descending, circumflex, and right coronary arteries) revealed no significant differences. **Conclusion**: In the short term, the switch from AZA to MMF does not appear by QCA to alter the progression of TCAD in heart transplant patients with newly diagnosed angiographic TCAD, however longer follow up is necessary to truly assess the efficacy of this therapy.

Abstract# 1455 Poster Board #-Session: P211-III

INCREASED CHEMOKINE GENE EXPRESSION IN HUMAN HEART GRAFTS.FOLLOWING ISCHEMIA. Nader Fahmy,[1] Mohamad Yamani,[2] Randall Starling,[2] Norman Ratliff,[3] James Young,[2] Patrick McCarthy,[2] Andrew Novick,[1] Robert Fairchild.[1] *[1]The Glickman Urological Inst., Cleveland Clinic Fdn., Cleveland, OH; [2]Dept. Cardiovascular Medicine, Cleveland Clinic Fdn., Cleveland, OH; [3]Dept. Anantomic Pathology, Cleveland Clinic Fdn., Cleveland, OH.*

Background. The development of ischemia following transplantation is followed by cellular infiltration and negatively affects graft outcome. Chemokines are cytokines with chemoattractant properties for leukocytes expressing specific receptors. Previous studies have shown that chemokine and receptor gene expression increase during acute rejection episodes in human heart grafts. The aim of the current study was to test chemokine gene expression in heart grafts having histopathologically proven ischemic lesions in the absence of rejection. Methods. The mRNA expression of IP-10, Mig, ITAC, RANTES, MCP-1, and IL-8 and the receptors CXCR3 and CCR5 was tested using quantitative (Taqman) real-time PCR in 76 endomyocardial biopsies taken from grafts without evidence of rejection from 16 patients. Sample biopsies were examined histologically for the presence of ischemic lesions. Results. 36.84 (n= 28) biopsies came from grafts that had evidence of ischemia. 6 patients showed evidence of ischemia within the first weeks following transplantation. Expression of IP-10, RANTES, MCP-1, and the receptor CCR5 was significantly increased during a documented histopathologic ischemic lesion in the absence of rejection ($P<0.01$). Conclusion. These results demonstrate significantly increased expression of T cell and macrophage chemoattractants in clinical heart allografts during ischemic episodes following transplantation. The results further indicate that increased chemokine gene expression is observed during graft inflammation and is not restricted to the occurrence of an acute rejection episode.

Abstract# 1456 Poster Board #-Session: P212-III

MORBIDITY AND MORTALITY OF CARDIAC SURGERY FOLLOWING RENAL TRANSPLANTATION. David G. Affleck,[1] Kristine J. Guleserian,[1] Michael K. Pasque,[1] Marc R. Moon,[1] Jennifer S. Lawton,[1] Rachel L. Herren,[1] Marci S. Bailey,[1] Ralph J. Damiano, Jr.,[1] Nader Moazami.[1] *[1]Cardiothoracic Surgery, Washington University School of Medicine, Saint Louis, MO.*

PURPOSE: With improved survival in renal transplantation (RT), the number of recipients undergoing cardiac surgery has increased. Outcomes of cardiac surgery requiring cardiopulmonary bypass (CPB) after RT have not been clearly defined. The purpose of this study was to review the morbidity, mortality and allograft function in RT patients undergoing major cardiac surgery. **METHODS:** We performed a retrospective database review of consecutive RT patients who underwent cardiac surgery requiring CPB from 1987 to 2002. Major morbidity, operative and long term mortality were examined and compared to the Society of Thoracic Surgeons database. **RESULTS:** Forty-seven RT patients (27 male, 20 female), with a mean age of 53 ± 11 years and LVEF of 40 ± 13% underwent cardiac surgery during the study period. Twenty-six (55%) patients underwent coronary artery bypass (CAB), 10 (21%) valve replacement, and 10 (21%) combined CAB/valve. One patient had a repair of a Type A aortic dissection. Mean time from RT to CPB was 4.9 ± 4.4 years. Twenty patients (42%) required preoperative dialysis (DIA) while 27 (58%) had stable allograft function (NDIA). Preoperative creatinine was 4.2 ± 3.4 mg/dl in the DIA group vs. 2.17 ± 1.0 mg/dl in the NDIA group. Patients with stable allograft function, had an immediate post-operative and discharge creatinine levels of 2.5 ± 1.1 mg/dl and 2.4 ± 1.5 mg/dl respectively. Operative mortality for the cohort was 8.5% (4/47). In the DIA group, the operative mortality was 15% (3/20) vs. 3.7% (1/27) in the NDIA group (p=0.298). Post-operative morbidities were not statistically different in the NDIA and DIA groups. Compared to the STS database, RT patients had higher rates of major morbidities as well as operative mortality. Actuarial 1, 3, and 5 year survivals were 62.2%, 62.2, and 13% in the DIA group vs. 79%, 69%, and 51% in the NDIA group (p = 0.09). **CONCLUSIONS:** Renal allograft function remains stable following major cardiac surgery. There is a trend towards improved long-term survival among those patients with stable allograft function. Perioperative morbidity is higher in the renal transplant population when compared to the STS database. Operative mortality is significantly higher for this select group of patients.

	RT Cohort (%)	STS (CAB)(%)	STS (CAB/valve) (%)
Operative Mortality	8.5	2.6	6.4
Pneumonia	8.5	2.7	4.7
Sepsis	8.5	1.0	2.1
Deep Sternal Infection	4.3	0.6	0.6
Reoperation-Bleeding	2.1	2.4	5.5

Abstract# 1457 Poster Board #-Session: P213-III

COMPARISON OF AZATHIOPRINE AND MYCOPHENOLATE MOFETIL IN YOUNG HEART TRANSPLANT RECIPIENTS. Aimee K. Armstrong,[1] Dennis C. Crowley,[1] Wei Wei,[2] Caren S. Goldberg,[1] Robert J. Gajarski.[1] *[1]Division of Pediatric Cardiology, University of Michigan Health System, Ann Arbor, MI; [2]Department of Biostatistics, University of Michigan, Ann Arbor, MI.*

Objective: To compare the frequency of rejection, leukopenia, and infection in young patients (pts) taking mycophenolate mofetil (MMF) or azathioprine (AZA) after orthotopic heart transplantation (OHT). **Methods:** Records were reviewed from pts under the age of 26 years who underwent OHT between 1/94 and 6/02, survived to discharge, and were followed in our transplant program. Immunosuppression for all pts included cyclosporine (CyA), prednisone, and either MMF or AZA. Analyzed variables included grades of rejection, treatment for rejection, WBC count <4,000, hospitalizations for infection, post-transplant lymphoproliferative disease (PTLD), and survival. **Results:** Sixty-two OHTs were performed in 61 pts, and 38 pts met the inclusion criteria. Sixteen pts received AZA; 11 received MMF; 9 switched from AZA to MMF; and 2 switched from MMF to AZA. Mean age at OHT was 11.6±7.5 years, and average follow-up time was 3.7±2.1 years. When accounting for variations in CyA levels, the number of pts experiencing rejection greater than or equal to grade 2 or 3A was not significantly different between pts on MMF vs. AZA during the first post-OHT year or for the duration of the follow-up period. Likewise, there was no difference in rejection episodes before and after pts switched from AZA to MMF. Both groups had similar numbers of treated rejection episodes. There was no significant difference in the number of hospitalizations for infection between pts on AZA vs. MMF. During the first year post-OHT, however, leukopenia occurred more often in pts receiving MMF vs. those on AZA (p=0.08). One pt taking MMF developed PTLD. Death occurred in 3 pts on MMF and in 1 pt taking AZA (p=NS). **Conclusions:** Optimal immunosuppression for cardiac allograft rejection has not yet been established in children. Adult studies show that MMF may reduce first year post-OHT rejection episodes and graft vasculopathy. MMF is now frequently used in place of AZA in both adult and pediatric pts. This is the first study to compare the two in young pts. These data demonstrate that, compared with AZA, MMF did not significantly reduce rejection frequency or associated co-morbidities but was associated with more leukopenia in the first OHT year. A prospective trial is needed to determine if MMF is a superior immunosuppressant or inhibitor of allograft vasculopathy in young heart OHT recipients.

Abstract# 1458 Poster Board #-Session: P214-III

CELL MEDIATED RESPONSIVENESS OF HEART TRANSPLANT RECIPIENTS. Ronald H. Kerman,[1] Hal Gibson,[1] Chris Garcia,[1] Eva McKissick,[1] Stephanie Rasmussen,[1] Chris Ballew,[1] Noriel Acorda,[1] Peter Pryzbylowski,[1] Cindy Thomas,[2] Barry D. Kahan,[1] Branislav Radovancevic.[2] *[1]Surgery, University of Texas Medical School-Houston, Houston, TX; [2]Texas Heart Institute, Houston, TX.*

Little information exists about the cell mediated responsiveness (CMR) of end stage heart disease (ESHD) patients and/or whether there is a correlation to the presence in their sera of HLA antibodies (Abs). We therefore evaluated the pretransplant (preTx) and serial post-Tx status of ESHD patients for the presence of HLA Abs and their CMR. HLA Abs were identified utilizing the Flow PRA assay (One Lambda, Inc., Canoga Park, CA.). CMR was determined utilizing a new Cylex Immune Function Assay (Cylex, Inc., Columbia, MD.) which measures ATP released from CD4+ T cells, following mitogenic stimulation with phytohemagglutinin (PHA). The amount of ATP is quantitatively measured using a luciferin-luciferase assay and may represent a surrogate of patient cellular immunity. The assay is performed within 24 hours and data are presented as the stimulation index, S.I., (stimulated cells/resting cells) representing the magnitude of increase in CMR following PHA stimulation. The CMR for normal healthy individuals (N=58) is 24 ± 13 ng/ml ATP. There were 9 ESHD patients with an HLA Ab Flow PRA of 51 ± 28% and a CMR of 35 ± 39 ng/ml ATP (which was significantly greater than the CMR for normals, $p < 0.05$). Thirteen ESHD patients with 0% HLA Abs had a comparable CMR of 30 ± 35 ng/ml ATP. Ten ESHD patients received cardiac allografts and were serially evaluated. Immediately post-Tx the CMR fell, then resolved equal to or below pre-Tx levels. Infectious episodes resulted in significant increases in CMR. Several clinically stable patients, treated by minimal immunosuppression, presented with low CMR values suggesting that a homeostatic immunoregulatory mechanism may be operative. In summary, then, this new immune function assay is user friendly, has twenty-four hour turn around time and may provide a sensitive assessment of the immune status of transplant recipients.

Abstract# 1459 Poster Board #-Session: P215-III

VENTILATORY EQUIVALENT FOR O_2 DURING EFFORT IN RECIPIENTS OF A HEART TRANSPLANT. Maria A. Montoliu,[1] Jose M. Urraca,[1] Blanca Rodriguez,[1] Jesus De La Iglesia,[2] Luis Palenciano.[3] *[1]Ergonomics Unit, Hospital Central de Asturias, Oviedo, Asturias, Spain; [2]Cardiology Service, Hospital Central de Asturias, Oviedo, Asturias, Spain; [3]Functional Biology, Universidad de Oviedo, Oviedo, Asturias, Spain.*

It is known that peak VO_2 (VO_2p) at a progressive effort in recipients of a heart transplant doesn't substantially differ from pre-transplant values. On the other hand, denervated implanted hearts respond with sluggish frequency increments at progressive effort tests; therefore, if the same protocol of external work rate increment is applied heart rate (HR) and VO_2 lag behind in comparison to before operation. VO_2p is thus attained later, when a higher external work is being carried out. As no changes in patient's mechanical efficiency can be expected after operation, a surplus of energy for that extra work must be provided by the anaerobic pathway. If that extra work is high enough, lactate accumulation in blood can be expected, this leading to a higher VO_2/ VCO_2 exchange ratio in the lung (RER) and a higher ventilatory equivalent for O_2 (VE/ VO_2). Both features could be identified at the progressive effort test. We tried to do so as some controversy exists on whether or not recipients of heart transplant reach high enough levels of exercise as to attain lactic acidosis. We examined pre and postoperative records from 11 recipients of heart transplant. Effort test were carried out on a treadmill. Variables were measured breath by breath and then averaged at 30 s intervals. The study group was made of 10 males and 1 female. Group´s mean (range) age was 52.0 (44-64) y, weight 76.5 (53-94) kg, height 1.68 (1.57-1.76) m. Differences was tested with the paired t-test, a $p<0.05$ was considered statistical significant. Mean (range) pre and post-VO_2p were respectively 1085.8 (612.0-1575) and 1109.2 (718.0-1714) (p: 0.76), HR 140.8 (108-205) and 126.5 (100-147) (p: 0.03), VE at VO_2p 36.0 (20.1-53.5) and 50.2 (42.1-62.0) ($p<0.001$), VO_2p/kg body weight 14.2 (8.8-21.3) ml/kg and 15.0 (9.8-26.0) (p:0.39), O_2puls (VO_2ml/heart beats) at VO_2p 7.8 (4.6-11.6) and 8.8 (5.8-13.5) (p: 0.30), exchange ratio at VO_2p 0.902 (0.738-1.009) and 1.125 (0.988-1.343) ($p<0.01$), ventilatory equivalent for O_2 (VE(l)/ VO_2(l)) at VO_2p 34.7 (22.3-48.4) and 46.6 (32.3-61.3) ($p<0.001$), index of used ventilatory capacity (VE(l)/FEV1(l)) 15.0 (7.9-26.5) and 22.7 (11.5-41.3) ($p<0.01$) and effort test duration 6.4 (3-10.5) and 11.2 (6-14.5) min. Our data (higher VCO_2/ VO_2 and VE for a similar VO_2) at the end of exercise suggest that a significant higher lactic acidosis developed after than before heart transplant.

Abstract# 1460 Poster Board #-Session: P216-III

COMPARISON OF RAPAMYCIN AND MYCOPHENOLATE MOFETIL THERAPY IN HEART TRANSPLANT RECIPIENTS TREATED WITH TACROLIMUS. Branislav Radovancevic,[1] Bojan Vrtovec,[1] Cynthia D. Thomas,[1] Edith L. Ford,[1] Guillermo Torre-Amione,[2] Aria P. Yazdanbakhsh,[1] O. H. Frazier.[1] *[1]Department of Cardiopulmonary Transplantation, Texas Heart Institute, Houston, TX; [2]Cardiology, Baylor College of Medicine, Houston, TX.*

Background: Both rapamycin (RPM) and mycophenolate mofetil (MMF) have been shown to reduce rejection in transplant recipients. We sought to compare their immunosuppressive effects in tacrolimus-treated heart transplant patients. **Methods:** Out of 18 heart transplant recipients enrolled, 10 were randomized to RPM (loading dose 10 mg, followed by 0.06±0.04 mg/kg/day), and 8 were treated with MMF (3 g/day). At 3.8±2.3 days after transplantation, RPM or MMF therapy was added to the standard tacrolimus (mean dose: 0.11±0.09 mg/kg/day) and prednisone regimen. Patients were followed for 230±178 days. Rejections with a score of ≥3A were considered hemodynamically significant if accompanied with pulmonary artery pressure elevation. **Results:** RPM and MMF groups did not differ in age (56±8 years in RPM group vs. 56±15 years in MMF group, p=0.92), gender (male: 70% in RPM group vs. 75% in MMF group, p=0.81), and mean plasma tacrolimus levels (10.6±2.6 ng/ml in RPM group vs. 9.2±1.5 ng/ml in MMF group, p=0.28). When compared to MMF group, the survival rates in the RPM group were higher, but the difference did not reach statistical significance (RPM group: 90%, MMF group: 63%, p=0.16). Also, the number of ≥3A rejections per patient (0.44±1.01 in RPM group vs. 0.33±0.50 in MMF group, p=0.78), and freedom from rejection (60 % in RPM group vs. 75 % in MMF group, p=0.50) was comparable in both groups. Mean post-transplant platelet counts (202±65 x 10^3/mm^3 in RPM group vs. 207±58 x 10^3/mm^3 in MMF group, p=0.88), white blood cell counts (9.7±3.1 x 10^3/mm^3 in RPM group vs. 12.6±3.6 x 10^3/mm^3 in MMF group, p=0.14), BUN levels (28.0±7.4 ng/ml in RPM group vs. 37.4±25.7 ng/ml in MMF group, p=0.31), and creatinine levels (1.33±0.37 ng/ml in RPM group vs. 1.55±0.60, ng/ml in MMF group, p=0.37) did not differ between the groups and remained stable throughout the treatment period. **Conclusions:** The immunosuppressive effects of RPM and MMF in tacrolimus-treated heart transplant recipients are comparable, and are not associated with leukopenia, thrombocytopenia, or worsening of renal function.

Abstract# 1461 Poster Board #-Session: P217-III

PROTECTION OF CADAVER DONOR HEARTS –EFFECT OF OXYGENATED FLUSHING/PRESERVATION SOLUTION AND WARM-INITIAL-FLUSHING.– Michiharu Suga,[1] Shigeaki Kaga,[1] Takeshi Nakatani,[2] Kazuhiko Yamada,[1] Soichiro Kitamura.[2] *[1]Dept. of Regenerative Medicine and Tissue Engineering, National Cardiovascular Center, Suita, Osaka, Japan; [2]Dept. of Cardiovascular Surgery, National Cardiovascular Center, Suita, Osaka, Japan.*

Purpose: The use of cadaver hearts could increase the number of heart transplantation. The period of in vivo warm ischemia and cold preservation, however, time-dependently injures cardiac grafts. In the current study, we tried to identify whether oxygenation of flushing/preservation solution and warm-initial-flushing contribute to protect cadaver donor hearts. **Methods:** Rat hearts were induced hypoxic arrest, sustained in vivo warm ischemia for 20 minutes, and then excised. Exp 1 (oxygenation of St. Thomas' Hospital II (ST) solution): The non-preserved control (NP) group hearts were immediately reperfused with a Langendorff apparatus for 60 minutes to evaluate cardiac functions. The other group hearts were flushed with 20 ml of normal (ST group) or oxygenated (OX group) ST solution at 4ºC, stored in the same solution for 2 hours, and reperfused in the same fashion. Exp 2 (warm-initial-flushing): After flushing with 40 ml of cold solution (4ºC) (C group), 20 ml of warm solution (35ºC) followed by 20 ml of cold solution (W group) or 20 ml of oxygenated warm solution followed by 20 ml of oxygenated cold solution (WO group), hearts were stored in cold solution for 2 hours and reperfused with a Langendorff apparatus for 60 minutes. **Results:** All hearts functioned throughout the period of reperfusion. Exp 1: Developed pressure (DP, mmHg) and +dP/dT (mmHg/sec.) in the NP, ST and OX groups were 173 ± 6, 83 ± 12 and 119 ± 34 ($P<0.05$; NP vs. ST, NP vs. OX and ST vs. OX), and 4020 ± 168, 1930 ± 291 and 2760 ± 895 ($P<0.05$; NP vs. ST, NP vs. OX and ST vs. OX), respectively. Exp 2: DP and +dP/dT in the C, W and WO groups were 76 ± 16, 127 ± 45 and 158 ± 15 ($P<0.05$; C and W, and C and WO), and 1438 ± 4948, 2725 ± 1196 and 5040 ± 644 ($P<0.05$; C vs. W, C vs. WO and W vs. WO), respectively. Furthermore, coronary flushing time (sec.) in the C, W and WO groups were 299 ± 21, 176 ±11 and 151 ± 12, respectively ($P<0.05$; C vs. W and C vs. WO). **Conclusions:** These results suggest that oxygenation of solution and warm-initial-flushing synergically enhance the functions of preserved cadaver hearts. Shorter flushing time in the treated groups implies that these modalities protect vasospasm of coronary vessels.

Abstract# 1462 Poster Board #-Session: P218-III

PROLONGED QTC INTERVAL PREDICTS MORTALITY IN HEART FAILURE PATIENTS TREATED WITH MILRINONE AND BETA-BLOCKER COMBINATION THERAPY. Bojan Vrtovec,[1] Reynolds M. Delgado,[1] Aria P. Yazdanbakhsh,[1] Aly Zewail,[1] Cynthia D. Thomas,[1] Rajko Radovancevic,[1] Branislav Radovancevic.[1] *[1]Department of Cardiopulmonary Transplantation, Texas Heart Institute, Houston, TX.*

Background: Long-term milrinone infusion has been associated with QTc interval prolongation and an increased risk of arrhythmias, while beta blockade is considered anti-arrhythmic and has been shown to shorten QTc interval. The significance of QTc interval prolongation in heart failure patients treated with combined milrinone and beta-blocker regimen has not been studied yet. **Methods:** We analyzed QTc interval duration in 51 patients with advanced heart failure (NYHA class IV, left ventricular ejection fraction <25%) who were treated with combined long-term intravenous milrinone infusion (0.375 mg/kg/min) and oral beta-blocker therapy. Before the initiation of milrinone, the mean QT interval was measured from standard ECG leads II and V4, and QTc interval duration was calculated with the Bazett formula. Patients were followed for 1 year after milrinone initiation. **Results:** QTc interval was prolonged (>440 ms) in 30 patients (59%) (Group 1), and normal in 21 (41%) (Group 2). Patients with and without QTc prolongation did not differ in age (60±12 years in Group 1 vs. 63±14 years in Group 2, p=0.24), gender (male: 70% in Group 1 vs. 75% in Group 2, p=0.58), or heart failure etiology (ischemic: 52% in Group 1 vs. 65% in Group 2, p=0.35). During follow-up, 15 of 51 patients (29%) died of cardiac causes (14 due to pump failure and 1 due to sudden cardiac death). The Kaplan-Meier cardiac mortality rate was 3.5 times higher in Group 1 than in Group 2 (p=0.002). **Conclusions:** Baseline QTc interval prolongation (>440 ms) is associated with increased cardiac mortality in patients with advanced heart failure who are treated with milrinone/beta-blocker combination therapy. This suggests the potential role of QTc interval in risk stratification of patients with advanced heart failure.

Abstract# 1463 Poster Board #-Session: P219-III

BACKGROUND AND PROGNOSTIC SIGNIFICANCE OF LOW LEFT VENTRICULAR EJECTION FRACTION AFTER HEART TRANSPLANTATION. Bojan Vrtovec,[1] Rajko Radovancevic,[1] Veronica V. Lenge,[1] Cynthia D. Thomas,[1] Aria P. Yazdanbakhsh,[1] O. H. Frazier,[1] Branislav Radovancevic.[1] *[1]Department of Cardiopulmonary Transplantation, Texas Heart Institute, Houston, TX.*

Background: Even though reduced left ventricular ejection fraction (EF) occurs relatively common after cardiac transplantation, its background and prognostic significance remain undefined. **Methods:** We reviewed all transthoracic echocardiograms of 158 cardiac transplant recipients transplanted between 1997 and 2001. EF was measured using modified Simpson's formula, and EF <40% was defined as low. Left ventricular mass was calculated using Devereux formula. Data on donor and recipient characteristics, post-transplant clinical course and immunosuppression were collected, biopsies were assessed for the presence of rejection and myocarditis, and angiograms were reviewed for the presence of transplant vasculopathy. **Results:** Low EF was present in 62 (39%) of patients. It occurred at 395±879 days after transplantation and lasted for 310±298 days. When compared to the remaining cohort, patients with low EF had increased number of HLA-B (1.88±0.35 vs. 1.59±0.67, p=0.018), and HLA- DR (2.00±0.14 vs. 1.55±0.67, p=0.001) donor/recipient mismatches, and a longer graft ischemic time (201±63 min vs. 188±53 min, p=0.05). Rejections (ISHLT grade IIIA or higher) occurred more frequently in low EF patients (1.62±2.10 rejections per patient) than in patients with normal EF (0.88±1.16, p=0.014). The 5-year cumulative survival of patients with low EF was no different from the survival of normal-EF group (78% vs. 80%, p=0.935). The survival rates in low-EF group were independent of the time of low EF occurrence, the duration of low EF, the degree of EF reduction, and the presence of left ventricular hypertrophy or transplant vasculopathy. **Conclusions:** Low EF after heart transplantation appears to be immune-mediated and related to prolonged graft ischemic time. It does not affect survival of cardiac transplant recipients.

Abstract# 1464 **Poster Board #-Session: P220-III**

A NOVEL TECHNIQUE FOR DONOR BONE MARROW PROCUREMENT FOR CLINICAL TOLERANCE TRIALS IN CHILDREN. Steven A. Webber,[1] Jayson R. Pereira,[1] Frank A. Pigula,[1] Sanjiv Gandhi,[1] Noriko Murase,[1] Jennifer E. Woodward,[1] Gerard J. Boyle,[1] Yuk M. Law,[1] Si Pham,[1] Adriana Zeevi,[1] Bartley P. Griffith,[1] John J. Fung.[1] *[1]Pediatrics and Surgery, University of Pittsburgh, Pittsburgh, PA.*

Background and methods: Donor bone marrow is used in many experimental, and more recently, clinical tolerance induction protocols. The standard approach has been procurement of vertebral bodies (VB). This approach has several limitations including difficulty obtaining consent from donor family, donor disfigurement, requirement for training of procuring surgeon and need to transport thoracic organs to recipient center before VB are obtained. We, therefore, developed an alternative strategy of sternal marrow procurement as part of a clinical trial of intrathymic donor bone marrow inoculation concomitant with pediatric heart transplantation. The feasibility and safety of this approach are reported. After sternotomy, each open edge of the sternum is vigorously curetted with sterile technique and the marrow and bone chips are placed in standard marrow procurement solution. Unmodified bone marrow is then prepared using standard techniques for VB, and cells are resuspended in 2 to 8 cc of lactate-ringers based media. **Results:** Bone marrow was obtained by this technique from 17 thoracic donors, ages 0.01-35 years (16 of 17 <18 years, median 2.3 years) with weight 3.7 to 61.4 kg (median 15 kg). Bone marrow preparation time was 2-1/2 to 3 hours. Mean total yield of cells was $90.7x10^7$ cells or $4.8x10^7$ cells / kg donor weight representing $5.6 x10^7$ cells / kg recipient weight. Sufficient bone marrow cells for infusion (minimum 75% of target dose of $8x10^7$ cells / kg recipient weight) were obtained from 10 of 17 (59%) procurements. Adequate cell collection correlated best with donor / recipient weight ratio (r=0.48; mean D/R ratio 1.6 vs 1.2 for sufficient and insufficient yields, respectively). Flow analysis of marrow cells was comparable to prior experience with VB marrow. The rapid preparation time did not delay recipient sternal closure. All marrow gram stains and cultures were negative. There was no mediastinal infection and no GVHD. **Conclusion**: The technique of sternal curettage is simple, less invasive and allows transport of marrow with the thoracic organs. It provides sufficient marrow for tolerance strategies requiring approximately $6x10^7$ cells / kg recipient body weight.

Abstract# 1465 **Poster Board #-Session: P221-III**

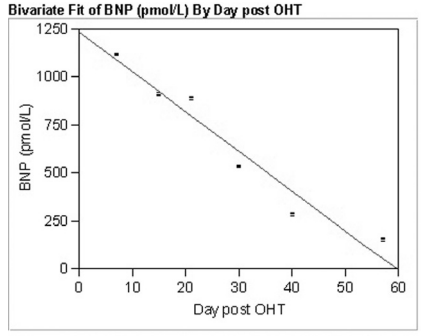
OBSERVATIONS ON THE USE OF 2-D ECHOCARDIOGRAMS IN EVALUATING CADAVER DONOR HEARTS. Martin F. Mozes,[1] George M. Mullen,[2] Maryl R. Johnson,[3] William R. Jacobs,[2] David McPherson,[3] Katrina Harmon,[1] Krystyna Malinowska.[2] *[1]Gift of Hope, Organ and Tissue Donor Network, Elmhurst, IL; [2]Heart Transplant Program, Loyola University Health Sysytem, Maywood, IL; [3]Cardiology, Northwestern Medical Center, Chicago, IL.*

Background: Correct timing and accurate interpretation of cadaver donor EC (echocardiogram) are critical in the appropriate and maximal utilization of hearts for transplantation. ***Purpose:*** To assess the accuracy of EC interpretations by comparison of 3 indpendent readings and to review the cadaver donor clinical parameter correlations with EC interpretation. ***Methods:*** 32 randomely selected cadaver donor EC tapes were retrospectively and independently reviewed by 2 expert cardiologists, other than the original donor hospital cardiologist. Only the left ventricular ejection fraction (EF) was used for quantitative correlation (≤5% difference=good) and also for determining hearts as GOOD (EF >45%) or POOR (EF<45%) for transplant. The reviewed clinical donor factors were age, gender, cause of death, duration from admission to brain death(BD) and from BD to EC and heart rate, arterial pressure, CVP and oxygenation at time of EC . ***Results:*** Complete data were available on 30 cases.There was agreement between all 3 reviewers in 16(53%)of EC's. There was 97% (29 of 30) agreement between the original reading and at least one of the subsequent independent reviews. The "GOOD" heart donors (n=23) differed form the "POOR" heart donors (n=7) in age (43±13 vs 28±15; p<.042) and in heart rate (130±17 vs 102±27; p<.005). There were trends in the correlation of male gender, trauma as cause of death, higher MAP and higher CVP with GOOD hearts. ***Summary:*** There was a strong agreement in interpretation of the EC's between the donor hospital reading and the independent readings.There is strong indication that POOR hearts (ie EF <45%) are associated with poor hemodynamic resuscitation ***Conclusions:*** Overall, LVEF readings in the cadaver donor setting are reliable. In order to avoid wastage of precious donor hearts, EC's should be performed at a time when the donor is optimally resuscitated. Alternatively, poor EC's should be repeated at such times. Additional monitoring (eg Pulmonary artery catheter) may be warranted to optimize heart recovery for transplantation.

Abstract# 1466 **Poster Board #-Session: P222-III**

TIME DEPENDENT DECLINE IN PLASMA BRAIN NATIURETIC PEPTIDE LEVELS OCCURS INDEPENDENT OF REJECTION AFTER ORTHOTOPIC CARDIAC TRANSPLANTATION. James O. O'Neill,[1] Andrew T. McRae,[1] David O. Taylor,[1] Kenneth Ng,[1] Hilal M. Yamani,[1] Randall C. Starling.[1] *[1]Kaufman Center for Heart Failure, Cleveland Clinic Foundation, Cleveland, OH.*

Introduction: Brain natiuretic peptide (BNP) levels are elevated in patients with heart failure. Serial levels of BNP early after orthotopic heart transplant (OHT) recipients have not been described. We measured serial BNP levels in patients who underwent OHT and sought correlation with other clinical and laboratory factors. Methods: With written, informed consent, 3 ml of venous blood was drawn from patients immediately before undergoing endomyocardial biopsy as part of their post-transplant regimen. BNP analysis was performed using a point of care kit (Biosite, San Diego). Concomitant central venous pressure (CVP) was recorded. Donor characteristics including age, ischemic time and echocardiographic data data were recorded. Patients received triple immunotherapy. Histological rejection was scored according to ISHLT guidelines. Results: Twelve patients were included (11 male), with a mean age of 56±8 years. Median BNP levels were elevated at 1120 (286-1300) pmol/L one week after OHT and showed a time dependent decline to 153 (28-638) pmol/l at 8 weeks. Bi-variate fit of time (days) against median BNP levels revealed r=0.97, p=0.0008 (see figure below). Three significant episodes of rejection (ISHLT grade 3A) occurred in 3 patients with BNP levels of 966,357 and 41 pmol/L at 2,5 and 6 weeks respectively. Mean ejection fraction 1 week post OHT was 55±5%. No factor analyzed (ischemic time, donor age, rejection, CVP, ejection fraction) other than time from transplant, showed a correlation with BNP levels. Conclusions: BNP levels show a time dependent decline in patients after OHT. They do not correlate with known clinical parameters, in particular with the degree of rejection or post –operative ejection fraction and the degree of reperfusion injury may explain this phenomenon. Further study is indicated including the relationship with the subsequent development of allograft vasculopathy.



BNP (pmol/L)=1233-20.6(Day post OHT), p=0.001, r=0.97

Abstract# 1467 **Poster Board #-Session: P223-III**

ASSESSMENT OF AN ACCELERATED HEPATITIS B VACCINATION PROTOCOL IN CONGESTIVE HEART FAILURE AND LEFT-VENTRICULAR ASSIST DEVICE PATIENTS AWAITING HEART TRANSPLANTATION. Steven Mawhorter,[1] Mona Nasrallah,[3] Randall Starling,[2] Judy Brakeman,[1] Rene Bennett,[2] Patrick McCarthy,[4] James Young.[2] *[1]Infectious Diseases, Cleveland Clinic Foundation, Cleveland, OH; [2]Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland, OH; [3]Case Western Reserve University, Cleveland, OH; [4]Cardiothoracic Surgery, Cleveland Clinic Foundation, Cleveland, OH.*

BACKGROUND: Hepatitis B is a serious, potentially vaccine-preventable disease, with increased mobidity and mortality in heart transplant recipients. Vaccination after transplant is not effective. Advanced age, obesity, renal and liver failure are known to reduce response to vaccination. There is no literature on vaccine response rates in patients with congestive heart failure (CHF), or with left-ventricular assist devices (LVAD) awaiting transplant. **METHODS**: Given limited time to transplant after evaluation and listing, an accelerated (0,1, 3 week) hepatitis B vaccine (20 mcg Engerix B, Glaxo SmithKline) protocol was employed. All participants tolerated vaccination well. Sequential anti-Hepatitis B surface antibody (anti-HBs) titers were monitored with ≥10 mIU/mL representing protective seroconversion in normal hosts. Participants were in one of three groups: I: CHF (n=9), II: LVAD (n=6), or III: age-matched controls (n=2). **RESULTS**: Mean age was 56.8 years (range 40 – 70 years). Median EF% was 15%. Mean peak serum creatinine was 2.1 (range 1.2 – 3.1). No statistical association was seen between antibody response level and age, body weight, serum creatinine, or transaminase levels. Four (26.6%) participants from Groups I and II developed a transient antibody response. Responders included 2 of the 5 who received their transplant before the third dose of vaccine. Refer to the table below. **CONCLUSION**: Heart failure is

associated with reduced hepatitis B vaccine response. LVAD placement does not favorably increase the rate of anti-HBs response. Alternative methods should be tested to determine if greater vaccine responsiveness could be obtained in this vulnerable population.

Group	Responders	Peak Anti-HBs Response §	Anti-HBs at 9 months §
I CHF (n=9)	2	110	0
II LVAD (n=6)	2	352	0
III Control (n=2)	1*	2026	1900

Anti-HBs units = mIU/ml; greater than 10 is protective §

* One control (age 49) had undiagnosed renal insufficiency (creatinine = 2.1), and did not respond to vaccination.

1468 Poster Board #-Session: P224-III

IDENTIFICATION OF EXPANDED CARDIAC DONORS USING HEART POSTTRANSPLANT MORTALITY OUTCOMES. Rami T. Bustami,[1] Susan Murray,[1,2] Keith P. McCullough,[1] Friedrich K. Port,[1] Frederick L. Grover,[3] W. Steves Ring,[4] Robert M. Merion.[1,2] *[1]SRTR/ URREA, Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI; [3]University of Colorado, Denver, CO; [4]University of Texas Southwestern Medical Center, Dallas, TX.*

Heart transplantation is limited by the shortage of suitable donors. There is growing interest in defining expanded criteria donor (ECD) hearts by comparing mortality outcomes of expanded donor hearts with outcomes of standard donor hearts. We analyzed data from the Scientific Registry of Transplant Recipients (SRTR) to identify ECD hearts using a cohort of 6241 heart transplant recipients who received a transplant between 02/01/1999 and 12/31/2001 and were followed through June 2002. A Cox proportional hazards regression model was used to analyze the relative risk (RR) of posttransplant mortality, with adjustments for 18 recipient and donor characteristics. Three donor factors were significantly associated with posttransplant mortality, including donor age, (0-17 years, RR = 1.34, P = 0.008 and ≥ 50 years, RR = 1.67, P < 0.001, vs age 18-39 years), donor smoking (RR = 1.21, P = 0.020) and CMV mismatch high risk (RR = 1.21, P = 0.023). Several recipient factors had significant impact on posttransplant mortality including age, heart diagnosis, life support use, infection requiring IV drug therapy, total serum bilirubin, serum creatinine, left ventricular remodeling, and mean PA pressure. The table shows those donor categories ("X") with RR of recipient mortality >1.6 (ECD hearts) compared with the ideal donor reference group age 18-39 without those donor factors. Using RR > 1.6, 19.1% of transplants in 1999-2001 would be defined as having used an ECD heart. During the study, 5.6% of recovered ECD hearts were not transplanted, compared with 2% of non-ECD hearts. This study identifies characteristics of ECD hearts by analyzing the RR of recipient mortality. These results may be used for allocating hearts and for discussions with heart transplant candidates.

Donor Condition X = RR> 1.6	**Donor Age Categories (Years)**			
	0-17	18-39	40-49	≥ 50
No conditions		Ref.		X
CMV high risk	X			X
CMV high risk and smoking	X		X	X

1469 Poster Board #-Session: P225-III

THE IMPACT ON INFECTION OF ROUTINE USE OF AN ABDOMINAL BINDER FOLLOWING IMPLANTATION OF A VENTRICULAR ASSIST DEVICE. Monica M. Colvin-Adams,[1] Sophia M. Ormaza,[2] Andrew J. Boyle,[1] Joseph A. Cytron,[1] Jeanne M. Thompson,[2] Kangxiong K. Liao,[2] Soon J. Park,[2] Leslie W. Miller.[1] *[1]Cardiology, University of Minnesota, Minneapolis, MN; [2]Cardiothoracic Surgery, University of Minnesota, Minneapolis, MN.*

BACKGROUND: Infection is the second most frequent complication following ventricular assist device(VAD) implantation. Most infections are related to inadequate skin seal at the drive line exit site. We evaluated the impact on infection rate of routine use of an abdominal binder that immobilizes the VAD drive line in patients who underwent VAD implantation as a bridge to transplant at our institution. **METHODS:** We retrospectively analyzed the infection rates of 64 patients who underwent VAD implantation between April 1995 and September 2002. Twenty-eight patients were managed without the use of an abdominal binder and 36 were managed with the abdominal binder. Infection sites, pathogens, clinical status, and duration of support were compared. Results were also compared before and after routine use of the binder. Infections were defined as positive cultures from one of the following sites: percutaneous drive line, intracorporeal (associated with the device), blood, and non blood-related (urine, pulmonary, etc.). **RESULTS**: The mean age was 51.5 years. 80% of patients in both groups were male. The mean duration of support was 183.78 +/- 160.8 days (114 days before routine use of the binder and 210 days after routine use; range 30-681 days). Forty-seven percent of all patients had infections (146 positive cultures). Seventeen percent of infections were percutaneous drive line infections, 5% intracorporeal, 17% blood-borne, and 61% non blood related. After 8/28/00, we began routine use of the abdominal binder. The number of infections and the percentage of patients who experienced infections decreased significantly after this date. The total infection rate prior to routine use of the abdominal binder was 75%. Following routine use of the binder, the infection rate decreased to 32% (p=0.005). In addition, there was a significant decrease in each type of infection.

	Rate of Infection Among Patients	
Infection Type	**Prior to Abdominal Binder**	**After Abdominal Binder**
Percutaneous Site	35%	10%
Blood-borne	45%	20%
Non Blood-related	70%	40%
Intracorporeal	15%	2.5%

CONCLUSIONS: Routine use of an abdominal binder that immobilizes the VAD drive line reduces clinical infection and prevents significant morbidity.

1470 Poster Board #-Session: P226-III

OKT3 VS. DACLIZUMAB FOR INDUCTION AND AZATHIOPRINE (AZA) VS. MYCOPHENOLATE MOFETIL (MMF) FOR ACHIEVING STERIOD FREE MAINTENANCE IMMUNOSUPPRESSION (SFMI) IN CARDIAC TRANSPLANT RECIPIENTS (CTxR). Scott A. Chapman,[1,2] Kristine Gilkerson,[1] Nancy Siemers,[1] Connie Rooney,[1] Bruce R. Lindgren,[3] Marc R. Pritzker,[1] Maria Teresa Olivari.[1] *[1]Department of Transplantation, Minneapolis Heart Institute/Abbott Northwestern Hospital, Minneapolis, MN; [2]Department of Experimental and Clinical Pharmacology, College of Pharmacy University of Minnesota, Minneapolis, MN; [3]Division of Biostatistics, School of Public Health University of Minnesota, Minneapolis, MN.*

Purpose: Since 1985, our immunosuppression protocol consists of induction and rapid steroid weaning to SFMI in CTxR at high risk for long term steroid exposure. Gradually, we have incorporated MMF over AZA for maintenance immunosuppression, and converted to daclizumab from OKT3 for induction. We have compared the ability to achieve SFMI and remain rejection free during three eras of immunosuppression: OKT3 induction pre-MMF approval (OKT3-1, n=50), OKT3 induction post-MMF approval (OKT3-2, n=36), and daclizumab induction post-MMF approval (DAC, n=16). **Methods:** Consecutive CTxR with > 6 month survival receiving induction and rapid steroid weaning were included. Demographics, immunosuppression agent use, survival, ability to achieve SFMI, and rejection incidence were compared. **Results:** There were no demographic differences between the 3 groups. Days post-transplant (post-Tx) differed between all 3 groups (p<0.001). CSA was used immediately post-Tx in most patients. FK was used at anytime post-Tx in 8% (526±831d), 25% (253±326d), and 25% (3±5d) of CTxR (p=0.07). AZA use immediately post-Tx was 100% of OKT3-1, 86% of OKT3-2, and 18.75% of DAC (p≤0.01 between all 3 groups). CTxR on MMF at any time post-Tx was 14% for OKT3-1, 55.5% for OKT3-2, and 81.25% for DAC (p<0.001 between OKT3-1 vs. OKT3-2 and OKT3-1 vs. DAC). MMF was initiated later post-Tx in OKT3-1 vs. OKT3-2 (1571±964 d vs. 245±410 d, p<0.001). 13 (81.25%) in DAC were started on MMF, all immediately post-Tx. SFMI was achieved in 92% of OKT3-1 (261±387 d), 83.3% OKT3-2 (282±354 d), and 81% DAC (157±43 d) (p=NSS). However, 24% in OKT3-1, 3.3% in OKT3-2, and 0% in DAC had to be restarted on steroid following rejection (p=0.004 OKT3-1 vs. OKT3-2 and p=0.018 OKT3-1 vs. DAC). More CTxR in OKT3-1 rejected compared to DAC (58% vs. 25%, p<0.001). Days to first rejection were 150±179, 52±52, and 28±28 (p=0.248), 3 were > 1yr post-Tx in OKT3-1. Total number of rejections were 60, 42, and 7, corresponding to 1.20(0-7), 1.17(0-9), and 0.44(0-3) rejections/ CTxR for OKT3-1, OKT3-2, and DAC (p=0.073). **Conclusion**: In CTxR, induction with daclizumab and maintenance immunosuppression with MMF reduces the number of rejections and improves the ability to remain on SFMI compared to OKT3 induction and AZA maintenance immunosuppression.

Organ Donation, Preservation and Policy II

1471 Poster Board #-Session: P227-III

GASTRIC BYPASS FOR REDUCTION OF CO-MORBID CONDITIONS IN PATIENTS WITH END-STAGE RENAL DISEASE. J. Wesley Alexander,[1] Keith S. Gersin,[1] Hope R. Goodman,[1] Michael A. Cardi.[2] *[1]Surgery, University of Cincinnati College of Medicine, Cincinnati, OH; [2]The Christ Hospital, Cincinnati, OH.*

Morbid obesity is a growing health problem that affects about 3% of the U.S. population. It is responsible for >300,000 deaths per year with a death rate of up to 10X or more for an age related population. The estimated yearly cost for this disease in the US is about $117 billion. Morbid obesity is especially prevalent in end-stage renal disease with resultant increases in morbidity and mortality. Moreover, obesity is a common contraindication for renal transplantation. Gastric bypass (GBP) has recently emerged as the gold standard for surgical weight loss, but has been used infrequently in ESRD/transplant patients. As a result, there is little published experience with GBP in this population. Methods: Retrospective analysis was made of clinical results of GBP procedures in dialysis and transplant patients using standard techniques. Results: A total of 23 patients underwent GBP: 6 with GBP after transplant (4>1 yr); 3 with GBP before transplant (6 mo to 5 yrs) and 14 in dialysis patients in preparation for transplant (7>1yr). Twenty-two patients had the open procedure and one was done laparoscopically. All patients are alive and have had marked reduction in their co-morbid conditions. In the dialysis patients awaiting a transplant, the one year reduction in weight has been 71% of excess BMI, with the number of patients receiving hypertension meds reduced by 80%, diabetic meds by 50% and hyperlipidemia meds by 67%. The patients followed for >1 yr who received a transplant prior to GBP had a reduction of their excess BMI by 68%, while those who had a GBP and then received a transplant had a reduction of 67%. Weight loss has been sustained throughout the periods of observation and co-morbid conditions have been reduced. Conclusions: Gastric bypass can be a safe and effective procedure for preparing morbidly obese patients for renal transplantation and for reducing co-morbid conditions, especially hypertension, hyperlipidemia and diabetes in both dialysis and transplant patients.

Abstract# 1472 **Poster Board #-Session: P228-III**

PRE-EXISTING COLON CARCINOMA IN SOLID ORGAN TRANSPLANT RECIPIENTS. T. L. Husted,[1] M. J. Hanaway,[1] T. M. Beebe,[1] J. Trofe,[1] T. G. Gross,[1] E. S. Woodle,[1] J. F. Buell.[1] *[1]Division of Transplantation, The University of Cincinnati, Cincinnati, OH.*

In the general population colon cancer (CA) represents 14% of all cancer cases and an equal percentage of cancer deaths. The risk of recurrence of pre-existing colon CA has not been defined in the transplant population, with occasional reports of tumor recurrence associated with immunosuppression. **Methods:** 56 cases of pre-existing colon cancer in solid organ transplant recipients were identified in our database. The organ transplanted, age at transplant, wait time to transplant, stage of disease, type of immunosuppression, and survival rates were reviewed. **Results:** Fifty-six pts with previously treated colon CA were transplanted: 34 kidney, 15 liver and 7 heart transplants. Mean age at transplantation for kidney recipients was 53.8 ± 9.3 years, for liver recipients 53.2 ± 11.0 years and for heart recipients 56.2 ± 4.9 years. All pts had adenocarcinoma of the colon. 16 of 34 kidney recipients (47%) had immunosuppression therapy which included antibody regimens (ALG or OKT_3), 6 of 15 liver recipients (40%) had antibody immunosuppression, and 4 of 7 heart recipients (57%) received antibody immunosuppression. There were no recurrences in the 9 patients (all organs) with a wait time of less than 2 years. 5 of 20 (25%) transplant recipients with a wait time of 2 – 5 years had recurrence of disease [kidney: 1 of 12 (8%); liver: 2 of 5 (40%); heart: 2 of 3 (66%)]. 4 of 23 (17%) transplant recipients with a wait time greater than 5 years had recurrence of disease [kidney: 2 of 15 (13%); liver 2 of 6 (33%); heart: 0 of 2]. Risk of recurrence was related to Dukes staging: A= 14% (n=1), B_1 or B_2 = 19% (n=7), and C_1 or D = 42% (n=5). The time to cancer related death transplant of the kidney pts was 21 mos, the mean for liver pts was 14.3 ± 19.0 mos and for heart pts was 32.0 ± 42.4 mos. **Conclusions:** Experience with pre-existing colon CA indicates that: 1) recurrence rates are low for renal (12%) recipients while cardiac (29%) recipients and especially liver (47%) recipients are at high to moderate risk, 2) excessive mortality after recurrence for both liver (71%) and heart (100%) transplant recipients and 3) recurrence is related to stages at diagnosis.

	Kidney	Liver	Heart	All
Mean Age at Dx (yrs)	47.7 +/- 9.3	48.8 +/- 10.9	52.1 +/- 4.9	47.2 +/- 9.5
Dukes Stage A	4	3	0	7
B1	19	3	2	24
B2	3	7	3	13
C	8	2	2	12
Mean Wait (mos)	73.8 +/- 67.8	53.9 +/- 48.6	50.2 +/- 25.1	75.4 +/- 70.3
Recurrence (mos)	n=4 (12%) 31 (10 to 45)	n=7 (47%) 14.4 (3 to 29)	n=2 (29%) 23 (16 to 29)	n=13 (23%) 23 (3 to 45)
5-yr Survival	94%	60%	71%	82%

Abstract# 1473 **Poster Board #-Session: P229-III**

A NOVEL APPROACH TO DECEASED DONOR ORGAN RECOVERY FURTHERS EFFICIENCY AND COST CONTAINMENT. Martin D. Jendrisak,[1] Keith A. Hruska,[1] Jessica Wagner,[2] Diane Chandler,[2] Dean Kappel.[2] *[1]Transplant Surgery, Washington University School of Medicine, St. Louis, MO; [2]Mid-America Transplant Services, St. Louis, MO.*

Background: We have previously reported significant cost benefit ($2,380 per donor) and improved process efficiency in deceased donor organ recovery when performed at the surgical facility at Mid-America Transplant Services (MTS).[1] The impact of subsequent process modifications to include unrestricted transport distance and elimination of anesthesia services are now reviewed. Methods: We compared time requirements for critical care unit (CCU) support following death declaration, operating room (OR) access delay, surgical time, organ and tissue recovery rates, organ discard and direct costs between MTS and hospital recoveries. Results: From 12/1/01 to 10/31/02, 55 MTS (60%) and 38 hospital (40%) recoveries were performed. Transfer exclusions included 25 for instability (27%), 7 for donor size (7%) and 6 for consent refusal (6%). There were 2 hospital and 1 MTS preoperative donor losses. In the MTS agroup, 14 hearts, 26 lungs, 52 livers, 88 kidneys and 22 pancreata were recovered. In the hospital group, 19 hearts, 20 lungs, 38 livers, 71 kidneys, 14 pancreata and 1 small intestine were recovered. There were no technical losses and all transplanted organs functioned. Group comparisons are shown in the table. Conclusion: A significant reduction in CCU time (3.5 hours) and OR delay (48 min.) was seen with MTS recoveries without compromise to donor organs and tissue for transplantation. Further substantial cost savings ($4,595 per donor) were also realized with the changes employed in current era practice. Jendrisak M, Hruska K, Wagner J, Chandler D, Kappel D. Cadaveric-donor organ recovery at a hospital-independent facility. Transplantation, Vol. 74, 978-82, 2002.

	MTS (N=55)	Hospital (N=38)	p value
CCU management (min.)	600 ± 251	816 ± 251	<.0001
MTS management (min.)	99 ± 40	-	-
OR delay (min.)	14 ± 13	62 ± 30	<.0001
OR time (min.)	187 ± 45	185 ± 49	NS
Organs per donor	3.9	4.2	NS
Organ discard/donor	.76	.47	NS
Tissue N(%)	32(58%)	18(47%)	NS
Cost/donor	$8,549	$13,144	<.0001

Abstract# 1474 **Poster Board #-Session: P230-III**

A PROSPECTIVE, RANDOMIZED TRIAL OF STEROID-FREE MAINTENANCE VS. DELAYED STEROID WITHDRAWAL, USING A SIROLIMUS/TACROLIMUS REGIMEN IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTS (SPK). Raja Kandaswamy,[1] Khalid Khwaja,[1] Angelika C. Gruessner,[1] Rainer W. Gruessner,[1] Lisa Pulkrabeck,[1] David E. R. Sutherland.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Intro: Rapid withdrawal (RW) of steroids used only to mitigate side-effects of anti-T-cell induction therapy has been purported to have advantages over delayed withdrawal (DW) on the incidence of adverse effects and rejection rates. We enrolled 39 (19 in RW and 20 in DW) recipients of primary enteric drained SPK transplants given Thymoglobulin (1.25 mg/kg x 1 if immediate kidney function, x 5-10 if delayed) and Daclizumab (1 mg/kg x 2) for induction and sirolimus and tacrolimus for maintenance immunosuppression (IS). The RW group received steroids for 4 days only, while the DW group had prednisone tapered to 5 mg at 3 mos and 0 mg at 6 mos. **Methods and Results:** Actuarial 1-year survival data and actual 6-month lab and side effect profile are presented. Recipient demographics, 1-year patient, kidney and pancreas graft survival were not different between groups. In the RW group, there were 2 px losses (1 thrombosis and 1 DWF) and 1 kidney loss (PNF) and 1 death (PTLD). In the DW group there were 3 px losses (2 thromboses and 1 DWF), 3 kidney losses (1 torsion, 1 thrombosis, and 1 PNF) and 1 death (MI/CHF). Side effects including nausea, vomiting, diarrhea, arthralgias, tremors, and headaches were not different between groups. The triglyceride level was higher in the SW group (200 vs. 128) at 6 months (p=0.04).

1 Year Survival Rates	**RDS**	**SW**
Pancreas graft survival	84%	81%
Kidney graft survival	92%	80%
Patient survival	92%	94%

	6 Month Data	
	RDS	**SW**
Hb	11.9 ± 1.6	13.3 ± 2.5
WBC	4.9 ± 2.2	4.8 ± 2.3
Creatinine	1.9 ± 1.9	1.3 ± 0.4
Amylase	54 ± 27	54 ± 16
Lipase	89 ± 88	66 ± 55
Hb A_1C	6.5 ± 1.9	5.8 ± 0.4
Cholesterol	188 ± 29	188 ± 30
TGL*	128 ± 29	200 ± 85
HDL	52 ± 11	44 ± 11
LDL	109 ± 47	104 ± 24
FK level	7.3 ± 2.8	8.4 ± 3.0

*p=0.04

Conclusion: Graft survival rates were similar in tacrolimus/sirolimus treated SPK recipients given or not given steroids for maintenance IS. Indeed, there were no rejection losses in those with RW vs. DW of steroids after anti-T-cell induction therapy. At 6 mos, the lipid profile was better in the group not given steroids. There appears to be no reason to add steroids for maintenance in tacrolimus/sirolimus treated SPK recipients given anti-T-cell as described for induction therapy.

Abstract# 1475 **Poster Board #-Session: P231-III**

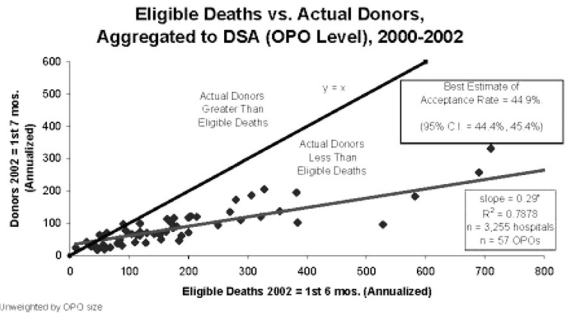
NEW METHODS FOR ESTIMATING TOTAL POTENTIAL (ORGAN) DONORS IN THE U.S. Joshua J. McGowan,[1] Mary K. Guidinger,[1] Keith P. McCullough,[1] Richard E. Pietroski,[2] Daniel S. Gaylin,[3] Akinlolu O. Ojo,[1,4] Friedrich K. Port,[1] Robert A. Wolfe,[1,4] Philip J. Held.[1] *[1]SRTR/URREA, Ann Arbor, MI; [2]Transplantation Society of Michigan, Ann Arbor, MI; [3]NORC, Washington, DC; [4]Univerisity of Michigan, Ann Arbor, MI.*

Background: Quantification of total potential donors (TPD) is difficult. We compared two estimates of TPD by hospital: 1) a projection based on hospital characteristics from a published model (Christiansen et al, AJPH 88,11, 1998); 2) an evaluation based on new data ("Eligible Deaths," ED). Methods: We estimated TPD using 1993 model betas, a set of hospital variables (AHA, 2000; CMS, 2001), and preliminary counts of ED (OPTN and OPO) potential brain dead donors reported by hospital and donation service area (DSA). Both estimates were compared with actual organ donors. Results: One credibility test for the models is the fraction of hospitals with more actual donors than the modeled TPD: Christiansen: 5.7%; ED: 4.8%. Another is the R^2 test shown in Table 1. These suggest the ED model is substantially more predictive of actual donors. The ED model estimates 11.8k TPD/year. Fig. 1 shows ED results aggregated to DSA level. Acceptance rate by DSA varied from 18% to 240%. The DSA with the highest acceptance rate had the fewest ED. Conclusion: Using actual donors as a benchmark, ED is a better predictor of donors by hospital. More complete data reporting (ED) will improve reliability of this new data source but is unlikely to change basic patterns reported here. ED nonresponse (~5%) suggests TPD could rise slightly. Variability in acceptance rates suggests practice patterns of hospitals, surgeons, and DSAs could lead to higher donor supply by raising overall acceptance rates.

Alternative Models of Actual Donors vs. Total Potential Donors (TPD) In These Sampled Hospitals (>50 Beds)

	n (Hospitals)	Actual Donors 2002	Acceptance Rate=Donors/PD	Total Potential Donors	Model R^2
Christiansen (projected)	3,399	5,313	31.2%	17.0k	0.43
Eligible deaths	3,255	5,296	44.9%	11.8k-12.4k	0.72

Abstract #1475 Figure



Abstract# 1476 Poster Board #-Session: P232-III

VARIATION IN RECIPIENT POST-TRANSPLANT DONOR-REACTIVE DTH RESPONSES OVER TIME. Ronald P. Pelletier,[1] Alice A. Bickerstaff,[1] Ronald M. Ferguson, Charles G. Orosz.[1,2,3] *[1]General Surgery, Ohio State University College of Medicine, Columbus, OH; [2]Pathology, Ohio State University College of Medicine, Columbus, OH; [3]Molecular Virology, Immunology and Medical Genetics, Ohio State University College of Medicine, Columbus, OH.*

We have used a newly developed delayed-type hypersensitivity (DTH) assay to directly determine the incidence of post-transplant donor-reactive T-cell sensitization in a large cohort of kidney (K) and simultaneous kidney-pancreas (SKP) recipients (AJT 2002; 2: 926-33). While this retrospective study was valuable for defining the incidence of post-transplant donor-reactive DTH reactivity in our transplant patients, a more detailed understanding of the dynamics of post-transplant donor-reactive T-cell sensitization following engraftment requires a longitudinal, prospective study. We are currently involved in such a study, and to date it includes (87) K and (37) SKP recipients. These patients were transplanted between 11/1987 and 10/2001 with a mean follow-up of (1223 +/- 783 days). There have been 268 tests performed in 124 total patients (median of 2, range 1 to 4). Patient sera were collected either during routine outpatient visits at 3, 6, and 12 months and annually thereafter, or hospital readmissions. For those patients tested within the first post-transplant year (n=78) we observed that 40% of study patients already had acquired donor-reactivity (DTH+) at the time of first testing, while 60% were non-donor reactive (DTH-). Upon repeat testing within that first post-transplant year, 50% of the DTH+ patients remained DTH+ and 50% became DTH-, while 49% of the DTH- patients remained DTH- and 51% of them became DTH+. For those patients tested after the first post-transplant year (n=46), 44% had acquired donor-reactivity (DTH+) at the time of first testing, while 56% were non-donor reactive (DTH-). Upon repeat testing, 78% of the DTH+ patients remained DTH+ and 22% became DTH-, while 54% of the DTH- patients remained DTH- and 46% became DTH+. Thus, we observed considerable changes in donor-reactive DTH reactivity in serially tested patients, especially within the first post-transplant year. We conclude that development of donor-reactive DTH reactivity can occur early (40% of patients at 3 to 6 months post-transplant) and continues to occur even after the first post-transplant year. Also, a significant percentage of patients develop and subsequently lose donor-reactive DTH reactivity, especially early post-transplant (within the first post-transplant year).

Abstract# 1477 Poster Board #-Session: P233-III

THE TISSUE DONATION EXPERIENCE: A COMPARISON OF DONOR AND NON-DONOR FAMILIES. James R. Rodrigue,[1] Michael P. Scott,[2] Anneliese R. Oppenheim.[3] *[1]Clinical & Health Psychology, University of Florida, Gainesville, FL; [2]Southeast Tissue Alliance, Alachua, FL; [3]Oppenheim Research, Tallahassee, FL.*

AIM AND METHOD: While there has been increase in research examining the experiences of organ donor and non-donor families, comparatively scant empirical attention has been directed toward tissue donation specifically. Our objective was to assess the experiences, perceptions, and attitudes of both donor and non-donor next-of-kin to whom a tissue donation request had been directed. Participants were 507 adults who consented to tissue donation and 507 adults who refused consent to tissue donation following the death of a family member. All donation requests had been made telephonically and by trained staff from the Southeast Tissue Alliance in Alachua, Florida. The primary outcome measure was the 42-item survey, which was created to examine donation-related perceptions and attitudes. Both qualitative and quantitative analyses were used to examine the survey data. RESULTS: Donor family participants were significantly more likely to be older, white, have more years of education, have higher family incomes, and be registered organ/tissue donors compared to non-donor family participants (all p's<.05). The vast majority of donor family participants were satisfied with the request/donation experience. Primary reasons for donation included: a desire to help others in need (48%), following the actual or perceived wishes of the deceased (35%), favorable attitude toward donation in general (8%), help to give meaning to family member's death (4%), and awareness of need for more donors (3%). Primary reasons for non-donation included: actual or perceived wishes of the deceased (33%), negative beliefs or misunderstanding about donation (28%), concerns about pain/suffering (16%), and negative perceptions of the request/donation process (15%). Also, there were important differences in attitudes and perceptions between donor and non-donor family members, with non-donors reporting more negative attitudes and beliefs about tissue donation (p's<.05). Finally, both donor and non-donor family participants desired more information about the preparation, distribution, and costs associated with tissue donation. CONCLUSION: There are important differences between donor and non-donor families regarding beliefs about tissue donation and perceptions of the requesting process. These differences emphasize the need to expand upon and improve current public education programs.

Abstract# 1478 Poster Board #-Session: P234-III

ELECTROPORATION-MEDIATED HGF GENE TRANSFER FOR PRESERVING GRAFT SURVIVAL. Yoshitaka Isaka,[1] Kazuhiko Yamada,[2] Enyu Imai,[1] Ryu Utsugi,[3] Yoshitsugu Takabatake,[1] Masayuki Mizui,[1] Naotsugu Ichimaru,[4] Koji Yazawa,[4] Akihiko Okuyama,[4] Shiro Takahara.[4] *[1]Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]National Cardiovascular Center, Suita, Osaka, Japan; [3]Urology, NIigata University, Niigata, Niigata, Japan; [4]Urology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

The annual rate of kidney graft loss caused by chronic allograft nephropathy (CAN) has not changed over the last decade. Recent reports suggest that antigen-independent factors such as acute renal ischemia are involved in the development of CAN. Hepatocyte growth factor (HGF) has been reported to exert mitogenic, anti-apoptotic, and anti-fibrotic actions. Recently, we developed a new gene transfer system by electroporation *in vivo*; infusing DNA solution via renal artery followed by electric pulses using tweezers type of electrode could introduce genes into glomerular mesangial cells and interstitial cells. This gene transfer approach may provide an efficient way to investigate the therapeutic potential of HGF gene transfer for long-term survival of allografted kidney. Therefore, we examined the renoprotective potential and safety of HGF gene transfer in porcine kidney transplant as pre-clinical trial. In order to assess the effect of the HGF gene transfection, an intentional 10 minutes of warm ischemic injury was introduced following left porcine kidney removal. The HGF gene expression vector was infused into the renal artery with clamping renal vein *ex vivo*. Thereafter, electric pulses were discharged using bath-type electrodes. Following electroporation-mediated ex vivo transfection, but prior to transplantation of HGF-transfected and control kidneys, the grafts were placed in an ice-bath for 10 minutes. Kidney grafts were transplanted after removing the contralateral right kidney. We observed immunoreactive HGF expression in transplanted kidney 7 days after transplantation. PCR analysis revealed that HGF expression vectors existed in transfected kidney 6 months after transfection, but they were not observed in other organs, including liver, lung and spleen. We observed initial tubular injury followed by tubulointerstitial fibrosis in vehicle-transfected kidney transplant. In contrast, HGF gene transfection rescued tubular damages, and thereby ameliorated interstitial fibrosis at 6 months post-transplant. In addition, interstitial α-SMA, a marker of phenotypic changes, expression was weak in HGF-transfected grafts compared with that in vehicle-transfected kidney transplant. In conclusion, electroporation-mediated HGF gene transfection may be a promising therapeutic tool for preserving graft survival.

Abstract# 1479 Poster Board #-Session: P235-III

KNOWLEDGE AND ATTITUDE OF HIGH SCHOOL STUDENTS TOWARDS ORGAN DONATION AND TRANSPLANTATION. Anil K. Mongia,[1] Ravinder Brar, Shella Mongia,[1] Cena Tejani, Noosha Baqi,[1] Anup Singh.[1] *[1]Pediatric Nephrology, Children's Medical Center of Brooklyn, Brooklyn, NY.*

Background: As of December, 2002 more than 80,000 people are on the waiting list for organ transplantation. It is a well-known fact that organ donation is affected by societal attitude towards organ donation. The purpose of this study was to identify knowledge and attitude of young adults towards organ donation. **Methods**: This study was conducted amongst 251 high school students in NYC by asking them to fill a questionnaire circulated during the year 2002. The questionnaire covered three broad areas (1) attitude and knowledge about transplantation, (2) willingness to donate and receive organs and (3) demographic data. **Results**: Forty-six percentage of students said they had received some sort of information on organ donation. The majority (57%) had received information from mass media while only a few (13%) had received it from school lectures. Seventeen percent said they had discussed organ donation with their family members. Fifty eight percent of students were aware of the shortage of donor organs. Forty five percent of students were willing to make a living kidney donation; 69% were willing to give it to their parents; 64% to their siblings; and 30% were willing to give a kidney to anybody; 75% were willing to donate blood. Forty two percent of students had considered donating their organ in case of death. The majority of students were willing to accept a organ donation if needed; 77% were willing to accept from a living donor; 44% would accept from a cadaver donor; while 6 % were not willing to accept a donated organ. A significantly higher percentage of young students were more likely to offer to donate if they had received some information regarding organ donation ($p<0.002$). Hispanic adolescents were more likely to donate an organ as compared to African American ($p<0.05$.) The attitude to donation did not depend on religion, level of education of the parents, or if a friend or family member needed an organ transplantation.Eighty four percent of students felt they were insufficiently informed about organ transplantation. The majority of students had concerns that future research in genetics was potentially dangerous.They also feared that hospital staff would remove their organs before their death if they signed donor cards. **Conclusion**: The study highlights the lack of information about organ donation and transplantation in young high school students. Educational campaigns focused on young students should be implemented with the help of school officials to raise awareness and increase organ donation.

Abstract# 1480 Poster Board #-Session: P236-III

HAND TRANSPLANTATION: UNIQUE DONOR AND RECIPIENT CRITERIA. Ruben N. Gonzalez,[1] Vijay S. Gorantla,[1] Diane J. Pidwell,[2] Linda Cendales,[1] Darla K. Granger,[3] Gordon R. Tobin,[4] Warren C. Breidenbach.[1] *[1]Hand and Microsurgery, Christine M. Kleinert Institute, Louisville, KY; [2]Pathology, Jewish Hospital, Louisville, KY; [3]Surgery, Wayne State University, Detroit, MI; [4]Surgery, University of Louisville, Louisville, KY.*

To date, 18 hands have been transplanted in 14 recipients with a 94% allograft survival rate. This early success means that more programs will enter into hand transplantation. It is paramount that transplant surgeons understand the differences in donor and recipient identification and management between hand and solid organ transplantation. The purpose of this abstract is to define these differences using criteria developed in our hand transplant program. Significant differences in donor criteria fall into 4 areas. 1) Donor selection; 2) Medical history; 3) Organ recovery; and 4) Consent. 1) Recipients normally request that skin color and sex are matched. Bone size must be matched for successful osteosynthesis. 2) Unlike solid organ transplants, we believe that any history of malignancy in the donor, even remote, is an absolute contraindication for hand transplants. The risk of transmitting a malignancy, no matter how minute, is unthinkable in this non-life-saving transplant. Donors with congenital or connective tissue disorders, peripheral neuropathy and traumatic deformities may also require exclusion. 3) The timing of donor hand procurement needs to be integrated in the sequence of solid organ procurement. Our protocol was to dissect last and harvest first. 4) As part of the consenting process, we offered families an arm prosthesis for open casket funerals. Recipient criteria differ in 4 areas. 1) Prosthesis; 2) Age; 3) Health state/co-morbid diseases; and 4) Amputation level. 1) Prospective recipients need to at least have attempted prosthetic use prior to transplant. This criterion relates to successful exit strategy in event of post-transplant complications. 2) Recipients <18 years of age are excluded due to issues of informed consent and the potentially increased risk of lymphoproliferative disorder. Recipients more than 65 years of age are excluded due to vascular concerns. 3) Severe co-morbid states, like major organ failure, are exclusion criteria in hand transplant. We consider blindness to be an absolute contraindication, since sensory return in a transplanted hand is delayed and/or absent and sight provides the only means of protecting the hand. 4) Finally, the level of amputation must be appropriate to allow motor function from the recipient musculature. In conclusion, transplant programs and OPOs should consider these criteria during donor and recipient selection and management when hand transplants are involved.

Abstract# 1481 Poster Board #-Session: P237-III

INCREASING ORGAN DONATION BY ENHANCING END-OF-LIFE CARE: A PILOT STUDY. Richard S. Luskin,[1] Kevin J. O'Connor,[1] Ellen H. Sheehy,[1] Deborah E. Sellers,[2] Judith A. Spross,[2] Erica Jablonski,[2] Mildred Z. Solomon.[2] *[1]New England Organ Bank, Newton, MA; [2]Center for Applied Ethics and Professional Practice, Education Development Center, Newton, MA.*

Objective: Increase organ donation by applying best practices in end-of-life care and quality improvement to hospital support of families of patients with grave neurologic prognoses. Methods: The intervention was implemented in 3 pilot hospitals with significant potential and actual donors, varied patient demographics and geographic locations (rural, urban). There were 17 matched comparison hospitals. Pilot hospitals 1) established Organ Donation Advisory Committees to customize and monitor the intervention; 2) developed clinical pathways initiated by poor neurologic prognosis as measured by Glasgow Coma Scale or comparable findings on routine neurologic exam; 3) integrated organ donation into hospital quality monitoring, and 4) recruited, trained and deployed Family Support Teams (FSTs) to provide emotional and practical support to families of severely neurologically compromised patients. The FST was trained NOT to raise the issue of organ donation with families. The intervention's impact was evaluated by comparing change in referral, consent, and donation rates, based on data limited to medically suitable potential donors, from baseline (1998-2000) to intervention (2001-2002) at pilot and comparison hospitals. Logistic regression controlled for age, sex, race, cause of death and concurrent efforts to increase organ donation. Qualitative data were gathered from interviews with clinicians and families affected by the intervention. Results: Referral rate in the pilot hospitals increased from 81% at baseline to 99% ($p<.05$) during intervention period compared with 87% to 94% in comparison hospitals ($p<.05$). The difference between pilot and comparison hospitals in change in referral rate was nearly significant ($p=.074$). Donation rates in pilot hospitals increased from 48% to 67% from baseline to intervention ($p<.05$). Consent rates were flat (66% to 68%, n.s.) Changes in consent and donation rates at the pilot vs. comparison hospitals were not statistically significant. Clinician and family interviews revealed that FSTs provided important psychosocial and logistical support to families in crisis and that the intervention was feasible and sustainable in intensive care units. Conclusions: After implementing a pathway with a clinical cue virtually every potential organ donor was referred to the local organ procurement organization. Assuring routine, timely referral of all potential donors coupled with increased family support should lead to increased donation.

TOLERANCE II

Abstract# 1482 Poster Board #-Session: P238-III

EARLY INHIBITION OF INNATE IMMUNITY INFLUENCES THE IMMUNE DECISION TOWARD ALLOGRAFT TOLERANCE IN NONHUMAN PRIMATES (NHP). Judith M. Thomas,[1] Anne Hutchings,[1] Jianguo Wu,[1] Clement Asiedu,[1] Devin Eckhoff,[1] Juan Contreras, William Hubbard,[1] Francis T. Thomas,[1] David M. Neville, Jr.[2] *[1]Surgery, Univ. of Alabama at Birmingham, Birmingham, AL; [2]Molecular Biology, NIHM, Bethesda, MD.*

Brief peritransplant treatment of NHP with FN18 immunotoxin (IT) plus 15-deoxyspergualin (DSG) has yielded an exceptional number (14/26; 54%) of tolerant kidney allograft recipients, currently surviving between 2 to 6 years (mean 3.8) without rejection, and without additional immunosuppression. As early as one week post-transplant, recipients that subsequently became tolerant could be distinguished from those that eventually rejected, on the basis of a remarkably polarized increase in the levels of plasma IL-10, and a reduction in IFN-γ. Further analysis suggested that the immune decision towards cytokine polarization and associated tolerance occured within a few hours of transplantation. Administering DSG within 5 hours of the transplant surgery resulted in a significantly higher incidence of rejection-free tolerance (76%) compared to administration >5 hours before or after transplant surgery (11% tolerant, $p<0.02$). A major mechanism of DSG action is inhibition of nuclear translocation of activated NF-κB, probably mediated through heat shock proteins (Hsp). We confirmed a significant reduction in cytoplasmic expression of Hsp70 and RelB, with almost complete inhibition of nuclear translocation of both proteins, in LN biopsies during DSG treatment of the tolerant NHP recipients. This early effect of DSG is consistent with the hypothesis that a crucial limitation to induction of stable tolerance is the activation of NF-κB and Hsp dependent innate responses to cells damaged by ischemia-reperfusion. This view was supported by studies in a murine mode of kidney ischemia-reperfusion injury, wherein a single dose of DSG given <5 hrs before injury protected kidney function, and reduced systemic levels of early innate response inflammatory cytokines to levels indistinguishable from those observed in sham-operated control mice. The narrow timing window for initiating DSG treatment suggests the innate immune system is poised to defeat allograft tolerance induction. Consequently, effective blockade of NFκ-B driven innate immunity must be in place immediately after allograft introduction to enable development of a tolerogenic environment and activation of IL-10 associated regulatory mechanisms in T cell depleted NHP. We postulate that the major role for DSG in promoting tolerance is the inhibition of immediate innate immune responses to the injured graft.

Abstract# 1483 Poster Board #-Session: P239-III

TOLERANCE INDUCTION AGAINST GAL-ALPHA(1-3)GAL AG IN CHIMERISM-BASED DRUG-INDUCED TOLERANCE. Yukihiro Tomita,[1] Ichiro Shimizu,[1] Toshiro Iwai,[1] Takashi Kajiwara,[1] Hisataka Yasui.[1] *[1]Department of Cardiovascular Surgery, Faculty of Medicine, Kyushu University, Fukuoka, Japan.*

We have previously reported a method of tolerance induction that comprises an i.v. injection of $1x10^8$ allogeneic spleen cells (SC) on day 0 and an i.p. administration of 200mg/kg of cyclophosphamide (CP) and 30mg/kg Busulfan (BU) followed by $1x10^7$ BMC from donor. By using this method, we were able to induce a long-lasting skin graft (SG) tolerance in H-2 mismatched combinations. By using alpha-Gal knockout mice which are preimmunized with alpha-Gal Ag and have anti-alpha Gal nAb, we evaluated the effectiveness of this tolerance system to induce B cell tolerance against Gal-alpha(1-3)Gal Ag. <Method> alpha-Gal knockout (alpha-Gal KO; H-$2^{b/d}$) and AKR (H-2^k) mice were used as recipients and donors. Group 1: untreated alpha Gal KO mice. Group 2: alpha-Gal KO mice were injected with $1x10^8$ AKR SC on day -2. Group 3: alpha-Gal KO mice were injected with $1x10^8$ AKR SC on day -2 and CP on day 0. Group 4: alpha-Gal KO mice were injected with $1x10^8$ AKR SC on day –2, CP and BU on day 0 and BMC on day 2. The level of anti-alpha-Gal IgM, IgG1, IgG2a, IgG2b, IgG3 was measured by FACS. AKR heart (HG) or skin (SG) grafting was performed 2 weeks after the treatments. Histological examination was performed in the transplanted HG. <Result> The production of anti-alpha-Gal IgM and IgG2a was increased two weeks after injection of AKR SC alone. However, the production of anti-alpha-Gal Ab was completely inhibited in Group 3 and 4. AKR heart grafts were rejected within 7 days after transplantation in untreated or AKR SC injected alpha-Gal KO mice. Histological analysis showed the hemorrhage within the cardiac muscle and thromboembolism in the coronary artery, i.e., the evidence of humoral rejection. In Group 3, on the other hand, survival of AKR HG were significantly prolonged and 70% of them survived over 100 days. Histological analysis showed no evidence of humoral rejection. However, SG were rejected within 14 days. In Group 4, all skin and heart grafts were permanently accepted without any evidence of both humoral and cellular rejections. <Conclusion> Our studies confirmed the effectiveness of our chimerism-based drug-induced tolerance system in the induction of tolerance in alpha Gal-knockout mice. The treatment with SC and CP can induce B cell tolerance against alpha-Gal Ag. For the induction of both B and T cell tolerance, however, four conditionings (SC/CP/BU/BMC) were required.

Abstract# 1484 Poster Board #-Session: P240-III

EFFECT OF NON-INHERITED MATERNAL ANTIGENS (NIMA) ON HEART ALLOGRAFT ACCEPTANCE IN MICE II: STRAIN DEPENDENCE OF T CELL SUPPRESSION INDUCED BY NIMA. J. Andrassy,[1] M. L. Molitor,[1] B. R. Marthaler,[1] L. D. Haynes,[1] H. W. Sollinger,[1] W. J. Burlingham.[1] *[1]Surgery, Transplant Division, University of Wisconsin, Madison, WI.*

Introduction: Exposure to non-inherited alloantigens of mother (NIMA) was shown to have a strong beneficial effect in transplantation. Recently we have replicated the NIMA-effect in a mouse heart transplant model. Here we tested the hypothesis that strain specific differences can influence the ability of mother to impact baby's alloimmunity. We compared two NIMA mouse breeding models, evaluating the heart graft survival and analysing the *in vitro* T-cell immune response. **Methods:** Mouse breedings consisting of a B6 father and either B6D2F1 (H-2^{bxd}) or B6C3F1 (H-2^{bxk}) mother were initiated. The resulting offspring homozygous for the "b" MHC antigens had been exposed to either NIMA of the "d" or the "k" haplotype *in utero* and via breast milk. Haplotypes of all offspring were tested using the PCR-technique as described by *Saha et. al.* Fully allogeneic heterotopic heart transplants from DBA/2 or C3H were performed in accordance to the NIMA exposure. Controls were either (bxb) F1 backcross from heterozygous males (either H-2^{bxd} or H-2^{bxk}) and homozygous B6 females or B6 inbred mice. The response of Th1-cytokine producing T-cells to allogeneic and semi-allogeneic donor splenocytes was tested using the ELISPOT-technique. **Results:** Exposure to NIMA^d resulted in long-term survival of DBA/2 heart allografts in 53% of the cases without any additional treatment, whereas all controls rejected their grafts by d11. Exposure to NIMA^k did not result in a prolongation of C3H hearts compared to the controls. ELISPOT experiments showed a dramatic decrease of IFN-g producing T cells of NIMA^d animals in response to allogeneic and semi-allogeneic stimulators compared to the non-exposed controls. For the NIMA^k mice, however, IFN-g producing T cells were suppressed to a much lesser extent (*Table 1*) thus correlating with the *in vivo* findings. **Conclusion:** There is a NIMA effect in the H-2^d but not in the H-2^k model. We observed a correlation between this effect *in vivo* and a downregulation of cytokine producing T-cells *in vitro*. At this point we cannot exclude the possibility that mother transmits the NIMA effect to the offspring by other background genes.

ELISPOT of IFN-g producing T cells

NIMA=d	358 ± 103	72 ± 21 (- 80%)	313 ± 3	113 ± 0 (- 64%)
NIMA=k	187 ± 40	130 ± 18 (-30%)	187 ± 90	123 ± 25 (- 34%)
Responder	**Non-exposed (bxb)**	**Exposed to NIMA**	**Non-exposed (bxb)**	**Exposed to NIMA**
Stimulator	DBA/2 **or** C3H **(allo)**		B6D2F1 **or** B6C3F1**(semi-allo)**	

Abstract# 1485 Poster Board #-Session: P241-III

NON-REGULATORY AND NKT-INDEPENDENT ALLOGRAFT TOLERANCE IS INDUCED FOLLOWING LIVER TRANSPLANTATION. Dengping Yin,[1] Ludmilla Deriy,[1] JiKun Shen,[1] Lianli Ma,[1] Chyung-Ru Wang,[2] Anita S. Chong.[1] *[1]Department of Surgery, The University of Chicago, Chicago, IL; [2]Department of Pathology, The University of Chicago, Chicago, IL.*

Non-arterialized orthotopic livers are spontaneously accepted in mice across MHC barriers without the need for immunosuppression or tolerance-inducing therapies. The mechanisms underlying liver transplantation tolerance remain unclear, although apoptotic alloreactive T cell death, immune deviation and active T cell regulation induced by donor liver-derived DC progenitors have been proposed. We here report on our investigations into the role of regulatory T and NK-T cells in liver-induced tolerance. We first confirmed that C3H mice spontaneously accepted allogeneic C57Bl/6 livers; 30-60 days after transplantation tolerance was formally demonstrated by the acceptance of donor-specific C57BL/6 hearts. Ex vivo analysis of splenocytes harvested from tolerant mice revealed primed alloreactive cells capable of producing large amounts of interferon-gamma and IL-2. Twenty-hour ELISPOT assays confirmed that primed IFN-gamma- and IL-2-producing cells were already present in the tolerant mice. Cell transfer of spleen cells from tolerance mice into Rag 2-deficient mice revealed that the transferred cells were capable of rejecting C57BL/6 hearts. These characteristics of liver-induced tolerance are in striking contrast to allograft tolerance induced by anti-CD40L blockade and intact bone co-transplantation in which suppressed IFN-gamma production was observed and tolerance was transferable into Rag 2-deficient mice. We next tested the hypothesis that the presence of allogeneic liver was actively and continuously maintaining tolerance. NKT cells have recently been implicated in the induction self and allogeneic tolerance and are highly enriched in the mouse liver, comprising up to 22% of total liver T cells. We tested whether donor liver-derived NKT cells were responsible for tolerance by transplanting livers from CD1d1-deficient mice. Livers from these mice, which lack the major subset of NK T cells that promptly secrete IL-4 following activation, were spontaneously accepted by C3H mice in a manner indistinguishable from wild-type C57BL/6 livers. Thus the mechanism of liver tolerance is not complete peripheral deletion of alloreactive cells, is not mediated by classic active regulatory cells and is independent of the effects of donor liver-derived NKT cells. These observations underscore the complexity of peripheral tolerance mechanisms that normally exist and which can be uncovered by different organ- or tissue-transplantation models.

Abstract# 1486 Poster Board #-Session: P242-III

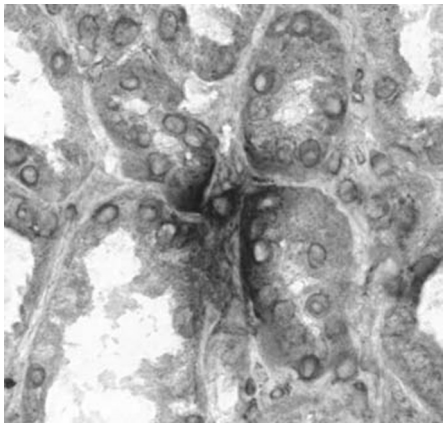
ACTIVE REGULATION OF ALLOREACTIVITY IN MICE TOLERIZED WITH ANTI-CD40L AND INTACT ACTIVE BONE CO-TRANSPLANTATION. Dengping Yin,[1] JiKun Shen,[1] Shimon Sakaguchi,[2] Anncy Varghese,[1] Lianli Ma,[1] Anita S. Chong.[1] *[1]Department of Surgery, The University of Chicago, Chicago, IL; [2]Department of Experimental Pathology, Kyoto University, Kyoto, Japan.*

$CD25^+$ T cells can function as professional regulatory cells in selected models of autoimmune disease and allograft tolerance. Here we test whether tolerance induced by anti-CD40L and intact active bone (IAB) is mediated by immuneregulatory T cells. BALB/c heart allografts, co-transplanted with IAB into C57BL/6 mice treated with anti-CD40L mAbs, were accepted for >150 days, while second BALB/c hearts transplanted on day 60-100 were accepted for >60 days. Transfer of naive C57BL/6 mice spleen cells (40 million/recipient) into tolerant C57BL/6 mice at the time of second BALB/c heart transplantation did not significantly alter the tolerant state. Transfer of 10 million naive C57BL/6 spleen cells into C57BL/6-$Rag2^{-/-}$ mice resulted in the rejection of BALB/c hearts within 13 days. When 10 million spleen cells from tolerant mice were transferred into $Rag2^{-/-}$mice, BALB/c hearts were accepted but third party hearts (from C3H mice) were rejected within 13 days. Co-transfer of tolerant with naive spleen cells revealed dominant tolerance at a 2:1 cell ratio. Tolerance in $Rag2^{-/-}$mice was maintained and enhanced by homeostatic proliferation. Thus naive cells (10 million) transferred on the day of tolerant cell transfer abrogated tolerance, but when naive cells were transferred on day 30, tolerance was maintained. Tolerance in $Rag2^{-/-}$mice was dependent on the transfer of CD4+CD25+ T cells, although co-transfer of CD8+ cells resulted in a more robust tolerance. The mechanism of tolerance in $Rag2^{-/-}$mice was probed with a series of reagents implicated in other models of T cell regulation. We observed that anti-IL10R and anti-glucocorticoid-induced tumor necrosis factor receptor family member (GITR) mAbs abrogated tolerance in $Rag2^{-/-}$mice, anti-TGFβ mAbs were partially effective, while anti-CTLA4 mAbs had no effect on tolerance. These results suggest that allograft tolerance induced by anti-CD40L and IAB is mediated by T cells sharing many features with classical regulatory T cells. Nonetheless caution must be exercised when data are based solely on experiments involving cell transfer into immune-deficient $Rag2^{-/-}$mice which permits homeostatic proliferation. Loss of tolerance may be due to inhibition of homeostatic proliferation of the regulatory cells rather than inhibition of regulation per se. Thus, experiments that inhibit T cell regulatory functions in the tolerant, but otherwise immune-competent mice, are currently underway.

Abstract# 1487 Poster Board #-Session: P243-III

TGFBETA-DEPENDENT IMMUNE REGULATION DURING RENAL ALLOGRAFT ACCEPTANCE II. HUMAN INTRA-GRAFT LATENT TGFBETA + CD4 T REGULATORY CELLS. William Burlingham,[1] Junglim Lee,[1] Satoshi Kusaka,[1] Ewa Jankowska-Gan,[1] Rachael Rigden,[1] Hans Sollinger,[1] Stuart Knechtle,[1] Anne VanBuskirk,[2] Jose Torrealba.[1] *[1]Departments of Surgery & Pathology, University of Wisconsin, Madison, WI; [2]Department of Surgery, Ohio State University, Columbus, OH.*

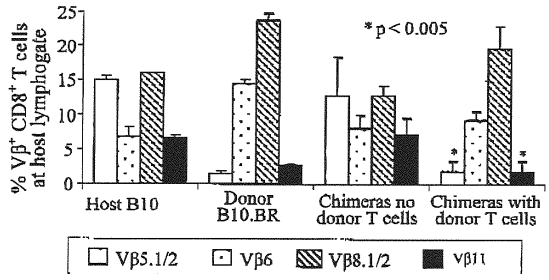
In part I of this study , we described a latent TGFbeta+ CD4 T cell present in the interstitium of monkey renal allografts during stable allograft acceptance. The disappearance of these unusual T cells coincided with onset of acute rejection and loss of peripheral TGFbeta - dependent inhibition of DTH response to donor soluble antigens . We wished to determine if the phenomenon of latent TGFbeta+ CD4 T cells in the graft interstitium is also a feature of human allograft acceptance. STUDY DESIGN: Peripheral blood samples from DS at 5-8 years post-transplant were tested for regulation by DTH in footpads of SCID mice. Frozen and paraffin-embedded tissue sections were subjected to immunohistochemistry using 2 different mouse anti-human TGFbeta mAbs to localize the TGFbeta-protein. RESULTS: We found using DTH assay that the CD4+CD25 dim subset of PBMC of kidney allograft acceptor DS contained T cells which could suppress the response to allopeptide derived from donor HLA class I; this suppression required both TGFbeta and CTLA4. Biopsy samples taken at yr 3.2 & 4.5 had previously been shown to contain abundant TGFbeta (but no IL-10) mRNA by RT/PCR. Anti-active TGFbeta mAb 1D11 detected normal positive staining in the renal tubules, but interstitial CD3+ T cells were uniformly negative. In contrast, anti- latent TGFbeta 1 mAb TB21 detected strongly positive interstitial T cells in frozen section that appeared to secrete TGFbeta-protein and coat the surrounding ECM and the basolateral aspect of adjacent tubules (see Fig 1— Interstitial latent TGFbeta+ T cell in between 4 renal tubules;year 4.5 biopsy; mAb= TB21;400x). CONCLUSION: Latent TGFbeta may be a marker for intragraft CD4 T-regulatory cells essential for allograft acceptance both in non-human primates and in humans.



Abstract# 1488 Poster Board #-Session: P244-III

CLONAL DELETION OF DONOR-SPECIFIC ALLOREACTIVE T CELLS IS CRITICAL FOR MAINTENANCE OF STABLE MIXED CHIMERISM AND INDUCTION OF DONOR-SPECIFIC TOLERANCE. Hong Xu, Paula M. Chilton, Yiming Huang, Loretta Doan, H. Leighton Grimes, Suzanne T. Ildstad. *[1]Institute for Cellular Therapeutics, University of Louisville, Louisville, KY.*

Pretreatment of the recipient with anti-αβ-TCR and anti-CD8 mAb reduced the total body irradiation from 700 to 300 cGy for establishing allogeneic chimerism in a mouse model (B10.BR to B10). However, a dissociation between chimerism and tolerance was observed when the threshold for nonmyeloablative conditioning was approached. Some chimeras rejected donor skin grafts with a time course similar to naïve controls even when they had significant levels (up to 70%) of donor chimerism. Donor-specific tolerance as well as durable chimerism occurred only in chimeras with donor peripheral T cells (DPTC), suggesting a critical role for donor T cells in the induction of durable chimerism and tolerance. Chimerism for donor B cells, NK cells, macrophages, granulocytes, and dendritic cells was present for both groups. To determine the mechanism for this dissociation, chimeras with or without DPTC were tested for their ability to mediate clonal deletion of TCR Vβ segments reactive to donor MHC. Significantly, deletion of recipient B10 Vβ 5.1/2$^+$ and Vβ 11$^+$ CD8 T cells occurred in chimeras producing donor T cells but not in chimeras without DPTC (Figure, $p < 0.005$). These results indicate that deletion of donor-specific alloreactive T cells is the most likely mechanism for tolerance induction. To evaluate T cell development, thymocytes were analyzed by flow cytometry. CD24 is a maker of T lineage commitment in CD4$^-$/8$^-$ thymocytes. Donor CD24$^+$ T cells were not detected in early stage pre-T cells (CD4$^-$/8$^-$) in chimeras devoid of DPTC. In contrast, donor CD24$^+$/4$^-$/8$^-$ cells were present in chimeras with DPTC. The pre-T cells from chimeras with DPTC represented all stages of T cell maturation (CD4$^-$/8$^-$ → CD4$^+$/8$^-$ or CD4$^-$/8$^+$). Taken together, these data demonstrate a strong correlation between early donor T cell lineage commitment and production in chimeras and the induction of tolerance.



Abstract# 1489 Poster Board #-Session: P245-III

THE BLOOD OF LONG TERM GRAFT SURVIVING GRAFT RAT RECIPIENTS OF CARDIAC ALLOGRAFT IS A RESERVOIR FOR REGULATORY T CELL POPULATIONS WITH SELECTED CDR3-LD AND HIGH Vβ/HPRT TRANSCRIPT RATIOS. David Lair,[1] Marina Guillet,[1] Sophie Brouard,[1] Jean-Paul Soulillou.[1] *[1]ITERT-INSERM U437, CHU Hotel Dieu,30 Bd Jean Monnet, Nantes, France.*

Pre-graft donor specific transfusion (DST) induces tolerance of allogeneic MHC incompatible heart graft in adult rat recipients. It has been shown that this state of tolerance could result from an active process involving some degree of clonal deletion and/or anergy and/or active regulatory cells. **Aims:** In this study, we investigated T cell selection and activation in long term graft survival (day 100) as well as the homing of cells able to transfer tolerance, in a model of DST-induced tolerance. **Materials and methods:** Quantitative TCR repertoire analysis was performed at the CDR3-LD level to identify T cell mobilisation in the graft, spleen and blood of long term surviving graft recipients of MHC incompatible heart graft. Cell phenotype was studied in the different immune compartments by flow cytometry. The capacity to induce graft survival prolongation in naive secondary hosts was analysed after cell transfer (from spleen and blood) from long term survivors, 100 days after transplantation. **Results:** Spleen cells (splenocytes, purified T cells, CD4$^+$ T cells) but also peripheral blood lymphocytes of long term graft recipients, were able to transfer long term or indefinite graft survival to naive hosts. Spleen cells (CD4^{+ve} or CD8^{+ve}) were always characterized by an unselected pattern (gaussian CDR3-LD) and a moderately altered pattern was found in the graft. However, blood T cells harbored a very altered CDR3-LD and a high Vb/HPRT transcript ratio. Furthermore, we observed an increase in CD4$^+$CD25$^+$ T cells, shown as potentially regulatory cells, in the blood and spleens of tolerant recipients. Taken together, these results corroborate recent data obtained in peripheral blood of human recipients tolerating a kidney graft. **Conclusion:** This is the first evidence that the blood of tolerant rats may concentrate highly selected T cell populations able, in a same manner as spleen T cells with unselected gaussian CDR3-LD, to transfer indefinite graft prolongation.

Abstract# 1490 Poster Board #-Session: P246-III

ALLOSPECIFIC MEMORY T CELLS UNDERGO APOPTOSIS IN AN IMMUNE PRIVILEGED SITE. Zhenhua Dai,[1] David M. Rothstein,[1] Fadi G. Lakkis.[1] *[1]Sections of Nephrology and Immunobiology, Yale University School of Medicine, New Haven, CT.*

Introduction: Memory T cells pose a challenge to tolerance induction because of their migratory and functional advantages over naive T cells. Like effector T cells, memory T cells home to non-lymphoid tissues where they are readily activated. However, it is not known whether memory T cells enter immune privileged sites or are subject to regulation or apoptosis at these sites. Methods: To address this question, we utilized two in vivo models of memory T cell recall. In the first model, BALB/c (H2d) pancreatic islet allografts were transplanted either under the kidney capsule (non-privileged site) or in the testes (immune-privileged site) of splenectomized alymphoplastic (aly/aly-spleen, H2b) mice. These mice fail to mount a primary alloimmune response but reject allografts promptly if they are adoptively transferred with memory T cells. In the second model, BALB/c islets were transplanted under the kidney capsule or in the testes of wildtype (B6, H2b) mice treated with costimulatory blockade (CTLA4Ig + MR1) to inhibit primary immune responses. These recipients accept allografts > 90 days but reject them promptly if they are adoptively transferred with memory T cells. In both models, 2.5 million CD8+CD44hi B6 memory T cells sensitized to BALB/c alloantigens were transferred 2 days after islet transplantation. Results: Transfer of CD8 memory T cells to mice transplanted with islets under the kidney capsule in either mouse model resulted in rapid allograft rejection (MST = 16 and 15 days, n = 5/model). In contrast, rejection mediated by the same CD8 memory T cells was significantly delayed if the islets were transplanted in the testes (MST = 37 and 44 days, n = 5/model). The same results were obtained if pure TCR-tg (2C) CD8+CD44hi memory T cells specific to the Ld BALB/c alloantigen were adoptively transferred. Analysis of 2C lymphocytes that infiltrated the kidney and testicular grafts one week after adoptive transfer demonstrated that delayed allograft rejection in the testes was not due to reduced proliferation of memory T cells. In contrast, 2C lymphocyte apoptosis was significantly increased in the testicular site (22%) vs the kidney site (7%). Memory T cell death in the testis was mediated by combined actions of Fas and CD30 death pathways but not by either pathway alone. Conclusion: Allospecific CD8 memory T cells undergo increased apoptosis upon recall by antigen in an immune privileged site. This finding suggests that the T cell memory hurdle to tolerance can be alleviated by transplanting cellular grafts into immune privileged sites.

Abstract# 1491 Poster Board #-Session: P247-III

FTY720 PREVENTS ANTI-CD4 INDUCED TOLERANCE IN A RAT KIDNEY TRANSPLANTATION MODEL. Kirsten Risch,[1] Grit Schroeder,[1] Katja Kotsch,[2] Anja Siepert,[1] Josef Brock,[1] Volker Brinkmann,[3] Thomas Ritter,[2] Manfred Lehmann,[1] Hans-Dieter Volk.[2] *[1]Inst. of Med. Biochem. & Mol. Biology, Univ. Rostock, Rostock, Germany; [2]Inst. of Med. Immunol., Humboldt Univ. Berlin, Berlin, Germany; [3]Transplant. Research, Novartis Pharma AG, Basel, Switzerland.*

FTY720 is a novel immunomodulator which is highly effective in models of transplantation and autoimmunity. Here, we investigated its efficacy in combination with a tolerance-inducing nondepleting anti-CD4 mab in a rat kidney transplantation model. Rats were treated with 0.3 mg/kg FTY720 from day -2 to 14 (or until rejection) and with 10 mg/kg (tolerance-inducing protocol) of the anti-CD4 mab RIB5/2 from day -1 to 3. The control groups received either RIB5/2 or FTY720 alone. Orthotopic kidney transplantation (DA to LEW) was performed in bilaterally nephrectomized recipients. Serum creatinine and blood lymphocyte counts were monitored, histology and TaqMan-PCR from grafts were performed at day 21. Moreover, the migration behavior of primed alloreactive (LEW to DA) EGFP+ T cells was analyzed under the cover of FTY720 therapy. These EGFP+ T cell lines were generated by a one way MLR in vitro using a MuMoLV based retroviral gene expression system. RIB5/2 given alone resulted in permanent graft survival (> 100 days) while FTY720 prolonged graft survival for one day (mean survival time (MST) 7.0 days) compared to untreated controls (MST 6.2 days) in this high-responder model. The group receiving combination therapy survived for 45.2 days only. Serum creatinine values were significantly increased from day 21 within this group. After FTY720 withdrawal histological evaluation of the graft (day 21) revealed significantly higher numbers of CD8+ cells, IL-2 receptor+ cells, macrophages and NK cells in comparison to RIB5/2 monotherapy. Moreover, we detected significantly elevated IL-2, IL-4, IFN-gamma but decreased bcl-2 expression compared to RIB5/2 monotherapy. To examine the mechanisms of FTY720 interaction with tolerance induction, we studied the homing of T cells to the graft. FTY720 therapy alone or in combination resulted in a profound decrease of circulating naive lymphocytes. On the other hand, the migration behavior of primed EGFP-labeled alloreactive T cells to the allograft was not altered. Very recent data of our group showed that the graft itself plays a key role in inducing regulatory T cells derived from recent thymus emigrants. In summary, FTY720 is powerful in preventing intragraft infiltration but this also affects the development of regulatory T cells which stabilize tolerance when the tolerance inducing drug (anti-CD4 mab) is tapered off.

Abstract# 1492 Poster Board #-Session: P248-III

TRANSFER OF TOLERISING CELLS IN TOLERANT SKIN GRAFTS (INDUCED BY DONOR SPECIFIC TRANSFUSIONS) ACROSS A RESTRICTED H-2 CLASS I DISPARITY PREVENTS SPECIFIC SKIN ALLOGRAFT REJECTION. Geoff Y. Zhang,[1] Min Hu,[1] Watson Debbie,[1] Alexander I. Stephen.[1] *[1]Centre for Kidney Research, Children's Hospital at Westmead, Westmead, NSW, Australia.*

Aim: To establish a murine model of donor specific transfusions (DST) induced skin allograft tolerance across a major Class I disparity. To investigate the mechanism of dominant tolerance by transferring tolerising cells from a tolerant skin graft into immunodeficient hosts. Methods: The recipient mouse strain used was C57BL/6 (B6)(H-2^b) . An H-2 class I mutant strain bm1(H-2^{bm1}) and F1(B6 x bm1) were used as donor mice. Bm1 has point mutation of 3 amino acids in the H-2 class I K molecule compared with the parent strain B6. Skin grafting between B6 and bm1 as well F1 to B6 cause acute rejection. To induce tolerance by DST, $3x10^7$ donor spleen cells were transfused via the tail vein 7 days before skin grafting. Results: Our data shows that a single DST to B6 mice leads to permanent acceptance of F1 skin grafts (6/6, MST >100days, controls MST 14 days). In the case of B6 grafted with bm1, DST can only prolong skin graft survival with a mean survival time of 38 days. To demonstrate the presence of CD4+/25+ regulatory cells, tolerant F1 grafts were regrafted onto a group of 4 RAG1 mice and the control autograft (B6) from same mice were grafted onto a second group of 4 RAG1 mice. Flow cytometry on Day 30 after regrafting showed CD4+/CD25+ T cells from grafts in both groups had colonised and expanded in RAG1 mice. CD25 expression was high on the transferred cells (55%). The RAG1 mice were then reconstituted with 10^7 naïve B6 spleen cells and challenged with a fresh F1 skingraft and a Balb/c skingraft as a third party graft. Control RAG1 mice reconstituted with 10^7 naïve B6 spleen cells and grafted, rejected F1 grafts by Day 14. At 30 days post transplant all third party grafts were rejected (MST=12 days), 1 of 4 F1 grafts on mice, which had been grafted with tolerated F1 skin had been rejected compared with 3 out of 4 F1 grafts that rejected on the mice with an autograft at Day14, 15 and 17. Conclusion: In this study, we have shown that DST can induce specific skin allograft tolerance across a restricted H-2 Class I disparity. Cells from a tolerant graft can colonise and expand in immune deficient RAG1 mice and prevent adoptively transferred naïve B6 spleen cells from rejecting F1 allografts. These results suggest that regulatory T cells exist within the tolerant graft and maintain dominant tolerance in this model. This implies that DSTs induce regulatory cells in addition to deleting alloreactive cells.

Abstract# 1493 Poster Board #-Session: P249-III

EFFECTS OF TISSUE RESTRICTED MINOR ANTIGENS IN KIDNEY AND SKIN ON IMMUNE TOLERANCE IN A LARGE ANIMAL MODEL. Christian S. Kuhr,[1,2,4] Murad Yunusov,[4] Marie-Terese Little,[4] Eustacia Zellmer,[4] Christopher L. Marsh,[1,2] Beverly Torok-Storb,[4] Rainer Storb.[3,4] *[1]Surgery, University of Washington, Seattle, WA; [2]Urology, University of Washington, Seattle, WA; [3]Medicine, University of Washington, Seattle, WA; [4]Clinical Transplantation, Fred Hutchinson Cancer Research Center, Seatlle, WA.*

Background: Mixed hematopoietic chimerism, induced through nonmyeloablative hematopoietic stem cell transplantation (HSCT), has proven safe and effective therapy for many hematological diseases. This strategy can be used to confer immune tolerance and offers potential for application to solid organ transplantation. Tissue specific minor antigens, not expressed by the engrafted hematopoietic cells, may evade this tolerance and incite an alloimmune response. We asked whether such tissue specific antigens, if present in either kidney or skin, would result in graft rejection in our pre-clinical mixed chimeric canine model. **Methods:** Mixed donor/host hematopoietic chimerism was established between five DLA identical littermates. This was accomplished through hematopoietic stem cell transplantation using nonmyeloablative conditioning with total body irradiation and a brief course of immunosuppression (35 days). Without additional immunosuppression, and after bilateral native nephrectomies, each chimeric animal received a kidney graft from their respective HSCT donor. Twelve months after kidney grafting, the chimeric animals received autologous, HSCT donor, and 3rd party full-thickness skin grafts. **Results:** All HSCT recipients developed and maintained mixed chimerism measured in peripheral blood (range 10-99%) with a median follow-up of 35 months (range 33 – 44). Following kidney and subsequent skin grafting no significant changes occurred in levels of chimerism. All HSCT donor dogs lost their littermates' kidneys; 4/5 through biopsy documented rejection and one from a technical complication. In contrast, renal function in the chimeric HSCT recipients remains stable and normal (serum creatinine <1.4 mg/dl) with a median follow-up of 26 months. No histological evidence of acute or chronic renal rejection is present on biopsies performed at 1, 6, 12, and 18 months post-transplantation. With a mean follow-up of 27 weeks autologous skin grafts were accepted in all of the chimeric animals while donor skin grafts show evidence of rejection in two of the four recipients. Third-party grafts were uniformly rejected within 21 days. **Conclusions:** In the outbred, DLA-identical, canine model of mixed hematopoietic chimerism, skin grafts, but not vascularized kidney grafts, contain tissue-restricted antigens with the immunodominance necessary to provoke a histologically demonstrable alloimmune response.

Abstract# 1494 Poster Board #-Session: P250-III

THE EFFECT OF CONVENTIONAL IMMUNOSUPPRESSANTS ON THE INDUCTION OF REGULATORY CELLS AFTER INTRATRACHEAL DELIVERY OF ALLOANTIGEN. Shintaro Shibutani,[1] Takurin Akiyoshi,[1] Humihiko Inoue,[1] Yoshinobu Akiyama,[1] Kenji Matsumoto,[1] Osamu Aramaki,[2] Nozomu Shirasugi,[2] Masaki Kitajima,[1] Masanori Niimi.[2] *[1]Department of Surgery, Keio University School of Medicine, Tokyo, Japan; [2]Department of Surgery, Teikyo University School of Medicine, Tokyo, Japan.*

Aim of Study: We have already reported that intratracheal delivery (ITD) of donor splenocytes could induce prolonged survival of a fully allogeneic cardiac graft resulting in the generation of regulatory cells. And furthermore, we reported that one of conventional immunosuppressants, FK506 has differential effects by its doses on the induction of regulatory cells after ITD of donor splenocytes (AST 2002). In this study, we examined the influence of other conventional immunosuppressants (cyclosporine, rapamycin, mycophenolate mofetil; MMF, azathioprine) following our protocol. **Methods:** Naive CBA ($H2^k$) mice were given $1x10^7$ C57BL/6 ($H2^b$) splenocytes into the trachea with intraperitoneal injection of conventional immunosuppressants for 7 consecutive days starting from the time of ITD. 7 days after the ITD, naive CBA mice were adoptively transferred with $5x10^7$ splenocytes from the pretreated mice and transplanted C57BL/6 cardiac grafts. **Results:** Naive CBA mice rejected C57BL/6 cardiac grafts acutely at a median survival time (MST) of 9 days. When CBA mice were given $1x10^7$ donor splenocytes into the trachea 7 days before transplantation, all of the grafts survived for over 40 days (MST = 81 days). Adoptive transfer study was shown in Table. MMF facilitated the generation of regulatory cells following our ITD model. On the other hand, rapamycin and low dose FK506 had no effect to generate regulatory cells. **Conclusion:** In the maintenance phase after organ transplantation, MMF in combination with low dose FK506 and/or rapamycin may be the best treatment to generate regulatory cells.

Adoptive transref study

Pretreatment	Dose (mg/kg)	Survival (days)	MST (days)
ITD alone		20, 20, 23, 49, 50, 62, 86, >100, >100	50
FK506	0.1	12, 26, 36, 55, 61, >100 x 2	55
	0.3	6, 6, 11, 11, 21	11
	0.5	5, 7, 7, 10, 15	7
	1.0	5, 5, 7, 8, 8	7
cyclosporine	5	6, 6, 9, 9, 10, 15, 24, 25, 31, 37	12.5
	10	5, 5, 7, 7, 11	7
	25	7 x 5	7
azathioprine	1.0	7, 7, 9, 11, 13, 16, 16, 17, 22	13
	2.0	7, 7, 10, 10, 10, 23	10
rapamycin	20	18, 24, 40, 50, >100 x 3	50
	40	63, 85 x 3, > 100 x 6	>100
MMF	20	70, 80, 93, >100 x 4	>100
	40	>100 x 7	>100

Abstract# 1495 Poster Board #-Session: P251-III

IN VITRO MONITORING OF RENAL ALLOGRAFT TOLERANCE INDUCED BY MIXED CHIMERISM AND COSTIMULATORY BLOCKADE IN NONHUMAN PRIMATES.

Tatsuo Kawai,[1] Siew-Lin Wee,[1] Svetlan Boskovic,[1] Ichiro Koyama,[1] Ognjenka Nadazdin,[1] Matthew Nuhn,[1] R. Neal Smith,[2] Megan Sykes,[3] Robert B. Colvin,[2] David H. Sachs,[3] A. Benedict Cosimi.[1] *[1]Surgery, Transplantation Unit, Massachusetts General Hospital, Boston, MA; [2]Pathology, Massachusetts General Hospital, Boston, MA; [3]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

Maintenance of long-term allograft survival following withdrawal of immunosuppression has been attempted using several tolerance inducing regimens. Major limitation of these studies has been the unavailability of reliable in vitro assays for detecting the return of damaging immunologic responses prior to the insidious development of chronic rejection (CR). In the current studies, we evaluated various immunological assays in long-term survivors treated with a mixed chimerism inducing nonmyeloablative regimen. **[Methods]** Cynomolgus monkeys were treated with low dose TBI (1.5GyX2), thymic irradiation (7 Gy), Anti-thymocyte globulin, donor bone marrow (DBM), a short course of CD154 blockade (20mg/kg up to day 10) and a one-month course of postoperative cyclosporine (group A, n=8). Control recipients were treated with the same regimen minus TI (group B, n=4), minus DBM (group C, n=2) or with substitution of donor splenocytes for DBM (group D, n=4). Sequential analyses of chimerism, MLR, CML and anti-donor antibody (ADA) and graft biopsies were performed. **[Results]** All recipients treated with the full regimen (group A) developed mixed chimerism and 7/8 survived long-term (>1392, >847, 837, 755, 401, 373, 206, 58 days). Among the long-term survivors, 4/8 recipients did not develop ADA nor CR, while 3/8 develop ADA within 100 days and eventually CR after 200 days. In contrast, all control recipients in groups B, C and D rejected their allografts acutely or chronically except for a single group B recipient that survived >300 days without ADA or CR . Induction of multilineage chimerism was confirmed to be essential to induce tolerance in this model as none of the recipients without DBM acquired tolerance. However, there was no significant difference in the level and the duration of chimerism as well as postoperative MLR or CML between tolerant recipients and rejectors within group A. In contrast, ADA against either donor T cells or B cells was consistently detected by flow cytometry in the three rejectors in group A that eventually developed CR with positive C4d staining. Similarly, all three long-term survivors(>200 days) in groups C and D developed ADA within 100 days and eventually CR after 200 days. **[Conclusion]** ADA monitoring by flow cytometry appeared to be a reliable assay for monitoring tolerance induction and stability in allograft recipients.

Abstract# 1496 Poster Board #-Session: P252-III

SPLENOCYTES FROM MICE WITH PERMANENT MIXED CHIMERISM AND TOLERANCE INHIBIT DONOR-SPECIFIC IMMUNE RESPONSE IN VITRO.

Yisheng Pan, Bin Luo, Hakan Sozen, Neal Heuss, David E. R. Sutherland, Bernhard J. Hering, Zhiguang Guo. *[1]Department of Surgery, University of Minnesota, Minneapolis, MN.*

Central deletion through mixed chimerism has been considered to be the mechanism of inducing tolerance. The role of peripheral tolerance through chimerism on inducing tolerance has not been well studied. We investigated whether in vitro anti-donor immune response was suppressed by regulatory cells from mice with permanent chimerism and donor tolerance. Donor Balb/c cells were injected into each C57BL/6 mouse at day -3, followed by cyclophosphamide at day -1. Bone marrow was infused at day 0. Anti-CD154 mAb and rapamycin were given from day 0 to day 14. Chimerism was measured by flow cytometry. Skin transplantation was performed at 6 weeks post-BMT. Splenocytes from mice with mixed chimerism and donor skin grafts over 280 days were harvested and tested for inhabitive effect on the frequency of INF-γ secreting cells against donor and third-party antigens by ELISPOT assay. C57BL/6 splenocytes, primed by both Balb/c and C3H skin grafts 2 weeks before ELISPOT, were used as responder and were cocultured with either Balb/c or C3H alloantigens. Splenocytes from chimeric mice were added at different tolerant cells/responder ratios. Without splenocytes from chimeric C57BL/6 mice, high frequency of INF-γ secreting cells was measured. When Balb/c allantigens were used as stimulator, the average numbers of INF-γ secreting cells were 263±53 per $3x10^5$ splenocytes, 580±163 per $6x10^5$ splenocytes, and 903±262 per $9x10^5$ splenocytes. Frequency of INF-γ secreting cells per $3x10^5$ splenocytes was significantly inhibited by splenocytes form mice with 8.8 ± 4.5% donor chimerism and the inhibition was dose-dependent. When splenocytes from chimeric mice were added at 0.5:1, 1:1, 1.5:1 and 2:1 ratio, the average numbers of INF-γ secreting cells were 216±93, 159±45, 140±42, and 96±47. When C3H alloantigens were used as stimulator, high frequency of INF-γ secreting cells was also detected. The average numbers of INF-γ secreting cells were 244±158 per $3x10^5$ splenocytes, 642±403 per $6x10^5$ splenocytes, and 962±284 per $9x10^5$ splenocytes. Inhibition of INF-γ secreting cells per $3x10^5$ splenocytes was not detected when chimeric splenocytes were added at 0.5:1, 1:1, 1.5:1 and 2:1 ratio. INF-γ secreting cells were 352±210, 348±185, 328±205, and 297±105. We demonstrated that splenocytes from chimeric mice suppressed donor-specific immune response in vitro. Our data suggest that peripheral tolerance through immunoregulation is induced in mice with permanent mixed chimerism and tolerance.

Abstract# 1497 Poster Board #-Session: P253-III

GENERATION AND EXPANSION OF REGULATORY CELLS: REQUIREMENTS, COMPARTMENT INVOLVED AND TIMING.

Hiroaki Kitade, Masaru Kawai, Takaaki Koshiba, Lut Overbergh, Annapaula Giulietti, Mark Waer, Chantal Mathieu, Jacques Pirenne. *[1]University Hospitals Leuven, Leuven, Belgium.*

There is a renewed interest for regulatory cells (RC) in nondeletional tolerance models. Requirements for RC development, compartments involved in RC generation/expansion and timing are poorly understood. We developed a donor-specific blood tranfusion (DSBT) induced transplantation (Tx) tolerance model in which RC operate. In this model, we search to clarify: 1) whether DSBT, graft, lymphoid tissues (thymus/spleen) are required; 2) in which compartment RC develop (graft and/or lymphoid tissues: spleen, thymus, lymph nodes (LN)); and 3) timing of these events. ***Methods.*** Heart Tx is done using fully mismatched RA (RT1p) and PVG (RT1c) rat as donor/recipient. Tolerance (indefinite graft survival-acceptance of 2ary donor-specific Tx) is induced by DSBT 12D preTx. Various cell types {splenocytes (Sple), thymocytes (Thy), LN cells and graft infiltrating cells (GIC)} are transferred from tolerant and control rats at various time points into naive irradiated (4.5Gy) PVG rats prior to heart Tx. Effect of thymectomy/splenectomy is studied. Mixed lymphocyte reaction (MLR) and phenotypic (FACS) analysis of lymphoid tissues in tolerant vs rejecting rats are performed. ***Results.*** *At 5D,* Sple and LN cells from tolerant rat transfer tolerance in 33% and 40% respectively whereas Thy and GIC are ineffective. *At 14D,* 100% Sple and LN cells transfer tolerance. *At 30D,* all cell types transfer tolerance: Sple, LN, GIC (100%), Thy (67%). Sple from naive rats, DSBT-treated non-Tx rat or DSBT-untreated rejecting rats do not transfer tolerance. Thymectomy and splenectomy prevent tolerance induction (graft survival: 13.2 and 25.5D, respectively). FACS analysis in spleen, LN, thymus and blood show no phenotypic difference (CD4, CD8, CD4/CD25, CD45) between tolerant vs rejecting rats. Anti-donor MLR show hyperresponsiveness at 7D and hyporesponsiveness at 14D postTx. ***Conclusions.*** RC are detectable early on (D5) in tolerant rats, but only in spleen + LN and at low concentration and hyperresponsiveness is present. Thereafter, RC gradually expand and become detectable in all compartments studied (graft/spleen/LN/thymus) and hyporesponsiveness develops. Combined effect of DSBT + graft, the presence of thymus and spleen are all prerequisites for RC generation/expansion. No particular phenotype is identified in RC-containing lymphoid tissues. Further phenotypical/functional characterization of RC is required.

Abstract# 1498 Poster Board #-Session: P254-III

TISSUE-ASSOCIATED FACTORS INFLUENCE $CD4^+$ T CELL ALLOIMMUNE RESPONSES.

Keri E. Lunsford,[1] Yue Wang,[1] Donghong Gao,[1] Ginny L. Bumgardner.[1] *[1]Department of Surgery, Division of Transplantation, The Ohio State University, Columbus, OH.*

In previous studies we found that FVB/N islets initiate CD4-dependent but not *(CD4-independent)* $CD8^+$ T cell rejection responses, whereas FVB/N hepatocytes (HCs) elicit both pathways. The purpose of the current study was to evaluate the influence of the same immunosuppressive strategy upon islet versus hepatocyte initiated alloreactive $CD4^+$ T cell immune responses. In previous studies we have determined that islet and hepatocyte rejection in CD8 KO mice is $CD4^+$ T cell dependent. **Methods:** FVB/N ($H\text{-}2^q$) islets or HCs were transplanted into CD8 KO ($H\text{-}2^b$) mice. Islets were transplanted under the kidney capsule of streptozotocin-induced diabetic mice, and survival was monitored by blood glucose. Recipient mice were treated with anti-LFA-1 mAb (0.15 mg for islets and 0.3 mg for HCs, ip, d0-6). **Results:** Treatment with anti-LFA-1 mAb induced long-term ***islet*** allograft survival >100 days in CD8 KO mice. In contrast, although anti-LFA-1 mAb prolonged ***HC*** survival in CD8 KO mice, HCs were uniformly rejected in < 60 days in all recipients. Three of 3 CD8 KO recipients with longterm islet survival (>100 days) induced by treatment with anti-LFA-1 mAb, maintained islet allograft function beyond an additional 70 days despite adoptive transfer of $5x10^6$ naive purified $CD4^+$ T cells (sufficient to cause islet rejection in SCID mice). Continued islet allograft survival despite adoptive transfer of naive T cells suggests that this treatment strategy induced the development of regulatory cells in islet allograft recipients.

Recipient	Cell Tx Type	Treatment	Islet Survival In days (n)	# $CD4^+$ T cells Transfered	Islet survival after $CD4^+$ T cell Transfer
CD8KO	Islet	Anti-LFA-1 mAb	>180 (3)	$5x10^6$ CD4	>70 (3)
CD8KO	HcTx	Anti-LFA-1 mAb	21, 35, 49, 70	nd	

Conclusions: These data show that (1) treatment with anti-LFA-1 mAb induces indefinite survival of allogeneic FVB/N islets but not FVB/N hepatocytes in CD8 KO mice. (2) Immunotherapy with anti-LFA-1 mAb and islet (but not MHC-matched hepatocyte) transplant appears to induce the development of regulatory cells which suppress $CD4^+$ T cell initiated rejection. In the current study, we demonstrate that tissue-associated factors significantly influence the activation of alloreactive $CD4^+$ T cells such that differential susceptibility to the same immunosuppressive strategy is observed.

Abstract# 1499 Poster Board #-Session: P255-III

IDENTIFICATION OF GENE EXPRESSION PROFILES THAT CORRELATE WITH ACUTE REJECTION VERSUS TOLERANCE INDUCED BY TACROLIMUS AND SIROLIMUS CO-ADMINISTRATION IN A NON-HUMAN-PRIMATE KIDNEY ALLOGRAFT MODEL. Mark J. Cameron,[1] Luoling Xu,[1] Cheryl M. Cameron,[1] Longsi Ran,[1] Ali Danesh,[1] Karoline Hosiawa,[1] Shijie Qi,[2] Jun Ouyang,[2] Anlun Ma,[2] Dasheng Xu,[2] Huifang Chen,[2] David J. Kelvin.[1] *[1]Department of Experimental Therapeutics, Toronto General Research Institute, UHN, Toronto, ON, Canada; [2]CHUM, Campus Notre-Dame, University of Montreal, Montreal, QC, Canada.*

To overcome the eventuality of donor organ failure due to progressive injury caused by acute and chronic allograft rejection, many promising immunosuppressive treatments have been recently introduced. We have previously shown that a 60-day treatment of Vervet monkeys with tacrolimus and rapamycin leads to extended survival of kidney allografts in the majority of animals (>90 d) without additional immunosuppression. The molecular basis of this induction of long-term operational tolerance (several >300 d) is unknown. With the hypothesis that genetic variation determines the fate of an allograft, one strategy is to perform a global analysis of gene expression profiles associated with transplantation injury versus tolerance using microarray technology. We used a 19200 human gene DNA microarray as a discovery tool to comprehensively identify the interrelated expression of immune- and non-immune-related genes during graft injury and tolerance induction. We set physiological baselines by comparing gene expression profiles of kidney biopsies (n≥3) from Vervet monkeys that underwent transplant surgery. We found that the number of genes with altered expression depended on the transplant modality. We recorded the percentages of altered genes as: (i) normal vs normal non-transplanted kidneys (same monkey) 0.3%, (ii) normal vs normal non-transplanted kidneys (different monkey) 4.2%, (iii) auto-transplanted kidneys (day 0 vs 7) 1.1%, and (iv) rejected kidneys (day 0 vs 7) 5.8%. Hierarchical clustering revealed that gene expression datasets from the same monkey non-transplanted kidneys clustered closely with the auto-transplanted kidneys and that these 4 clustered at a medium distance from the different monkey non-transplanted kidneys and at a long distance from the rejected kidneys. Partitional clustering was performed to identify higher order relationships between these gene clusters and those accumulated from tolerant grafts. Detailed analysis revealed several co-regulated families of genes including inflammatory vs regulatory cytokines/chemokines and ESTs. Once the validation of selected genes by real-time PCR is complete, this study may facilitate diagnosis and the tailoring of tacrolimus and rapamycin co-administration for human and non-human-primate transplantation. Supported by Genome Canada and the CIHR.

Abstract# 1500 Poster Board #-Session: P256-III

PERIPHERAL ROLE OF NK T CELLS FOR THE ESTABLISHMENT OF TOLERANCE IN CYCLOPHOSPHAMIDE (CP)-INDUCED TOLERANCE. Toshiro Iwai,[1] Yukihiro Tomita,[1] Takashi Kajihara.[1] *[1]Department of Cardiovascular Surgery, Faculty of Medicine, Kyushu University, Fukuoka, Japan.*

[Purpose] Recent reports provided the evidence that NK T cells play important roles in the induction of transplantation tolerance. We have previously reported a method of CP-induced tolerance. In this method, we have demonstrated the three major mechanisms, i.e., clonal destruction for tolerance induction, intrathymic clonal deletion and the appearance of regulatory T cells for the maintenance of tolerance (JI 1990). The unsolved mechanism of CP-induced tolerance system is the regulatory role against the effector T cells which are generated in the thymus from clonal destruction in the periphery through the establishment of intrathymic clonal deletion. The aim of the present study was to investigate these regulatory roles by using NK T KO mice. [Methods] Balb/c (WT; H-2^d, Ly1.2, Mls-1^b) or NKT KO (Balb/c background) mice were primed with 1×10^8 DBA/2 (H-2^d, Ly1.1, Mls-1^a) spleen cells(SC) on day 0 and treated with 200mg/kg CP on day 2. In some group, recipients were thymectomized on day –14 (ATx). Donor skin grafting was performed at 4weeks. Chimerism was evaluated using FITC-anti-Ly1.1 and PE-anti-Ly1 (1.1+1.2) mAb at various times. Clonal destruction was examined by analyzing the expression of Mls-1a-reactive $CD4^+CD8^-V\beta6^+$ T cells in the spleen or thymus. [Results] In WT mice treated with SC and CP, skin allograft tolerance and mixed chimerism were induced. In the NKT KO mice treated with SC and CP, however, survival of skin grafts were moderately prolonged (median 35). When NK T KO mice treated with ATx, skin grafts were permanently accepted. Donor derived T ($Ly1.1^+$) cells were detectable from 2-14 weeks after treatments in NKT KO mice treated with ATx, SC and CP. On the other hand, chimerism were detectable at 2 weeks and became undetectable by 14 weeks in NKT KO mice treated with SC and CP. The level of chimerism in the NKT KO mice treated with ATx, SC and CP were significantly higher than that in the NKT KO mice treated with SC and CP. As to the mechanisms, clonal destruction was induced in SC of all recipients. On the other hand, however, clonal destruction in the thymus of WT or NKT KO mice treated with SC and CP was not observed. [Conclusion] The present study strongly indicated the regulatory role of NKT cells. Effector T cells in the thymus were not destructed by the treatment with SC and CP. New effector T cells migrate from the thymus into the periphery until clonal deletion (associated with mixed chimerism) occurs. Without the existence of NKT cells, these effector T cells gradually break the tolerant state.

Abstract# 1501 Poster Board #-Session: P257-III

TRANSFER OF MUCOSAL TOLERANCE BY A MURINE LIVER TRANSPLANT. Wei Li,[1] Sonja T. Chou,[1] Celso Wang,[1] Jennifer Ra,[1] Christian S. Kuhr,[1] James D. Perkins.[1] *[1]Department of Surgery, Division of Transplantation, University of Washington School of Medicine, Seattle, WA.*

Antigen administration via a mucosal route favors the induction of peripheral tolerance. The mechanism of the liver's role in this form of peripheral tolerance is unknown. Using a murine liver transplant model, we studied the liver's role in inducing and maintaining peripheral tolerance. **Methods:** BALB/c mice were divided into 3 groups according to the dosage of ovalbumin (OVA) delivered via gastric lavage, low dose (0.5 mg/d for 5 days), high dose (500 mg/d for 5 days), and non-fed (control). Livers from these 3 groups were then transplanted into OVA naive BALB/c mice. A standard delayed-type hypersensitization (DTH) reaction was documented 7 days after transplant by measuring the thickness of stimulated footpads. Further study involved adoptive transfer of the liver non-parenchymal cells (NPC) from the 3 groups into naive mice. In addition, *in vitro* proliferative response assays and cytokine profiles were conducted on NPC from the 3 groups. Finally, livers from OVA naive mice were transplanted into the 3 groups. **Results:** The transplanted livers from either low dose or high dose OVA fed BALB/c mice transferred tolerance to OVA naive mice. The mean footpad thickness was 0.35 mm for the low-dose fed donors and 0.2 mm for the high-dose fed donors, compared with 0.8 mm for the controls ($p<0.01$). Adoptive transfer of liver NPC from low dose and high dose OVA fed mice further induced OVA tolerance by significantly decreasing the footpad thickness to 0.42±0.09 mm ($p<0.05$) and 0.27±0.13 mm ($p<0.001$), respectively, from the control of 0.9±0.17 mm. The *in vitro* proliferative response of the NPC to OVA revealed a decreased response to both dosage groups over the control group with the high dose having the lowest response. The cytokine profile of the NPC revealed decreased concentration of IL-2 and INF-γ of both OVA fed groups compared to controls, and an increasing concentration of IL-4 correlating with an increasing dose of OVA. Transplanting livers from naive mice into either low dose or high dose OVA fed BALB/c mice did not break the established tolerance. **Conclusions:** The liver is sufficient to induce peripheral tolerance in a mucosal tolerance model feeding OVA. The NPC can induce this tolerance. From the adoptive transfer and the proliferative assays of NPC, the high dosage of OVA appears to be more tolerizing. This tolerizing effect could be related to the increasing concentration of IL-4 with increasing dose of OVA. Finally, the liver is not necessary for the maintenance of peripheral tolerance in this model.

Abstract# 1502 Poster Board #-Session: P258-III

ALLOREACTIVITY OF LYMPHOCYTES DERIVED FROM KIDNEY TRANSPLANT PATIENTS TREATED WITH CAMPATH-1H: SUBSET ANALYSIS USING CSFE-LABELING. Debra D. Bloom,[1] Huaizhong Hu,[1] John H. Fechner,[1] Stuart J. Knechtle.[1] *[1]Surgery, University of Wisconsin-Madison, Madison, WI.*

Campath-1H is a humanized monoclonal antibody that imposes rapid depletion of T and B lymphocytes, NK cells, and monocytes when administered in vivo. In an effort to facilitate immune acceptance of their graft, kidney transplant recipients were given Campath-1H at day-1 and day 0 of transplant, followed by long-term treatment with low-dose rapamycin. At month 12 post-transplant, recovery of lymphocyte populations is still incomplete, as T cell numbers are 10-20% that of pre-transplant levels. To gain insight into the alloreactivity of these repopulating lymphocytes, and to achieve the sensitivity needed to study low lymphocyte numbers, one-way MLR was performed using CSFE-labeling and flow cytometry. METHODS: Peripheral blood lymphocytes (PBL) of six Campath-treated kidney allograft patients were examined for their ability to proliferate in response to donor antigen. Responder lymphocytes were labeled with CSFE and cultured with irradiated donor or third party PBL for 6 days. The cells were then incubated with PE-conjugated antibodies to CD3, CD4, CD8, CD19, and CD56 and subsequently analyzed by flow cytometry. RESULTS: In five of six patients studied, CD8+ cells proliferated less well to donor antigen compared to that of third party antigen (see Table for range of % CSFE-low cells). In three of six patients, a reduction in proliferation to donor antigen was seen for CD4+ T cells. Interestingly, CD56+ cells did indeed proliferate in response to alloantigen, but proliferation did not differ significantly between donor and third party antigen. CONCLUSION: This preliminary study suggests that donor-specific unresponsiveness to kidney allografts in Campath-treated patients may perhaps be beginning by 12 months post-transplant. Other assays to test for signs of unresponsiveness are needed to support this initial finding.

CSFE-Low Lymphocytes in MLR

	$CD3^{csfe\ lo}/CD3$	$CD4^{csfe\ lo}/CD4$	$CD8^{csfe\ lo}/CD8$	$CD56^{csfe\ lo}/CD56$
Donor (n=6)	6-16*	5-26	0-12	4-19 (n=3)
3rd Party 1 (n=6)	9-41	8-28	4-45	4-18 (n=3)
3rd Party 2 (n=5)	15-44	13-46	8-47	22-24 (n=2)

*Indicates range of percentages seen.

Abstract# 1503 Poster Board #-Session: P259-III

CHARACTERIZING RENAL TRANSPLANT REJECTION AND IMMUNOSUPPRESSION BY GENE PROFILING OF BIOPSIES AND PERIPHERAL BLOOD LYMPHOCYTES. Sunil Kurian,[1] Stuart M. Flechner,[1,2] Steven Head,[1] Starette Sharp,[1] Thomas Whisenant,[1] Jie Zhang,[3] Jefferey Chismar,[1] Tony Mondala,[1] Daniel J. Cook,[2] John Walker,[3] Daniel Salomon.[1] *[1]Molecular and Experimental Medicine/ DNA Array Core, The Scripps Research Institute, La Jolla, CA; [2]Transplant Center/Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, OH; [3]Genomics Institute, Novartis Research Foundation, San Diego, CA.*

Background: Strategies that promote the development and refinement of immunosuppressive drug regimens will improve their safety, and enhance the quality of life for transplant recipients. We have used DNA Microarrays to obtain gene expression profiles from kidney biopsies and peripheral blood lymphocytes (PBL) in an attempt to define predictable and reproducible functional class patterns which define the state of the graft. Methods: Patient samples (>60) included 15-gauge needle core biopsies and simultaneous peripheral blood obtained from healthy kidney donors undergoing nephrectomy, transplant recipients with biopsy confirmed acute rejection, those with non-rejection renal dysfunction, and those with stable graft function. Additional PBL were obtained from non-immunosuppressed healthy blood donors. Biopsies were scored using the Banff system. Samples were stored in RNAse free solution at -80C until utilized. After RNA extraction, Affymetrix GeneChip Microarrays (U95A) were generated for each sample. Results: Hierarchical clustering of samples (dnaChip) and statistical analysis of individual gene expression signals demonstrated clear differences in gene expression patterns in both kidney tissue and PBL that correlated with the patient's renal function and state of immunosuppression. The gene expression data showed significant differences in patients undergoing acute rejection relative to patients with kidney dysfunction not related to graft rejection, and patients with well functioning grafts. Differences in functional class prediction algorithms also applied to gene expression data from PBL of transplant recipients, which successfully predicted rejection status and use of immunosuppression in the transplanted patients. Conclusion: This study provides preliminary data suggesting that PBL can serve as a surrogate marker to monitor immunosuppression and characterize graft function. Such analysis may help to define states of over or under immunosuppression, and help to monitor progression of chronic allograft nephropathy. The potential for non-invasive and dynamic monitoring of the graft allo-immune response and immunosuppression may have a profound impact on patient morbidity and eventual graft outcome.

Abstract# 1504 Poster Board #-Session: P260-III

ANALYSIS OF THE MHC IN GRAFT REJECTION REVISITED BY GENE EXPRESSION PROFILES. Kenneth Christopher,[1] Yurong Liang,[1] Rachel DeFina,[1] He Hongzhen,[2] Kathleen J. Haley,[2] Mark A. Exley,[3] Patricia W. Finn,[2] David L. Perkins.[1] *[1]Laboratory of Molecular Immunology, Brigham and Women's Hospital, Boston, MA; [2]Pulmonary Division, Brigham and Women's Hospital, Boston, MA; [3]Cancer Biology Program, Beth Israel Deaconess Medical Center, Boston, MA.*

The role of MHC molecules in graft rejection is incompletely understood. We performed a comparative study of the functions of Class I, Class II, Minor antigens and CD1 in murine cardiac allograft rejection by investigating the expression of a panel of immune genes. For Class I, we analyzed allograft recipients deficient in β2M, a component of the Class I heterodimer. We included CD1 KO recipients to differentiate β2M KO strain effects due to CD1 deficiency. The serum cytokines TNFα, IL6, IFNγ and IL1β are evaluated post transplant by ELISA. The intragraft expression of 55 immune genes are measured by RPA. Data is analyzed via hierarchical clustering dendrograms and self-organizing maps. Mean graft survival in days: β2M deficient (BALB→β2M KO)(19.3), Class II deficient (BALB→Class IIKO)(21.8), minor antigen disparate (BALB→B10.D2)(29.0), CD1 deficient (BALB→CD1KO)(6.8), allogeneic (BALB→B6)(7.5), alymphoid (BALB-RAGKO→B6-RAGKO)(>100), and syngeneic (B6→B6)(>100). High levels of IFNγ are noted on day 5 in the β2M KO, CD1 KO, and minor antigen disparate recipients. The Class II KO recipients had <1% of the IFNγ levels of the allogeneic control. The data indicate that a Class II response may be necessary to generate the increased IFNγ in the serum. The dendrogram at day 1 separates the transplant groups according to total gene expression into three clusters: 1)syngeneic, alymphoid, Class II KO, and allogeneic groups; 2) CD1 KO, minor antigen disparate, and β2M KO groups; 3) the untransplanted control. This observation suggests that even as early as day one following transplantation, each group is generating differences in the intragraft alloimmune response. Despite β2M and Class II KO recipients both having prolonged survival, the β2M and Class II KO recipients show widely divergent subsets of differentially expressed genes. The Self Organizing Map generated a similar pattern to the day 7 dendrogram, with an expression profile of increased expression in the allogeneic, Class II KO, and minor antigen disparate, and decreased expression in the β2M KO and CD1 KO groups. The genes in the Map included MIP1α, MIP1β, MIP2, MCP1, TCA3, IP10, RANTES, TCRα, CD3ε, CD8α, CD8β,and CD45 The analysis indicates that each classical and nonclassical MHC gene deficiency induces both the upregulation and the downregulation of distinct subsets of genes, and that similar kinetics of rejection can be attributed to different molecular mechanisms.

Abstract# 1505 Poster Board #-Session: P261-III

EXPRESSION OF ALLOGRAFT INFLAMMATORY FACTOR-1 IN T-LYMPHOCYTES: A ROLE IN T-CELL ACTIVATION AND ALLOGRAFT REJECTION. Sheri E. Kelemen,[1] Howard J. Eisen,[1] Michael V. Autieri.[1] *[1]Advanced Heart Failure Center, Temple University School of Medicine, Philadelphia, PA.*

The transcriptional profile of gene expression during the allograft response is an important approach in characterization of the molecular processes responsible for transplant rejection. Messenger RNA expression in transplanted human left ventricle and allografted rat left ventricle hearts were examined by cDNA microarray analysis. The mRNA expression of one gene, Allograft Inflammatory Factor-1 (AIF-1), was increased 4.4 fold compared with normal human hearts, and 5.2 fold compared with isografted rat hearts. AIF-1 is a cytokine responsive protein whose expression in endomyocardial biopsies correlates with rejection and development of coronary artery vasculopathy. Immunohistochemical analysis of these samples and endomyocardial biopsies from transplant patients demonstrates that AIF-1 is expressed in CD3 positive T lymphocytes and to a smaller degree, in cardiac myocytes where large numbers of inflammatory cells are present. AIF-1 expression in either of these cell types has not been reported and is a novel finding. To characterize the function of AIF-1 in T lymphocytes, AIF-1 was over expressed in the human T lymphoblastoid cell line MOLT-4 by retroviral (R.V.) gene transduction. Over expression of AIF-1 in these cells lead to an 8.7 fold increase in AIF-1 R.V. transduced MOLT-4 cell number (552.7+/-21.6 Vs 57.2+/-3.0) ($p<0.001$) for AIF-1 R.V. versus empty vector controls. This study suggests that AIF-1 expression plays an important part in T lymphocyte activation, and infiltrating T lymphocytes affect AIF-1 expression in cardiac myocytes.

Abstract# 1506 Poster Board #-Session: P262-III

CALCINEURIN INHIBITOR SPARING IMMUNOSUPPRESSION: A SELECTIN ANTAGONIST PLUS LOW DOSE SIROLIMUS AND CSA PREVENTS EARLY AND LATE ALLOANTIGEN INDEPENDENT INJURY TO RENAL TRANSPLANTS. Martin Gasser,[1] Ana Maria Waaga-Gasser,[1] Miriam S. Lenhard,[1] Gray D. Shaw,[2] Wayne W. Hancock,[3] Nicholas L. Tilney.[4] *[1]Dept. of Surgery, Julius Maximiliams University, Wuerzburg, Bavaria, Germany; [2]Genetics Institute, Cambridge, MA; [3]Dept. of Pathology, Children's Hospital, Philadelphia, PA; [4]Dept. of Surgery, Surgical Research Lab., Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Brain death, a putative risk factor for chronic rejection, upregulates inflammatory factors in the donor organs. After transplantation, P-/E-selectins on endothelial cells mediate early leukocyte adhesion and subsequent antigen-independent changes. These changes then trigger induction of specific host immunity. We studied the effects of a new strategy to improve long-term behavior of renal allografts from brain dead (BD) F344 donors in LEW rat recipients. Initial cellular events were inhibited using recombinant P-selectin glycoprotein (rPSGL-Ig, 50μg given to the donor 3hrs after BD and to the recipient immediately after transplantation). High or low-dose sirolimus (SRL) and cyclosporine (CsA) were used to suppress subsequent antigen-dependent responses. Recipient groups (n=8-12/Gp) included:

Group (Gp)	Donor	Therapy
1-syn control	LD	no
2-allo control	LD	SRL 0.1mg/kg/d 4-13+CsA 1mg/kg 10d
3-allo control	BD	SRL 0.4mg/21d
4-allo treatment Gp	BD	SRL 0.4mg/21d+rPSGL-Ig
5-allo control	BD	SRL 0.4mg/21d+CsA 1mg/10d
6-allo treatment Gp	BD	SRL 0.4mg/21d+CsA 1mg/10d+rPSGL-Ig
7-allo treatment Gp	BD	SRL 0.1mg/d 4-13+CsA 1mg/10d+rPSGL-Ig

Syn, syngeneic; allo, allogeneic; LD, living donor; BD, brain dead donor

At 150 days after transplantation, LD control allografts (Gp 2), but not isografts (Gp 1), showed functionally and histologically moderate signs of chronic rejection. BD controls treated with SRL alone (Gp 3) experienced progressive chronic rejection. Addition of rPSGL-Ig (Gp 4) protected the kidneys substantially. With CsA plus high dose SRL, however, grafts from Gp 5 and 6 demonstrated severe tubular injury. Best overall results were seen when rPSGL-Ig was given with low dose SRL and CsA (Gp 7); gene expression of IL-2, IFN-γ, and TGF-β were comparable to Gp 1 isografts although elevated in the other groups (RNAse protection assay and Real Time PCR). In conclusion, high dose SRL plus CsA without and with rPSGL-Ig produces severe chronic changes with profound tubular atrophy in renal allografts. rPSGL-Ig inhibits early inflammatory events associated with donor BD and acts synergistically with low dose SRL and CsA to prevent chronic rejection. This new immunosuppressive strategy seems therefore to be promising in patients receiving transplants, particularly from marginal donors, to improve the longterm outcome.

Abstract# 1507 Poster Board #-Session: P263-III

PROLONGATION OF MURINE CARDIAC ALLOGRAFT SURVIVAL BY DOWN-REGULATION OF MATRIX METALLOPROTEINASE-2 ACTIVITY. Andres Jaramillo,[1] Wei Lu,[1] Lacey G. Campbell,[1] J. Michael Shipley,[2] Robert M. Senior,[2] Shigeyoshi Itohara,[3] T. Mohanakumar.[1,4] *[1]Department of Surgery, Washington University, St. Louis, MO; [2]Department of Cell Biology & Physiology, Washington University, St. Louis, MO; [3]Brain Science Institute, Wako, Japan; [4]Department of Pathology & Immunology, Washington University, St. Louis, MO.*

Purpose: The role of matrix metalloproteinases (MMP) in the pathogenesis of cardiac allograft rejection has not been addressed. The goals of this study were 1) to determine whether MMP-2 and MMP-9 play a role in the pathogenesis of cardiac allograft rejection. **Methods:** BALB/c cardiac allografts were transplanted into 129/SvEv, MMP-2-KO, and MMP-9-KO mice. Rejection was defined by cessation of a palpable heartbeat. The intra-graft levels of MMP-2 and MMP-9 mRNA were determined by RT-PCR and real time RT-PCR. The intra-graft levels of MMP-2 and MMP-9 enzymatic activity were determined by gelatin zymography and immunohistochemistry. **Results:** 129/SvEv isografts did not show any histopathological signs of rejection at day 40. These isografts revealed no MMP-2 or MMP-9 mRNA expression or enzymatic activity. BALB/c allografts were rejected by 129/SvEv and MMP-9-KO mice at days 15.2 ± 2.6 and 10.5 ± 4.5, respectively. In contrast, an allograft transplanted into a MMP-2-KO mouse survived >29 days. In addition, treatment of 129/SvEv recipients with doxycycline, a potent inhibitor of MMP enzymatic activity, prolonged the survival of the allografts to 34.0 ± 2.4 days (P=0.00008). Rejected allografts showed severe mononuclear cellular infiltration and collagen deposition (fibrosis), and significant levels of MMP-2 mRNA transcription and enzymatic activity. In contrast, functioning allografts harvested at day 15 from doxycycline-treated recipients revealed mild mononuclear cellular infiltration and no fibrosis. Analysis of graft-infiltrating cells in rejected allografts revealed that >90% of these cells expressed MMP-2 and <2% of these cells expressed MMP-9. **Conclusion:** These results indicate an important role for MMP-2 in the pathogenesis of cardiac allograft rejection and suggest that this enzyme is a suitable target for therapeutic intervention for the treatment of allograft rejection.

Abstract# 1508 Poster Board #-Session: P264-III

INNATE IMMUNE AMPLIFICATION OF T CELL EFFECTOR FUNCTION AND CARDIAC ALLOGRAFT REJECTION. Tarek El-Sawy,[1,2] John Belperio,[3] Robert Strieter,[3] Robert Fairchild.[1,2] *[1]Dept. Immunology, Cleveland Clinic Fdn., Cleveland, OH; [2]Dept. Pathology, Case Western Reserve Univ. Schl. Medicine, Cleveland, OH; [3]Dept. Medicine, UCLA Schl. Medicine, Los Angeles, CA.*

INTRODUCTION: Neutrophils are the first leukocytes observed infiltrating grafts following organ reperfusion and the principal mediators of the early inflammatory response in allografts. Following this initial inflammatory response neutrophils are no longer observed within the graft parenchyma but return 2-3 days prior to completion of rejection. This reappearance correlates with the T cell mediated induction of neutrophil chemokines, KC and MIP-2, within the graft in the days prior to rejection. The goal of the current study was to test the role of neutrophils in promoting cardiac allograft rejection late in the process. METHODS: C57BL/6 (H-2b) mice received complete MHC mismatched, A/J (H-2a) cardiac grafts heterotopically. To deplete neutrophils, allograft recipients received 100 ug of anti-Ly-6G mAb beginning day 3 post-transplant and then every day until rejection. Graft frozen sections were stained for CD4+ cells and CD8+ T cells, macrophages and neutrophils. Total graft RNA was analyzed by ribonuclease protection assay to test the levels of IFN-g, FasL and TNF-a expressed in the allografts. RESULTS: Control Ig-treated recipients rejected cardiac allografts around day 7-8. At this time, intense graft infiltration with T cells, macrophages and neutrophils was observed. Antagonism or depletion of neutrophils after early inflammation resolved resulted in a doubling in allograft survival. Grafts from all neutrophil depleted recipients were strongly beating when harvested at day 7. At this time, infiltration with macrophages, CD4+ and CD8+ cells, but not neutrophils, was observed at equivalent levels to rejected control allografts and continued to day 12-14 with the exception that low numbers of neutrophils were observed at the later times post-transplant. Analysis of mRNA expression demonstrated significant decreases in the expression of IFN-g, FasL and TNF-a in neutrophil depleted recipients both at day 7 post-transplant and at the time of rejection compared to controls. CONCLUSIONS: In addition to a prominent role in early inflammation, neutrophils play a significant role at later times in the rejection process by amplifying T cell effector function in the allograft. Innate immune mechanisms are able to accelerate the rejection process through interaction with the alloreactive adaptive immune compartment in the allograft.

Abstract# 1509 Poster Board #-Session: P265-III

CONTRIBUTION OF MHC CLASS II EXPRESSION BY DONOR HEMATOPOIETIC CELLS IN CD4-MEDIATED CARDIAC ALLOGRAFT REJECTION. Todd J. Grazia,[1] Ronald G. Gill,[2] Jan Jensen,[2] Jan N. Jensen,[2] Biagio A. Pietra.[3] *[1]Department of Pulmonary Sciences and Critical Care Medicine, University of Colorado/Health Sciences Center, Denver, CO; [2]Barbara Davis Center for Childhood Diabetes, University of Colorado/Health Sciences Center, Denver, CO; [3]Department of Pediatric Cardiology, The Children's Hospital/University of Colorado, Denver, CO.*

Background and Purpose: In allo-transplantation, antigen presentation can occur via two distinct pathways: the *direct* (donor MHC-restricted) and the *indirect* (host MHC-restricted). Recently, the critical importance of the direct pathway in CD4-mediated cardiac allograft rejection has been demonstrated. However, it is unclear whether such CD4+ T-cell recognition requires MHC class II expression on donor hematopoietic or non-hematopoietic cells. We hypothesized that hematopoietic-derived 'professional' antigen presenting cells (APCs) are necessary for CD4-dependent cardiac allograft rejection. To test this hypothesis, we tested the ability of CD4+ T-cells to reject cardiac allografts that expressed MHC class II exclusively on hematopoietic cells. ***Methods:*** C57Bl/6 (B6) MHC class II-deficient (C2D) mice were lethally conditioned (1200R) followed by grafting with MHC class II positive (wild type) B6 bone marrow tagged with a ubiquitous transgenic green fluorescent protein (GFP) marker. Complete donor chimerism (B6—>C2D) heterotopic cardiac allografts were transplanted into immune-deficient BALB/c $Rag1^{-/-}$ recipients. Purified BALB/c CD4+ T-cells were then transferred into heart-grafted BALB/c $Rag1^{-/-}$ hosts and graft function was monitored by daily palpation. ***Results:*** Flow cytometric analysis of peripheral blood demonstrated complete (>99%) donor chimerism in B6—>C2D hosts with GFP+ CD45+ cells. CD4+ T-cells triggered acute rejection in B6 control allografts (n=2) in less than 14 days. In contrast, MHC class II deficient (C2D) allografts (n=3) survived >50 days after CD4+ T-cell reconstitution. Chimeric (B6—>C2D) hearts, in which MHC class II was expressed only on hematopoietic cells, did not vigorously restore acute allograft rejection with 2 of 3 allografts surviving >50 days post-reconstitution. ***Conclusions:*** Isolated expression of MHC class II to the hematopoietic-derived APCs did not vigorously restore acute CD4-mediated cardiac allograft rejection. These results suggest that hematopoietic-derived APCs may not be sufficient to illicit acute direct CD4 T-cell rejection and implies that other candidate cell types (e.g. endothelial cells) may have a significant role in acute cardiac rejection.

Abstract# 1510 Poster Board #-Session: P266-III

DENDRITIC CELL PRIMING IS NOT SUFFICIENT TO INCREASE ALLO-REACTIVE T CELL RESPONSES TO MEDIATE ACUTE REJECTION OF CLASS II MHC DISPARATE HEART ALLOGRAFTS. Soren Schenk,[1] Chunshui He,[2] Qi-wei Zhang,[2] Danielle Kish,[2] Tarek El-Sawy,[2] Kiyotaka Fukamachi,[1] Peter Heeger,[2,3] Robert Fairchild.[2,3] *[1]Dept. Biomedical Engineering, Cleveland Clinic Fdn., Cleveland, OH; [2]Dept. Immunology, Cleveland Clinic Fdn., Cleveland, OH; [3]The Glickman Urological Inst., Cleveland Clinic Fdn., Cleveland, OH.*

INTRODUCTION: Single MHC disparate skin allografts are rapidly rejected. In contrast, vascularized cardiac allografts expressing such differences are not acutely rejected although the graft survival is limited by the eventual development of transplant associated vasculopathy. Skin allografts contain many more dendritic cells (DC) than cardiac allografts. The goal of this study was to test the ability of allogeneic DC priming to promote the rejection of cardiac grafts expressing single class II MHC differences. METHODS: C57BL/6 (B6) (H-2b) mice received heterotopically transplanted heart or skin grafts from B6.H-2^{bm12} (bm12) or A/J (H-2a) donors. Donor bone marrow was cultured for 5 days in IL-4 /GM-CSF and DC were isolated and used to prime mice 3-4 days before transplant. Recipients received skin allografts 14 days prior to cardiac allograft transplantation. Recipient spleen cells were tested for the frequency of alloantigen-specific T cells producing IFN-g by ELISPOT assays. RESULTS: bm12 skin allografts were rejected by day 15 whereas 80% of heart allografts survived to day 100. Control B6 recipients rejected complete MHC-mismatched cardiac grafts at day 7-8, recipient priming with donor DC reduced allograft survival to day 5-6. In contrast, DC priming before bm12 cardiac transplantation resulted in only a loss of 60% of grafts by day 60. Similar results were observed priming with skin allografts before cardiac allografts. At days 7-14 post-transplant, allografts from control recipients had mild cellular infiltration (ISHLT grade 1A-1B) whereas grafts from DC or skin allograft primed recipients had severe cellular infiltrates (3A-3B). Allogeneic DC priming increased numbers of alloreactive T cells producing IFN-g approximately 2-fold (from 73.8/6 x 10^5 cells in control recipients to 110.1 cells/6 x 10^5 in DC primed recipients). However, the numbers in each group were considerably smaller than the number observed in the spleens of recipients of complete MHC mismatched cardiac grafts (950/6 x 10^5). CONCLUSIONS: DC priming increases the intensity of cellular infiltration into class II MHC disparate cardiac allografts but is not sufficient to mediate acute rejection of the majority of the grafts. One factor that is likely to influence this result is the low precursor frequency of alloreactive T cells participating in this rejection model.

Abstract# 1511 Poster Board #-Session: P267-III

INDIRECT ALLORECOGNITION OF MHC CLASS II PEPTIDES ACCELLERATES CARDIAC ALLOGRAFT REJECTION IN MINIATURE SWINE. Douglas R. Johnston,[1] Ruediger Hoerbelt,[1] Joshua D. Mezrich,[1] Louis C. Benjamin,[1] Tsuyoshi Shoji,[1] Richard S. Lee,[1] Stuart L. Houser,[1] Levi G. Ledgerwood,[1] Rebecca S. Hasse,[1] James S. Allan,[1] Mohamed H. Sayegh,[2] Joren C. Madsen.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA; [2]Department of Nephrology, Brigham and Womens' Hospital, Boston, MA.*

INTRODUCTION: The role of MHC class II antigens in chronic rejection is unclear. We have examined the role of indirect recognition of polymorphic donor class II allopeptides in the rejection of class II-disparate cardiac allografts using tacrolimus-treated miniature swine. METHODS: Heterotopic heart transplants were performed across class II barriers in MHC defined MGH miniature swine treated with a 12-day course of tacrolimus. Recipients were immunized with 7 polymorphic peptides spanning the α1 domains of SLAcc genes DR and DQ in complete Freund's adjuvant on POD -21. Sensitization was confirmed by positive response to DTH testing on POD -7 and by the development of in vitro proliferative response to the immunized peptides. Recipients were evaluated by serial histologic analysis and in vitro MLR peptide proliferation assays. RESULTS: Hearts transplanted into unimmunized control animals (n=3) survived >100 days (POD 113, 120, 134), and developed lesions of CAV on POD 75, 92 and 99 (n=3). Animals immunized with class IIc peptides developed positive DTH responses and in vitro proliferative responses to certain peptides. In contrast to controls, hearts in class IIc peptide-immunized hosts (n=4) rejected significantly earlier (POD 35, 44, 46, 54; P<0.01) and developed accelerated CAV (POD 22, 24, 28, 22; P<0.01). Immunization with a control, rat MHC peptide did not lead to accelerated rejection (n=1). Development of CAV was accompanied by increased in vitro responses to peptide in unimmunized animals, and by a shift in peptide response toward DQ antigens in immunized animals (shifting immunodominance). CONCLUSIONS: Indirect recognition of polymorphic MHC class II allogeneic peptides can accelerate acute rejection and CAV in class II-disparate swine. The pattern of peptide responses changes over time and with progression of rejection. This finding may have important implications for clinical tolerance protocols.

Abstract# 1512 Poster Board #-Session: P268-III

TOLL-LIKE RECEPTOR 4 MUTATIONS DO NOT AFFECT THE REJECTION OF SKIN ALLOGRAFTS IN MICE. Benjamin Samstein,[1,2] Jeffrey L. Platt.[1] *[1]Surgery, Mayo Clinic, Rochester, MN; [2]Surgery, New York Presbyterian Hospital, New York, NY.*

We sought to determine the extent to which alloimmune responses to major and minor antigens depend on TLR4 signaling.**Methods**: Male and female 6-12 wk-old, Balb/c, C3H/HeJ, C3H/HeSnJ, C57BL10ScNcr and C57BL10SnJ were obtained. Orothotopic tail skin grafts were transplanted. Grafts were scored using a three-category system including hair loss, scaling and scarring. Grafts meeting all three categories were determined to have rejected.**Summary of Results**: To investigate the role of toll-like receptors in the rejection of allografts, we carried out skin transplants from Balb/c (H-2^d) to C3H/HeJ (H-2^k). C3H/HeJ mice have a single amino acid substitution, which renders the TLR4 incapable to interacting with its downstream signaling molecule, MyD88. Alteration in TLR4 did not impact on the immune response to fully MHC-mismatched skin allografts, MST 11.1days versus 11.5 days (see Table I). To test whether a defect in TLR4 and not some other C3H/HeJ specific defect was responsible for this result, we tested allografts from another TLR4 mutant mouse strain C57BL10ScNCr (H-2^b). The C57BL10ScNCr mouse has a naturally occurring deletion of chromosome 4 resulting in no expression of TLR4. Like the C3H/HeJ mice, no differences were noted between the TLR4 mutant and wild type strains, MST 9.5 days versus 10.0 days (see Table I). To determine if the role of TLR4 might be more easily seen with minor antigen differences, we next performed skin transplants in mice differing only by H-Y antigen. The donor and the recipients had identical TLR4 function. There was again, no significant difference between TLR4 normal and mutant mice. In C57BL10 background mean graft survival was 27.5 days versus 25.0 days, while in the C3H background the survival was 89.9 days versus 94.4 days (see Table II). **Conclusions**: There is much interest in inhibition of TLR pathway for modulation of immune responses. While TLRs other than TLR4 may contribute to initiation of alloimmune responses, our results suggest that rejection of allografts occurs without perturbation regardless of TLR4 function.

MHC-mismatched Skin Grafts in TLR4 Normal and Mutant Mice

Donor	Recipient	MST (days)	number
Balb/c	C3H/HeJ	11.1 d	11
Balb/c	C3H/HeSnJ	11.1 d	6
Balb/c	Balb/c	>25 d	5
Balb/c	C57BL10ScNCr	9.5 d	5
Balb/c	C57BL10SnJ	10 d	5

H-Y mismatched Skin Transplants in TLR4 normal and mutant mice

Background	Sex	MST (number)
C57BL10ScNcr	M—F	27.5 d (5)
C57BL10SnJ	M—F	25 d (9)
C57BL10SnJ	F—F	> 100 d (8)
C3H/HeJ	M—F	89.9 d (15)
C3H/SnJ	M-F	94.4 d (18)
C3H/SnJ	F-F	> 100 d (10)

Abstract# 1513 Poster Board #-Session: P269-III

PRESERVATION OF RENAL ALLOGRAFT FUNCTION BY VITAMIN D. Debra A. Hullett,[1] Joseph M. Yracheta,[2] Hans W. Sollinger,[1] Byran N. Becker.[2] *[1]Department of Surgery, University of Wisconsin-Madison, Madison, WI; [2]Department of Medicine, University of Wisconsin-Madison, Madison, WI.*

Introduction: $1,25(OH)_2D_3$, the active metabolite of vitamin D, regulates immune responses and prevents both acute and chronic rejection. We have previously shown that 1,25(OH) prolongs allograft survival in rat model of chronic allograft nephropathy (CAN). The development of interstitial fibrosis and glomerulorsclerosis, common features of CAN, are associated with elevated levels of TGFβ-1 expression. Recent studies have suggested crosstalk between the TGFβ-1 and vitamin D signaling pathways. TGFβ-1 regulates gene transcription through the Smad signaling proteins. In previous work we have shown that $1,25(OH)_2D_3$ treatment of allograft recipients inhibits interstitial fibrosis. In this study we examined the ability of $1,25(OH)_2D_3$ to prevent glomerulosclerosis. **Methods:** The Fisher to Lewis rat model of CAN was used. Recipients were treated with $1,25\text{-}(OH)_2D_3$ (1000 or 500 mg/kg/day) orally. Urinary protein was monitored until graft harvest. Histological sections and lysates were prepared at 24 weeks and analyzed by immunoblotting, immunohistochemistry and ELISA. **Results:** Monotherapy with $1,25\text{-}(OH)_2D_3$ significantly (p=0.004) lowered urinary protein in comparison to untreated allogeneic controls or allogeneic recipients treated with low dose CSA (1.5 mg/kg/d;10 days). Histological examination of Mason's trichrome stained section revealed decreased collagen deposition in the glomeruli. Bioactive TGFβ-1 levels in renal lysates of $1,25\text{-}(OH)_2D_3$-treated recipients as determined by ELISA were not different from untreated allogeneic controls. However, immunhistochemistry for TGFβ-1 revealed significantly decreased TGFβ-1 expression in the glomeruli in treated recipients. No difference in tubular staining was observed. Immunoblotting of renal lysates showed a dramatic reduction in receptor-activated Smad 2 expression in $1,25\text{-}(OH)_2D_3$- treated allogeneic recipients (>200 fold p=0.04). Immunostaining for Smad 2 showed significantly reduced tubular staining and no glomerular staining in $1,25\text{-}(OH)_2D_3$-treated recipients. **Conclusions:** Our data demonstrate that in the setting of CAN $1,25\text{-}(OH)_2D_3$ decreases fibrosis and maintains glomerular structure and function. The data also suggest that $1,25\text{-}(OH)_2D_3$ through its receptor may prevent CAN by interacting with the TGFβ signaling pathway. These results are consistent with our observation of stabilized renal function in transplant recipients placed on calcitriol. Finally, $1,25\text{-}(OH)_2D_3$ may provide a significant advance in immuosuppressive therapy by prolonging graft survival in addition to preventing bone loss.

Abstract# 1514 Poster Board #-Session: P270-III

THE EFFECT OF IN VIVO TREATMENT WITH ANTI-ASIALO GM1 ANTIBODIES ON THE SURVIVAL OF RAT LIVER ALLOGRAFTS. Hideaki Obara,[1] Christine L. Hsieh,[1] Yasuhiro Ogura,[1] Carlos O. Esquivel,[1] Olivia M. Martinez,[1] Sheri M. Krams.[1] *[1]Department of Surgery, Stanford University School of Medicine, Stanford, CA.*

The role of NK cells in allograft rejection after solid organ transplantation remains unclear. While cytotoxic T lymphocytes are generally thought to be important in the process of rejection, we have shown that depletion of $CD8^+$ T cells does not prevent rejection in a fully MHC-mismatched model of rat liver transplantation (DA to Lewis). Moreover, a significant population of recipient derived NKR-$P1^+$ NK cells found in the graft early after transplantation express IFN-γ, granzyme B and Fas ligand. To study the role of NK cells in allograft rejection we used rabbit anti-asialo GM1 antisera (AGM1) and the anti-NK3.2.3 monoclonal antibody (NK3.2.3) to eliminate NK cell activity in vivo. In preliminary experiments peripheral blood mononuclear cells were isolated from control Lewis rats or Lewis rats treated with either rabbit AGM1 or NK3.2.3 and analyzed by flow cytometry. NK3.2.3 binds to both rat NK and NKT cells and selectively, but only partially, depletes NKR-$P1^+$ cells. In contrast asialo GM1 is expressed on all NKR-$P1^+$ cells and most (90%) TCR$\alpha\beta^+$ cells and treatment with AGM1 depletes all NK cells, some $CD8^+$ T cells, but not $CD4^+$ T cells. To define the role of NK cells after liver transplantation we studied the effect of AGM1 or NK3.2.3 on allograft survival. Lewis recipients of DA allografts treated with NK3.2.3 (100 μL) on either day -2, -1, 1 or day -2, -1, 1, and every 3 days thereafter did not have prolonged survival compared with untreated recipients (MST 12 vs. 11 days; n=5). Similarly, recipients treated with multiple doses of AGM1 (100 μL/day) did not have prolonged survival. In contrast allografted recipients treated with a single dose of AGM1 (50 μL) on day -1 had significantly prolonged survival (MST 33 days; n=8). In an AGM1-treated recipient who survived for more than 100 days, we performed tail skin grafts from both DA (donor), and PVG (third party) to investigate whether donor-specific tolerance was induced. As expected, the PVG (third party) graft was rejected at 14 days. In contrast the DA (donor) graft was accepted suggesting that donor-specific tolerance was present. Taken together these data suggest that $AGM1^+$ cells play a role in liver allograft rejection and that modulation of this population may promote long-term graft survival.

Abstract# 1515 **Poster Board #-Session: P271-III**

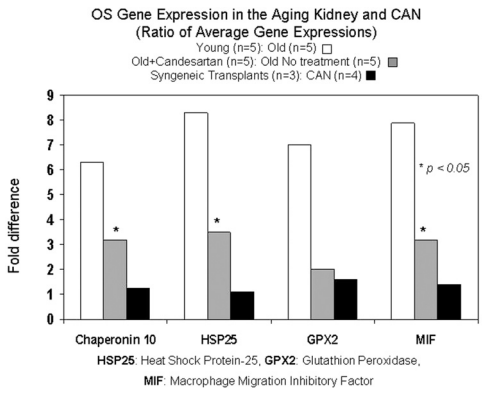
THE ANTIOXIDANT VITAMIN C SYNERGIZES WITH CYCLOSPORINE TO IMPROVE CARDIAC DYSKINESIS VIA AN INOS-INDEPENDENT PATHWAY. Thanhhang K. Nguyen,[1] Allan M. Roza,[1] Christopher C. Johnson,[1] Mark B. Adams,[1] Gail Hilton,[1] Galen M. Pieper.[1] *[1]Transplant Surgery, Medical College of Wisconsin, Milwaukee, WI.*

Graft rejection may involve oxidant stress with increased reactive oxygen production and/or decreased antioxidant defenses, leading to myocardial contractile dysfunction. **PURPOSE:** In the present study, we examined cardiac contractility by sonomicrometry and the mechanism of action of an antioxidant vitamin C (VitC) in allograft rejection. **METHODS:** Allogeneic (WF→Lew) or isogeneic (Lew→Lew) transplantation of donor rat hearts was performed. Allogeneic recipients were untreated or received 120 mg/100g VitC, 2.5 mg/kg CsA (post-operative day 0-7) or VitC + CsA. Graft function was monitored daily by palpation and by measuring myocardial % segment shortening (%SS) by sonomicrometry. Nuclear binding activity of redox-sensitive transcription factors, nuclear factor κB (NF-κB) and activator protein (AP-1), was determined by electrophoretic mobility shift assays (EMSA). Western blot analysis was used to evaluate the NF-κB-dependent gene, inducible nitric oxide synthase (iNOS). Plasma NO metabolites were measured by Griess reaction. **RESULTS:** There was a significant decrease in %SS at post-operative day 6 (3.70 ± 0.47 isograft; 0.56 ± 0.17 allograft, $P < 0.0002$) that was improved with treatment (2.01 ± 0.19 VitC + CsA, $P < 0.005$ vs. allograft). NF-κB nuclear binding activity was increased in allograft and was blocked by VitC + CsA therapy but not VitC alone. However, VitC alone blocked AP-1 binding activity (in densitometry arbitrary units: 123,070 ± 4486 isograft;186,597 ± 25,620 allograft; 128,968 ± 7530 VitC, $P < 0.05$). iNOS protein was present in allografts at post-operative day 4 but not isografts or native hearts of allograft recipients. iNOS protein was unaltered by VitC alone but decreased in the presence of VitC + CsA (in densitometry arbitrary units: 54,123 ± 2010 allograft; 21,031 ± 3989 VitC + CsA, $P < 0.0001$). Plasma NO metabolites (in μM) were increased in allografts but inhibited with CsA only (8.87 ± 0.86 isograft; 39.50 ± 3.50 allograft; 12.10 ± 0.87 CsA; 43.81 ± 1.94 VitC; 16.27 ± 1.57 VitC + CsA). **CONCLUSIONS:** These findings demonstrate that VitC alone may have protective effects through a NO-independent mechanism. Furthermore, VitC appears to preferentially inhibit activation of the redox-sensitive transcription factor AP-1 vs. NF-κB pathway in cardiac allograft rejection.

Abstract# 1516 **Poster Board #-Session: P272-III**

OXIDATIVE STRESS IN THE AGING KIDNEY AND CHRONIC ALLOGRAFT NEPHROPATHY. Arjang Djamali,[1] Joe Yracheta,[1] Lynn Jacobson,[1] Jenifer Sprague,[1] Kurt Saupe,[3] Debra Hullett,[2] Bryan N. Becker.[1] *[1]Medicine, Nephrology Section, University of Wisconsin, Madison, Madison, WI; [2]Surgery, Transplant Division, University of Wisconsin, Madison, Madison, WI; [3]Medicine, Cardiology Section, University of Wisconsin, Madison, Madison, WI.*

Chronic allograft nephropathy (CAN) manifests histopathologic features of accelerated senescence and oxidative stress (OS). This invokes the pathogenesis of aging in CAN. To directly address the association of OS with kidney aging and CAN and to assess the effect of angiotensin receptor blockers as anti-aging treatment, we performed 2 sets of experiments. First, young (6m old) and old (18m old) Fisher344 (F344) rats were given Candesartan (10mg/kg/d) or no treatment for 3 months (n=5-7 in all 4 groups). Next, in an established model of CAN, F344 to Lewis (L) (n=4) and L to L kidney transplants (n=3) were performed in 6m old animals. Allograft recipients were treated with low dose cyclosporine A (1.5mg/kg/d) for 10 days. Animals were all sacrificed at 9m except the old rats in experiment #1. These were sacrificed at 21m. HE staining of kidneys in the aging animals revealed significant interstitial fibrosis, tubular atrophy and glomerular thrombosis in old untreated animals versus old Candesartan-treated and young untreated animals ($p < 0.01$ for each). A 96 gene Stress Toxicity micraoarray (GeArray) was then utilized to analyze the OS gene profile in all groups. Data were normalized to the background and GAPDH, the housekeeping gene. The ratios of average gene expression between different groups demonstrated significant differences in HSP25 and chaperonin 10 levels.



Immunohistochemical staining for HSP25 demonstrated increased levels of glomerular and tubular protein expression in old Candesartan-treated vs untreated rats and in syngeneic vs allogeneic grafts. Our data demonstrate that (1) OS is associated with kidney aging and that treatment with Candesartan reverts the OS gene profile to a youthful genotype. (2) Senescence, at least as manifested by OS, appears to be associated with CAN, though the extent of its impact on the evolution of CAN seems to be less than in aging non-transplanted kidney.

Abstract# 1517 **Poster Board #-Session: P273-III**

A20 PROTECTS THE GRAFT ENDOTHELIUM AGAINST DEATH RECEPTOR (TNF AND FAS) INDUCED APOPTOSIS, NK CYTOTOXICITY AND COMPLEMENT MEDIATED NECROSIS. Soizic Daniel,[1] Maria B. Arvelo,[1] Virendra I. Patel,[1] Christopher R. Longo,[1] Tala Shukri,[1] Jerome Mahiou,[1] Shane T. Grey,[1] Christiane Ferran.[1] *[1]Immunobiology Research Center. Department of Surgery, Beth Israel Deaconess Medical Center. Harvard Medical School, Boston, MA.*

Objective: Endothelial cells (EC) are the first targets that encounter the host immune attack in a vascularized graft. This immune attack often leads to the loss of EC through apoptotic and necrotic cell death. Loss of this anti-inflammatory and anti-thrombotic barrier has been implicated in the pathophysiology of acute and chronic allograft rejection as well as delayed xenograft rejection. In this study, we analyzed the effect of the cytoprotective protein A20 in EC. Methods and Results: Our data demonstrates that overexpression of A20 by means of recombinant adenoviral mediated gene transfer protects EC from TNF mediated apoptosis (FAC analysis of DNA content) by inhibiting proteolytic cleavage of apical caspase 8 and 2 and executioner caspases 3 and 6, as assessed by colorimetric assays and Western blot analysis. A20 also blocked Bid processing and release of cytochrome c (western blot) hence preserving mitochondrion integrity (mitochondrial transmembrane potential). Beyond protection from TNF, A20 protects EC from Fas mediated apoptosis by inhibiting caspase 8 activation and significantly blunts natural killer cells mediated cytotoxicity by blocking the perforin/granzyme B pathway (calcein release). In addition to protecting EC from apoptotic stimuli, A20 safeguards EC from complement mediated necrosis (LDH release). This is the first demonstration that the cytoprotective effect of A20 in EC extends beyond protecting from TNF to significantly shutting down death pathways associated with cell mediated toxicity (Fas/FasL and perforin/granzyme B) as well as complement mediated necrosis. Conclusion: These results strongly suggest that expression of A20 in EC would positively impact upon graft survival and validate the rationale for pursuing A20 as a gene therapy candidate for the protection of vascularized grafts against inflammatory and immune based attack.

Abstract# 1518 **Poster Board #-Session: P274-III**

INTERPLAY BETWEEN PERFORIN AND IFN-γ IN KIDNEY TRANSPLANT REJECTION. EVIDENCE THAT PERFORIN IN CTL CAN MEDIATE MICROVASCULAR INJURY AND GRAFT NECROSIS. Philip F. Halloran,[1] Joan Urmson,[1] Adis Tasanarong,[1] Lin-Fu Zhu.[1] *[1]Medicine, University of Alberta, Edmonton, AB, Canada.*

Kidney allograft rejection is accompanied by infiltration with perforin/granzyme +ve CD8 T cells, with tubulitis and arteritis. Nevertheless, the tubulitis and arteritis are independent of perforin and antibody (ATC 2002). However, during rejection, kidneys from donors lacking IFN-γ receptors (GRKO mice) also develop microvascular congestion and ischemic necrosis of the parenchyma. We studied the role of perforin and alloantibody in the aggressive injury in kidneys lacking IFN-γ receptors. Donors were either wild type CBA or CBA lacking IFN-γRs, transplanted into host B6 mice that were wild type, immunoglobulin deficient (IgKO), or perforin deficient (PerfKO). Kidneys were studied at days 7 and 21.

Days Post Tx	Day 7				Day 21		
Donor	CBA	GRKO	GRKO	GRKO	CBA	GRKO	GRKO
Host	B6	B6	IgKO	PerfKO	B6	B6	PerfKO
(# of mice)	(20)	(15)	(13)	(5)	(5)	(5)	(6)
# of mononuclear cells in interstitium	162	108	98	195	149	14	172
Necrosis %	**0.25**	**22**	**49**	**3.0 ***	**26**	**86**	**8 ***
Peritubular capillary congestion %	**1.8**	**46**	**67**	**15 ***	**18**	**30**	**1.7 ***
Tubulitis %	26	36	35	46	68	45	78
Interstitial Infiltrate	53	51	50	62	53	33	53
Arteritis	1.3	0.1	0.6	0	1.4	1.0	1.2
Donor Class I	+++	-	-	-	+++	-	-

* significant difference between GRKO into B6 vs GRKO into PerfKO

Results: As previously reported, kidney allografts developed tubulitis, interstitial infiltrate, and arteritis in PerfKO hosts, indicating perforin is not required for these lesions. IFN-γR KO (GRKO) kidneys in B6 hosts underwent necrosis and peritubular capillary congestion (PTC) whereas those from donors with intact IFN-γRs did not. The grafts into hosts lacking immunoglobulins (IgKO) still underwent necrosis and capillary congestion, proving these lesions were not due to antibody. However, GRKO grafts into hosts lacking perforin did not undergo necrosis and PTC. Conclusion: Although not necessary for tubulitis and arteritis perforin has a unique role in microvascular injury when IFN-γ cannot act on the graft. Thus the mechanism of aggressive destruction of IFN-γ deficient grafts is T cell mediated and perforin dependent. Perforin dependent microvascular injury is normally inhibited by IFN-γ, indicating a powerful interplay between host Perforin + graft IFN-γRs at the level of rejecting graft. This is the first non redundant role identified for perforin in kidney graft rejection.

Abstract# 1519 Poster Board #-Session: P275-III

RENAL TUBULAR EPITHELIAL CELLS (TEC) CAN MEDIATE Fas-FasL DEPENDENT "SELF APOPTOSIS" AND AUGMENT RENAL ALLOGRAFT INJURY. Caigan Du,[1,3,4] Qiunong Guan,[2] Ziqin Yin,[2] Mark Masterson,[2] Robert Zhong,[1,2,3,4] Anthony M. Jevnikar.[1,2,3,4] *[1]Transplantation, Immunity and Regenerative Medicine, Lawson Health Research Insititute, London, ON, Canada; [2]Transplantation, Robarts Research Insititute, London, ON, Canada; [3]Multi Organ Transplant Program, London Health Sciences Centre, London, ON, Canada; [4]University of Western Ontario, London, ON, Canada.*

The role of Fas/FasL interactions in kidney allograft injury may be complex as renal tubular epithelial cells (TEC), like T cells, can express both Fas and FasL. The role and regulation of TEC "self" injury by expression of Fas/FasL has not been reported. Firstly we tested whether limiting the expression of either Fas or FasL alters renal injury in a transplant model. C57BL/6 (B6) mice were transplanted (n = 9-12/group) with Fas deficient C3H-Faslpr (lpr), FasL deficient C3H-FasLgld (gld), or normal (wild type, WT) C3H/Hej kidneys. No treatment was given and mice were sacrificed at 15±1 days. lpr and gld recipient survival was equivalent but improved compared to WT donors ($p < 0.05$). lpr and gld recipients had lower serum creatinines (41±8 and 52±7 umol/l) than WT (84±8 umol/l, $p<0.001$). As Fas and FasL engagement can alter T cell activation we co-cultured T cells with cloned WT, gld and lpr TEC for 24-48 hours. B6 T cell expressed equivalent levels of CD25, CD45, and CD69 suggesting allograft survival differences were not due to changes in TEC mediated T cell activation. We then tested whether attenuation of injury in Fas or FasL deficient kidneys might have been related to attenuated "self" injury of TEC. Apoptosis was measured using Annexin V and specific TEC were identified in co-cultures using DIL membrane labeling. FasL bearing / Fas deficient lpr TEC were able to induce apoptosis in Fas bearing TEC including WT (15.2±2.4 %) and gld (6.4±0.3 %) after 24 hours of co-incubation. They were unable to induce apoptosis in Fas deficient TEC. Furthermore FasL deficient gld-TEC could not induce apoptosis in either lpr-TEC or "self". TEC may require injury (anoikis) or cytokine activation to become susceptible to "self apoptosis". TEC monolayers treated for 24h with IFN-g (250U/ml) in serum free-conditions lead to apoptosis in WT (26.9±7.5%) but not in Fas or FasL deficient TEC, which were equivalent to non treated controls (< 10%). Results were similar using TNF-a. These results demonstrate the complexity of Fas/FasL interactions in kidney allograft injury. Fas deficiency may reduce TEC injury by FasL on T cells during rejection. However, during stress or cytokine activation, TEC may commit a novel form of self injury ("fratracide") which requires TEC expression of Fas/FasL. These data suggest that inhibition of renal Fas or FasL might be a useful strategy to prevent TEC loss during rejection.

Abstract# 1520 Poster Board #-Session: P276-III

DOES DONOR AGE AFFECT IMMUNOGENICITY OR TISSUE FRAGILITY? EVIDENCE THAT OLD KIDNEYS DEVELOP EQUIVALENT INFLAMMATION BUT MORE TUBULITIS AND TUBULAR ATROPHY. Anette Melk,[1] Bernhard M. W. Schmidt,[1] Joan Urmson,[1] Kim Solez,[2] Philip F. Halloran.[1] *[1]Division of Nephrology and Immunology, University of Alberta, Edmonton, AB, Canada; [2]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada.*

Kidneys from older donors show decreased survival, but it is not clear whether this reflects increased immunogenicity or increased tissue susceptibility to injury. We therefore compared the pathology of rejection in transplants from young versus old donors. We studied vascularized mouse kidney transplants (CBA into B6) at day 7 after transplantation. Donor age was 3 (n=4) or 18 (n=3) months. Banff lesions were graded semi-quantitatively. MHC expression and cytology of the infiltrate were determined by immunoperoxidase staining. Cytotoxic molecules were analyzed by Taqman RT-PCR. Tubular diameter was measured using ImageJ computer software. Parameters of infiltration and inflammation were similar in young versus old kidneys (table 1). Donor and recipient MHC class I and II, cell counts for $CD45^+$, $CD3^+$, $CD4^+$ and $CD8^+$ and mRNA levels for cytotoxic molecules (granzyme B, perforin) were the same. However, older transplants showed more tubulitis (table 2). In rejecting older donor kidneys, tubular crossections were smaller and the tubular basement membrane showed intense wrinkling. These features suggest tubular atrophy and were not present in age-matched normal control mice. Conclusion: Older kidneys show no difference in MHC induction, T cell infiltration, or cytotoxic molecule expression. However, they rapidly developed features of tubular atrophy and tubulitis when they experienced rejection. Thus our findings indicate that the problem with older donor kidneys is not more immunogenicity, but greater susceptibility of the old parenchyma to tubular invasion, deterioration and atrophy when rejection occurs.

	Donor MHC I	Donor MHC II	CD45+ [#]	CD3+ [#]	CD8+ [#]	Granzyme B	Perforin
young (3 mo)	3.3 ± 0.5	4.0 ± 0.0	210 ± 28	170 ± 16	174 ± 25	175 ± 34	226 ± 117
old (18 mo)	2.0 ± 2.0	2.7 ± 2.3	215 ± 15	177 ± 40	148 ± 19	179 ± 62	224 ± 60

	tubulitis [%]	inter. infiltrate [%]	arteritis [%]	tubular size	tubular wrinkling [%]	cells/tubular cross section [#]
young (3 mo)	28 ± 5	55 ± 10	1.0 ± 0.8	1.4 ± 0.2	4 ± 6	4.3 ± 0.7
old (18 mo)	47 ± 12*	60 ± 17	1.7 ± 2.1	1.0 ± 0.1*	48 ± 29*	3.8 ± 0.4

* $p<.05$ vs. young, Mann-Whitney U test

Abstract# 1521 Poster Board #-Session: P277-III

SENESCENCE MARKER $P16^{INK4A}$ IS INDUCED IN ACUTE REJECTION: EVIDENCE THAT REJECTION INDUCES EPITHELIAL CELL SENESCENCE. Anette Melk,[1] Bernhard M. W. Schmidt,[1] Oki Takeuchi,[1] Joan Urmson,[1] Philip F. Halloran.[1] *[1]Division of Nephrology and Immunology, University of Alberta, Edmonton, AB, Canada.*

Donor age is the major predictor of function and survival of kidney transplants. This could represent the effect of cell senescence due to age, coupled with accelerated senescence due to transplant stresses such as rejection. In the present experiments we sought robust markers for somatic cell senescence *in vivo*, and examined the effect of rejection on those markers. We surveyed the expression of genes previously associated with cell senescence *in vitro* in mice of different ages (n=37). The genes were $p16^{INK4a}$, $p19^{ARF}$, regucalcin, metallothioneins 1 and 3, HIC-5, TGF-β and Hsp70 with expression measured by real-time RT-PCR. Only $p16^{INK4a}$ was very low in development and strongly induced in aging, making it a unique candidate marker for somatic cell senescence. $P16^{INK4a}$ expression increased significantly with age in nuclei of tubules, with less interstitial and glomerular staining. We then studied how acute rejection affected $p16^{INK4a}$ expression in young (3 months, n=4) vs. old (18 months, n=3) donor kidneys. In rejecting kidneys at day 7 (CBA into B6) , $p16^{INK4a}$ mRNA and protein increased in young and old donors. Increased $p16^{INK4a}$ protein were found in tubular, interstitial and glomerular cells. The increases in glomerular staining were striking since this was rare in normal aging. Interstitial staining probably reflects infiltrating lymphocytes that show $p16^{INK4a}$ expression, suggesting that these lymphocytes underwent senescence in the graft with no difference between young and old donor kidneys. Conclusion: Acute rejection induces the senescence marker in cells of young and old kidneys, adding to senescence due to age. Overall $p16^{INK4a}$ expression was highest in rejecting kidneys from old donors. Thus, older kidneys not only show more age-related senescence, but the number of senescent cells increases with acute rejection. This may limit their capability to repair peritransplant injuries and to maintain organ function and mass.

	p16INK4a mRNA [units]	p16INK4a in tubules [% of nuclei]	p16INK4a in glomeruli [% of nuclei]	p16INK4a in interstitium [% of nuclei]
normal young (3 mo)	6.5 ± 3.0	8.4 ± 6.6	2.8 ± 1.6	2.1 ± 0.5
tx young (3 mo)	18.1 ±10.8†	23.9 ± 4.3†	24.2 ± 9.6†	17.3 ± 5.0†
normal old (18 mo)	82.0 ± 35.9*	44.3 ± 7.1*	9.1 ± 7.0	7.3 ± 2.7*
tx old (18 mo)	148.2 ± 96.9**	56.5 ± 8.4	36.3 ± 5.5‡	18.8 ± 6.0

* <.05 vs. normal young; **<.05 vs. all groups; †<.05 vs. normal young; ‡<.05 vs. normal old

Abstract# 1522 Poster Board #-Session: P278-III

SIGNIFICANT REDUCTION IN SERUM CITRULLINE LEVELS IN INTESTINAL ISCHEMIA/REPERFUSION INJURY AND SMALL BOWEL GRAFT REJECTION MODELS. T. Fukumori,[1] S. Santiago,[1] S. Salgar,[1] P. Ruiz,[1] N. Wasserberg,[1] W. De Faria,[1] P. Tryphonopoulos,[1] C. Gandia,[1] P. Pappas,[1] J. M. Saudubray,[2] V. Esquenazi,[1,3] J. Miller,[1] A. Tzakis.[1] *[1]University of Miami School of Medicine, Miami, FL; [2]Pediatric Genetics, Hospital Necker, Paris, France; [3]VA Medical Center, Miami, FL.*

Background: An assay for detecting acute cellular rejection after small bowel transplantation (SBTx) has been a goal of several studies. Serum citrulline is almost exclusively derived from citrulline synthesized by the intestinal mucosa. In this study, citrulline levels were measured in a rat intestinal ischemia/reperfusion (I/R) injury model and in orthotopic intestinal transplant (OIT) rejection model. **Materials and Methods:** I/R injury was induced in male LEW rats by clamping superior mesenteric artery for 60 min. Animals were sacrificed at 3, 5, 12, 24, 48, and 72 h after I/R, and intestinal specimens were collected for histopathology (HP) and blood for citrulline analysis. Mucosal damage was determined by Park's score 0 - 8. OITs were performed (DA to LEW) without any immunosuppression. Pre- and Post-Tx blood samples were collected for serum citrulline analysis. Rats were sacrificed on day 7 and intestinal specimens were collected for HP. **Results:** The Park's score observed at 3, 5, 12, 24, 48, and 72 h after I/R were 3.7 (denuded villi), 3.0 (epithelial lifting along the villous side), 1.5 (extended subepithelial space), 1.25 (subepithelial space), 1.5, and 1.25, respectively. Marked mucosal damage was observed at 3 and 5 h after I/R ($P < 0.05$), and the mucosa rapidly returned almost to normal thereafter. The mean serum citrulline levels were 80, 69, 63, 49, 52, 74, and 87 µmol/L in serum obtained before fasting, after fasting, 5, 12, 24, 48, and 72 h post-I/R, respectively. Serum citrulline levels significantly reduced at 12 and 24 h after I/R, and returned to normal (before fasting) levels at 48 h after I/R. The severity of mucosal damage preceded the onset of reduced levels of citrulline in the serum. In OIT recipients, citrulline concentration was 97, 52, 67, and 46 µmol/L in pre-Tx, 3, 5, and 7 days post-Tx serum samples, respectively. Post-Tx citrulline levels were significantly ($P<0.001$)lower than pre-Tx level. Mean biopsy proven rejection on day 7 post-Tx was 2.0; correspondingly citrulline level was low (46 µmol/L). **Conclusions:** The serum citrulline levels were inversely proportional to the intestinal mucosal damage that occurred due to I/R injury, and were significantly reduced ($P<0.001$) during SB graft rejection.

Abstract# 1523 **Poster Board #-Session: P279-III**

***IN VITRO* IMMUNOSUPPRESSIVE EFFECTS OF GANCICLOVIR IN COMBINATION WITH MYCOPHENOLIC MOFETIL.** Christopher M. Bearden,[1] Benita K. Book,[1] Richard A. Sidner,[1] Edward F. Srour,[2] Mark D. Pescovitz.[1,2] *[1]Department of Surgery, Indiana University School of Medicine, Indianapolis, IN; [2]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN.*

Introduction: Cytomegalovirus (CMV) infection has been implemented as a direct cause of acute rejection in kidney transplant patients. Patients treated prophylactically for CMV infection have been noted to have lower acute rejection rates and when combined with mycophenolate mofetil (MMF) also have a higher rate of myelosuppression. It has been hypothesized that the reduced rejection rate results from the reduced rate of CMV. The effects of ganciclovir (GCV) and MMF on lymphocyte proliferation and neutrophil maturation have not been studied. We propose as an alternative hypothesis for the reduced rejection rate with anti-CMV treatment and increased myelosuppression when GCV is used with MMF that there is additive inhibition of cell growth. **Methods:** Phytohemagglutinin (PHA) responses and allogeneic stimulation were measured in MHC class II disparate individuals with mycophenolic acid (MPA), the active form of MMF, and GCV in clinically relevant concentrations. Data represent percent inhibition ± SD and were determined using [^{3}H]-thymidine incorporation. CD34+ hematopoietic precursor cells were prepared from bone marrow aspirates of CMV negative samples. Cells were cultured for 7 days then exposed to MPA and GCV. After 2 weeks, they were manually counted and analyzed by flow cytometry for CD11b, CD14, CD15, CD33, and CD34. Comparisons were performed using Student's t-Test. **Results:** Compared to controls, GCV at 5, 10, and 15μg/ml significantly decreased PHA responses by 23%±15, 46%±11, and 60%±7 ($P<0.005$) and allogeneic responses by 26%±16, 54%±11, and 67%±8 ($P<0.005$). MPA alone at 30ng/ml decreased PHA stimulation by 33%±8. The addition of GCV at 5, 10, and 15μg/ml further decreased this proliferation by 45%±8, 53%±7, and 60%±6($P<0.005$ vs MPA alone). Compared to controls, GCV significantly decreased bone marrow cell proliferation by 44%±9, 58%±6, and 81%±4 at each dose. MPA alone at 30 ng/ml decreased proliferation by 56%±10 which was not different than GCV at 10 or 15μg/ml. Flow cytometry revealed no differences in cell phenotypes between treated and untreated cultures. **Conclusion:** GCV had similar *in vitro* immunosuppressive activity compared with MPA within pharmacologic ranges. Combined, the drugs had additive immunosuppressive and antiproliferative effects. The reduction in acute rejection rates seen with GCV prophylaxis may be through its immunosuppressive activity rather than through prevention of CMV disease.

Abstract# 1524 **Poster Board #-Session: P280-III**

CO DELIVERY IN THE DONOR IMMEDIATELY PRIOR TO ORGAN HARVESTING PREVENTS ISCHEMIA/REPERFUSION INJURY AND CHRONIC GRAFT DETERIORATION. Paulo Ney A. Martins,[1] Anja Reutzel-Selke,[1] Kirstin Atrott,[1] Anke Jurisch,[1] Johann Pratschke,[1] Roland Buelow,[2] Peter Neuhaus,[1] Hans-Dieter Volk,[3] Stefan G. Tullius.[1] *[1]Dept. of Surgery, CharitéVirchow-Clinic, Humboldt-University, Berlin, Germany; [2]SangStat Inc., Fremont, CA; [3]Dept. of Med. Immunology, CharitéHumboldt-University, Berlin, Germany.*

We and others have shown the beneficial effects of HO-1 induction for the prevention of both acute and chronic rejection before. HO-1 degrades Heme into CO, Fe and Biliverdin. We followed the effects of CO delivery by methylene chloride (MC/100mg/kg/p.o.) on chronic graft rejection and prolonged cold ischemia. F-344 renal allografts were grafted into Lew recipients after a 6h cold ischemia. Recipients received a short term CyA treatment (1.5 mg/kg/d x 10 d). 4 groups (n=6/group) were followed for 180 days: Donor treatment with MC at -4h prior to organ harvesting (group 1), recipient short (10 days) or long-term (180 d) MC administration (groups 2 and 3). Controls in group 4 did not receive MC. Animals in all groups survived the observation period. CoHb peaked at 6% by 3.5 hours after MC application, returning to norm values by 24 h (2%). Proteinuria was reduced significantly following MC treatment both, after a long-term application in the recipient or after a single treatment in the donor (control: 60±20 mg/24h vs. MC recipient long-term: 11±2 mg/24h and MC donor short: 15±1 mg/24h, respectively; $p < 0.05$). Morphological alterations characteristic of chronic graft deterioration had improved in all groups following CO delivery. Improvements were particularly impressive following long-term application in the recipient and even more pronounced following a single donor treatment (glomerulosclerosis by 180 days: 20±5 % following donor treatment; 30±8% following long-term MC application, and 80±10% in controls/$p < 0.0001$ and 0.001, respectively). While ED1+ MØ, CD4+ T cells and MHC II expression were significantly ($p < 0.01$) reduced by 180 days in animals receiving MC long-term, cellular infiltrates and MHC II expression were comparable in grafts receiving MC as donor-treatment, over a short-term period in recipients or in controls without CO delivery. Thus, CO delivery by MC administration inhibited I/R injury and prevented chronic graft deterioration. Those effects were particularly impressive following a single CO delivery in the donor prior to organ harvesting demonstrating the relevance of I/R on chronic graft rejection. MC application for the delivery of CO may represent a novel clinical approach which could be initiated in the donor following the diagnosis of brain death and the decision for organ donation.

Abstract# 1525 **Poster Board #-Session: P281-III**

ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKER IN COMBINATION WITH CYCLOSPORINE A REDUCES THE INCIDENCE AND SEVERITY OF TRANSPLANT VASCULOPATHY IN A HETEROTOPIC CARDIAC TRANSPLANT MODEL. Markus Richter,[1] Friedrich Mohr.[1] *[1]Heart Center of Leipzig, University of Leipzig, Leipzig, Germany.*

The renin-angiotensin-system (RAS) plays a pivotal role in proliferation of vascular smooth muscle cells (VSMC) contributing to the development of transplant vasculopathy (TVP). We investigated the effects of an angiotensin II blocker, losartan (AT1-blocker), and an ACE inhibitor, enalapril, on the severity and the incidence of TVP in a rat cardiac transplant model. Methods: After cardiac transplantation from Lewis to Fisher rats, recipients were randomly divided into 6 groups, 1: no therapy, 2: 3mg/kg/d s.c. cyclosporine (CSA), 3: CSA and 10mg/kg/d p.o. losartan, 4: CSA and 40mg/kg/d p.o. enalapril, 5 and 6 as group 4 and 5 but additionally pre-treated with losartan or enalapril 7 days prior to transplantation. Eighty days after grafting the incidence and the extent of TVP was assessed by digitizing morphometry and is expressed as the mean vessel occlusion of all arteries. Results: CSA and CSA/enalapril post-treatment significantly reduced mean vessel occlusion (mvo), but not incidence of TVP. Additional reduction of mvo (significant to CSA and controls) was achieved in CSA/enalapril pre-treatment and both CSA/losartan pre- and post-treatment groups. Both the incidence of TVP and the mvo were significantly reduced only by losartan post-treatment in combination with CSA. Conclusions: Our results validate the important role of the renin-angiotensin system in neointimal proliferation after cardiac transplantation. Although we achived our results in an animal model, we think that AT1 blocker after cardiac transplantation in humans should be proven to prevent the development of TVP.

Abstract# 1526 **Poster Board #-Session: P282-III**

SPECIFIC DYSREGULATION OF TROPONINS AND INFLAMMATORY MARKER GENES IN ENDOMYOCARDIAL BIOPSIES FROM HEART TRANSPLANT RECIPIENTS. Sheri E. Kelemen,[1] Daniel P. Bednarik,[2] Michael V. Autieri,[1] Kenneth B. Margulies,[1] Howard J. Eisen.[1] *[1]Advanced Heart Failure Center, Temple University School of Medicine, Philadelphia, PA; [2]Genelogic, Inc., Gaithersburg, MD.*

BACKGROUND: Graft surveillance by protocol biopsies can reflect the immunological status of the graft as well as the efficacy of anti-rejection therapy. However, standard morphological features of endomyocardial biopsies do not necessarily correlate with graft function or prognosticate future rejection episodes. The goal of this work is to identify objective, specific, and quantitative surrogate markers of graft rejection and function. METHODS: A novel and proprietary micro sample amplification (MSA) technique has been developed to reliably amplify mRNA in a non-anomalous and non-disproportionate manner from nanogram quantities of RNA. MSA was used to generate a transcriptional profile of gene expression from endomyocardial biopsies from different International Society of Heart and Lung Transplant (ISHLT) rejection grades. RESULTS: This study demonstrates that expression of Troponin I, Troponin T2, and Troponin C are proportional to the ISHLT rejection grade, with more mRNA of each transcript being detected in grade 3A versus 1A biopsies. This is significant in that the Troponin family of proteins regulate muscle contraction by cyclin sequestration. Similarly, expression of several IFNg-inducible genes are also expressed in a rejection grade manner. In particular, the IFNg-inducible gene IFI30 was specifically detected only in grade 3A samples. IFI30 has been characterized as a lysosomal thiol reductase that is important in antigen processing. CONCLUSIONS: MSA is novel technique to identify gene expression in limited tissue samples such as biopsies. Specifically, the Troponins and IFI30, in conjunction with other genes may represent surrogate molecular markers of the status of allograft rejection and function in cardiac transplant recipients.

Abstract# 1527 Poster Board #-Session: P283-III

BID CLEAVAGE IN LIVER ALLOGRAFT REJECTION AND HEPATOCYTE APOPTOSIS. Erica D. Riddle,[1] Hideaki Obara,[1] Elena Fabrikant,[1] Olivia M. Martinez,[1] Sheri M. Krams.[1] *[1]Surgery, Division of Transplantation, Stanford University, Stanford, CA.*

Despite advances in transplantation, acute rejection remains a significant obstacle in the survival of the grafted organ and the recipient. Previous studies have shown that acute rejection in the liver is accompanied by apoptosis in the graft. We sought to determine the important intracellular mediators of apoptosis during acute liver allograft rejection, specifically focusing on Bid. Bid is a cytosolic protein integral in the crosstalk between granule, and death receptor-mediated pathways of apoptosis. In hepatocytes, Bid's importance is indicated by previous studies in the Bid knock-out mouse: these mice are resistant to hepatocyte apoptosis induced by death receptor ligation. To study apoptosis during allograft rejection, our laboratory utilizes a well-established rat model of orthotopic liver transplantation with Lewis and Dark Agouti (DA) rats. Here we have observed acute rejection, accompanied by apoptosis of hepatocytes, in the Lewis rat 9-13 days after receiving a DA liver. We have generated a unique polyclonal antibody that recognizes both the zymogen and cleaved forms of rat Bid. This antibody has detected truncated Bid in a western blot with liver tissue protein lysates isolated from rejecting allografts, indicating that Bid cleavage is a physiological process and a part of apoptosis during transplant rejection. To further support this data, primary hepatocytes isolated from healthy adult Lewis rats, cultured *in vitro*, readily undergo apoptosis after treatment with TNF-α and actinomycin D and Bid cleavage can be detected in these cells. Similarly, the FaO rat hepatoma cell line undergoes apoptosis, determined by cell-cycle analysis, following treatment with TNF-α and cyclohexamide, and Bid cleavage can be detected by western blot in these cells. Having shown Bid cleavage during hepatocyte apoptosis and allograft rejection, hepatocyte apoptosis may be blocked by inhibiting Bid cleavage. It has been shown that caspase 8 and granzyme B cleave Bid at specific aspartic acid residues, 59 and 75, respectively. Using site-directed mutagenesis, we have created Bid cleavage mutants, changing the caspase 8 and granzyme B cleavage sites from aspartic acid residues to glutamic acid residues (D59E, D75E). Adenoviral delivery of these cleavage mutants into the donor liver may provide a way to abrogate Bid cleavage, resulting in protection from hepatocyte apoptosis. This dominant-negative effect can protect against ischemia/reperfusion injury and prolong allograft survival.

Abstract# 1528 Poster Board #-Session: P284-III

EXPRESSION OF THE GRANZYME B INHIBITOR PI-9 IN HUMAN RENAL ALLOGRAFTS EXPLAINS STABLE RENAL FUNCTION IN SPITE OF CYTOTOXIC INFILTRATES. Ajda T. Rowshani,[1,2] Sandrine Florquin,[3] Frank N. J. van Diepen,[4] Angela M. Wolbink,[5] J. Alain Kummer,[6] Frederike Bemelman,[1] C. Erik Hack,[5] Ineke J. M. ten Berge.[1,2] *[1]Renal Transplant Unit ,Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands; [2]Division of Clinical Immunology and Rheumatology, Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands; [3]Department of Pathology, Academic Medical Center, Amsterdam, Netherlands; [4]Laboratory for Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands; [5]Department of Immunopathology, Sanquin Research at the CLB, Amsterdam, Netherlands; [6]Department of Pathology, VU Medical Center, Amsterdam, Netherlands.*

Background Granzyme B positive T-lymphocytes are known to infiltrate renal allografts during acute cellular rejection and cause graft injury by mediating apoptosis of tubular cells. Protease Inhibitor 9 (PI-9) is a human intracellular serpin that binds to granzyme B and inhibits its function. We hypothesized that expression of PI-9 in renal tubular cells may provide protection from the cytotoxic effect exerted by granzyme B positive T-lymphocytes. For that purpose, we compared the expression of granzyme B and PI-9 in renal transplant biopsy specimens from patients with acute cellular rejection and patients with subclinical rejection. Methods Renal allograft biopsy specimens were obtained from patients with a subclinical rejection defined as the presence of a mononuclear infiltrate without deterioration of renal function (n=9), acute rejection type I (n=6), acute rejection type II (n=3), acute rejection type III (n=6) and no-rejection (n=9) Monoclonal antibodies (Ab) used for staining were anti-granzyme B and anti-PI-9. The secondary Ab was biotinylated rabbit-anti-mouse F(ab)2Ig which was detected by avidine-biotine-HRP complex. Immunostainings were scored on a scale of 0-4 by two of us (S.F. and A.T.R.) independent of each other. Findings PI-9 was expressed by tubuli in the majority of biopsies with acute rejection and in all biopsies with subclinical rejection. Granzyme B was expressed by infiltrating mononuclear cells in all biopsies. For each individual biopsy, PI-9 score for tubuli was subtracted by the score for granzyme B in inflammatory cells. This resulted in a median score of 0.0 (range: -1.0-0.0) for the subclinical rejection group; 1.0 (range: 0.0-4.0) for the acute rejection group; and -0.5 (range: -1.0-1.0) for the group with stable function due to a significant shortcoming production of PI-9 by tubular epithelial cells during acute rejection ($p < 0.01$). Interpretation These data support the idea that PI-9 expression in renal tubular epithelial cells protects human renal allografts from granzyme B-induced apoptosis. This may explain the stable renal function in spite of histologically apparent inflammatory infiltrates.

Abstract# 1529 Poster Board #-Session: P285-III

MOTILITY AND NEURONAL POPULATION AS PARAMETERS OF GRAFT VIAVILITY ALTER IN DIFFERENT GRADES OF ACUTE REJECTION IN SMALL BOWEL TRANSPLANTAION. Toshihiko Watanabe,[1] Ken Hoshino,[1] Naoki Shimojima,[1] Shigeru Kawachi,[1] Minoru Tanabe,[1] Go Wakabayashi,[1] Motohide Shimazu,[1] Yasuhide Morikawa,[1] Masaki Kitajima.[1] *[1]Department of Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjyukiu-ku 160-8582, Tokyo, Japan.*

Acute rejection is a major cause for graft loss after small bowel transplantation and an evaluation of the motility of the graft is thought to be one means of assessing rejection together with histological examination. The aim of this study was to elucidate the morphological changes in the enteric nervous system of wholemounts in the intestinal transplants to understand the physiological function and effectiveness of motility examination of the acutely rejected transplants. Heterotopic small bowel transplantations in Thiry-Vella loop fashion were performed in the following experimenral grouping. sham operated Brown Norway (group I), Syngenic transplants (BN to BN) (group II), Allogenic transplants (BN to LEW) with FK506 0.64mg/kg/d (group III), Allogenic transplants (BN to LEW) without FK506(group IV). Three strain gauge transducers were sutured to the jejunum in group I or to the Thiry-Vella loop in the other groups. The viability of the intestine was estimated whether migrating motor complex (MMC) was observed at 5,7,10 days after transplantation. And we also stained wholemounts of transplanted graft with Cuplolinic Blue to evaluate complete neuronal population of myenteric neurons. In group II and III, MMC frequency is markedly decreased with an interval of 11.2±2.3 munutes, but increased with an amplitude of 119.8±35.8 (x1/1000g) compared with group I. In group IV at day 7, there appeared low amplitude cluster without migrating motor complex and then no contraction was seen in day 10. After labeling the entire neuronal population of the myenteric plexus, number of neurons/ganglion and neurons/μm²ganglionic area have been measured quantitatively in order to compare with the intestinal motility which was evaluated with strain gauge transducers. In quantitative analysis, 20% and 60 % neuronal loss was observed in group IV at day 7(4.9±0.9/x1000μm²) and 10(2.4±0.3/x1000μm²) respectively, whereas no significant alteration of neuronal population was seen in group I, II , III and group IV at day 5. We conclude that acute rejection cause the changes of graft motility and neuronal population after transplantation in the moderately and severely rejected transplants. We consider that evaluation of the motility and neuronal population in the small bowel transplants is a useful method to monitor the viability of the grafts.

Abstract# 1530 Poster Board #-Session: P286-III

INTRACORONARY INTERFERON-γ PROMOTES VASCULOPATHY IN TOLERANT MINIATURE SWINE BEARING CLASS I DISPARATE HEARTS. Ruediger Hoerbelt,[1] Louis C. Benjamin,[1] Douglas R. Johnston,[1] Stuart L. Houser,[1] Tsuyoshi Shoji,[1] James S. Allan,[1] Levi G. Ledgerwood,[1] Rebecca S. Hasse,[1] David H. Sachs,[1] Joren C. Madsen.[1] *[1]Department of Surgery, Transplantation Biology Research Center, Boston, MA.*

Purpose: The role of IFN-γ in the process of allograft rejection remains controversial. In a previous study, we found that delivery of IFN-γ into the coronary circulation of class I disparate cardiac allografts accelerated acute rejection and cardiac allograft vasculopathy (CAV). Here, we evaluated the effect of intracoronary on the development of vasculopathy in miniature swine rendered tolerant to the heart graft. **Methods:** We have shown previously that tolerance to donor antigens is induced when class I disparate hearts are co-transplanted with a donor-derived kidney transplant in recipients treated with 12-days of cyclosporine (10-13mg/kg/day). An Alzet osmotic mini-pump was implanted in the experimental group of animals, which delivered IFN-γ directly into the graft coronary vasculature at a rate of 100 ng/d. After approximately 100 days, animals were graftectomized and morphometry was performed. All animals were followed by cell mediated lympholysis (CML) assays at regular intervals. **Results:** A control animal (n=1) that received a heart/kidney transplant and CyA but no INF-γ developed long term graft survival with donor specific unresponsiveness in CML assays. Morphometry of the cardiac graft showed vasculopathy in only 0.1% (1/738) of vessels and mild cellular infiltrates (ISHLT 1a/4) after 100 days. Heart/kidney recipients that were treated with CyA and intracoronary IFN-γ (n=2) also accepted their grafts long term and maintained donor specific unresponsiveness in CML assays. However, they developed significant (grade 3/3) vascular lesions in 3.3% (15/451) and 9.9% (50/503) of vessels with only mild cellular infiltrates (1a/4). MHC class II on graft endothelial cells was up-regulated in IFN-γ treated animals as compared to controls. **Conclusion:** Local delivery of IFN-γ appears to promote the development of vascular lesions in otherwise tolerant recipients. These data suggest that local IFN- γ levels are critical in the generation of chronic vascular lesions and that this cytokine may need to be specifically blocked in tolerance protocols to completely prevent chronic rejection.

Abstract# 1531 Poster Board #-Session: P287-III

CYCLOOXYGENASE INHIBITION PROMOTES REJECTION OF MOUSE RENAL ALLOGRAFTS. Troy J. Plumb,[1] Paulo N. Rocha,[1] Beverly H. Koller,[2] Thomas M. Coffman.[1] *[1]Internal Medicine, Duke University, Durham, NC; [2]Genetics, University of North Carolina, Chapel Hill, NC.*

Rejection of a kidney transplant is characterized by an intense local inflammatory response. This response is complex and is shaped by the actions of a number of soluble inflammatory mediators. Among these mediators, prostanoid products of the cyclooxygenase (COX) pathway of arachidonic acid metabolism appear to play a role. The purpose of these studies was to determine the global actions of COX metabolites in kidney allograft rejection. We first examined the role of the individual prostanoids PGE_2 and TXA_2 in model cellular immune responses in vitro. Addition of PGE_2 from 1 nM to 1 μM caused dose proportional inhibition of lymphocyte proliferation in response to mitogens such as Con A and PHA as well as anti-CD3 antibody, which stimulates proliferation directly through the T cell receptor complex. PGE_2 also inhibited proliferation in the mixed lymphocyte response (MLR), an in vitro model of the cellular alloimmune response. However, proliferation triggered by PMA and ionomycin was not affected by PGE_2 suggesting that the actions of PGE_2 to attenuate T cell activation are proximal to the calcium signal. By contrast, TXA_2 acting through TP receptors stimulates cellular immune responses. For example, in the MLR, proliferation of TP-deficient lymphocytes is substantially impaired compared to $TP^{+/+}$ controls (1588±564 vs. 3952±93 cpm; p=0.002). Thus, these two prostanoid products of the COX pathway have opposing effects on the immune response. To determine how the actions of COX metabolites affect immune injury in vivo, we examined the effects of pharmacological inhibition of COX on survival of mouse kidney transplants. (C57BL/6 x 129)F1 mice were transplanted with kidneys from MHC-disparate donors followed by bilateral native nephrectomy. The kidney transplant recipients were treated with vehicle or the COX inhibitor indomethacin (3.5 mg/kg/day). At 35 days after transplantation, graft survival was 100% in the vehicle group and 28% in the group receiving indomethacin (p=0.013). Thus, global inhibition of COX enzymes is detrimental to graft survival, suggesting a dominant effect of immunosuppressive prostanoids in this model. The relative impact of these prostanoid actions may play a critical role in determining the balance between rejection and long-term survival of organ allografts.

Abstract# 1532 Poster Board #-Session: P288-III

IMPACT OF MHC CLASS II INCOMPATIBILITY ON CHRONIC LUNG ALLOGRAFT REJECTION AND GENE PROFILES. Shinji Nakashima,[1] Sabrina M. Tom,[2] Zhiping Qian,[1] Barbara A. Wasowska,[1] Salma Rahimi,[1] E. Rene Rodriguez,[1] Joe G. N. Garcia,[2] William M. Baldwin III.[1] *[1]Transplantation Laboratory, Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD; [2]Division of Pulmonary and Critical Care Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD.*

Lungs have a greater incidence of chronic rejection than other organ transplants. The unique aspect of rejection in lungs is that it centers on the bronchioles and produces an obliterative bronchiolits (OB). Many potential risk factors associated with the development of OB have been reported, including graft ischemic time, cytomegalovirus pneumonitis, episodes of moderate or severe acute rejection, late acute rejection, and degree of HLA compatibility. We examined the relative role of MHC class I and II antigens in causing bronchiolitis in orthotopic lung transplants between congenic PVG rats treated with sustained cyclosporin A (CsA) immunosuppression. Transplants mismatched for MHC class II antigens had significantly more peribronchiolar infiltrates than MHC class I incompatible transplants. No significant increase in infiltrates was found between MHC class II and MHC class I plus II incompatible lung transplants. Continuous treatment with CsA was effective in limiting mononuclear cell infiltrates in the perivascular compartment, but less effective in decreasing mononuclear cell infiltrates in the bronchial compartment. Immunohistochemistry demonstrated that MHC class II antigen expression was upregulated on airway epithelium, but not vascular endothelium. DNA microarray analysis revealed that MHC class II incompatible transplants upregulated IL-9 receptor and two downstream chemokines: macrophage inflammatory protein (MIP-1α) and regulated on activation, normal T cells expressed and secreted (RANTES) chemokines. Activated bronchial epithelial cells have been shown to upregulate IL-9R expression and MIP-1α and RANTES secretion. These results indicate that MHC class II incompatibility in lung transplants augments chronic peribronchial infiltrates in spite of CsA immunosuppression, and also increases bronchial epithelial cell expression of MHC class II antigens and selected chemokines.

Abstract# 1533 Poster Board #-Session: P289-III

CD39/NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE-1 (NTPDase-1) MODULATES ALLOGRAFT REJECTION AND CELLULAR IMMUNE RESPONSES. Yongsheng Li,[1] Eva Csizmadia,[1] Jean Sevigny,[1] Keiichi Enjyoji,[1] Simon C. Robson. *[1]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

CD39 (NTPDASE-1), the major endothelial and Langerhans cell (LC) ectonucleotidase, hydrolyzes ATP and ADP to AMP to the ultimate product adenosine. Pericellular ATP is increased upon T-cell activation and mediates cellular activation pathways. In cutaneous inflammation models, LC-associated cd39 regulates cellular immune responses to both direct chemical stimuli and haptens. We hypothesized that cd39 plays a key role in regulating nucleotide-mediated dendritic cell (DC)-T cell communication that occurs during alloantigen presentation. BALB/c, B6/129 wild type and B6/129 cd39 null mice were studied as donors and recipients in standard heterotopic cardiac allograft models. Under certain conditions, recipients were treated with anti-CD154 mAb. The graft survival times are summarized in Table 1. In the anti-CD154 treated groups, cd39 null mice maintained mismatched BALB/c grafts for prolonged survival times, relative to wild type mice. The null B6/129 grafts were rejected faster than the untreated control hearts in the wild type combinations; in keeping with loss of cd39 protective thromboregulatory properties. In the untreated groups, all grafts were rejected at equivalent times.

Table 1: Cardiac Allograft Survival

Recipient	Donor	Treatment	Cardiac Graft Survival(days)	MST
B6/129	BALB/c	Untreated	6, 6, 7, 7, 7, 8, 8, 9	7
B6/129	BALB/c	Anti-CD154	8, 13, 14, 15	14
BALB/c	B6/129	Untreated	6, 7, 7, 8	7
BALB/c	B6/129	Anti-CD154	8, 12, 16	12
cd39KO	BALB/c	Untreated	6, 6, 6, 7, 7, 7, 7	7
cd39KO	BALB/c	Anti-CD154	20, 22, 24	22
BALB/c	cd39KO	Untreated	6, 7, 8, 9	8
BALB/c	cd39KO	Anti-CD154	5, 5, 8, 8	7

We further quantitatively analyzed proliferation of alloreactive T cells in vivo. Splenic lymphocytes from B6/129 wild type (WT) or cd39 null mice were labeled with a tracking fluorochrome 5-carboxyfluorescein diacetate succinimidyl ester (CFSE) and then were injected into lethally irradiated BALB/c mice. Three days after adoptive transfer, cells were recovered from the hosts. In untreated control and mutant mice, approximately 20% of CFSE labeled T cells proliferated in the host spleen. With prior anti-CD154 treatment, 12% of CFSE labeled cd39 null T cells proliferated, in contrast to 16% CFSE labeled wild type T cells. In conclusion, cd39/NTPDase1 plays an important role in allograft rejection, under conditions of costimulation blockade. We suggest these effects, in a similar way to type IV responses to haptenic cutaneous inflammation, may be mediated by disordered T-cell and DC interactions.

Lymphocyte Activation

Abstract# 1534 Poster Board #-Session: P290-III

INHIBITION OF SRC KINASES PROLONGS MURINE CARDIAC ALLOGRAFT SURVIVAL. Qi-Wei Zhang,[1] Tarek El-Sawy,[1] Hiroyki Amano,[1] Danielle Kish,[1] Robert Fairchild,[1] Geraldine Miller.[2] *[1]Dept. Immunology, Cleveland Clinic Fdn., Cleveland, OH; [2]Dept. Medicine, Vanderbilt Univ., Nashville, TN.*

INTRODUCTION: Src family tyrosine kinases play critical roles in T and B cell activation. To test the hypothesis that Src family kinase activation contributes to allograft rejection, the effect of a pharmacological inhibitor of Src kinase activation on acute rejection of heart allografts in mice was tested. METHODS: C57BL/6 (H-2b) mice received heterotopically transplanted heart grafts from syngeneic or A/J (H-2a), donors. An inhibitor of Src family kinase activation, CGP77675 (CGP), was given s.c. to graft recipients 2x/day/3.5 mg/kg beginning on the day of transplant (day 0). Anti-CD40L mAb MR1 was given 250 ug i.p./day on days 0 or on days 0 and +1. Recipient spleen cells were tested for frequency of alloantigen-specific T cells producing IFN-g by ELISPOT assays. Graft sections were stained with antibodies to assess macrophages, CD4+ and CD8+ T cell infiltration. RESULTS: Allografts in control recipients were rejected on day 7-8 and CGP had minimal effect when given alone extending survival to 10-13 days post-transplant. Rejected allografts from control recipients had extensive perivascular and parenchymal mononuclear cellular infiltrates. Rejected allografts from CGP treated recipients had moderate perivascular mononuclear cell infiltration with low levels of CD8+ T cell parenchymal infiltration. Spleen cells from control recipients had high levels of alloantigen-specific cells producing IFN-g whereas recipients treated with CGP had low levels indicating inhibition of alloreactive T cell priming. Single and doulbe doses of MR-1 prolonged allograft survival to day 9-11 and to day 20 post-transplant, respectively. In combination with CGP, the single dose of MR-1 extended allograft survival to 17-35 days. Most strikingly, the allografts rejected at day 18 from recipients treated with the single dose of MR-1 plus CGP had virtually no cellular infiltration. CGP given with two doses of MR-1 extended allograft survival to days 35 and 56 in two recipients. In three other recipients receiving this combiantion, CGP treatment was stopped at day 60 and the allografts continued to beat strongly beyond 100 days post-transplant. Histological examination indicated no cellular infiltration or the development of neointimal formation or vasculopathy. CONCLUSIONS: The Src kinase activation inhibitor CGP inhibits T cell priming to heart allografts and in combination with anti-CD40L mAb is effective at promoting long-term survival of the allografts without development of graft vasculopathy.

Abstract# 1535 Poster Board #-Session: P291-III

PORCINE-DERIVED EMBRYONIC STEM CELLS ARE IMMUNE-PRIVILEGED AND RESISTANT TO NATURAL KILLER CELL LYSIS. Sabrina Bonde,[1] Afshin Varzavand,[1] Gang-Ming Zou,[1] Elizabeth Field,[1] Eric Walters,[2] Matthew Wheeler,[2] Nicholas Zavazava.[1] *[1]Internal Medicine, University of Iowa & VA Medical Center Iowa City, Iowa City, IA; [2]Beckman Institute for Advanced Science and Technology & Department of Animal Sciences, University of Illinois, Urbana, IL.*

Embryonic stem (ES) cells are unique due to their high degree of plasticity and ability to differentiate into various tissues, when cultivated under optimal conditions. However, due to their low immunogenicity, ES cells are ideal for use in repair of damaged tissue and for the induction of mixed chimerism, which may ultimately lead to transplantation tolerance. Here, we used a Meishan-derived ES cell line to characterize porcine-derived ES cells. Porcine ES cells express very low MHC class I molecules, moderately express the undiffrentiation marker SSEA-1 (stage-specific embryonic antigen 1) and alkaline phosphatase, but no MHC class II molecules or any other CD markers expressed by mature hematopoietic cells. In addition, ES cells express high levels of FasL (CD95L), which they shed into the supernatant. To test whether porcine ES cells are protected from the innate immune system, undifferentiated ES cells were tested for their susceptibility to NK cell lysis. They were found to be resistant to swine- or human-derived NK cells indicating either lack of receptors for NK cells or the ability to evade lysis by cytotoxic T cells. To test whether MHC expression by ES cells can be up-regulated, ES cells were further treated with swine-derived IFN-g. Porcine ES cells up-regulated MHC class I expression, but remained negative for class II antigens. Thus, these data indicate that ES cells derived from a large domestic animal share the same characteristics as ES cells from rodents and that they appear to be immune-privileged through low MHC expression and resistance to NK cell lysis.

Abstract# 1536 Poster Board #-Session: P292-III

EXPANSION OF THE CYTOMEGALOVIRUS-SPECIFIC CD27$^-$ EFFECTOR CD8$^+$ T CELL POOL BY REPETITIVE ANTIGENIC STIMULATION. Ester M. M. van Leeuwen,[1,2] Laila E. Gamadia,[1,2] Rene A. W. van Lier,[2] Ineke R. M. ten Berge.[1] *[1]Div. of Nephrology, Clinical Immunology & Rheumatology, Dept. of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands; [2]Laboratory for Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands.*

In persistent viral infection, like cytomegalovirus (CMV) infection, memory cells can display either a non-cytotoxic CD27+ phenotype or a cytotoxic CD27- phenotype. CD27 is a TNF-receptor like molecule, and interaction with its ligand CD70, expressed on B and T cells upon activation, leads to expansion and differentiation of CD8+ T cells to the CD27- cytotoxic phenotype. In latently infected individuals, CD27+ and CD27-CD8+ T cells seem equally capable of controlling overt viral replication. Under conditions of impaired T cell immunity, like in transplantation settings, human cytomegalovirus can reactivate from lifelong latency, to be controlled again after adaptation of the immunesystem to a new balance between host and persistent virus. To analyse the role of antigenic burden in the determination of CD27+ or CD27-CD8+ T cell phenotypes, renal transplant recipients with distinctly different CMV-specific non-cytotoxic or cytotoxic memory CD8+ T cells prior to transplantation were longitudinally studied during reactivation of CMV. The expression of the ligand of CD27, CD70, was analysed directly ex-vivo. CMV-specific CD8+ T cells were analysed for the subset markers CD27, CD28, CD45RA and R0 and CCR7, perforin and granzyme B content, and cell-cycle molecules. Furthermore, in vitro experiments stimulating CMV-specific CD8+ T cells with CMV-peptide in combination with CMV-antigen, IL2, IL-15 or IL-21 were performed. Our results show that the expression of CD70 is dependent on the level of antigen and the concurrent cytokine environment. Ex-vivo, expression of CD70 could be detected on B-cells, CD4+ T cells, CD8+ T cells and CMV-specific CD8+ T cells in relation with viral replication. Concomitant analysis of CMV-specific CD8+ T cells during viral reactivation showed that these cells increase in number and simultaneously loose CD27. Furthermore, we show that the expression of CD70 and subsequent differentiation of CMV-specific CD8+ T cells to a CD27- cytotoxic phenotype is not only regulated by the presence of antigen but also by environmental cytokines. Thus, in viral reactivation, CD70-CD27 interaction drives both CD8+ T cell expansion and differentiation, where the number of antigen-specific CD27- effector CD8+ T cells is linearly related to the total number of antigen specific CD8+ T cells.

Abstract# 1537 Poster Board #-Session: P293-III

DOUBLE GENETIC MODIFICATION OF ADENOVIRUS FIBER WITH RGD AND POLYLYSINE MOTIFS ENHANCES GENE TRANSFER, REDUCES INFLAMMATION AND TOXICITY TO ISOLATED HUMAN PANCREATIC ISLETS. Juan L. Contreras,[1] Hongju Wu,[2] Cheryl Smyth,[1] Christopher Eckstein,[1] Carlton J. Young,[1] Toshiro Seki,[2] Guadalupe Bilbao,[1] David T. Curiel,[2] Devin E. Eckhoff.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL; [2]Human Gene Therapy, University of Alabama at Birmingham, Birmingham, AL.*

The ability to transfer immunoregulatory or cytoprotective genes into pancreatic islets may allow enhanced islet engraftment and survival after transplantation (Tx). Adenoviral vectors (Ad5) have been used widely to deliver therapeutic genes to different tissues. Limitations associated with the use of Ad5 for gene therapy are related to the virus reliance on the presence of its primary receptor, transient nature of the transgene expression, as well as immediate inflammatory and immune response elicited by the infection.Since the RGD and polylysine (pK7) motifs have been shown to enhance Ad5 infection via Ad5 receptor-independent pathway, we hypothesized that they could act additively to improve infectivity and reduce toxicity to isolated human pancreatic islets (IHPI). Methods: Hand-picked IHPI (4 preparations) were infected with non-modified Ad5, single modified Ad5 with RGD (Ad5RGD) or pK7 (Ad5pK7) and Ad5RGDpK7. Transfection efficiency was evaluated by green fluorescent protein expression in dissociated single islet cells, apoptosis by a quantitative assay, NF-κB nuclear translocation using a promoter-Luciferase NF-κB responsive construct, and RANTES by ELISA. *In vivo* functionality was evaluated after Tx into diabetic NOD-SCID mice (intraportal, 2000 IEQ). Results: Compared to unmodified and singly-modified Ad5 vectors, Ad5RGDpK7 demonstrated the highest infectivity (at 0.1 viral particles (VP) /cell=82.3±7.9% of the iselts cells *vs* Ad5=6.7.4%, $P<0.001$), significantly reduced apoptosis (7.83±2.56% of islet cells *vs* Ad5=16.2±2.3, $P<0.05$), NF-kB nuclear translocation (2345±845 RLU/mg protein *vs* 13889±1564, $P<0.001$) and the expression of RANTES (17.2±2.45 vs 103.23±8.9 pg/mL, $P<0.001$). 500 VP/cell of Ad5 were required to infect > 85% of the iselt cells. Higher glucose disposal rate was demonstrated 8 days post-Tx in animals that received Ad5RGDpK7-infected islets(3.5±0.7 vs Ad5=2.0±0.3, n=8, $P<0.05$). A significant reduction in Ad5-driven specific Th1 and antibody response was observed in immunocompetent animals with syngeneic islets infected with Ad5RGDpK7. Conclusions: Ad5RGDpK7 exhibited higher transfection efficiency and reduced inflammatory responses related to Ad5 infection. This strategy may thus be used to overcome toxicity and inflammation of Ad5 vectors to successfully modify isolated pancreatic islets.

Abstract# 1538 Poster Board #-Session: P294-III

CD4$^-$CD8$^-$ REGULATORY T CELLS PROTECT MICE FROM GRAFT-VERSUS-HOST DISEASE. Kevin J. Young,[1] Barb DuTemple,[1] M. James Phillips,[1] ZhuXu Zhang,[1] Li Zhang.[1] *[1]Department of Laboratory Medicine and Pathobiology, Multi Organ Transplantation Program, Toronto General Research Institute, University Health Network, University of Toronto, Toronto, ON, Canada.*

Numerous studies have indicated that regulatory T (Treg) cells are important for controlling autoimmune diseases and allograft rejection. We have demonstrated an important role for CD4$^-$CD8$^-$, double negative (DN) Treg cells in prolonging allograft survival. The goal of this study was to determine if DN Treg cells can regulate graft-versus-host disease (GVHD) in immunoincompetent recipients following the infusion of haematopoitic stem cells. METHODS: Immunoincompetent Scid or lethally irradiated mice were infused with splenocytes that were mismatched at a single class I major histocompatibility complex (MHC) locus with the recipient. The body weight of recipients, as well as the number of anti-donor lymphocytes were monitored, and the suppressive function of donor-derived lymphocytes was tested *in vitro* using proliferation and cytotoxicity assays. Furthermore, GVHD was induced in mice by infusing allogeneic CD8$^+$ T lymphocytes, and the capacity of DN Treg cells to prevent GVHD was assessed by co-infusing *in vitro* generated DN Treg cells. RESULTS: Immunoincompetent mice that were transplanted with single class I MHC locus mismatched splenocytes did not develop GVHD (n=55). The number of anti-host CD8$^+$ T cells rapidly increased, but then quickly decreased and remained barely detectable following transplantation. Moreover, when naïve anti-donor CD8$^+$ T cells were infused into mice these cells failed to proliferate in contrast to third party control CD8$^+$ T cells. These data indicate that anti-host CD8$^+$ T cells were likely suppressed *in vivo*. Interestingly, donor-derived DN T cells increased 10-fold and formed the majority of lymphocytes in the spleen of recipient mice. The DN Treg cells could dose-dependently inhibit the proliferation of CD8$^+$ T cells *in vitro*, and were able to attenuate GVHD that was caused by the infusion of single MHC class I locus mismatched CD8$^+$ T cells. Together, these data indicate that DN Treg cells are able to specifically suppress donor-reactive T lymphocytes, and thereby prevent or attenuate GVHD without abrogating immune responses to other antigens. These findings may lead to novel cellular therapies for the prevention and/or treatment of GVHD.

Abstract# 1539 Poster Board #-Session: P295-III

INDUCTION OF ANTI-LYMPHOMA ACTIVITY IN THE ABSENCE OF GRAFT-VERSUS-HOST DISEASE BY CD4$^-$CD8$^-$ REGULATORY T CELLS. Kevin J. Young,[1] Barb DuTemple,[1] M. James Phillips,[1] ZhuXu Zhang,[1] Li Zhang.[1] *[1]Department of Laboratory Medicine and Pathobiology, Multi Organ Transplantation Program, Toronto General Research Institute, University Health Network, University of Toronto, Toronto, ON, Canada.*

Allogeneic lymphocytes are potent mediators of leukemia remission, although their beneficial effects are often hampered by graft-versus-host disease. The goal of this study was to determine if the beneficial graft-versus-leukemia activity of allogeneic lymphocytes could be harnessed in the absence of graft-versus-host disease. METHODS: Immunoincompetent Scid or lethally irradiated mice were challenged with a lethal dose of A20 lymphoma cells, together or in the absence of an infusion of single Major Histocompatibility Complex (MHC) class I locus mismatched splenocytes. The number of donor derived $CD4^+$, $CD8^+$ and $CD4^-CD8^-$ double negative (DN) T cells was monitored in each group of animals. Eight weeks after transplantation donor lymphocytes were purified from mice that did not develop lymphoma, and the ability of these cells to kill A20 lymphoma cells was assessed. Furthermore, DN Treg cells were generated *in vitro* and tested for their ability to suppress A20 cells *in vitro* and *in vivo*. RESULTS: Scid or lethally irradiated mice that were challenged with A20 cells succumbed to lymphoma between 34-50 days after infusion. In contrast, >75% of mice that were co-infused with single class I MHC locus mismatched splenocytes survived indefinitely (n=20). Our data show that donor derived $CD8^+$ T cells are not involved in protecting mice from A20 lymphoma. Interestingly, the number of DN Treg cells increased 15 fold in mice that did not develop lymphoma, and DN Treg cells isolated from the spleen of these animals were cytotoxic to A20 lymphoma cells *in vitro*. DN Treg cells that were generated *in vitro* were also cytotoxic to A20 lymphoma cells, indicating that both *in vitro* and *in vivo* activated DN Treg cells can suppress A20 cells. When DN Treg cells were infused into naïve mice together with A20 lymhoma cells, 86% of recipient mice were protected from lymphoma onset. Together, these results demonstrate that an anti-lymphoma activity can be generated in mice without causing graft-versus-host disease. Furthermore, DN Treg cells can suppress lymphoma cells *in vivo* and *in vitro,* suggesting that DN Treg cells could be used as a novel strategy for the treatment of lymphoma.

Abstract# 1540 Poster Board #-Session: P296-III

OVEREXPRESSION OF Bcl-2 CONFERS CYTOPROTECTION *IN VIVO* TO ISOLATED ADULT PORCINE PANCREATIC ISELTS (PI)EXPOSED TO XENOREACTIVE ANTIBODIES (XA) AND COMPLEMENT (C). Juan L. Contreras,[1] Stacie Jenkins,[1] Guadalupe Bilbao,[2] Xiao Xiang,[1] Francis Thomas,[1] Devin Eckhoff,[1] David Curiel,[2] Judith Thomas.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL; [2]Human Gene Therapy, University of Alabama at Birmingham, Birmingham, AL.*

Exposure of PI to fresh human or primate serum results in acute islet damage mainly mediated by XA and C. Under certain circumstances, when XA and C-mediated immune responses are inhibited for a few days, grafts can survive indefinitely despite the return of XNA and C, a phonomenon referred as "accomodation". Expression in the graft of anti-apoptotic or protective genes, such as A20, Bcl-xl and Bcl-2 make the graft resistant. In the present study, we evaluated the possibility of PI cytoprotection by gene transfer of Bcl-2 agains XA and C *in vivo* after transplatnation (Tx) in NOD-SCID mice previously reconstituted with primate lymphocytes. Methods: Male NOD-SCID mice were reconstituted with primate lymphocytes ($5x10^8$) 15 days before PI Tx. PI were infected with an adenovirus encoding Bcl-2 (AdBcl-2) or an irrelevant gene (AdLacZ). *In vitro* islet cytotoxicity was evaluated by LDH release. 24 hours after the infection, 5000 IEQ were infused into the portal vein in STZ-induced previously reconstituted diabetic NOD-SCID mice (n=6). Results: Reconstitution of NOD-SCID mice was confirmed by inmunohistochemical detection of rhesus T and B cells in BM and spleen. Serum obtained from these mice induced significant cytotoxicity to PI *in vitro* (complete serum 1:2, LDH release=80.2±3.4%, 1:16= 35.8±4.78%) compared with serum obtained from non-reconstituted controls (1:2=12.4±2.7% and 1:16=8.34±4.78, P>0.05). Gene transfer of Bcl-2 significantly reduced LDH release *in vitro* following PI exposure to serum from reconstituted mice (28.9±5.6%) comapred with AdLacZ controls (86.4±4.2%, P<0.05). Immediate destruction of AdLaz or Mock infected PI was demonstrated after Tx in reconstituted mice (day 3, glucose levels >250mg/dL in 100% of the recipients). In contrast, 75% of the animals that received PI overexpressing Bcl-2 presented non-fastig glucose leves < 250mg/mL. 8 days after the Tx glucose disposal rates were significantly higher in recipients that received AdBcl-2 infected PI (1.52±0.3) *versus* AdLacZ or Mock infected PI (0.31±0.2 and 0.4±0.1, respectively, P<0.05). Histological analyses confirmed the presence of intact PI in liver samples from reconstituted mice. Conclusions: Overexpression of Bcl-2 allowed porcine islets to survive and function in vivo after continuous exposure to XNA and C. This strategy has potential to improve survival of pancreatic iselt xenotransplants.

Abstract# 1541 Poster Board #-Session: P297-III

HEME OXYGENASE-1 MEDIATES CYTOPROTECTIVE AND ANTIAPOPTOTIC FUNCTIONS OF Ad-IL-13 GENE TRANSFER. Bibo Ke,[1] Xiu-Da Shen,[1] Charles R. Lassman,[2] Feng Gao,[1] Ronald W. Busuttil,[1] Jerzy W. Kupiec-Weglinski.[1] *[1]Dumont-UCLA Transplant Ctr., Dept. Surgery; [2]Pathology, UCLA School of Medicine, Los Angeles, CA.*

Background: We have shown that Ad-IL-13 gene tranfer prevents liver injury induced by ischemia/reperfusion (I/R), decreases production of pro-inflammatory cytokines and prevents apoptosis in endothelial cells by upregulating the expression of "protective" molecules, including anti-oxidant heme oxygenase-1 (HO-1). This study was designed to test a hypothesis that HO-1 mediates Ad-IL-13 cytoprotection both in vivo and in vitro. **Methods:** SD rats were infused with Ad-IL-13 or Ad-βgal reporter gene ($2.5x10^9$pfu i.v) with or without adjunctive tin protoporphyrin (SnPP), a competitive HO enzymatic activity inhibitor. 24 h later, livers were harvested, stored for 24 h at 4°C in UW solution, and then transplanted into syngeneic hosts. Animals were followed for survival, liver tissue and blood samples were harvested for future analyses. For in vitro study, HUVEC were transfected with Ad-IL-13 or Ad-βgal. After 36-48 h, HUVEC were incubated with TNF-α with or without adjunctive SnPP. Cytotoxicity assay and TUNEL staining were performed. **Results**: 100% of orthotopic liver transplant (OLT) recipients treated with Ad-IL-13 survived >14 days. In contrast, adjunctive SnPP decreased 14-day survival to 57%, comparable with Ad-βgal controls. This was accompanied by increased sGOT levels in Ad-IL-13 + SnPP, as compared with Ad-IL-13 gr. (day 1: 721 ± 42 vs. 103 ± 9, p<0.005). OLTs treated with Ad-IL-13 + SnPP revealed severe hepatocyte necrosis and sinusoidal/vascular congestion (Suzuki's score = 3.3 ± 1.2 vs. 0.67 ± 0.8 without SnPP, p<0.005). SnPP treatment was not hepatotoxic on its own, as sGOT levels and liver histology remained normal in naive rats treated with SnPP. HO-1 depression has led to an increased frequency of TUNEL+ cells in OLTs (25.5 ± 12.1 cells, vs. 5.5 ± 4.1 cells without SnPP; p<0.005), and suppressed expression of A20, Bcl-2, Bcl-xl, and HO-1. In in vitro studies, SnPP-induced inhibition of HO-1 in Ad-IL-13 cultures markedly increased the number of TUNEL+ cells (130 ± 35 with SnPP, vs. 25 ± 11 TUNEL+ cells without SnPP; p<0.0002). In parallel, Western blot-assisted detection of HO-1, A20 and Bcl-2/Bcl-xl in HUVEC cultures decreased after adjunctive SnPP. **Conclusion:** This study is the first to provide evidence for the key role of HO-1 in the cytoprotective and anti-apoptotic functions of virally induced IL-13 both in vivo and in vitro. Based on results of HO-1 neutralization studies, we propose that HO-1 represents one of putative IL-13 downstream effectors.

Abstract# 1542 Poster Board #-Session: P298-III

ENGRAFTMENT AND TUMORIGENICITY OF EARLY EMBRYONIC STEM CELLS IN MYOCARDIAL SCAR TISSUE-A COMPARATIVE STUDY IN SYNGENEIC, ALLOGENEIC AND SCID MICE. Theo Kofidis,[1] Leora Balsam,[1] Jorg L. De Bruin,[1] Toshiyuki Yamane,[2] Grant Hoyt,[1] Irving L. Weissman,[2] Robert C. Robbins.[1] *[1]Cardiothoracic Surgery/Falk Research Center, Stanford Medical School, Stanford, CA; [2]Dept of Pathology, Stanford Medical School, Stanford, CA.*

Purpose: A variety of primordial cells have been used to replace infarcted myocardium but the majority have demonstrated poor survival as well as inability to restore cardiac function. In this new comparative approach, we address the potential of early embryonic stem cells (ESC) to remodel infarcted heart muscle. **Methods:** SV129-H2B mouse embryonic stem cells were transfected with the GFP(green fluorescent protein) gene and harvested on plating day 7. $5x10^6$ cells or culture medium only were injected into acutely ischemic myocardium of mice that had undergone ligation of the left anterior descending coronary artery. Three mouse strains (n=6 each) were studied: syngeneic (sv129), allogeneic(C57) and immunodeficient (SCID). Hearts were harvested two weeks later and stained with Masson's Trichrome to assess for scar and tumor formations. Immunofluorescent staining for GFP and cardiac markers (connexin 43, α-sarcomeric actin) was also performed. Cardiac function, including fractional shortening and overall contractility, was evaluated by echocardiography. **Results:** The injected cells formed extensive GFP-expressing grafts within the myocardial scar. There was colocalization of connexin 43 and α-sarcomeric actin with GFP in a large portion, though not all of the cells, suggesting differentiation of ESC to cardiomyocytes. No teratomas were found, although nuclear irregularity was evident after two weeks in vivo. Nuclear irregularity was most prominent in the SCID group, moderate in the syngeneic group and least in the allogeneic group, indicating distinct immune response to the grafted cells in every group. Fractional shortening was best in SCID mice (34.3±6.2%), followed by syngeneic (30.6±4.3%) and allogeneic mice (24.1±5.4%), but was significantly better in all treated groups compared to controls (18.9±3.9%). **Conclusions:** Early embryonic stem cells engraft, survive in large populations and restore the function of infarcted myocardium. Host immunologic factors influence their tumorigenic and restorative potential.

Abstract# 1543 Poster Board #-Session: P299-III

LONG-TERM EXPRESSION AND TOLERANCE INDUCTION AFTER ADENO-ASSOCIATED VIRAL-MEDIATED IN UTERO GENE TRANSFER. Gerald S. Lipshutz,[1] Wendy Mahler,[2] Karin L. Gaensler.[2] *[1]Department of Surgery, University of California, San Francisco, San Francisco, CA; [2]Department of Medicine, University of California, San Francisco, San Francisco, CA.*

Background: The development of gene transfer in utero will make possible the amelioration and cure of diseases with pre- and post-natal morbidity. Stem cells may be transduced more easily in utero than in adult tissues and expression of genes prior to immune maturation may allow for tolerance induction. Recombinant adeno-associated viruses (rAAV) have been demonstrated to transfer genes in adults but with immune consequences. We hypothesized that long-term expression could be obtained and tolerance induced to a non-self protein after expression is begun prenatally. Materials and Methods: rAAV containing the luciferase (luc) reporter gene and the elongation factor 1-α (EF1α) promoter were constructed. At gestation day 15, three pregnant female mice were anesthetized and $3x10^{11}$ genomes of rAAV-EF1α-luc were injected by intraperitoneal route (IP) into each fetus (n=41). Animals were euthanized and tissues analyzed for luciferase while others underwent serial examination by in vivo bioluminescent imaging (BLI) of transmitted photons with detection by a charge coupled device camera. Western analysis was performed by standard methods. Anti-rAAV and anti-luciferase ELISA and lymphocyte proliferation assay were performed to examine the immune response to luciferase. Results: Survival of animals to birth was 87.5%. Luciferase was primarily detected in the peritoneum and liver. Other tissues, including heart, lung, and thymus demonstrated lower levels. Real-time, in vivo bioluminescent imaging demonstrated expression to 18 months (length of study). Western analysis demonstrated 0.007 ng of luciferase/25 mg tissue. No humoral response to rAAV or luciferase was detected. Lymphocyte proliferation assay demonstrated no thymidine uptake compared to controls. Subsequent adult IP injection into in utero AAV-injected mice resulted in stable postnatal boosting of expression, further indicating the lack of previous development of immunity. Conclusion: These studies demonstrate that rAAV can provide life-long expression after in utero injection. In addition, functional tolerance to non-self proteins may be achieved by expression in utero. These studies have implications for both gene and cellular therapy and organ transplantation and the induction of tolerance.

Abstract# 1544 Poster Board #-Session: P300-III

ROLE OF GRAFT PARENCHYMA ON STABLE MACROCHIMERISM IN RECIPIENTS OF SMALL INTESTINAL ALLOGRAFTS AND DONOR BONE MARROW. Atsunori Nakao,[1] Kei Kimizuka,[1] Tetsuma Kiyomoto,[1] Joao Seda,[1] Anthony J. Demetris,[1] Michael A. Nalesnik,[1] Noriko Murase.[1] *[1]Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

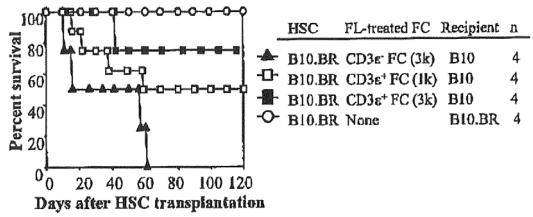
We have recently shown that stable macrochimerism can be established in unmodified recipients of intestinal allografts with simultaneous donor bone marrow (BM) infusion. Although numerous studies analyzed hematopoietic stem cell engraftment in cytoablated recipients, little is known about the origin and behavior of donor stem cells in non-cytoablated organ allograft recipients. In this study, we analyzed origin of macrochimerism after intestine transplants. **Methods**: Sex-mismatched transplantation was performed in BN to LEW rat strain combination under tacrolimus (1.0 mg/kg/day, d0-13, 20, and 27). Levels and lineages of chimerism were studied by flow cytometry using mAbs for LEW and BN class I antigens and by real time PCR with Y chromosome probes. **Results**: When small intestine was transplanted alone, there was an early high level chimerism; however it decreased with time and disappeared by d150. Similarly, when BM was infused without organ grafts, long-term chimerism was not obtained in conventionally immunosuppressed recipients. However, after co-transplantation of intestine and BM, long-term stable multilineages macrochimerism (8-12%), including donor T, B, and NK cells, was established for >150d. To examine the possibility that donor leukocytes from intestinal grafts play a role in establishing long-term macrochimerism, intestinal graft passenger leukocytes were eliminated by donor ALS pretreatment or *ex vivo* graft irradiation (10 Gy). Although these procedures significantly decreased early chimerism, long-term macrochimerism was not eradicated when donor BM was infused, suggesting an important function of graft parenchyma in maintaining chimerism. Origin of macrochimerism was further studied by transplanting male intestine + female BM or female intestine + male BM from BN donors into female LEW recipients. By analyzing male DNA in donor population, BM-derived donor cells were shown to slowly increase with time and reached to 40-50% of donor population by d40. **Conclusions**: These results suggest that donor hematopoietic progenitor cell engraftment occurs in conventionally immunosuppressed recipients when BM was transplanted together with intestinal parenchyma. Allgraft parenchyma may be important in providing syngenic microenvironment for infused donor BM.

	allograft		% donor cells		
Group	intestine	BM	d7	d40	d150
1	no	yes	0.7	5	0
2	yes	no	9.9	3.9	0
3	yes	yes	12.7	14.1	9.3
4	depletion w/rad	yes	0.7	5.1	1.9
5	depletion w/ALS	yes	3.3	6.9	5

Abstract# 1545 Poster Board #-Session: P301-III

CD3Ε+ FLT3-LIGAND -EXPANDED FACILITATING CELLS FACILITATE ENGRAFTMENT OF ALLOGENEIC HSC. Yiming Huang,[1] Francine Rezzoug,[1] Carrie L. Schanie,[1] Hong Xu,[1] Suzanne T. Ildstad.[1] *[1]Institute for Cellular Therapeutics, University of Louisville, Louisville, KY.*

$CD8^+/TCR^-$ facilitating cells (FC) enhance engraftment of purified hematopoietic stem cells (HSC) in allogeneic recipients. The presence of a CD3ε-TCR-β-Fcp33 complex directly correlates with the ability of FC to facilitate allogeneic HSC engraftment. However, only 5% of FC are $CD3ε^+$. The biologic function of $CD3ε^+$ FC therefore could not be evaluated until now due to low cell number. We previously reported that treatment of mice with Flt3 ligand (FL) induces significant expansion of FC and HSC in the bone marrow and peripheral blood (PB). In the present study we evaluated the function of FL-expanded FC on the basis on the expression of $CD8^+/TCR^-/CD3ε^+$ ($CD3ε^+$ FC) or $CD8^+/TCR^-/CD3ε^-$ ($CD3ε^-$ FC). B10.BR ($H\text{-}2^k$) mice were injected with 10 mg FL/day for 10 days. Control animals received saline only. HSC ($Sca\text{-}1^+/c\text{-}kit^+/lin^-$) were sorted from bone marrow of untreated B10.BR mice and $CD3ε^+$ FC or $CD3ε^-$ FC from PB of FL-treated donors after 10 days of treatment. Allogeneic (C57BL/10: $H\text{-}2^b$) recipient mice were conditioned with 950 cGy total body irradiation and reconstituted with 5000 untreated HSC plus 1000 or 3000 FL-treated $CD3ε^+$ FC or $CD3ε^-$ FC from PB. The biologic activity of FC was most potent in the $CD3ε^+$ subpopulation. 50% of recipients of 1000 $CD3ε^+$ FC and 75% of mice given 3000 $CD3ε^+$ FC engrafted. In striking contrast, the $CD3ε^-$ FC population was significantly less efficient at facilitation as reflected by impaired engraftment. In animals that engrafted, the absolute counts of WBC, RBC and Hb recovered with a kinetic similar to syngeneic control. None of the recipients exhibited evidence for graft-versus-host disease (GVHD). These data demonstrate that $CD8^+/TCR^-/CD3ε^+$ FC are the most efficient subpopulation in facilitating allogeneic HSC engraftment yet avoiding GVHD.



Abstract# 1546 Poster Board #-Session: P302-III

ADENOVIRAL iNOS GENE TRANSFER TO DONOR LIVER GRAFTS: POSSIBLE PROTECTION MEDIATED BY HO-1 INDUCTION. Takashi Kaizu,[1] Noriko Murase,[1] Yoshihito Takahashi,[2] Brian T. Bucher,[1] Kaye M. Reid,[1] Lifang Shao,[1] David A. Geller.[1] *[1]Surgery, Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Surgery, University of Kitasato, Sagamihara, Kanagawa, Japan.*

We have previously shown that the L-arginine/nitric oxide synthase (NOS) pathway has a protective role in hepatic cold ischemia/reperfusion (I/R) injury. Further, we found that donor liver pretreatment with adenovirus encoding inducible NOS (AdiNOS) ameliorates I/R injury after rat liver transplantation. This study was designed to determine the mechanisms by which AdiNOS pretreatment protects against hepatic I/R injury. **Methods:** Orthotopic syngeneic LEW rat liver transplantation (OLT) was performed after 18 hrs preservation in cold UW. AdiNOS or control gene vector (AdLacZ) ($2x10^9$/pfu) was delivered to the liver by donor i.v. pretreatment 4 days before harvesting. Uninfected grafts also served as control. In the first series of experiments, donor rat livers were assessed for iNOS or HO-1 protein expression 4 days after adenoviral injection and prior to OLT. In the second series, the AdiNOS pretreated donor livers underwent OLT, and serum AST levels and histopathology were assessed after reperfusion. **Results:** AdiNOS pretreatment induced striking hepatic iNOS protein expression 4 days after injection, whereas iNOS protein was not detected in the AdLacZ-treated liver, confirming the successful iNOS gene transfer. A significant expression of HO-1 protein was also detected in AdiNOS-treated, but not AdLacZ-treated donor liver, indicating that hepatic HO-1 expression was possibly induced through AdiNOS gene transfer, but not by adenoviral vector-mediated stress. AdiNOS pretreatment markedly improved serum AST levels and tissue necrosis compared with untransfected or AdLacZ-treated group. (Table, mean ± SE, n = 2-6 animals per group, $*P < 0.05$ v.s. AdLacZ-treated group) **Conclusion:** These results demonstrate that AdiNOS pretreatment leads to protection against hepatic cold preservation injury. Hepatic HO-1 induction, which is concomitant with iNOS expression, may account for part of the protective effect of donor pretreatment with AdiNOS.

	iNOS expression (/control)	HO-1 expression (/control)	AST (IU/L)		
Transplant Group	Before harvest	Before harvest	6h	24h	48h
Uninfected Control	1.0	1.0	3317±578	5547±979	1992±467
AdLacZ ($2x10^9$/pfu)	1.2	1.4	2314±350	3433±879	1064±438
AdiNOS ($2x10^9$/pfu)	24.9	3.9	1427±210	1547±352*	328±60*

Abstract# 1547 Poster Board #-Session: P303-III

IMPROVED GLUCOSE-DEPENDENT HEPATIC INSULIN PRODUCTION BY USE OF A TRANSLATIONAL ENHANCER. Philipp C. Nett,[1] Hans W. Sollinger,[1] Tausif Alam.[1] *[1]Department of Surgery, Division of Transplantation, University of Wisconsin Hospital and Clinics, Madison, WI.*

Gene-therapy based hepatic insulin production is a promising strategy in the treatment of insulin dependent diabetes mellitus (IDDM). We have previously shown that hepatocytes transduced with an insulin gene construct (Ad.SAM) containing three units of glucose inducible regulatory (GIR) elements, an albumin promoter and a modified human insulin cDNA, synthesize and release functional insulin in physiological range in response to glucose challenge. *In vivo* experiments showed that Ad.SAM treatment was able to correct fasting hyperglycemia in streptozotocin (SZ)-induced diabetic rats. However, due to insufficient amount of insulin production, the blood glucose levels during oral glucose tolerance tests were only partially corrected. Reasoning that adding a translational enhancer (TE) to the insulin construct may lead to increased insulin production, we have generated a new insulin gene construct (Ad.SATEM) that contains a TE from vascular endothelial growth factor. Results from primary hepatocytes ($1x10^6$ cells) transduced with comparable amount of either Ad.SAM or Ad.SATEM, *ex vivo*, showed that while the insulin production was similarly dependent on glucose concentration and time, the amount of insulin produced from cells transfected with Ad.SATEM was much higher than with Ad.SAM. Thus, the culture medium from Ad.SATEM transfected hepatocytes contained 445 ng/ml and 120 ng/ml insulin at 27.5 and 3.5 mM glucose, respectively, whereas Ad.SAM, under similar conditions, caused 66 ng/ml and 23 ng/ml insulin production at high and low glucose, respectively after 27 hours. Therefore, in comparison to Ad.SAM, Ad.SATEM with a TE caused a 6.7-fold increase in insulin output at high glucose. Our data suggest that if 10-20% of hepatocytes in liver produce similar amount of insulin *in vivo*, correction of hyperglycemia in fed ad libitum diabetic rats might be possible. This study provides novel information on the potential use of translational enhancement in an insulin gene construct, that yields a therapeutically relevant amount of insulin to correct diabetic hyperglycemia in IDDM.

Abstract# 1548 Poster Board #-Session: P304-III

OVER EXPRESSION OF CYCLIN INHIBITOR p21 IN p21-/- MICE PREVENTS LYMPHOCYTE PROLIFERATION, EXPRESSION OF CYCLINS AND PRO-INFLAMMATORY CYTOKINES IFN-g AND TNF-aγα. Ashwani K. Khanna.[1] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Introduction: We have demonstrated that the over-expression of p21 results in a reduced proliferation of lymphocytes, this study was designed to explore if deficiency of p21 results in increased lymphocyte proliferation. Methods: We compared the proliferation of lymphocytes from p21-/- mice and wild type mice to inr esponse to anti-CD3 mAb and alloantigen in MLR cultures. We over-expressed p21 in p21-/- mice by intramuscular injection with p21 sense plasmid DNA into p21 knockout mice. Two Groups of mice (n = 8 each) were injected intramuscularly twice at an interval of 5 days either with p21 sense plasmid DNA or empty vector plasmid DNA. Four mice from each group were sacrificed 7 and 21 days after the last injection. To understand the role of lymphocyte proliferation on inflammation, we also studied the expression of cyclins, IFN-g and TNF-a in these p21-/-mice Results: The results from wild type (n =9) and p21-/- mice (n = 9) demonstrate that lymphocytes from p21-/- mice proliferated statistically significantly ($p<0.01$) higher compared to wild type mice. In MLR assay using stimulator lymphocytes either from p21-/- or wild type mice indicated that increased proliferation was seen only when the lymphocytes from p21-/- mice were used as stimulators. Results demonstrate that the transfection of p21 sense plasmid DNA but not empty vector plasmid DNA in p21-/- mice resulted in a significantly decreased proliferation in response to mitogens. Also, mRNA expression of Cyclin G, IFN-g and TNF-a was significantly decreased in anti-CD3 stimulated splenocytes from p21 transfected p21-/-mice compared to control p21-/- mice. Conclusions: These results suggest that the a) the cyclin inhibitor p21 can potently inhibit lymphocyte activation in response to allo-and mitogenic stimuli and b) prevents the expression of cyclins pro-inflammatory cytokines. These properties of p21 can potentially be exploited as immunosuppressive agent in organ transplantation.

Abstract# 1549 Poster Board #-Session: P305-III

THE HUMAN CD8α TRANSGENE DOES NOT RESTORE FACILITATIVE POTENTIAL TO NON-FACILITATING $CD4^+$ AND $B220^+$ CELLS. Kimberly L. Gandy,[1] Dana Giangiacomo,[1] George Rofaiel,[1] Jos Domen.[2] *[1]Surgery and Immunology, Duke University Medical Center, Durham, NC; [2]Medicine and Immunology, Duke University Medical Center, Durham, NC.*

Multiple labs have demonstrated that cells which express CD8α facilitate the engraftment of hematopoietic cells across allogeneic barriers. We have shown that CD8α cells enhance the engraftment of purified hematopoietic stem cells (HSC) across allogeneic barriers in lethally irradiated recipients. We have shown that different CD8α expressing subpopulations of whole bone marrow facilitate, and that bone marrow that lacks CD8α is deficient in facilitative potential. We have also shown that facilitator cells must be genetically disparate from the host to facilitate, and that cells devoid of lytic potential have decreased but present facilitative potential. These studies seek further understanding of the mechanisms by which CD8 cells facilitate with the premise that the engraftment/rejection phenomena involved with this limited and homogeneous cellular population may have generalized implications to transplant biology. We have used cells from mice which express the human CD8α transgene under the control of the CD2 promotor, a condition which has resulted in the expression of CD8α in $CD4^+$ and $B220^+$ peripheral blood and bone marrow. These populations usually do not express CD8α and have been previously shown to be devoid of significant facilitative potential. BALB/c mice were lethally irradiated and reconstituted with 2000 purified c-Kit^+, Thy-1.1^{lo}, Lin^{-lo}, Sca-1^+ (KTLS) stem cells with or without other sorted bone marrow subpopulations. All populations were purified to >90% purity and the source of origin of the three different cellular populations could be distinguished by cell surface markers as previously described. Mice were bled for analysis of cell counts at 2 weeks, and bled for FACS analysis of donor-derived reconstitution at 4 weeks. Analysis at 4 weeks of reconstitution in mice given HSC alone (n = 5), HSC and CD8 (n = 5), and HSC and $B220^+$hCD8α cells (n = 9) showed donor-derived reconstitution of 18%, 88.8% ($p=0.01$), and 37.9% ($p = 0.02$) respectively. Similar results were suggested by analysis of mononuclear cell counts in mice reconstituted HSC alone (n = 4), HSC and CD8 (n = 5), and HSC and $CD4^+$hCD8α cells (n = 10). Mononuclear counts of $2 X 10^6$ /ml, $6 X 10^6$ ($p =0.02$), and $4.2 X 10^6$ ($p = 0.1$) respectively, were detected. These data suggest that human CD8α is not sufficient to confer full facilitative potential to non-facilitating $B220^+$ and $CD4^+$ populations of cells.

Abstract# 1550 Poster Board #-Session: P306-III

***EX VIVO* ADENOVIRAL GENE TRANSFER OF CONSTITUTIVELY ACTIVATED STAT3 REDUCED ISCHEMIA-REPERFUSION INDUCED INJURY AND PROMOTED LIVER REGENERATION IN 20% RAT PARTIAL LIVER TRANSPLANT MODEL.** Michitaka Ozaki,[1] Lei Guo,[1] Sanae Haga,[1] Keita Terui,[1] Hui Qi Zhang,[1] Shin Enosawa,[1] Seiichi Suzuki. *[1]Deparment of Innovative Surgery, National Research Institute for Child Health and Development, Setagaya, Tokyo, Japan.*

Purpose. Stat3 is one of the most important transcription factors involved in liver regeneration. Recetnly Stat3 has been reported to target both mitogenic genes and anti-apoptotic genes. This study was designed to see the protective effect of constitutively activated form of Stat3 (Stat3-C) against post-Tx liver injury and the promotive effects toward liver graft regeneration in rat 20% partial liver transplant (PLTx) model by *ex vivo* adenoviral gene transfer method. **Method.** Adenovirus vector coding Stat3-C gene was generated in our laboratory. Stat3-C construct was made by substituting cysteine residues for A661 and N663 of the murine Stat3. Lewis rats (male, 250g) were used for the PLTx experiments. Physiological saline(PS) or adenoviruses, encoding inert bacterial LacZ gene (AdLacZ) and Stat3-C (AxCAS3-C) were intraportally introduced to 20% liver graft and clamped for 30min during cold preservation period(2.5x10[8]pfu/graft). Prior to orthotopic liver Tx, 20% liver graft was flushed out with cold saline to reduce the risk of viral infection to the recipient rat. In post-Tx periods, liver graft weight and serum levels of GOT/GPT/Bilirubin were measured, and histological study and electromobility gel shift assay (EMSA) of the liver tissue were performed. **Results.** Stat3-C expression in liver tissue was confirmed by western blot analysis. Serum levels of GOT/GPT and Bilirubin were reduced in the group of rats with over-expressed Stat3-C, compared to both PS and AdLacZ groups. Stat3-C induced anti-apoptotic proteins such as Bcl-2, $Bcl-_{XL}$ and also reduced caspase activities (caspase3 & 8). EMSA revealed that Stat3-C introduction remarkably activated Stat3 DNA binding, and interestingly reduced pro-apoptotic Stat1 activity at the same time. Stat3-C promoted liver regeneration that was examined by measuring graft weight and mitotic index. **Conclusions.** Stat3-C adenovirally introduced to the liver graft by *ex vivo* method successfully over-expressed Stat3-C protein and activated its DNA binding in liver tissue early after Tx. By expressing anti-apoptotic proteins and increasing mitosis, Stat3 reduced ischemia-reperfusion-induced injury and promoted liver regeneration.

sGOT/sGPT and Liver weight after 20% PLTx

	LacZ	Stat3-C
sGOT (IU/L)	456 +/- 152	148 +/- 15
sGPT (IU/L)	333 +/- 109	48 +/- 14
liver/body wt ratio (%)	2.11 +/- 0.85	2.72 +/- 0.51

Abstract# 1551

DONOR KIDNEY EXCHANGE FOR INCOMPATIBLE RECIPIENTS. Francis L. Delmonico,[1] Paul E. Morrissey,[1] George S. Lipkowitz,[2] William Harmon,[1] Jonathan Himmelfarb,[1] Jeffrey Stoff,[1] Martha Pavlakis,[1] Helen Mah,[1] Jane Goguen,[1] Richard Luskin,[1] Edgar Milford,[1] Richard J. Rohrer.[1] *[1]New England Organ Bank, Newton, MA; [2]LifeChoice Donor Services, Windsor, CT.*

Motivated individuals have historically been thwarted in their attempt to donate a kidney to a family member or friend due to biological barriers, such as ABO blood group incompatibility. However, a new approach to this problem effectively doubles the rate of transplantation for waiting recipients. **Methods:** This UNOS Region has devised a system that would enable either a simultaneous live donor exchange or a live donor/cadaver donor list-paired exchange to incompatible recipients. The list-paired exchange is accomplished as follows: the incompatible living donor provides an allograft to a patient on the center cadaver waiting list in exchange for the Region cadaver donor pool providing a priority allograft to the incompatible recipient of the live donor. The priority does not supercede emergency kidney allocation alone or with liver or heart transplants, nor 0 mm sharing. Each proposed exchange is reviewed by Region oversight. **Results:** Two live donor exchange kidney transplants (4 recipients) and 8 list paired exchange kidney transplants (16 recipients) have been performed.

List Paired Exchange Data:

Live Dnr Relationship	Live Dnr ABO	Live Dnr Age	Date Live Dnr Tx	List Recip Age	List Recip Wait Time	Time LD to CD Tx	CD Recip Age	CD Donor Age
mother	O	43	2/23/01	37	725 days	17 days	12	32
wife	A	51	3/20/01	30	812 days	56 days	40	17
wife	A	50	8/27/01	54	410 days	10 days	53	27
neighbor	A	69	9/18/01	63	501 days	15 days	30	47
wife	A	62	12/11/01	53	396 days	22 days	65	31
brother	A	39	3/12/02	38	716 days	29 days	40	22
mother	B	56	7/17/02	31	2126 days	5 days	37	56
friend	AB	51	10/1/02	37	458 days	5 days	36	16

All List Recipients of LD were Blood type identical; all CD Recipients were O Blood type.

All CD recipients (who had established care relationships with Region transplant centers) were unsensitized and on dialysis. None had undergone transplantation previously. **Outcomes:** All list paired exchange kidney transplants are functioning. One of the live donor exchange transplants has failed because of rejection. **Conclusions:** This innovative system yields an additional donor source for patients awaiting a deceased donor kidney. As every paired exchange transplant removes a patient from the waiting list, it also precludes the incompatible recipient from having to go on the waiting list. Therefore, this approach also increases access to donor kidneys for the remaining transplant candidates on the list.

Abstract# 1552

SIX-MONTH RESULTS OF THE FIRST PROSPECTIVE, RANDOMIZED, MULTI-CENTER KIDNEY TRANSPLANT STUDY COMPARING TACROLIMUS+RAPAMUNE VS TACROLIMUS+MMF COMBINATION THERAPY. Robert Mendez,[1] for the Fujisawa Study Group. *[1]National Institute of Transplantation, Los Angeles, CA.*

Abstract: Following are the 6-month results of this study conducted at 27 U.S. centers. One year data will be analyzed and available for presentation at the meeting. **Objective:** To compare the safety and efficacy of combination therapy of tacrolimus(Tac)+sirolimus(Srl) vs. Tac+mycophenolate mofetil(MMF). Prior to transplant, adult recipients of a primary renal transplant were randomized 1:1 to receive Tac+corticosteroids with either Srl(N=185) or MMF(N=176). The primary endpoint was the incidence of biopsy confirmed acute rejection. Patient(pt.) and graft survival, renal function and adverse events were also evaluated. Target trough levels were 5-15 ng/mL for Tac and 4-12 ng/mL for Srl. There were no significant differences between the groups regarding PRA%, cold ischemia time, gender, race or donor demographics. Pts. in the MMF group were older(mean 47.8 vs 45.3;p=0.05) and there was a trend toward more zero antigen mismatches in the Srl group (10.3% vs 5.1%;p=0.07). More MMF pts.(31%) had delayed graft function than Srl pts.(22.7%;p=0.07), of these 22 Srl(12%) and 37 MMF(21%) pts. had dialysis in the first week(p=0.02). More pts. discontinued Srl, primarily for hyperlipidemia. Dose modifications were more frequent with MMF, primarily for GI complaints and leukopenia, for Srl the most common reasons were hyperlipidemia and wound healing.

RESULTS AT 6-MONTHS

	Tac/Srl N=185	Tac/MMF N=176
Pts. with biopsy-confirmed acute rejection	13%	11.4%
Graft survival	93%	95.5%
Pt. survival	97.3%	97.7
Median Tac levels (ng/mL)	8.5	8.7
Median Srl/MMF dose (mg)	3.0	1500
Mean serum creatinine (mg/dL)*	1.77	1.44
Serum creatinine >1.5 mg/dL*	45.1%	33.3%
Serum creatinine >2.0 mg/dL*	19.7%	7.9%
Srl/MMF discontinuations*	21.1%	10.8%
Srl/MMF dose changes for adverse events*	25.9%	53.4%
New onset insulin dependent diabetes mellitus	7.6%	7.7%
Mean total cholesterol (mg/dL)*	218	192
Mean LDL cholesterol (mg/dL)*	111	98
Pts. treated with lipid lowering therapy*	60%	37%
Mean diastolic blood pressure (mmHg)*	80	77

*p<0.05

Conclusion: The incidence of biopsy confirmed acute rejection was similar in both treatment groups. Pt. and graft survival was excellent and equivalent. More pts. discontinued Srl and there were more dose changes with MMF. These data demonstrate that Tacrolimus is safe and effective when combined with either Srl or MMF. The tacrolimus-MMF combination may optimize renal function and minimize cardiovascular risk factors.

Abstract# 1553

24-MONTH RESULTS OF A MULTICENTER STUDY OF EVEROLIMUS FOR THE PREVENTION OF ALLOGRAFT REJECTION AND VASCULOPATHY IN *DE NOVO* CARDIAC TRANSPLANT RECIPIENTS. Donna Mancini,[1] Mario Viganò,[2] Luis A. Pulpon,[3] Christoph Bara,[4] Robert B. Love,[5] Howard J. Eisen,[6] Jane Murphy,[7] Kamal H. Abeywickrama,[8] Peter Bernhardt,[8] the RADB253 Study Group. *[1]Medical Center, Columbia Presbyterian, New York, NY; [2]IRCCS, Policlinico San Matteo, Pavia, Italy; [3]Cardiol. Dept., Clinica Puerta de Hierro, Madrid, Spain; [4]Klinik für Thorax-, Herz- und Gefässchirurgie, Medizinische Hochschule, Hannover, Germany; [5]University Hospital, University of Wisconsin, Madison, WI; [6]School of Medicine Cardiology Section, Temple University, Philadelphia, PA; [7]Business Unit Transplantation, Novartis Pharmaceuticals, East Hanover, NJ; [8]Business Unit Transplantation, Novartis Pharma AG, Basel, Switzerland.*

Everolimus (Certican™, RAD), an investigational new drug, is a proliferation inhibitor that targets primary causes of chronic rejection. **Purpose:** A 24-month analysis of an international, double-blind trial of the safety and efficacy of everolimus vs azathioprine (AZA) in *de-novo* heart transplant recipients. **Methods:** Patients (N=634) were randomized to either everolimus 1.5 mg/day (N=209), everolimus 3 mg/day (N= 211) or AZA 1-3 mg/kg/day (N=214), with cyclosporin microemulsion, steroids and statins. The incidence of efficacy failure (biopsy-proven acute rejection (BPAR) ≥3A, BPAR with hemodynamic compromise, graft loss, death or lost to follow-up) was determined. Incidence of intimal thickening and allograft vasculopathy was assessed by intravascular ultrasound (IVUS). **Results:** At 24 months, the efficacy failure rate was significantly lower in the everolimus 1.5 mg (p=0.016) and 3 mg (p<0.001) groups vs AZA (incidence: 45.9%, 36% and 57.5%, resp.). The incidence of BPAR was also significantly lower with everolimus (1.5 mg: 34.9%, 3 mg: 22.7%, AZA: 48.1%). Patient survival showed no differences (90%, 86.3% and 88.8%). Viral infection occurred less frequently in both everolimus groups, and CMV infection rates were significantly lower (8.6% and 8.1%) than in the AZA group (22.4%). There were more bacterial infections with 3 mg (40.3%) and 1.5 mg everolimus (37.3%, both p<0.05) vs AZA (25.7%). Mean serum creatinine was higher with everolimus (p<0.001) but stabilized after Month 12. LDL and HDL were not different between the groups. Triglycerides and cholesterol were elevated in both everolimus groups (p<0.05). IVUS data demonstrated significant less change in mean maximal intimal thickness for everolimus (0.07mm, 0.06mm and 0.15mm, respectively, p<0.05). The incidence of allograft vasculopathy was better in both everolimus groups compared to AZA (33.3%, 45.5% and 58.3%, respectively, p<0.05 for everolimus 1.5mg vs AZA). **Conclusion:** Everolimus demonstrated superior efficacy compared to AZA. Survival was excellent in all three groups. The incidence of allograft vasculopathy was lower with everolimus. Thus, everolimus has the potential to offer an important new benefit to cardiac transplant patients.

Abstract# 1554

EVALUATION OF INTRAVENOUS IMMUNOGLOBULIN (IVIG) AS AN AGENT TO LOWER ALLOSENSITIZATION AND IMPROVE TRANSPLANTATION IN HIGHLY-SENSITIZED ADULT ESRD PATIENTS: REPORT OF THE NIH IG02 TRIAL.

Stanley C. Jordan,[1] Dolly Tyan,[1] Don M. Stablein,[2] Matthew McIntosh,[2] Ashley Vo.[1] *[1]Kidney Transplantation & Transplant Immunology, Cedars-Sinai Medical Center, LA, CA; [2]EMMES Corp., Rockville, MD.*

Introduction: IVIG is an immunomodulatory agent with potential applications to solid organ transplantation. Here we report on the ability of IVIG vs placebo to reduce anti-HLA antibody levels and improve transplantation in a NIH controlled clinical trial. Patients & Methods: Between 1997 and 2000, twelve U.S. transplant centers entered 101 adult highly-sensitized ESRD patients (PRAs> 50% monthly X3) into the IG02 trial. Patients were randomized 1:1 (49 IVIG; 52 Placebo) to receive IVIG (Gamimune N 10% SD or placebo 0.1% albumin, Bayer Corp.). Subjects received IVIG 2gm/kg (maximum dose 180gm) monthly x 4 or equivalent volume of placebo with additional infusions at 12 and 24 months. If transplanted, additional infusions were given monthly X4. The primary objective of the study was to determine if IVIG reduced the number of months on dialysis by improving transplantation. Other parameters such as the ability to reduce anti-HLA antibody levels and infection rates were examined. Results: Baseline PRA levels were similar in both groups (IVIG 81.2%±2.1 vs. 83.8%±1.9). IVIG significantly reduced PRA levels in study subjects over the 3 year study period compared with placebo (p=0.005). 18 (37%) IVIG and 9 (17%) placebo pts. were transplanted (p=0.03). More frequent transplants were observed in the IVIG group regardless of prior transplant history (p=0.02): prior transplant, 34% vs 10%, no prior transplant 43% vs 26%. The maximum likelihood estimate of annual transplant rate is 24% for IVIG and 12% for placebo. 22/27 were cadaver transplants (16 IVIG, 6 placebo). Thirteen rejection episodes were reported (12 IVIG vs 1 placebo) in 8 of 18 IVIG and 1 of 9 placebo subjects. Five graft failures occurred (3 IVIG, 2 placebo). The remaining viable transplants have mean SCrs of 1.7±1.1 (IVIG) at 529 days post-tx vs 1.3±0.4 mg/dl for placebo at 607.4 days post-tx. Adverse events were similar in both groups (23 IVIG, 24 placebo). Headache was the most common AE (p=0.005 IVIG (43%) vs placebo (17%)). Conclusions: From this multi-center, double-blinded, placebo controlled trial we conclude that IVIG is superior to placebo in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized ESRD patients. The 2 year allograft survival and mean SCrs were similar in both groups. Thus, IVIG treatment offers significant benefits to highly-sensitized ESRD patients awaiting transplantation.

Basic Plenary II

Abstract# 1555

TARGETING THE CHEMOKINE MCP-1/CCR2 CHEMOKINE PATHWAY INDUCES PERMANENT SURVIVAL OF ISLET ALLOGRAFTS THROUGH A PD-L1-DEPENDENT MECHANISM.

Iris Lee,[1] Liqing Wang,[1] Rongxiang Han,[1] Andrew D. Wells,[1] Qunrui Ye,[1] Lieping Chen,[2] Wayne W. Hancock.[1] *[1]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA; [2]Immunology, Mayo Clinic, Rochester, MN.*

Little is known regarding the mechanisms by which host T cells and macrophages are recruited to sites of islet transplantation and mediate islet allograft rejection or, indeed, how islet and other allografts survive long-term in the presence of an intact immune system. We have begun to explore the contribution in vivo of various chemokines and chemokine receptors to leukocyte recruitment and activation following islet allografting, beginning with MCP-1 which is known to be produced de novo by human islets, and whose expression is increased by pro-inflammatory cytokines. We now present data that targeting of the MCP-1/CCR2 pathway in vivo induces prolonged islet graft survival in fully mismatched allograft recipients:- BALB/c islets were rejected by 10-13 days in B6/129 recipients which were (i) untreated, or treated for 2 weeks with (ii) a low dose of rapamycin (RPM, 0.2 mg/kg/d), or (iii) anti-MCP-1 mAb alone (100 µg three times/week), whereas (iv) recipients given 2 weeks of anti-MCP-1 mAb and low-dose RPM therapy had prolonged graft survival (>60 days so far, p<0.001). The inhibitory surface receptor PD-1, a CTLA-4 homolog, delivers a negative signal to T-cells upon ligation by PD-L1, and we showed by flow cytometry induction of PD-1 and PD-L1 by CD4+ and CD8+ T cells upon in vitro activation by CD3 plus CD28 mAbs, as well as following in vivo alloactivation induced by adoptive transfer of parent->F1 CFSE-labeled lymph node cells. Interestingly, analysis of islet allografts harvested at 60 days following anti-MCP-1 mAb plus RPM therapy showed intact islets with strong insulin staining, and a peri-islet infiltrate of CD4+ and CD8+ T cells plus macrophages, in conjunction with PD-L1 expression by a subset of peri-islet mononuclear cells. We therefore tested whether PD-L1 expression was key to the beneficial effects of targeting the MCP-1/CCR2 using the same therapeutic protocol as before plus addition of a neutralizing anti-PD-L1 mAb. We found that PD-1/PD-L1 blockade abrogated long-term survival and led to islet allograft rejection by day 25 (p<0.01). Data from our in vivo and in vitro studies lead to 2 key conclusions; (i) targeting the MCP-1/CCR2 chemokine pathway can result in long-term islet allograft survival, and (ii) maintenance of islet survival using such a protocol is an active event requiring induction and ongoing expression of the recently recognized novel immunoregulatory costimulatory ligand, PD-L1.

Abstract# 1556

HEME OXYGENASE-1 PROMOTER GENE POLYMORPHISM IS ASSOCIATED WITH GRAFT SURVIVAL OF CADAVERIC KIDNEY DONORS.

Annemiek Peeters, Joke Roodnat, Francine Lemos, André Uiterlinden, Willem Weimar, Carla Baan. *[1]Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.*

Tissue damage of the graft by allo-antigen independent and dependent factors seems to be the key event in processes that result in renal dysfunction. Recent data suggested that the outcome of the graft is influenced by its ability to protect itself from injury. Tubular cells attenuate to injury by the anti-inflammatory and anti-apoptotic effects of heme oxygenase (HO)-1. Gene transcription of HO-1 is modulated by a (GT)n dinucleotide repeat in the promoter. After activation, short (≤27) repeats are associated with increased HO-1 production. To determine whether the cytoprotective effect of HO-1 has a genetic basis after clinical kidney transplantation we examined this functional HO-1 gene polymorphism in 387 consecutive recipients that were transplanted between September 1995 and March 2001 and in 307 donors. Of the patients 60% (233/387) and of the donors 56% (171/307) were carriers of the short (GT)n repeats (p=0.24). Analysis showed that graft failure was associated with donor and not with recipient HO-1 gene polymorphism. The 3-year graft survival of donors kidneys with short (GT)n repeats, the high producers, was significantly higher than that of donor kidneys with long (GT)n repeats, the low producers: 92% vs 84%, p=0.006, Kaplan Meier. This difference in graft survival was caused by a subgroup of donors as the effect of the HO-1 gene polymorphism was seen in cadaveric donor kidneys (n=171) but not in living donor kidneys (n=136). Graft survival of cadaveric grafts with short (GT)n repeats (n=89) was significantly better than that of the cadaveric kidneys with long (GT)n repeats (n=82): 89% vs 77%, p=0.02, respectively. Further, we found an association between the reason for graft loss and the presence of long (GT)n repeats. 77% (10/13) of the donor kidneys that failed because of chronic rejection (biopsy proven) were carriers of the long (GT)n repeats vs 54% (12/22) of the other failures, and 43% (59/136) of the functioning grafts (p=0.05). In conclusion, our results suggest that cadaveric donor kidneys that are carriers of short alleles, and therefore have the ability of high HO-1 production, are less vulnerable to tissue injury resulting in significantly less chronic allograft dysfunction and better graft survival.

Abstract# 1557

IMPORTANCE OF LIGHT FOR ALLOIMMUNE RESPONSES MEDIATED BY CD4$^+$ T CELLS.

Zhong Guo,[1] Ying Dong,[1] Jun Wang,[1] Marvin Newton-West,[1] Nikos Emmanouilidis,[1] Yang-Xin Fu,[2] Kenneth A. Newell.[1] *[1]Surgery, Emory University, Atlanta, GA; [2]Pathology, University of Chicago, Chicago, IL.*

Introduction. A number of costimulatory molecules have been shown to augment CD4$^+$ T cell activation thereby potentiating alloreactive T cell function. LIGHT is an inducible costimulatory molecule expressed by T cells and DC. Engagement of its ligand HVEM, which is expressed constitutively by T cells, delivers a costimulatory signal. The aim of this study was to determine the role of LIGHT in the CD4$^+$ T cell response to alloantigens. **Methods.** The effect on T cell function of disrupting LIGHT was first examined *in vitro* in a CFSE proliferation assay using LIGHT$^{-/-}$ or wild type (WT) T cells treated with the blocking protein HVEMIg. The effect of disrupting LIGHT on T cell function *in vivo* was determined by reconstituting B6 RAG$^{-/-}$ mice bearing C3H skin grafts with 10 x 10^6 purified CD4$^+$ WT or LIGHT$^{-/-}$ T cells. The function of WT and LIGHT$^{-/-}$ CD4$^+$ T cells isolated from RAG$^{-/-}$ mice bearing skin allografts was assessed by CFSE MLR (proliferation), Elispot assay (priming), and CBA assay (cytokine quantification). **Results.** Disruption of the LIGHT pathway by HVEM-Ig or using LIGHT$^{-/-}$ T cells reduced the proliferation of CD4$^+$ T cells to plate-bound anti-CD3 mAb (51% and 42% reduction respectively vs. controls). Rejection of skin allografts by LIGHT$^{-/-}$ CD4$^+$ T cells was significantly impaired (MST of 46±11 days vs. 11±4 days for WT T cells, p = 0.0002). The number of T cells recovered from transplanted RAG$^{-/-}$ mice reconstituted with WT and LIGHT$^{-/-}$ CD4$^+$ T cells was equivalent (14.4±0.14 x 10^6 vs. 12.5±0.13 x 10^6, p=ns). The proliferation to donor-strain allogeneic DC *in vitro* of LIGHT$^{-/-}$ CD4$^+$ T cells isolated from transplanted RAG$^{-/-}$ mice was impaired relative to WT CD4$^+$ T cells (4.3±3.2% vs. 48.7±6.3%, p<0.01). The frequency of primed alloreactive CD4$^+$ T cells was also reduced in the LIGHT$^{-/-}$ group (2733±3004 vs. 16350±2277 spots/10^6 T cells, p<0.01). Lastly production of TNFα, IFNγ, and IL-2 was reduced in the LIGHT$^{-/-}$ group vs. the WT group (1.7, 5.8, and 7.1 fold respectively). **Conclusions.** The absence of LIGHT does not significantly affect homeostatic proliferation. LIGHT expressed by T cells can serve as a costimulatory molecule for other T cells that express HVEM (no APC in anti-CD3 mAb coated wells). LIGHT appears to be important for the response of CD4$^+$ T cells to alloantigens expressed by skin grafts. The decreased proliferation, priming, and cytokine production upon re-exposure to alloantigen *in vitro* of LIGHT$^{-/-}$ CD4$^+$ T cells suggests a defect in the initial activation of these cells.

Abstract# 1558

DICHOTOMY IN ACTIVATION OF ALLOREACTIVE CD4+ AND CD8+ T CELLS IN VIVO. Farhana Amanullah,[2] Gulcin Demirci,[1] Yon Su Kim,[1] Wenda Gao,[1] Terry B. Strom,[1] Xian C. Li.[1] *[1]Department of Medicine, Division of Immunology (Harvard Medical School), Beth Israel Deaconess Medical Center, Boston, MA; [2]Department of Medicine, Division of Nephrology (Harvard Medical School), Children's Hospital, Boston, MA.*

T cell activation and its effector function is greatly influenced by costimulatory signals and growth factor signals. However, exactly how these costimulatory signals and growth factor signals interact with each other and integrate into the general activation program of T cells, especially in allograft rejection, is largely unknown. In the present study we examined the effect of CD28/CD154 costimulation and gamma chain (γc) signals on T cell activation in vivo. We found that both CD4 deficient and CD8 deficient recipient mice can vigorously reject fully MHC mismatched skin allografts with a mean survival time of about 10 days (n=7,4). Treatment of recipient mice with rapamycin, a drug that blocks growth factor triggered signaling events, markedly prolonged the skin allograft survival in CD4 deficient recipients (MST= 60 days) but not in CD8 deficient recipients (MST=12 days). In vivo studies using CFSE labeled lymphocytes proliferating in irradiated allogeneic hosts revealed that both rapamycin and anti-common γc mAb (γc is a shared receptor component by all known T cell growth factor receptors) selectively blocked in vivo expansion of CD8+ T cells without affecting the proliferation of CD4+ T cells, thus suggesting that CD4+ and CD8+ T cells have different sensitivity to growth factors. In vitro experiments showed that both CD4+ and CD8+ T cells are extremely sensitive to growth factor stimulation as IL-2 and IL-15 stimulated vigorous proliferation of CD4+ and CD8+ T cells. Interestingly, IL-15 stimulated proliferation of CD4+ and CD8+ T cells was completely abolished by blocking the γc in vitro. In contrast, anti-γc failed to block in vitro IL-2 stimulated CD4+ T cell proliferation. Studies using IL-2 deficient T cells showed that while anti-γc inhibited in vitro proliferation of CD4+ T cells, the in vivo expansion of IL-2 deficient CD4+ T cells was not affected by anti-γc or rapamycin. These experiments suggest that γc dependent signals or rapamycin sensitive signals are dispensable for in vivo expansion of CD4+ T cells but not CD8+ T cells. In striking contrast, blocking CD28 and CD154 costimulatory molecules markedly inhibited the in vivo expansion of CD4+ T cells whereas the in vivo expansion of CD8+ T cells was not significantly affected. We conclude that activation of CD4+ and CD8+ T cells is likely governed by distinct mechanisms. While activation of CD8+ T cells is dependent on T cell growth factors, activation and expansion of CD4+ T cells is dependent primarily on costimulatory signals.

LIVER: POST-TRANSPLANT COMPLICATIONS/ PRE-TRANSPLANT MANAGEMENT/MALIGNANCY

Abstract# 1559

DIFFUSE BILE DUCT INJURY AFTER ORTHOTOPIC LIVER TRANSPLANTATION - A MULTIFACTORIAL PROBLEM. Gerd Otto,[1] Christian Moench,[1] Anja Uhrig.[1] *[1]Transplantation and Hepatobiliary Surgery, Johannes Gutenberg University, Mainz, Germany.*

Introduction: Diffuse bile duct injury is a major complication following orthotopic liver transplantation (OLT). Multiple factors have been claimed to cause ischemic type biliary lesions (ITBL). In this study we present two new major factors influencing the development of ITBL. **Material and Methods:** The high viscosity of UW solution may lead to an ineffective preservation of the bile ducts. We tried to prevent ITBL by an arterial back-table pressure perfusion (APP). 178 orthotopic liver transplantations (OLT) performed between 9/1997 and 5/2002 were analyzed with regard to ITBL. All grafts were preserved with UW solution: 120 by in situ standard perfusion including portal perfusion, 58 by APP (300ml UW-solution at a pressure of 150 mmHg). The CC Chemokine receptor 5 (CCR5) of 106 recipients was analyzed in order to detect CCR5D32 mutation. **Results:** In the standard perfusion group 17 (14%) of 120 patients developed ITBL. One of 58 patients following APP had ITBL. This difference was highly significant ($p<0.01$). Maximal AST and ALT levels within the first 3 days were significantly lower in the APP group (AST $p<0.01$, ALT $p<0.001$). No primary non-function occurred in the APP group. 90 patients (84.9%) had a normal CCR5 (wild type) whereas 16 patients suffered from the CCR5D32 mutation (15 heterozygote, 1 homozygote). ITBL occurred in 5 of 90 patients with normal CCR5 and in 6 of 16 cases with CCR5D32 mutation ($p=0.001$, Fisher test). Other donor related factors in patients w/o vs. w/ CCR5 mutation were comparable (gGT $p=0.615$, donor age $p=0.210$, cold ischemia time $p=0.382$, and warm ischemia time $p=0.333$). The rate of acute rejection (34% vs. 31%, respectively; $p=1.0$) and the CMV infection rate (25% vs. 37,5%; $p=0.356$) were identical in both groups. In the multivariate analysis the development of ITBL was significantly influenced by donor age ($p=0.007$), CCR5D32 mutation ($p=0.0001$) and the use of APP as a protective factor ($p=0.001$). There was no influence of the recipient age ($p=0.311$), warm ($p=0.762$) and cold ischemia time ($p=0.746$). **Conclusion:** Diffuse bile duct injury following OLT is a multifactorial problem. APP is an easy and reliable method to prevent ITBL following OLT and it should be mandatory in liver procurement. Even though donor age and CCR5D32 mutation may contribute to the development of ITBL, their influence seems to be of minor significance in the clinical setting.

Abstract# 1560

THE EFFECT OF ISCHEMIA-REPERFUSION INJURY ON THE INCIDENCE OF EARLY ACUTE CELLULAR REJECTION AND THE RECURRENCE OF HEPATITIS C POST CADAVERIC LIVER TRANSPLANTATION. Anil S. Paramesh,[1] Gabriel E. Gondolesi,[1] Lawrence Liu,[3] Swan Thung,[2] Arief Surianwinata,[2] Eugene Nguyen,[1] Sander Florman,[1] Sasan Roayaie,[1] Nancy Kreiger,[1] Thomas Fishbein,[1] Sukru Emre,[1] Leona K. Schluger,[1,3] Myron E. Schwartz,[1] Thomas Schiano.[1,3] *[1]Recanati/Miller Transplantation Institute, The Mount Sinai School of Medicine, New York, NY; [2]Department of Pathology, Mount Sinai School of Medicine, New York, NY; [3]Division of Hepatology, Mount Sinai School of Medicine, New York, NY.*

Aim: There is some evidence that ischemia-reperfusion injury (IRI) may predispose to the development of acute cellular rejection (ACR) and the recurrence of Hepatitis C (HCV).We performed this study to identify and correlate the incidence of ACR and recurrence of HCV with IRI post liver transplantation (OLT). **Materials & Methods:** Between 01/94 through 12/01, all HCV patients who underwent primary whole graft cadaveric OLT were included in this study. Patients were divided into 4 groups based on their post OLT peak ALT values (<400, 400-1000, 1000-2000 and >2000 IU/ml, respectively). The degree of IRI in the post reperfusion allograft biopsies were graded from 0-6 based on neutrophilic infiltration and centrilobular necrosis. The degrees of IRI was correlated with the study groups. These groups were analyzed to determine the incidence and grade of first ACR episodes as well as the incidence of biopsy proven recurrence of HCV. $p < 0.05$ was considered significant. **Results:** 431 patients with complete biochemical and histologic data were included in this study. No patients recieved HCV+ allografts. There were 179 patients in Group I, 140 in II, 64 in III and 48 in IV. The mean peak ALT values were 240.6, 619.13, 1413.2 and 3556.65 IU/ml, respectively. There was a significant association of higher histologic degree of IRI with higher ALT values ($p=0.001$). Protocol biopsies were not performed. No significant difference was found in the incidence, time to development or grade of first ACR between the 4 groups.There was a significant difference in the incidence of recurrence of HCV between the 4 groups ($p=0.018$). Actuarial survival curves for time to recurrence showed a trend towards earlier recurrence with increasing IRI (mean of 293.7 days in Group I and 198.7 days in Group IV). Survival curves also showed a trend towards lower patient survival with earlier recurrence. **Conclusions**: The degree of IRI in liver transplant allografts does not significantly influence the incidence or grade of ACR. IRI does significantly influence the incidence of recurrence of HCV and appears to decrease the time to recurrence. Patients with earlier recurrence appear to have lower survival rates.

Abstract# 1561

INTRACRANIAL PRESSURE AND LIVER TRANSPLANTATION IN PATIENTS WITH FULMINENT HEPATIC FAILURE: INDICATION FOR PRE-OPERATIVE INVASIVE MONITORING. Omar Barakat,[1] Christopher P. Snowden,[1] Digby Roberts,[1] Phillip Bayly,[1] Liesl T. Smith,[1] David Talbot,[1] Bryon C. Jaques,[1] Derek M. Manas.[1] *[1]Liver Unit, Freeman Hospital, Newcastle upon Tyne, United Kingdom.*

Despite the Favorable outcome of Liver Transplantation (LT) in patients with Fulminent Hepatic Failure (FHF), cerebral edema and brain stem herniation, remain the major contributors of death in up to 80% of patients awaiting LT. Although, invasive monitoring of Intracranial Pressure (ICP) is a recognized method to detect early rise in ICP and guide medical therapy, there are no clear consensus regarding the indication for their use. **Objective:** to identify patients with FHF who will benefit the most from such a monitoring technique, as part of their management while awaiting LT. **Methods**: Between January 1994- January 2002, 63 patients were placed on the United Kingdom national super-urgent waiting list after they met the King's College criteria for FHF. Intracranial Pressure (ICP) monitoring via a Camino Bolt, was used in 31 patients following sedation and ventilation. Paracetamol overdose was the main cause of liver failure in 97% of patients (30/31). **Results**: Twenty-three patients, in whom the ICP was monitored, underwent OLT within 22±3.3 hour (mean ± SE) from listing, which was significantly shorter compared to 9 patients who died on the waiting list before an organ could be found (57± 18 hour, Mean ± SE)($P<0.001$). While on the waiting list, 16 patients (Group I) with severe metabolic acidosis (PH£ 7.3) at the time of listing, despite aggressive resuscitation, demonstrated significantly higher ICP, and lower Cerebral Perfusion Pressure values compared to 15 patients (Group II) with normal acid-Base Balance, (38±12mmHg vs. 13.0 ±1.1mmHg $P<0.03$, and 45.8+6.5mmHg vs. 64+2.7mmHg $P<0.01$, respectively). In addition, they underwent significantly more therapeutic interventions to normalize the ICP (2.4±0.6 vs. 0.6± 0.3, respectively. $P<0.01$). Importantly, only 30% of patients in GII required therapeutic intervention for a transient rise in ICP with 100% response rate compared to 93% of patients in GI who required intervention with only 65% response rate. There was no difference in 1-year survival of patients who underwent OLT in both groups (60% vs 69% respectively). **Conclusion**: 1- Patients with persistent acidosis despite aggressive medical therapy could benefit from ICP monitoring while awaiting liver transplantation to optimize their preoperative management. 2- Patients with normal PH and hemodynamic stability may be followed safely without such an invasive monitoring technique.

Abstract# 1562

ALPHA-FETO PROTEIN IS A STRONG PREDITOR OF DE-LISTING DUE TO TUMOR PROGRESSION - AN ANALYSIS OF LIVER TRANPLANT CANDIDATES WITH HEPATOCELLULAR CARCINOMA WITH COMPETING RISKS APPROACH. Noriyo Yamashiki,[1] Tomoaki Kato,[1] Rajendar Reddy,[3] Jeffrey Gaynor,[1] David Levi,[1] Seigo Nishida,[1] Juan Madariaga,[1] Jose Nery,[1] Eugene Schiff,[2] Andreas Tzakis.[1] *[1]Liver and GI Transplant, University of Miami, Miami, FL; [2]Hepatology, University of Miami, Miami, FL; [3]Gastroenterology, University of Pennsilvania, Philladelphia, PA.*

De-listing due to tumor progression in liver transplant (OLT) candidates with hepatocellular carcinoma (HCC) has been a disturbing problem because successful OLT can a curative therapy in early stage HCC. Under a recent change in UNOS policy, which incorporates the Model for End-Stage Liver Disease (MELD) Scoring System, a higher priority status is assigned to HCC patients according to their modified TNM stage. However, it is possible that other patient characteristics, possibly in combination, may be stronger predictors of risk of delisting than stage by itself. In order to determine factors affecting de-listing, we reanalyzed the data with competing risks approach. **Method:** The clinical outcomes of 93 patients who were listed for transplantation with HCC or were diagnosed with HCC following listing were analyzed. Factors that predict delisting were identified. **Results:** The patients were seen at the our institution between January, 1997 and June, 2001. The modified TNM stage was I/II in 81 patients and III/IV in 12 patients. Sixty-nine of 93 patients (74%) were transplanted with a median waiting period of 3.4 (1-23.2) months, and 22 (24%) patients were delisted after a median follow-up of 5.5 (1-13.7) months due to tumor progression (14), non-compliance (5) and death from liver failure (3). By Cox regression analysis, a higher alpha-fetoprotein (AFP) ≥100 ng/ml was associated with a significantly higher hazard rate of delisting due to tumor progression (p=.00003), whereas 4 separate factors were independently associated with a significantly lower hazard rate of transplantation: a more recent listing year (1999-2001, p=.012), blood type O (p=.013), Stage I HCC (p=.030), and serum bilirubin <4 mg/dl (p=.037). By logistic regression analysis, AFP ≥100 ng/ml was the only factor that significantly influenced the overall probability of delisting due to tumor progression (p=.0011). **Conclusion:** The initial AFP level at the evaluation should be considered in addition to tumor stage in any future refinement of the urgency score for liver transplant candidates with HCC.

Abstract# 1563

OUTCOMES OF PRE-TRANSPLANT LOCAL TREATMENT FOR HEPATOCELLULAR CARCINOMA IN THE SETTING OF CIRRHOSIS. Kim M. Olthoff,[1] Kate Timmins,[1] Kirti Shetty,[2] Weijing Sun,[2] Emma E. Furth,[3] Michael Soulen,[4] Mark Rosen,[5] Abraham Shaked.[1] *[1]Department of Surgery, University of Pennsylvania, Philadelphia, PA; [2]Department of Medicine, University of Pennsylvania, Philadelphia, PA; [3]Department of Pathology, University of Pennsylvania, Philadelphia, PA; [4]Interventional Radiology, University of Pennsylvania, Philadelphia, PA; [5]Department of Radiiology, University of Pennsylvania, Philadelphia, PA.*

Pre-transplant treatment modalities for hepatocellular carcinoma (HCC) include surgical resection, ablative procedures, and embolization. We analyzed the efficacy of non-resection therapy in a group of patients with HCC who were accepted as transplant candidates and received pretransplant tumor-directed intervention. Pathologic outcome was studied once the liver explant became available. **Patients and methods:** 28 patients with known HCC and liver cirrhosis underwent local pre-transplant tumor-directed therapy between 2/1996 and 12/2002 (f/u 1 – 64 months). Patients received therapy in a range from 1 day to 2 years prior to OLT. Interventions included hepatic artery chemoembolization (HACE), open or percutaneous radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), radioembolization (Therasphere), or a combination (table). Explants were examined for tumor necrosis and viability, multifocality, satellite lesions, tumor grade and vascular invasion.

HACE	RFA	HACE + RFA	PEI	HACE + PEI	Therasphere
11	8	3	1	1	4

Results: Nineteen of the 28 explants demonstrated some degree of necrosis or infarction. Viable tumor in the treated mass was seen in 25/28 cases, moreover, development of new lesions distant from treated lesions was found in 12/28, and satellite lesions close to treated tumor in 7/28. Only 1 explant had no viable tumor found. There were 4 complications of ablative therapy including diaphragmatic perforations (2) segmental portal vein thromboses (2), and recanalized hepatic artery thrombosis (1). **Conclusions:** Local control of tumor growth in cirrhotic livers using non-surgical treatment modalities fails to eradicate the primary lesion, with the majority having persistent viable tumor and development of de novo new lesions. In theory, liver resection may be effective in achieving local control, but not the development of de novo lesions. Liver transplantation may be the only option, as it removes the entire cirrhotic liver with all foci of HCC. It remains to be determined whether non-surgical ablative therapy will affect recurrence after transplantation.

Abstract# 1564

CUMULATIVE DROP OUT FROM THE UNOS WAITING LIST FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC): IMPLICATIONS FOR ORGAN ALLOCATION POLICY. Y. K. Maddala,[1] L. M. Stadheim,[1] J. C. Andrews,[1] C. B. Rosen,[1] G. J. Gores.[1] *[1]Mayo Clinic, Rochester, MN.*

UNOS organ allocation policies are based on Model for End Stage Liver Disease (MELD) scores to assign a priority for cadaveric orthotopic liver transplantation (OLT). However, many HCC patients do not have a calculated MELD score which provides them with an urgent priority for OLT. Patients with HCC are now assigned arbitrary MELD scores to provide timely access to OLT. An equitable policy would equate HCC progression beyond acceptable transplantation criteria with death on the waiting list. However, limited information is available regarding HCC progression over time, especially in patients receiving pre-OLT therapy (e.g., chemoembolization). Thus, the **AIMS** of the current study were to analyze drop out rates on the waiting list due to disease progression for patients with HCC treated with chemoembolization. **METHODS:** Between January 1994 and August 2001, 55 patients with HCC were listed for OLT. All patients met the current European Association for the Study of Liver Disease (EASL) consensus criteria (J Hep 35:421-430,2001) for the diagnosis of HCC and UNOS indications for transplantation. Patients with HCC were chemoembolized at the time of listing for OLT and 3 months later. Subsequent chemoembolizations were performed for viable tumors (i.e., persistent contrast enhancement on CT or MR scans) and/or a rising alpha-fetoprotein. All patients underwent a CT scan of the abdomen and chest every 3 months on the waiting list to assess for HCC progression. **RESULTS:** Forty (73%) patients had unicentric lesions while 15 (27%) had multicentric disease. A total of 6 (11%) patients had disease progression on the waiting list. One patient developed pulmonary metastases, two developed vascular invasion with portal vein thrombosis, one developed adrenal metastases, and two developed intrahepatic disease progression exceeding transplantation criteria. Cumulative drop out on the waiting list due to disease progression was 2 (4 %) over the first 3 months, 5 (9%) over 6 months and 6 (11%) over 12 months. For HCC patients who were transplanted, the median time on the waiting list was 211 days (range 28-1099 days). There were no significant differences in age, gender, tumor characteristics (size, multicentricity) and serum alpha-fetoprotein levels in those who underwent OLT vs. those who dropped out. In **CONCLUSION,** patients with HCC pre-treated with chemoembolization should receive MELD scores equivalent to a mortality rate of 9% over 6 months.

Abstract# 1565

LONG-TERM TUMOR-FREE SURVIVAL AFTER LIVER TRANSPLANTATION COMBINING PANCREATICODUODENECTOMY AND RADIOTHERAPY FOR EARLY STAGE CHOLANGIOCARCINOMA IN PRIMARY SCLEROSING CHOLANGITIS. Youmin Wu,[1] Frederick C. Johlin,[2] Chris S. Jensen,[3] Stephen C. Rayhill,[1] Daniel A. Katz,[1] Rou-Yee Chenhsu,[1] Frank A. Mitros.[3] *[1]Surgery, University of Iowa, Iowa City, IA; [2]Internal Medicine, University of Iowa, Iowa City, IA; [3]Pathology, University of Iowa, Iowa City, IA.*

Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease associated with cholangiocarcinoma (CC). Early diagnosis of CC and removal of all tissue at risk remains a challenge. We reported our initial experience with surveillance cytology, radiotherapy, total hepatectomy with partial pancreaticoduodenectomy en bloc and liver transplantation for stages I or II CC with multilevel dysplasia. **Methods**: PSC patients underwent surveillance ERCP cytologic evaluation. Suspicious lesions were followed by repetitive multi-level cytologic re-examinations. Published validated cytologic features of CC with sensitivity of 83% and specificity of 98% were used by at least 2 cytologists. From 1996, lesions were also staged by endoscopic ultrasonography (EUS) plus fine needle aspiration, with sensitivity of 90% and specificity of 93%. After external-beam irradiation and iridium-192 brachytherapy, patients with stage I or II CC and multifocal dysplasia underwent staging laparotomy when a donor liver became available. Total hepatectomy with partial pancreaticoduodenectomy en bloc, followed by liver replacement. Extended lymphadenectomy was not performed. **Results**: Between 1988 and 2001, 42 PSC patients were followed. CC was detected in 7 (16.7%) (stage I: n=5, stage II: n=2). One refused transplantation and developed local recurrence 35 months after brachytherapy. The other 6 completed radiotherapy and underwent surgery 140 ± 59 days after diagnosis. Their explanted specimen revealed obliteration of the CC but residual dysplastic lesions after radiotherapy. Postoperative complications included intra-abdominal infection requiring conservative treatment (n=2) and pancreatic leak requiring pancreatic duct reconstruction (n=1). One patient died 55 months posttransplant from diabetes without tumor recurrence. The other 5 were alive and well without recurrence at 14, 30, 50, 51, and 67 months after surgery. **Conclusions**: EUS plus ERCP surveillance cytology increase our ability of earlier detection and more accurate preoperative staging. Radiotherapy followed by extended surgery to remove the entire biliary duct epithelium en bloc may offer promising tumor-free survival and is well tolerated. This protocol appears to eliminate early stage CC and further studies in more patients are needed.

Abstract# 1566

INCIDENCE OF ADVERSE EVENTS WITH LIPID LOWERING AGENTS IN LIVER TRANSPLANT PATIENTS. J. E. Martin,[1] R. Moradia,[1] A. Sweeney,[1] L. Trumbull,[4] M. Bass,[4] F. L. Weber, Jr.,[2] J. F. Buell,[3] M. J. Hanaway,[3] S. M. Rudich,[3] E. S. Woodle,[3] J. Aranda-Michel.[2]
[1]College of Pharmacy; [2]Internal Medicine-Digestive Disease; [3]Surgery, University of Cincinnati; [4]Transplant Services, The University Hospital, Cincinnati, OH.

With escalating awareness of cardiovascular complications among transplant (txp) patients (pts), cholesterol-lowering agents are utilized more frequently. The purpose of this study was to evaluate the incidence of adverse events (AE) with lipid lowering therapies (LLT) in liver txp pts. **Methods:** As part of a comprehensive cholesterol management protocol liver pts receiving a LLT were identified and complications documented. Complications were defined as 1 of the following: myalgia(pain without ↑CPK), myosits (↑CPK without pain), myopathy (↑CPK with pain), or rhabdomyolysis. Lab abnormalities include changes in Cr, BUN, CPK, UA, K, Ca, and LFT's.**Results:** There were 69 pts on LLT. Mean age was 54±9. There were 45 M, 24 F; 64 C, 5 AA. Mean f/u after starting LLT was 18±16 months. Overall, LDL and Tri ↓ was 45% ±59 and 8% ± 46 respectively, with 90% pts at goal. Among pts who started on a single agent, 31(65%) were on pravastatin (P), 7(15%) on gemfibrozil (G), 4(8%) on simvastatin (S), 4(8%) on atorvastatin (A), 1(2%) on lovastatin (L). Five (7%) pts were on combination. One pt on G + P, 3 pts on G + P and 1 pt on G + S. Sixteen (23%) pts were switched to different agent at least once due to intolerance or inability to meet desired chol. goal on prior agents. Eight patients (50%) were switched P to A. Twenty-nine (42%) pts were on CYP 3A4 inhibitors other than CSA. Six (9%) pts out of 69 had some form of muscular damage. Five (7%) had myalgia, and 1 (1.44%) pt had myopathy. There was no incidence of myositis or rhabdomyolysis. Among pts with myalgia, 3 were receiving P and 2 pts A. The pt with myopathy was on P and diltiazem a CYP 450 inhibitor. All 6 pts were on CSA but 5 pts with myalgia were not on CYP 450 inhibitors other than CSA. Mean time for AE was 4±3 months from the initiation of LLT. None of these pts required hospitalization. All AEs resolved by stopping the LLT. All lab values were unchanged during events except for 1 pt with myopathy who had an ↑ CPK (366 U/L). **Conclusion:** Use of LLT in liver txp pts had a low incidence of AE's and no episodes of rhabdomyolosis. The primary use of P may have contributed to this outcome. The total number of pts who had myopathy without rhabdo was 1of 69 (1.44%) which is slightly higher than the average in the general population (<0.1%). While the difference in complications among the LLT in txp pts needs continued study, this data supports safe use of LLT in liver transplantation.

Abstract# 1567

LIVER TISSUE AND SERUM VIRAL LOAD DO NOT PREDICT SEVERITY OF RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION. Victor I. Machicao,[1] Julio Mendez,[1] Murli Krishna,[1] Hugo Bonatti,[1] Raj Satyanarayana,[1] Barry Rosser,[1] Bashar Aqel,[1] Denise Harnois,[1] Jeffery L. Steers,[1] Rolland C. Dickson.[1]
[1]Department of Transplantation, Mayo Clinic, Jacksonville, FL.

Background: High hepatitis C (HCV) viral load in serum before and early after liver transplantation (LT) have been associated with decreased survival after LT. However, there is conflicting evidence that elevated viral load affects the severity of HCV recurrence after LT. The role of HCV viral load in hepatic tissue before LT, as a predictor of HCV recurrence is unknown. The aim of our study was to assess the value of hepatic and serum viral load predicting the clinical and histological outcome of recurrent HCV after LT. Methods: Longitudinal cohort study involving all pts undergoing first LT at our Center between March 1998 and December 2001 for HCV-related liver disease. Protocol liver biopsies were obtained from the explanted liver, at 4 and 12 months after LT, and yearly thereafter. Hepatic HCV RNA quantitation was performed with a polymerase chain reaction (Amplicor HCV Monitor Test, v2.0). Liver and serum viral load were expressed as $\log_{10}$ IU/mL. A single liver pathologist reviewed all biopsy specimens in a blinded fashion. Ishak's score was used to quantify fibrosis in biopsy specimens. Moderate to severe HCV recurrence was defined as either re-LT/listing for HCV recurrence, or presence of fibrosis score >3. Results: 167 required LT for HCV-related liver disease during the study period. 135 pts were included in the study. Baseline liver tissue and serum viral load before LT were unable to predict neither worse fibrosis progression after LT (Table 1), nor graft loss due to recurrent HCV (Table 2). Serum viral load reached maximal value at 4-months after LT, but lack correlation with worse clinical or histological HCV-recurrence. Conclusions: Elevated hepatic and serum viral load before LT did not predict severity of HCV recurrence. The role of serum viral load in HCV recurrence appears overestimated.

Table 1: Advanced Fibrosis According to Tissue and Serum Viral Load

Median Viral Load ($\log_{10}$ IU/mL)	Fibrosis 0-3	Fibrosis >3	P-value
Liver Tissue - Day 0	4.27	3.99	0.74
Serum - Day 0	5.54	5.36	0.97
Serum - 4 mo.	6.35	6.7	0.63
Serum - 1 yr.	6.19	6.2	0.18

Table 2: Graft Loss Secondary to Recurrent HCV According to Tissue and Serum Viral Load

Median Viral Load ($\log_{10}$ IU/mL)	No Graft Loss	Graft Loss	P-value
Liver Tissue - Day 0	4.25	4.09	0.25
Serum - Day 0	5.53	5.29	0.8
Serum - 4 mo.	6.37	6.53	0.83
Serum - 1 yr.	6.22	6.17	0.59

Abstract# 1568

STABLE RENAL TRANSPLANT PATIENTS ON MYCOPHENOLATE MOFETIL CAN BE SAFELY SWITCHED TO ENTERIC-COATED MYCOPHENOLATE SODIUM: 12-MONTH DATA OF A PROSPECTIVE RANDOMIZED TRIAL. K. Budde,[1] J. Curtis,[2] G. Knoll,[3] L. Chan,[4] H. H. Neumayer,[1] C. Panis,[5] Y. Seifu,[5] M. Hall for the Myfortic Maintenance Renal Transplant Study Group.[5]
[1]University Hospital Charite, Berlin, Germany; [2]U of Alabama, Birmingham, AL; [3]Ottawa Hospital, Ottawa, Canada; [4]U of Colorado Health Science Center, Denver, CO; [5]Novartis Pharmaceuticals, East Hanover, NJ.

Enteric-coated mycophenolate sodium (EC-MPS, *myfortic*™) is an advanced formulation of mycophenolate, designed to release mycophenolic acid (MPA), the active moiety, into the small intestine, unlike mycophenolate mofetil (MMF), which releases MPA in the stomach. This delayed MPA release is designed to help protect the upper gastrointestinal (GI) tract. **Purpose:** This study evaluated whether maintenance renal transplant patients taking MMF could be converted to EC-MPS therapy without compromising tolerability or efficacy. **Methods:** This was phase III, double-blind, randomized, study in renal transplant patients ≥ 6 months post-transplant being maintained on 1000 mg b.i.d. MMF in combination with cyclosporine with/without corticosteriods. Patients were randomized to receive equimolar amounts of MPA: either 720 mg b.i.d. EC-MPS (n = 159) or 1000 mg b.i.d. MMF (n = 163). The 12-month intent-to-treat analysis of safety, GI tolerability (using a GI severity score) and efficacy is presented. **Results:** The 0-12 month incidence of overall AEs (EC-MPS 93.7% and MMF 92.6%; p = ns), and of GI AEs (EC-MPS 60.0% and MMF 61.0%; p = ns) was similar in both groups. Relative to baseline, increases in GI AE severity scores were lower with EC-MPS (3-fold increase vs. 9-fold increase for MMF, p = ns). Fewer serious AEs were reported in the EC-MPS group (23.3% vs. 30.1%; p = ns). Although the incidence of infections was similar in both groups (EC-MPS 58.5% and MMF 58.9%), the incidence of serious infections was significantly lower with EC-MPS (8.8% vs. 16.0%; $p \leq 0.05$). Efficacy failure (BPAR, graft loss, death) was 2.5% with EC-MPS and 6.1 % with MMF (p = ns). **Conclusions:** In this 12-month trial, 720 mg b.i.d. EC-MPS was at least as effective as 1000 mg b.i.d. MMF in maintenance renal transplant patients, but with reduced GI severity scores, serious AEs and infections. The trends favoring EC-MPS for GI AE severity scores, serious AEs and serious infections ($p \leq 0.05$) suggest that the enteric coating improves the delivery of MPA, with efficacy failure being rare. Thus, stable renal transplant patients receiving MMF, or patients who require dose adjustments or drug withdrawal due to GI intolerance, can be safely converted to EC-MPS with no efficacy or tolerability compromise.

Abstract# 1569

CALCINEURIN-INHIBITOR NEPHROTOXICITY AND EFFICACY STUDY: THE CANNES TRIAL. Eduard M. Scholten,[1] Ajda Rowshani,[2] Janto Surachno,[2] Erik van Kan,[3] Serge Cremers,[4] Leendert C. Paul,[1] Ineke J. ten Berge,[2] Johan W. de Fijter.[1] *[1]Nephrology, Leiden; [2], Amsterdam; [3]Clinical Pharmacy and Toxicology, Amsterdam Medical Center, Amsterdam, Netherlands; [4]Leiden University Medical Center, Leiden, Netherlands.*

Methods: In this prospective, randomized multicenter study we compared AUC-guided dosing of cyclosporine (CsA) with tacrolimus (Tac) in renal transplant recipients. To maintain efficacy and minimize non-immune toxicity low target AUCs were defined under basiliximab prophylaxis (20 mg d0 and d4). CsA AUC(0-12 hr): 5400 ng.h/ml, after six weeks 3250 ng.h/ml. Tac AUC (0-12 hr): 210 ng.h/ml, after six weeks 125 ng.h/ml. Dose adjustments were guided by a population based pharmacokinetic model with a maximum a posteriori Bayesian fitting method and limited sampling, developed and validated for both CsA and Tac. Other immunosuppressive drugs were steroids and mycophenolate mofetil (1000 mg b.i.d. in the CsA group and 500 mg b.i.d. in the Tac group). Primary read-out will be graft structure in protocol biopsies at 6 and 12 months, scored by morphometry of interstitial fibrosis. Here we report the preliminary evaluation of clinical outcome parameters at six months. **Results:** We included a total of 124 patients in the study of whom110 had at least six months follow-up. Baseline demographic and transplant-related characteristics were not significantly different in the groups. Cumulative incidence of biopsy-proven acute rejection episodes (ARE) at six months was 13,8% in the CsA group and 9,6 % in the Tac group. Non-immune toxicity parameters, including mean blood pressure (CsA 140/81 mmHg vs Tac 138/82 mmHg) and mean ratio of total cholesterol over HDL-cholesterol (CsA 4,69 vs Tac 4,22) were comparable. CsA treated-patients tended to use more antihypertensive drugs (1,78 vs 1,56 drugs / patient) and statins (30 vs 22% of patients on statins). De novo DM occurred in 5,6 % of the patients in the CsA group and in 18,5 % in the Tac group. None of the CsA-treated patients were insuline dependent versus 40 % of Tac-treated patients. The calculated GFR was comparable in both groups. **Conclusion:** A calcineurin-inhibitor sparing regimen with basiliximab prophylaxis and controlled systemic exposure resulted in lower acute rejection rates in tacrolimus treated patients, but the high incidence of post-transplant DM may preclude long-term treatment. Non-immune toxicity profiles including the awaited morphometric analysis of protocol biopsies may furhter substantiate a patient-oriented choice for either CsA or Tac.

Abstract# 1570

SIROLIMUS TREATMENT IS ASSOCIATED WITH TESTOSTERONE DEFICIENCY IN MALE KIDNEY TRANSPLANT RECIPIENTS. Lutz Fritsche,[1] Klemens Budde,[1] Duska Dragun,[1] Fritz Diekmann,[1] Gunilla Einecke,[1] Markus Giessing,[2] Hans-Hellmut Neumayer.[1] *[1]Nephrology, Charité Campus Mitte, Berlin, Germany; [2]Urology, Charité Campus Mitte, Berlin, Germany.*

BACKGROUND: The detrimental effect of sirolimus on the testes is well established in animal models. So far it is unknown whether this effect is also present in human subjects. METHOD: The influence of a sirolimus-based immunosuppressive regimen on testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels was assessed in a retrospective case-control study. Data on all sirolimus treated kidney graft recipients in whom a hormone profile had been performed as part of the routine laboratory assessment were extracted from our electronic patient record. A control patient on a rapamycin-free regime matched for sex, age and time between kidney transplantation and hormone profile was identified for each sirolimus patient. RESULTS: Hormone profiles were available for 24 male sirolimus patients (SIR). The 24 matched male controls (CON) were of comparable age (SIR: 47.9 ± 14.0 vs. CON: 47.2 ± 12.5 years) and time since transplantation was similar (SIR: 7.5 ± 5.2 vs. CON: 7.5 ± 5.5 years). In the SIR group testosterone levels were significantly lower (SIR: 10.3 ± 7.5 vs. CON: 17.1 ± 7.5 nmol/l; p=0.001) while FSH (SIR: 23.2 ± 31.4 vs. CON: 9.0 ± 10.4 IU/l; p=0.046) and LH (SIR: 30.3 ± 44.9 vs. CON: 10.8 ± 9.0 IU/l; p=0.047) were higher. The testosterone level was below the normal range in 14 of 24 SIR patients compared to 2 of 24 CON patients ($p < 0.001$). Testosterone concentration correlates inversely with the sirolimus dose ($r= -0.50$; $p < 0.001$). This correlation was also significant in the multivariate regression analysis ($p= 0.001$) while age, time since transplantation, steroid dose, creatinine and urinary protein showed no correlation with testosterone levels. CONCLUSION: This is the first report on testosterone suppression in sirolimus-treated males. The concurrent elevation of FSH and LH is consistent with the negative feedback regulation between testosterone and these hormones. This observation requires prospective verification in larger patient numbers. Clinical consequences of these findings remain to be determined.

Abstract# 1571

DOES CONVERSION TO MYCOPHENOLATE MOFETIL IN CHRONIC ALLOGRAFT NEPHROPATHY PROLONG GRAFT SURVIVAL? Keith P. Graetz,[1] Jonathon O'Dair,[1] Anita Boswell,[1] Keith M. Rigg,[1] Magdi Shehata.[1] *[1]The Transplant Unit, City Hospital, Nottingham, United Kingdom.*

Calcineurin inhibitors are increasingly implicated in the pathogenesis of chronic allograft nephropathy (CAN), the leading cause of renal allograft loss after the first year post transplantation. We first reported our early results of Cyclosporin (CyA) withdrawal and its substitution with either Tacrolimus or Mycophenolate Mofetil (MMF) in patients with CAN and deteriorating renal function in early 2000. Both drugs improved renal function initially. On follow up it became apparent that only MMF sustained this improvement. All patients with CAN, worsening transplant function and CyA based immunosuppression are now switched to MMF based therapy. We report long term follow up of these patients. 54 patients (15 from the original cohort) were included. Primary diseases were 8 reflux nephropathy, 8 glomerulonephritis, 5 adult polycystic kidney disease, 4 renovascular, 3 IgA nephropathy, 2 diabetes, 7 other and 16 unknown. MMF was commenced and the dose increased as tolerated over 2 weeks. Complete CyA withdrawal followed in dose increments of 1/3rd over 3 months. Biopsy was performed routinely in the first 15 patients. Data is expressed as mean±standard deviation.

Male:Female ratio	35:19
Age at transplantation	39.7±11.9
Time post transplantation to conversion (years)	6.8±4.3
Serum Creatinine at conversion (µmol/L)	224±73
Cockroft Gault GFR at conversion (ml/min)	40.8±15.8
Rate of deterioration of serum creatinine in 6 months prior to conversion (µmol/L/yr)	72±89

Mean follow up was 21.2±12.8 months. No acute rejection was seen in the conversion period. One graft was lost to progression of CAN and 3 patients died with a functioning graft. 2 patients failed conversion and were converted back to CyA (diarrhoea not managed by dose splitting or dose reduction). Other side effects noted included diarrhoea, pneumonia, other infections and anaemia Improvements in serum creatinine and the GFR were seen at all time points after conversion. The greatest improvement occurred in the first 3 months after conversion (during CyA withdrawal). Significant improvements were also seen in systolic and diastolic blood pressures and in lipid profiles.

	At Conversion	3 months	6 months	1year	2 years	3 years
Serum creatinine (µmol/)	224±73	183±53	165±44	184±51	171±76	140±27
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

This data demonstrates the benefit of withdrawing CyA in patients with CAN and that MMF can then be used to maintain the improvement with prolongation of graft survival.

Abstract# 1572

FTY720 IN COMBINATION WITH EVEROLIMUS: A NOVEL CNI-FREE REGIMEN AS EFFECTIVE IN AFRO-AMERICANS AS IN CAUCASIANS AT HIGH RISK FOR DGF. Barry D. Kahan,[1] Helio Tedesco,[2] Marc I. Lorber,[3] Clarence Foster,[4] Hans Sollinger,[5] Rafael Mendez,[6] Deise R. Carvalho,[7] Jennifer Dehlinger.[8] *[1]Department of Surgery, Univ. of Texas-Houston Medical School, Houston, TX; [2]Hospital do Rim e Hipertensao, Sao Paulo, Brazil; [3]Yale University School of Medicine, New Haven, CT; [4]Division of Transplantation, Univ. of Maryland Medical Center, Baltimore, MD; [5]Department of Surgery, Univ. of Wisconsin Hospital, Madison, WI; [6]National Institute of Transplantation, Los Angeles, CA; [7]Unidad de Transplant Renal, Hospital Geral De Bonssucesso, Rio de Janeiro, Brazil; [8]Novartis Pharmaceuticals Corporation, East Hanover, NJ.*

Delayed graft function (DGF) and acute rejection (AR) exert detrimental effects on graft function and survival after renal transplantation (RTx). USRDS 12-month graft survival in patients without AR is 91% vs. 75% in patients with DGF. FTY720 is a sphingosine-1-phosphate receptor agonist, highly potent for rejection prevention in combination with everolimus in animal models. **Aim of the study**: A regimen combining FTY720 and everolimus with no calcineurin inhibitors (CNIs) may present a novel and optimized immunoprophylaxis. The aim of this ongoing proof-of-concept study was to evaluate the efficacy and safety of this regimen in RTx patients at high risk for DGF. **Methods**: Eligibility was determined pre-RTx using a DGF risk index based on 5 factors: donor age/donor cause of death/cold ischemia time/recipient race/history of prior RTx. All patients received 5 mg FTY720 + 4 mg everolimus prior to RTx. Post-transplant dosage was 2.5 mg FTY720 OD + 2 mg everolimus BID (C0 targets: 4-8ng/mL) + corticosteroids. CNIs and antibody induction were prohibited. **Results**: Fifty-six patients were enrolled. The Intent-to Treat population consists of 52 patients who were transplanted and who received at least one maintenance dose of study medication. Twenty (38.4%) patients were Afro-American (AA). Twenty five patients experienced DGF (48%); 5 patients had acute tubular necrosis on renal biopsy. The major efficacy endpoints at month 12 are the following:

Efficacy Results

Endpoint	DGF (n=25)	No DGF (n=27)	All patients (n=52)
BPAR	9 (36%)	10 (37%)	19 (36.5%)
Graft loss	6 (24%)	4 (14.8%)	10 (19.2%)
Death	2 (8.0%)	1 (3.7%)	3 (5.8%)

Two graft losses were preceded by BPAR. Despite a higher rate of DGF (70%) in the AA patients, the rate of BPAR was 35% in these patients. Overall, thirty-two patients (57%) reported at least one infection and the rate of bradycardia was 16%. **Conclusion**: FTY720 combined with everolimus, without induction or CNI therapies, compares favorably for graft survival and rejection prophylaxis with conventional rigimens in patients at risk for DGF. These promising results, obtained with a CNI-free regimen, warrant further investigation in the general renal population.

Abstract# 1573

THE IMPACT OF SIROLIMUS (SRL), MYCOPHENOLATE MOFETIL (MMF), CYCLOSPORINE (CsA), and AZATHIOPRINE (AZA) ON LYMPHOCELE FORMATION AND TREATMENT AFTER KIDNEY TRANSPLANTATION. Stuart M. Flechner,[1] Lingmei Zhou,[1] Barbara Mastroianni,[1] Kathy Savas,[1] Ithaar Derweesh,[1] Rosemaree Fisher,[1] Pratek Patel,[1] Mahesh Goel.[1] *[1]Transplant Center/Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, OH.*

Background: More potent immunosuppressive drugs, which reduce the incidence of acute rejection, may result in a greater number of post transplant wound healing problems. We compared the occurrence of lymphocele formation and treatment after kidney transplantation among 513 recipients given three different maintenance drug regimens. Methods: Consecutive series of adult patients initially treated with SRL/MMF/P (3/00-8/02) vs. CsA/MMF/P (1/96-6/00) vs. CsA/AzA/P (1/93-6/97) were analyzed for post transplant lymphocele formation. All available imaging studies were reviewed (US, CT, MRI, etc.) and those with peritransplant fluid collections >2cm were identified. The were no significant differences in demographic characteristics between the 3 groups in recipient sex, cause ESRD/diabetics, retransplants, donor sex, or cold ischemia time; but differed, respectively, in recipient age (49.2, 47.1, 42.4 years p=.0002) race (28.9, 16.1, 16.9% non-white, p=.0078), BMI >30kg/m2 (26.9, 23.8, 13.9%, p=.0061), use induction therapy (99.3, 87.5, 73.6%, p=.001), donor age (45.5, 42, 40.2 years, p=.005), and incidence of 1-year acute rejection (8.9, 14.8, 51.3%, p<.0001). The incidence of fluid collections in the 3 groups were, respectively, 45.3, 33.93, and 24.87%, p=.014. Results: While the majority of fluid collections were observed and not treated, the frequency (%) of different methods of treatment are compared in

N=513	SRL/MMF (152)	CSA/MMF (168)	CSA/AZA (193)	p-Value	p-Value
Aspirated Only	6.57	4.76	3.10	.316	
Tube Drained	2.63	2.97	1.03	.408	
OR-Open	8.55	1.78	4.66	.019	
OR-Lap	5.26	2.97	4.14	.646	
None-Observe	22.36	21.4	11.91	.017	
					I vs. II .013
Total Treated	23.0	12.50	12.95	.014	I vs. III .014
					II vs. III .890

Multivariate analysis of the risk factors associated with the need to treat a lymphocele revealed BMI >30 kg/m2 RR 1.49 (CI 1.02-2.04); treated Acute Rejection RR 1.45 (CI 1.02-2.06); and use of SRL 3.08 (CI 1.99-4.75) were significant independent factors; while the other drugs were not. Conclusions: Obesity, the use of sirolimus, and the treatment of acute rejection are independent risk factors for the need to treat post transplant lymphoceles. These findings should be considered in counseling recipients who are older, non-white, more obese, receive older donor organs, yet experience fewer rejection episodes than in previous eras.

Abstract# 1574

CADAVERIC RENAL ALLOGRAFT LOSS FROM DONORS > 50 YEARS OLD IS DIMINISHED WITH PROGRAF. Carlton J. Young,[1] Clifton Kew,[2] Sharon Hudson,[1] Michael Gallichio,[1] Bruce Julian,[2] John Curtis,[2] Mark Deierhoi,[1] Robert Gaston.[2] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL; [2]Medicine, University of Alabama at Birmingham, Birmingham, AL.*

Marginal Renal Allografts (MRG), age >50, constitute a significant percentage of available organs for transplant. This study evaluated current immunosuppressive therapy and its effect on MRG graft survival (GS). **METHODS:** From 1/96 to 5/02, 964 CAD transplants were performed at our institution. 223 (23%) were MRG. Maintenance immunosuppression was Mycophenolate Mofetil (MMF) and Steroids with 148 patients receiving Prograf (FK) and 816 Neoral (N). For MRG, 29 received FK and 194 Neoral. Induction therapy was OKT3 or ATGAM prior to 5/98. Daclizumab (two dose regimen) was utilized for induction after 5/98. Data on 511 donors of 954 CAD grafts was available for analysis. There were no significant demographic differences between African-Americans (AA, N=509) and Caucasians (C, N=455); but more females received MRG (p>0.0001). Actuarial analysis was produced by Kaplan-Meier. Survivorship and a hazard function was provided by a three phase parametric model. **RESULTS:** Acute Rejection (AR) rate, by one year, was higher for AA compared to C (47% v. 34%; p=0.004); and for recipients of Non-Heartbeating donor allografts (p=0.03). GS was significantly better for allografts <50 years old.

TABLE 1: GS DONORS <50 VERSUS MRG

	N=	1-YR	3-YR	5-YR	P value
Donor <50	741	92%	85%	73%	
MRG	223	89%	78%	62%	p=0.03

Multivariate analysis showed that Donor Age >60 was a significant risk factor for worse long-term graft function (p<0.0001).

TABLE 2: GS BY DONOR AGE

Category	DONOR AGE	N=	1-YR	3-YR	5-YR	P value
1	<50	741	92%	85%	73%	1 v. 4; p=0.002
2	50-54	83	92%	88%	72%	2 v. 4; p=0.009
3	55-59	64	90%	78%	70%	3 v. 4; p=.15
4	60-64	42	88%	62%	43%	all (1-5) v. 6; p<0.01
5	65-69	28	89%	80%	65%	all other combinations p>0.2
6	70+	6	50%	33%		

Graft loss was associated with Transplant Year (p=0.02) where later years had improved GS. MRG had 25% (55/223) graft loss compared to 18% (135/741) for grafts <50 years over this time period (p=0.03). Risk of graft loss was no different with FK between MRG and grafts < 50 yrs. [10% (3/29) v.13% (15/119); p=0.74]. Neoral had increased risk of loss for MRG versus grafts <50 yrs. [27% (52/194) v. 19% (120/622); p=0.03]. **CONCLUSIONS:** (1) There is an increased risk of graft loss with MRG >60 years old. (2) FK conferred the same risk of graft loss for MGR recipients as recipients of grafts <50 yrs. (3) MMF + D improved overall GS for MRG. (4) MRG should be used with caution when donor age >60.

Abstract# 1575

FREEDOM FROM REJECTION AND STABLE RENAL FUNCTION ARE EXCELLENT CRITERIA FOR STEROID WITHDRAWAL IN TACROLIMUS THERAPY. Zbigniew Wlodarczyk,[1] Janusz Walaszewski,[2] Ferenc Perner,[3] the COSTAMP Study Group. *[1]Renal Transplantation Unit, District Hospital, Poznan, Poland; [2]Dept. of General and Transplantation Surgery, Medical University, Warsaw, Poland; [3]Transplantation and Surgical Clinic, Semmelweis University, Budapest, Hungary.*

Objectives: This open, prospective, multicenter, randomized, parallel group study was designed to examine the safety and efficacy of two tacrolimus-based regimens, when steroids are selectively withdrawn 3 months after kidney transplantation. **Methods:** A total of 496 adult kidney transplant recipients were randomly assigned to receive either tacrolimus (Tac), MMF and steroids (n=243), or Tac, azathioprine and steroids (n=246). The initial oral dose of Tac was 0.2 mg/kg/day. Subsequent doses were adjusted according to whole blood target trough levels of 15 ng/mL on days 0-21, 10-15 ng/mL on days 22-41, and 5-10 ng/mL on days 42-183. MMF dosing was 1 g/day, azathioprine was administered with 1-2 mg/day. Steroids were tapered from 20 mg/day to 5 mg/day during months 1-3. From month 3 onwards, steroids were withdrawn in patients who were free from steroid-resistant acute rejection and who had serum creatinine concentrations <160 µmol/L. Study duration was 6 months. **Results:** At the end of the study, graft survival was 95.1% (Tac/MMF) and 93.5% (Tac/Aza). Patient survival was 98.4% in both treatment groups.The 6-month incidences of biopsy-proven acute rejection were 18.9% (Tac/MMF) compared with 26.9% (Tac/Aza), p=0.038. The incidence of steroid-resistant acute rejection was 2.1% and 4.9%, respectively. At the end of month 3, steroid withdrawal was performed in 60.5% (Tac/MMF) and 48.8% (Tac/Aza) of patients, p<0.01. During months 4-6, biopsy-confirmed acute rejection was observed in 2.7% (Tac/MMF) compared with 0.8% (one patient) in the Tac/Aza group. In patients who continued to receive steroids, the incidences of biopsy-proven acute rejections during months 4-6 were 3.5% (Tac/MMF) and 7.1% (Tac/Aza). At the end of the study, the steroid-free patients had an excellent kidney function with a median serum creatinine concentration of 119.5 µmol/L (Tac/MMF) and 120.7 µmol/L (Tac/Aza); the median serum creatinine in all patients of the treatment groups were 130.1 µmol/L and 132.8 µmol/L, respectively. **Conclusion:** Both tacrolimus regimens are safe and effective. The combination of Tac and MMF permitted a higher proportion of steroid-free maintenance treatments with a low incidence of acute rejection, and with a very good renal function.

Abstract# 1576

LONG-TERM COMPARISON OF THYMOGLOBULIN VERSUS ATGAM FOR INDUCTION IN ADULT RENAL TRANSPLANTATION: EVIDENCE OF IMPROVED ALLOGRAFT SURVIVAL AT 5 YEARS. Karen L. Hardinger, Mark A. Schnitzler, Brent Miller, Jeffrey Lowell, Surendra Shenoy, Daniel C. Brennan. *[1]Transplantation, Barnes-Jewish Hospital at Washington University, St. Louis, MO.*

Controversy exists surrounding the long term safety and efficacy of induction therapy in renal transplantation. At one-year, results of a randomized double-blinded trial of Thymoglobulin versus Atgam for induction therapy in renal transplant patients revealed that the composite end point of freedom from death, graft loss, or rejection, the "event-free survival," was superior with Thymoglobulin (94%) compared with Atgam (63%; $P = 0.0005$). While Thymoglobulin patients had similar patient survival to Atgam patients, Thymoglobulin patients had better graft survival, less graft rejection, and less CMV-disease. The purpose of this study was to examine the long-term safety and efficacy of antithymocyte induction in renal transplantation. **Methods**. Transplant recipients (n=72) were randomized 2:1 in a double-blinded fashion to receive Thymoglobulin (n=48) 1.5 mg/kg IV or Atgam (n=24) 15 mg/kg IV, intraoperatively, then daily for at least 6 days. Maintenance immunosuppression consisted of cyclosporine, azathioprine or mycophenolate mofetil, and prednisone. **Results**. At five years after transplantation, the composite endpoint of freedom from death, graft loss, or rejection, "event-free survival", was superior with Thymoglobulin (73%) compared to Atgam (37%; $P < 0.001$). Patient survival was similar between the groups. Despite a small number of patients treated, graft survival was significantly better in the Thymoglobulin arm (79%) versus the Atgam arm (58%; P=0.026). The incidence of CMV disease was less with Thymoglobulin than Atgam at 5 years (13% vs. 33%; P=0.03). There were two cases of PTLD in the Atgam arm and no cases in the Thymoglobulin arm. The mean 5 year serum creatinine was 1.9±0.7 mg/dL in the Thymoglobulin arm (n=35) and 1.5±0.7 mg/dL in the Atgam arm (n=13; P=NS). **Conclusions.** Five-year follow-up of induction with Thymoglobulin resulted better event-free survival and better graft survival without a risk of PTLD and CMV disease when compared to Atgam.

ORGAN DONATION, PRESERVATION AND POLICY I

Abstract# 1577

BLACKS AS DONORS FOR TRANSPLANTATION: A MULTIVARIATE ANALYSIS OF THEIR IMPACT ON TRANSPLANT OUTCOMES. Clive O. Callender,[1] Patrice V. Miles,[1] Wida S. Cherikh.[2] *[1]MOTTEP, Howard University, Washington, DC; [2]Research, UNOS, Richmond, VA.*

Purpose. Cadaveric kidney transplant recipients for Black donors have been reported to have poor graft survival. We compared the consequences of donations for all ethnic donors for kidney (KI), liver (LI) and heart (HR) transplantation. In this study, we compared graft/patient survival of different donor-to-recipient ethnic combinations. **Methods.** A total of 118,769 transplants including 77,689 living and cadaveric KI, 26,124 cadaveric LI, and 14,956 cadaveric HR recipients from the OPTN/UNOS database during 4/01/1994-12/31/2000 were analyzed. A multivariate Cox regression model was used to analyze the effect of donor-recipient ethnicity combination on graft survival in KI and LI recipients, and patient survival in HR recipients. White, Black, Hispanic, Asian and other minority groups were examined. The analysis also included other donor, recipient and transplant characteristics. Results are presented as relative risk (RR) of graft loss/mortality. For comparison purposes, White to White combination was used as the baseline. **Results.**

Ethnicity Combination	RR of graft loss for KI (p-value)	RR of graft loss for LI (p-value)	RR of mortality for HR (p-value)
Black to White	1.213 (<0.001)	1.215 (<0.001)	1.067 (0.29)
Black to Black	1.509 (<0.001)	1.366 (<0.001)	1.513 (<0.001)
Black to Hispanic	1.091 (0.28)	1.095 (0.34)	1.340 (0.13)
Black to Asian	0.837 (0.27)	1.105 (0.62)	0.650 (0.23)*
Black to Other Minority	0.717 (0.21)	1.042 (0.85)	--

* Asian and other minorities were combined due to small number

The analyses also show the following: 1. Organs (KI, LI, HR) donated from Blacks perform more poorly than organs donated from other ethnic groups. 2. Black recipients of cadaveric or living donor kidneys were associated with a significantly poorer graft survival regardless of recipient ethnicity. Black recipients of cadaveric livers and hearts had a significantly poorer graft survival when receiving organs from Whites, Blacks or Hispanics. 3. Among all donor-recipient ethnic combinations, Black to Black was associated with the highest RR of graft loss (for KI and LI) or mortality (for HR) (P<0.001). **Conclusion.** These analyses demonstrate unique differences in the Black donor-recipient populations from those of other ethnic groups and mandates the need for further research to understand these differences, which are more of an enigma now than ever before.

Abstract# 1578

THE DIFFERENT REASONS FOR NON-DONATION AMONG ETHNIC SUB-POPULATIONS. David A. Laskow, Daniel Concepcion, Disha Patel, Shannon Lynch, Kathie Soroka, Bill Reitsma. *[1]Dept.of Surg.Divsion of Kidney/Pancreas, Robert Wood Johnson Univ. Hospital, New Brunswick, NJ; [2]NJ Organ and Tissue Sharing Network, Springfield, NJ.*

Currently, the annualized growth rate of the national organ waiting list (13.5%) far outstrips the growth rate of transplants performed (5.3%), resulting in a severe organ shortage. From Jan. 1999 to Dec. 2000 the N.J. Organ and Tissue Sharing Network consented 52% of the possible donors. The ethnic origins of the 260 non-consented donors (NCD) are 43% Caucasian, 25% African American (AA), 20% Hispanic (H), 9% Asian (A) and 3% others. A 20-30 min. interview in the family's primary language ensued, covering 40 questions concerning donation. Listed below are the significant differences among the ethnic groups. H (78%) and AA (74%) NCD are most likely to be male compared to Asians (56%). Among Hispanics, 75% of the time a female made the decision concerning donation compared to 30% of Asians. 62% of the Asians interviewed consider themselves religious and 59% feel their religion forbids donation, compared to 30% and 17% in Hispanics. 60% of AA NCD families mistrust the medical system and 20% have had a poor hospital experience, compared to 5% and 5% among Hispanics and 7% and 5% among Asians. All NCD families felt that the organ bank representatives were sympathic; however, only 50% of Asians understood the process of organ donation compared to 100% of Hispanic and 90% of AA NCD. In addition, 66% of Asians felt that language was a barrier to donation as compared to 35% of Hispanics. All the groups were asked to donate the majority of the times (>80%) by a Caucasian. No group demonstrated that they understood the difference between brain death and cardiac arrest. 52% AA, 23% Hispanics, and 33% Asians described their understanding as "a moderate amount" or "a large amount." The final decision not to donate was likely to be made by the family in the Hispanic and AA groups (65% and 80%), where as in the Asian NCD 75% of the time a single individual made the final decision. All groups were comfortable (90%) with their decision not to donate. Less than 10% of the ethnic NCD families thought paying 1000 dollars for funeral expensive would have altered their decision. Although if asked to donate now 65% of Hispanic NCD families said they would consider donation as opposed to only 16% among Asians and 10% among AA NCD families. In conclusion, there are considerable differences among the ethnic groups surveyed for denying organ donation. A more select approach, addressing the cultural differences among the ethnic groups could result in a higher donor consent rate.

Abstract# 1579

SUCCESSFULL KIDNEY AND LIVER TRANSPLANT OUTCOMES FROM 150 NON-HEART BEATING DONORS (NHBD) IN A SINGLE OPO. Richard Hasz,[1] Howard Nathan,[1] John Edwards,[1] John Abrams,[1] Michael Moritz,[3] Cosme Manzerbetia,[2] David Reich,[2] Abraham Shaked,[4] Kim Olthoff,[4] Harold Yang.[5] *[1]Gift of Life Donor Program, Philadelphia, PA; [2]Albert Einstein Medical Center, Philadelphia, PA; [3]Hahnemann University Hospital, Philadelphia, PA; [4]Hospital of the University of Pennsylvania, Philadelphia, PA; [5]Harrisburg Hospital, Harrisburg, PA.*

Purpose: This organ procurement organization evaluated kidney and liver donation and transplant outcomes from NHBDs over 7 years to demonstrate an effective increase in the donor pool. **Method:** A standard NHBD protocol was established for donor selection. Every non- brain dead (NBD) referral was evaluated for NHBD suitability. From June 1995 to July 2002, 2,510 NBD donor referrals were evaluated. NHBD recoveries were attempted on 158 patients which resulted in 150 donors with 290 kidneys and 76 livers recovered. Organs were preserved utilizing UW and simple cold storage. An analysis was performed to evaluate how NHBD increased organ availabilty and kidney and liver transplant outcomes. The Kaplan-Meier method was used to ascertain patient and graft survival rates. **Results**:

NHBD Donation Outcomes

	NBD Referrals	Total Donors	NHBD	Kidneys txp/proc.	Livers txp/proc
1995	198	222	2	2/4	1/1
1996	236	261	12	17/23	2/4
1997	301	292	14	21/28	3/6
1998	307	298	25	36/47	9/14
1999	416	331	24	43/48	9/12
2000	484	298	23	40/42	9/12
2001	383	315	32	51/62	13/17
2002 *	185	180	18	35/36	6/10
Totals	2510	2200	150(6.8%)	245/290 (84%)	52/76 (68%)

* January- July 9th

NBD referrals comprised 25% of all organ donor referrals to the OPO and approx. 6% were considered suitable for NHBD. NHBD represented 6.8% of total donors recovered. Kidneys and livers recovered resulted in transplant rates of 84% and 68% respectively.

Kidney and Liver Transplant Outcomes

	Kidney Graft Survival	Kidney Patient Survival	Kidney Median Creat.	Liver Graft Survival	Liver Patient Survival
1 yr.	77 %	95 %	1.45	76 %	82 %
3 yr.	73 %	90 %	1.40	68 %	73 %
5 yr.	70 %	85 %	1.40	68 %	73 %

There were 245 kidneys transplanted into 239 recipients (6 recipients received 2 kidneys) and 52 livers transplanted. One kidney had PNF and 72 (30%) developed ATN (dialysis within first week of transplant). Kidney graft and patient survival was 77% and 95 % at one year and 70% and 85% at five years. Liver graft and patient survival was 76% and 82% at one year and 68% and 73% at five years. **Conclusions:** This OPO effectively increased the donor pool by 7% over 7 yrs. and 10% in the last two years utilizing a standard NHBD protocol yielding acceptable kidney and liver transplant outcomes comparable to other expanded donors.Widespread acceptance of NHBD could increase the US donor pool.

Abstract# 1580

EARLY FUNCTION OF CADAVERIC KIDNEY, PANCREAS, AND LIVER TRANSPLANTS PERFUSED USING IN SITU LR FOLLOWED BY BACKTABLE ORGAN PERFURSION WITH UW. G. L. Bumgardner,[1] M. L. Henry,[1] E. Elkhammas,[1] E. A. Davies,[1] R. P. Pelletier,[1] A. Rajab,[1] R. M. Ferguson.[1] *[1]Department of Surgery, Division of Transplant, Ohio State, Columbus, OH.*

The ***purpose*** of this retrospective study was to determine immediate graft function after kidney (KTx), combined kidney and pancreas (PKTx), or liver transplantation (LTx) at a single center using a standard *in situ* cold Lactated Ringers (LR) flush followed by backtable flush with Viaspan (UW). *In situ* perfusion with LR, which does not have the viscosity of UW, is used to achieve rapid organ cooling. **Methods:** Data for 1/93-9/02 was collected for multiorgan procurement of 492 cadaveric donors from organ procurement and transplant databases. **Group I** (80%) underwent *in situ* perfusion with LR and backtable perfusion and packaging with UW. **Group II** (20%) underwent *in situ* perfusion with UW alone (or in conjunction with LR) and backtable perfusion and packaging with UW. Perfusion data was available on 82% of donors. 987 recipients (490 KTx, 300 PKTx, 199 LTx) received these organs and were transplanted at a single center. LR and UW volume used to *in situ* perfuse, backtable perfuse, and package organs were used to calculate estimated pefusate cost based on the mean volume and pricing for LR ($2.35/L) and UW ($239/L). **Results:** Group I used 8.9±2.3L LR and 3.2±1.2L UW resulting in estimated perfusate cost of $786. Group II used 5.8±2.0L UW resulting in estimated perfusate cost of $1451. Serum creatinine (S Cr) for KTx; S Cr, fasting blood glucose (FBS), serum amylase (S amy), and urine amylase (U amy) for PKTx; AST and ALT for LTx; and 1 yr graft and pt survival were analyzed. ~80% of recipient chemistries for organs transplanted after *in situ* LR flush were available (median values shown in Table I). No significant differences in recipient chemistries were observed for organs transplanted from Group I or II (not shown).

Table I. Organ Tx	**Day 1**	**Day 3**	**Day 7**	**Day 14**	**Day 30**	**1 yr Graft**	**1 yr Patient**
PKTx (S Cr)	3.6	1.9	1.5	1.6	1.7	85% KTx	92%
PKTx (S amy)	244	129	106	126	107	82% PTx	
PKTx (U amy)	3687	3216	17435	24523	30818		
PKTx (FBS)	121	109	104	92	96		
KTx (S Cr)	5.5	2.9	2.0	1.7	1.9	87%	93%
LTx (AST)	569	147	50	43	40	78%	81%
LTx (ALT)	424	300	150	110	67		

Conclusions: *In situ* perfusion of kidney, pancreas, and livers with LR followed by backtable perfusion and packaging in UW is 1) associated with immediate graft function as demonstrated by prompt decrease in S Cr after KTx, decrease in serum amylase, increase in urine amylase, euglycemia after PKTx, and decrease in serum AST and ALT after LTx, 2) is more economical than *in situ* UW perfusion.

Abstract# 1581

PRESENCE OF HLA ANTIBODIES IN BLOOD COMPONENTS: AN UNAPPRECIATED RISK FACTOR FOR TRANSPLANT PATIENTS? Shealynn B. Harris, Robert A. Bray, Cassandra D. Josephson, Christopher D. Hillyer, Howard M. Gebel. [1]*Departtment of Pathology, Emory University Hospital, Atlanta, GA.*

One of the more aggressive approaches in renal transplantation is the use of intravenous immunoglobulin and plasmapheresis (PP) as pre-emptive or rescue therapy for patients with HLA alloantibodies. As a result of PP, patients can become hypocoaguable, requiring supportive transfusions with fresh frozen plasma (FFP) and/or cryoprecipitate (Cryo). HLA alloantibodies in these components have been linked to post-transfusion complications such as transfusion-related acute lung injury (TRALI). This association raises the spectre of other complications resulting from HLA alloantibodies transfused into transplant patients, including graft loss/dysfunction (solid organ recipients) or failed stem cell engraftment. The incidence of complications would be dependent, in part, upon the frequency of HLA alloantibodies in the blood donor population. To address this issue, we screened segments from randomly selected units of FFP, Cryo, and red blood cells (RBCs) for HLA Class I and Class II alloantibodies. Testing was performed using FlowPRA (One Lambda Inc), an HLA antigen-specific flow cytometric assay.

Blood Components with HLA Alloantibodies

Components	Class I only	Class II only	Class I +II	Total
RBCs (n=106)	7 (7%)	8 (8%)	3 (3%)	18 (18%)
CRYO(n=66)	3 (5%)	3 (5%)	10 (15%)	16 (20%)
FFP(n=77)	9 (12%)	4 (5%)	9 (12%)	22 (29%)
All (n=249)	19 (8%)	15 (6%)	22 (9%)	56 (23%)

(%) = % of samples tested

Our data reveal that 1:4 to 1:5 blood components contain HLA alloantibodies. We speculate that this unappreciated frequency of HLA antibodies in blood components may lead to other post-transfusion complications such as organ dysfunction or loss. Of specific concern are patients undergoing PP to eliminate HLA antibody who then require blood component support to manage their hypocoaguable state. The infusion of products containing unrecognized HLA alloantibodies may pose a significant risk factor to these patients. Thus, the identification of HLA antibody-containing blood components may be an important consideration for transplant patients requiring transfusion support.

Abstract# 1582

TRANSPLANT ECONOMICS IN A COMPLEX PAYOR MARKET. E. Y. Zavala,[1,3] B. S. Marshall,[1] K. Bankston,[1] J. Hurst,[1,2] E. S. Woodle.[2] [1]*Transplant Services, The University Hospital;* [2]*Dept. of Surgery;* [3]*College of Pharmacy, The University of Cincinnati, Cincinnati, OH.*

There are currently 261 hospitals in the United States with solid organ transplant programs, representing approximately 4% of the acute care hospitals in the U.S. A number of evolving issues have complicated third party payor contracting, particularly managed care contracting; and as a result, the processes of assuring accurate transplant revenue capture has become extremely problematic. Our institution identified a potential problem in the Patient Accounting department's ability to properly manage and bill for transplant services under manage care contracts. We, therefore, analyzed the transplant economics and revenue management performance for our institution. **Methods:**A multidisciplinary team (MDT) was formed in August 2001 consisting of Transplant Services, Patient Accounting, Payor Relations, Registration and Organizational Effectiveness. There were 22 individuals in the MDT and an estimated total cumulative 588 hours invested in evaluating the revenue capture process. The MDT was charged with identifying problems in the transplant billing process and developing a systemized solution to consistently and effectively manage the processes. **Results:**Only 78% of expected payment over an 18 month period had been collected which represented $2,120,073.00 in underpayments. The underpaid transplant cases were: Liver-48%, Kidney-33% and Heart-19%. The primary reasons for underpayments were: original and followup accounts billed incorrectly-38%, payor requested additional information that was never sent-25%, payor had no record of claim being received-22%. Days to zero out transplant patient accounts exceeded 203 days. The transplant billing and payment processes were concluded to be ineffective. The MDT developed a standardized transplant payment process, transplant financial management database, and an Underpayment Recovery Unit (URU). The core of the improved system was a new key position of a Transplant Operations Analyst (TOA) which was designated as the hub for all transplant payments and accounting processes. The URU was established in September 2001, and the TOA was hired in November 2001. The URU collected $2,290,430.00 in underpayments. The new system and processes reduced days to zero to 63.1 days, and as of August 2002, collected 97.6% of all transplant dollars expected. **Conclusion:**Transplant programs must maintain a focus on their economic efficiency and business processes to ensure fiscal viability in this era of multiple payors, complicated payment mechanisms, and competitive transplant reimbursement rates for transplant hospitals and physicians.

Abstract# 1583

LOW VOLUME TRANSPLANT CENTERS ARE ASSOCIATED WITH WORSE OUTCOME FOLLOWING RENAL AND HEPATIC TRANSPLANTATION. David A. Axelrod,[1] Randy Webb,[2] Alan Leichtman,[1] Jeffery Punch,[1] Robert M. Merion.[1,2] [1]*Department of Surgery, University of Michigan, Ann Arbor, MI;* [2]*Scientific Registry of Transplant Recipients, Ann Arbor, MI.*

Introduction: Outcomes of some complex surgical procedures performed at centers with higher volumes have been shown to be better than those performed at low volume hospitals. The impact of volume on transplant outcome has not been addressed using national, risk-adjusted data. Methods: Data from the Scientific Registry of Transplant Recipients were analyzed for renal and hepatic transplant recipients from 1/96 to 12/99. Center volume was used to assign centers into volume quartiles (kidney) or terciles (liver). Logistic regression models were constructed to examine the impact of transplant center volume quantile on one-year patient (liver) and graft (kidney) survival adjusting for 12 donor and recipient clinical characteristics, ischemic time, HLA-matching (kidney), and transplant center clustering. Results: The range of renal transplant volume (N=44,755) at 258 transplant centers was 1 to 1389 (interquartile range 226-624) over 5 years. Among renal transplant recipients, the unadjusted rate of graft loss within one year, was significantly lower at high volume centers (8.4%) when compared with very low (9.6%), low (9.8%), and medium (9.8%) volume centers (p<0.001). After adjustment, kidney transplant at very low volume centers was associated with significantly worse outcome (odds ratio [OR] 1.21; P<0.05) when compared to high volume centers (Table). Liver transplant volume (N=14,816) at 126 centers ranged from 1 to 1485 (interquartile range 233-610) over 5 years. Unadjusted one-year mortality rates did not differ significantly (p=0.21) between high volume centers (16.3%) and low (16.7%) or medium (15.5%) centers. However, after adjustment, significantly higher odds of one-year mortality were observed at low volume centers (OR 1.23; P<0.02).(Table) Conclusions: Outcomes of kidney and liver transplantation at centers with the lowest volumes are significantly worse in a risk-adjusted analysis. Further research should examine surgical and medical management practices that differ between high and low volume centers.

Association between Volume Quantile and Transplant Outcome

Category	Transplants per year	Odds Ratio	P-Value
Kidney Transplant			
Very Low	<45	1.21	0.05
Low	46-75	1.17	0.09
Medium	76-125	1.13	0.22
High	>125	1.0 (Reference)	NA
Liver Transplant			
Low	<60	1.23	0.02
Medium	60-112	1.02	0.82
High	>112	1.0 (Reference)	1.0

Renal transplant outcome= one year graft failure (includes death with function). Hepatic transplant outcome= one year mortality.

Abstract# 1584

BODY MASS INDEX AND CADAVERIC ORGAN DONOR POTENTIAL. Kevin O'Connor,[1] James Whiting,[2] James Bradley,[1] Paul Morrissey,[3] Francis Delmonico.[1] [1]*New England Organ Bank, Boston, MA;* [2]*Surgery, Maine Medical Center, Portland, ME;* [3]*Surgery, Rhode Island Hospital, Providence, RI.*

Background. The importance of body mass index (BMI) as a factor in determining cadaveric organ donor potential has not been critically examined. Nine hundred and forty five cadaveric organ donors procured over a five year period were reviewed. Seventy-four children, 9 patients where a BMI could not be determined and 24 non-heartbeating donors were excluded from the analysis, leaving 838 donors. The mean BMI of all donors was 26.6 ± 6 (range 14-60). Twenty-four (2.8%) of organ donors had BMIs >40, 45 (5.4%) had BMIs between 35 and 40 and 114 (13.6%) had BMIs > 30 qualifying them as obese. Linear regression failed to reveal a significant association between BMI and organs recovered or organs transplanted. Segregating donors into BMI categories demonstrated that donors with very high BMIs were significantly less likely to produce certain organs as seen below. In addition, donors with extremely high BMIs produced fewer transplantable organs per donor again as seen below. **Conclusions.** Although very high BMIs are significantly associated with fewer organs recovered and transplanted per organ donor, obesity is not a contraindication to organ donation. Obese deceased patients remain an important source of transplantable organs.

Organs Recovered

BMI	<30 (N=652)	30-35 (N=114)	35-40 (N=45)	>40 (N=27)	Total (N=838)
Liver	590 (90%)	100 (88%)	39 (87%)	19 (70%)*	748 (89%)
Pancreas	231 (35%)	39 (34%)	13 (29%)	2 (7.4%)*	285 (34%)
Heart	285 (44%)	41 (36%)	11 (24%)*	10 (37%)	347 (41%)
Kidney	1230 (94%)	211 (93%)	80 (89%)	45(83%)	1566 (93%)
Total/Donor	3.9±1.5	3.6±1.3	3.3±1.3	2.9±1.3*	3.8±1.5

* p < 0.04 as compared to the rest of the cohort

Organs Transplanted

BMI	<30 (N=652)	30-35 (N=114)	35-40 (N=45)	>40 (N=27)	Total (N=838)
Liver	508 (78%)	98 (86%)	32 (71%)	8 (30%)*	646 (77%)
Pancreas	152 (23%)	30 (26%)	2 (4%)*	1 (3.2%)*	185 (22%)
Heart	285 (44%)	40 (35%)	11 (24%)*	10 (37%)	346 (41%)
Kidney	1041 (80%)	194 (85%)	59 (66%)	37 (68%)	1331 (80%)
Total/Donor	3.3±1.7	2.8±1.6	2.5±1.6*	2.1±1.6*	3.1±1.7

*p<0.04 compared to the rest of the cohort

Abstract# 1585

PREDICTING POSITIVE CROSSMATCHES BY IDENTIFYING UNACCEPTABLE ANTIGENS USING HIGH DEFINITION FLOW CYTOMETRIC BEADS IN HIGHLY SENSITIZED RECIPIENTS.

Walter F. Herczyk,[1] Barbara O. Burgess,[1] Janet Tuttle-Newhall,[2] Nancy L. Reinsmoen.[1] *[1]Clinical Transplantation Immunology Laboratory, Duke University Medical Center, Durham, NC; [2]Department of Surgery, Duke University Medical Center, Durham, NC.*

Highly sensitized (>80% PRA) patients on the solid organ wait lists receive additional points for possible organ allocation; however, the crossmatches are usually positive since unacceptable antigens are difficult to identify in these cases. The high definition single antigen flow cytometry beads in combination with the specificity analysis flow cytometry beads (One Lambda, Inc.) allow for the accurate identification of antibody specificities for these highly sensitized patients. We have used these flow bead reagents for PRA analysis to identify unacceptable antigens for our sensitized kidney, heart, and lung patients on the wait list. To determine if the PRA analyses accurately predicted the crossmatch results, we analyzed the results for 14 consecutive highly sensitized patients called in for final crossmatches. Of the 14 patients, 8 had PRAs >80% and 6 had PRAs ranging from 46 – 67% (anti-class I +/or II). A total of 18 donor specimens was tested by CDC-AHG and/or flow cytometry crossmatches for these patients over a 6-month period. The high definition unacceptable antigen analysis accurately predicted 11 positive crossmatches and 7 negative crossmatches (100% concordance). Based on these results, we have used the unacceptable antigens defined by high definition flow cytometry beads to select patients for final crossmatches. During the past 6 months, the average positive crossmatch rate was 16% (19/97 donors tested) compared with 26% (27/103) for the same 6-month period during the previous year. Locally, 30% of recipients transplanted had PRAs >20% compared with the national average of 11.26% (p=0.0001). Thus, identifying the unacceptable antigens by flow beads has reduced the positive crossmatch rate by 39%, while allowing for a significantly higher percentage of sensitized patients to be transplanted locally than the national average. An accurate assessment of unacceptable antigens for these patients will decrease the cost for crossmatching, facilitate rapid organ allocation, and decrease the time and cost associated with patients traveling to the transplant center unnecessarily.

Positive Crossmatch Rate (Over Same 6-Month Period)

	Sensitized Patients XM Neg	Sensitized Patients XM Pos	Total # of Donors	% Pos XM
2001	5	27	103	26
2002	12	16	97	16

(p-value = 0.048, 1- tailed analysis)

Regulatory Cells in Tolerance Induction

Abstract# 1586

TIM-3 CONTROLS THE ALLOANTIGEN-SPECIFIC IMMUNOSUPPRESSIVE FUNCTION OF CD4+CD25+ REGULATORY T CELLS IN TRANSPLANTATION TOLERANCE.

Alberto Sanchez-Fueyo,[1] Jane Tian,[2] Christoph Domenig,[1] Xin Xiao Zheng,[1] Anthony J. Coyle,[2] Terry B. Strom.[1] *[1]Medicine/Immunology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Millenium Pharmaceuticals, Cambrdige, MA.*

TIM-3 is an Ig superfamily member selectively expressed on terminally differentiated Th1 cells and capable of inhibiting Th1 responses. To investigate the role of TIM-3 on the generation of immunoregulatory networks in transplantation, we have used a TIM-3/Fc fusion protein to block this pathway in an islet allograft model in which C57BL/6 recipients are rendered tolerant to DBA/2 islets by the combination of donor specific transfusion (DST) plus anti-CD40L. The administration of TIM-3/Fc together with DST plus anti-CD40L abrogated tolerance induction and resulted in rapid graft rejection. In addition, DST plus anti-CD40L treatment failed to ensure engraftment in TIM-3KO recipients. Given our finding that TIM-3/Fc preferentially binds CD4+CD25+ regulatory T cells (T regs), which are required for the tolerizing effect of DST plus anti-CD40L, we hypothesized that T regs might use TIM-3 to inhibit alloaggressive Th1 responses. This was tested in vivo by sorting $4x10^5$ wild type T regs and transferring them into Scid skin allograft recipients together with $1x10^5$ CD4+CD25- T cells from wild type or TIM-3KO mice. Skin allograft rejection was prevented in both cases, indicating that regulatory T cells can suppress Th1 clones in the absence of TIM-3. To test whether TIM-3 blockade, while not eliminating the suppressive capacity of T regs, could compromise the generation of alloantigen-specific T regs, we then used the adoptive transfer model to assess the impact of DST plus anti-CD40L treatment on CD4+CD25+ T cells. T regs harvested from treated hosts, but not those obtained from naive mice, were capable of protecting skin allografts when transferred into Scid recipients together with naive CD4+CD25- T cells at a 1:1 ratio, and this effect did not occur when DST was obtained from a third party donor. Moreover, this enhanced alloantigen-specific suppressive phenotype conferred by DST plus anti-CD40L was abolished by the concurrent administration of TIM-3/Fc, since T regs harvested from hosts treated with DST, anti-CD40L and TIM-3/Fc did not exert greater suppressive effects than naive T regs. In short, our results indicate that the tolerizing effect of DST plus anti-CD40L depends on the capacity of T regs to acquire powerful alloantigen-specific immunosuppressive function, and that this effect is regulated by TIM-3. We propose that TIM-3 curtails Th1 responses by bolstering the induction of antigen-specific T regs.

Abstract# 1587

GENERATION OF $CD25^+CD4^+$ REGULATORY T CELLS IN A TRANSPLANTATION MODEL: DEVELOPMENT IS THYMUS-INDEPENDENT AND DOES NOT REQUIRE $CD25^+$ PRECURSORS.

Mahzuz Karim,[1] Cherry I. Kingsley,[1] Andrew R. Bushell,[1] Birgit S. Sawitzki,[1] Kathryn J. Wood.[1] *[1]Nuffield Department of Surgery, University of Oxford, Oxford, United Kingdom.*

$CD25^+CD4^+$ regulatory T cells (Treg) play an important role in the control of immune responses *in vivo*. It has been demonstrated that naturally-occurring autoreactive $CD25^+CD4^+$ Treg undergo positive selection within the thymus and leave as committed $CD25^+$ Treg. We have shown that $CD25^+CD4^+$ Treg with the capacity to prevent the rejection of skin allografts can be generated by pre-treatment with donor-specific blood transfusion (DST) given under the cover of anti-CD4 therapy. We wished to establish whether these Treg develop via a similar thymus-dependent pathway to naturally-occurring Treg. Thymectomised adult CBA ($H2^k$) mice were pre-treated with blood from C57BL/10 ($H2^b$) donors under the cover of anti-CD4 antibody, and $CD25^+CD4^+$ cells isolated for adoptive transfer. T cell-deficient (CBA-$Rag^{-/-}$) recipients were reconstituted with $CD45RB^{high}CD4^+$ effector cells from naive mice with or without $CD25^+CD4^+$ cells from the pre-treated animals, and then received a C57BL/10 skin allograft. Reconstitution with effector cells alone resulted in acute skin allograft rejection whereas, in contrast, co-transfer of the $CD25^+CD4^+$ cells prevented this rejection and allowed long term survival of skin grafts, demonstrating that these Treg were generated in a thymus-independent process. We next wished to establish whether these $CD25^+CD4^+$ Treg arise from the peripheral expansion of naturally-occurring $CD25^+CD4^+$ Treg that cross-react with alloantigen, or by the conversion of mature peripheral T cells (which may be CD25 positive or negative) to a Treg phenotype. We therefore reconstituted CBA-$Rag^{-/-}$ mice with CD25 *negative* $CD4^+$ cells, treated these animals with C57BL/10 blood and anti-CD4 antibody, and administered $CD45RB^{high}CD4^+$ effector cells prior to performing a C57BL/10 skin allograft. These mice all accepted their skin allografts long term, whereas animals reconstituted in the same way but without the tolerising anti-CD4 / DST protocol all rejected their grafts acutely. These results demonstrate that, unlike the development of naturally-occurring autoreactive $CD25^+CD4^+$ Treg, anti-CD4 / DST pre-treatment does not generate alloreactive Treg through a thymus-dependent process or by the expansion of pre-existing $CD25^+CD4^+$ Treg populations, but rather by the conversion of mature peripheral T cells to a regulatory phenotype. These observations may have important implications for the design of clinical protocols to induce allograft tolerance in adult recipients.

Abstract# 1588

IL-15 AND COGNATE ALLOGENEIC STIMULATION SUCCESSFULLY EXPAND DE NOVO INDUCED HUMAN DONORSPECIFIC REGULATORY CD4+ T-CELLS THAT ONLY SHOW SUPPRESSION UPON ALLOSPECIFIC ACTIVATION.

Hans J. P. M. Koenen,[1] Esther Fasse,[1] Irma Joosten.[1] *[1]Department for Bloodtransfusion and Transpantation Immunology, University Medical Center Nijmegen, Nijmegen, Netherlands.*

Immunosuppressive regulatory T-cells are considered relevant for immunotherapy. To obtain sufficient cell numbers for clinical application, ex vivo expansion without loss of suppressor function is crucial. Previously, we reported expansion of human anergic regulatory T-cells without loss of anergy by allospecific stimulation in the presence of exogenously added IL-2. IL-15, like IL-2, is a T-cell growth factor that in contrast to IL-2 stimulates survival of T-cells. Here, we studied whether IL-15 could indeed be exploited as a superior growth factor of de novo induced human CD4+ anergic regulatory T-cells. Anergic regulatory CD4+ T-cells were generated by costimulation blockade of primary mixed lymphocyte reactions. Next, IL-15 as compared to IL-2, was investigated with respect to expansion and function of these anergic regulatory CD4+ T-cells. Optimal expansion required cognate allogeneic stimulation in the presence of exogenous IL-15. IL-15 resulted in enhanced survival that was paralleled by an increased number of Bcl-2 expressing cells. Moreover, IL-15 induced a distinct type of anergy characterized by hyper-reactivity to IL-15, and hence resulted in improved expansion. Notably, IL-15 expanded regulatory CD4+ T-cells were superior immunosuppressors of both naive and memory T-cells. Immunosuppression required alloantigen specific stimulation, appeared γ -irradiation resistant and was independent of IL-10, TGFβ or CTLA-4 interactions. IL-15 expanded regulatory CD4+ T-cells were stable suppressors that did not affect autologous recall responses in the absence of allogeneic MHC. Thus, IL-15 expanded de novo induced human anergic regulatory CD4+ T-cells have the potential to be used for antigen specific immunotherapy.

Abstract# 1589

ACTIVATION OF REGULATORY T CELLS IN ACCEPTED ALLOGRAFTS. Erica L. Altman,[1] Sharon Germana,[1] David H. Sachs,[1] Christian Leguern.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

Immune tolerance to MHC class II-matched solid organ transplants proceeds through the regulatory pathway in miniature swine and likely involves T-regulatory cells (T-reg). Human and murine T-reg are $CD4^+CD25^{high}$ T cells that have the capacity to suppress immune responses both *in vitro* and *in vivo*. We have recently described a phenotypically and functionally corresponding population from the peripheral blood of MHC-defined miniature swine. Swine $CD4^+CD25^{high}$ T-reg are also $CD8^+$, and express intracellular CTLA-4. These studies were undertaken in order to elucidate the potential role of MHC class II molecules in the emergence and activity of swine T-reg in the induction of tolerance to renal allografts. The parameters of T-reg activation and the tolerogenic capacity of class II^+ immature dendritic cells (iDC) as antigen presenting cells (APC) were evaluated using *in vitro* co-culture systems. T helper (Th) cells and T-reg were sorted from peripheral blood T cells, and iDC were generated from the plastic-adherent population of peripheral blood cells by culture with IL-4 and GM-CSF and were irradiated before use. Proliferation was measured by assessing [^{3}H]-thymidine incorporation. Highly sensitive spectratyping analysis of Vβ transcript usage was used to trace the activation of T-reg both *in vitro* and *in vivo*, from individual allograft recipients over time. Our studies demonstrated dose-dependent suppression of polyclonal Th proliferation by T-reg *in vitro* in the presence of autologous iDC. Although T-reg were non-proliferative when stimulated by either autologous or allogeneic iDC, intracellular CTLA-4 was up-regulated and TCR Vβ clonal dominance developed. These phenotypic changes in the T-reg population suggest that autologous iDC may naturally activate T-reg. In tolerant renal allograft recipients, marked TCR Vβ clonal dominance was detected in graft infiltrating lymphocytes. Similar TCR Vβ lengths were also present in *in vitro*-activated peripheral regulatory populations from the same recipients. This suggests the presence of activated T-reg intragraft. Furthermore, we have shown that $CD4^+CD8^+$ T cells accumulate in tolerated but not in rejected renal allografts. Taken together, our results are consistent with a mechanism of peripheral tolerance induction and/or maintenance that operates through the selective triggering of T-reg that traffic to the graft. Studies are underway to investigate the specific role played by autologous class II molecules and/or derived peptides in this process.

Abstract# 1590

HIGH DOSE OF ANTITHROMBIN III INDUCED INDEFINITE SURVIVAL OF FULLY ALLOGENEIC CARDIAC GRAFTS AND GENERATED REGULATORY CELLS. Osamu Aramaki,[1] Nozomu Shirasugi,[2] Tadatoshi Takayama,[1] Masanori Niimi.[2] *[1]Department of Surgery, Nihon University, Tokyo, Japan; [2]Department of Surgery, Teikyo University, Tokyo, Japan.*

Background. Antithrombin III (AT-III) is a physiologic inhibitor of thrombin and other serine proteases in the clotting cascade. The clotting cascade is also activated after organ transplantation. AT-III was found to inhibit inflammatory reactions independent of its anti-coagulant activity when given in high-dose. In this study, we examined whether AT-III could induce unresponsiveness to fully allogeneic cardiac grafts. Methods. CBA ($H2^k$) mice were given intravenous injection of 50 or 500 U/kg AT-III or control plasma the same day as transplantation of a heart from C57BL/6 ($H2^b$) mouse. To examine the existence of regulatory cells, we conducted adoptive transfer study. Results. Naive CBA mice rejected C57BL/6 cardiac grafts acutely (median survival time [MST] = 9 days). The 50 U/kg dose of AT-III induced a moderate increase in graft survival (MST = 25 days), whereas control mice rejected their graft acutely (MST = 7 days). With the 500 U/kg dose of AT-III, all grafts survived indefinitely (> 100 days). Histologic findings in the allogeneic cardiac grafts 100 days after transplantation were similar to those in syngeneic grafts. However, marked leukocyte infiltration was observed on 7 days after transplantation in this group, indicating that the anti-inflammatory effect induced by AT-III was not completely operative in the grafts. On the other hand,when 5 X 10^7 of splenocytes from mice treated with 500 U/kg dose of AT-III were adaptively transferred into naive second recipients 30 days after transplantation, these second recipients accepted their grafts indefinitely, suggesting that regulatory cells were generated after 30 days. In vitro, proliferation of splenocytes from CBA recipients given an intravenous injection of 500 U/kg AT-III was markedly suppressed compared with that of splenocytes from CBA recipients given either no treatment, 50 U/kg AT-III, or control plasma. AT-III suppressed generation of interleukin-2. Conclusion. High dose of AT-III induced indefinite survival of fully allogeneic cadiac grafts and generated regulatory cells. AT-III can be not only anti-thrombotic and anti-inflammatory agents but also a strong immunomodulating agent when used at high dose.

Abstract# 1591

THE ALLOGRAFT BY ITSELF INDUCES REGULATORY T-CELLS (Treg) INDEPENDENT FROM TOLERANCE INDUCTION PROTOCOLS. Stefan G. Tullius,[1] Manfred Lehmann,[2] Alexander Filatenkov,[1] Holger Schmidt,[1] Anja Reutzel-Selke,[1] Martina Seifert,[3] Birgit Sawitzki,[3] Kirsten Risch,[3] Peter Neuhaus,[1] Hans-Dieter Volk.[3] *[1]Dept. of Surgery, Virchow-Clinic, CharitéHumboldt-University, Berlin, Germany; [2]Dept. of Med. Biochemistry, University Rostock, Rostock, Germany; [3]Dept. of Med. Immunology, CharitéHumboldt-University, Berlin, Germany.*

Both, CTLA4-Ig and non-depleting anti-CD4 antibodies (RIB 5/2) induce long-term survival of allografts in high-responder combinations (DA to LEW). Although CTLA4-Ig cannot prevent acute rejection completely (temporary rise of serum creatinine/intragraft expression of TH1 transcripts) the later course in both protocols is comparable: tolerance can be adoptively transferred by splenocytes at day 60. We speculated that the graft by itself may be more important to induce Treg than tolerance induction protocols. Based on this hypothesis, Treg should accumulate in the graft early post-Tx. In fact, tolerance can be transferred following RIB 5/2 by graft infiltrating cells (GIC) earlier (day 14) and more efficiently (20 x less cells) than by splenocytes. Moreover, prevention of graft infiltration by FTY 720 (0.3 mg d2 to 14) prevented tolerance induction suggesting the importance of an early contact of the graft with (pre)-Treg. We then tested the role of the graft itself for the induction of Treg in the absence of tolerance inducing protocols. In fact, DA renal or F-344 heart grafts transplanted into LEW that rejected their first graft acutely resulted in an alloantigen-specific long-term graft survival in the absence of immunosuppression. All 2nd grafts (n=7/group) survived >100 days compared to 8±1 days in the high-responder and 24±5 days in the low-responder combination. While 1st grafts demonstrated characteristic signs of acute rejection and high titers of IgG alloantibodies (376±8 MCS at 1:100), 2nd grafts demonstrated only minor signs of chronic rejection (minor degrees of glomerulo- and arteriosclerosis) with strong cellular infiltrates (ED1+ MØ and CD4+ T-cells) by d 100; almost no allo-antibodies were detectable (1.5±2 MCS). Tolerance could be adoptively transferred by both splenocytes and GIC (less cells) 28 days following the 1st graft. All animals (n=7) survived >100 days. Most interestingly, they demonstrated a normal histology with only few cellular infiltrates and almost no allo-antibodies. These data demonstrate that the graft by itself induces Treg. During the initial engraftment the activation of Treg may be too late to influence the rejection process initiated by effector T-cells. Tolerance protocols need to anergize/delete effector T-cells while sparing T-reg activated by the graft itself to maintain tolerance.

Abstract# 1592

THYMUS DEPENDENT REGULATORY T CELLS MEDIATE TRANSPLANTATION TOLERANCE INDUCED BY ANTI-CD45RB. Xiaolun Huang,[1] Daniel Moore,[1] Moh-Moh Lian,[1] Kent M. Lee,[1] Haiying Chen,[1] Robert Zhong,[2] James F. Markmann,[1] Shaoping Deng.[1] *[1]Surgery, University of Pennsylvania Health System, Philadelphia, PA; [2]Surgery, University of Western Ontario, London, ON, Canada.*

Background: Selective interference with CD45RB isoform of leukocyte common antigen (CD45) by mAb therapy is able to induce transplant tolerance probably through induction of Th2 T cells and/or up-regulation of CTLA4 on T cells. In this study, we investigated the role of recipient thymus in anti-CD45RB induced tolerance. **Methods:** Heart grafts from C3H mice were transplanted to either normal or thymectomized C57/B6 (B6) mice. Recipients were untreated or treated with anti-CD45RB mAb (100ug ip days 0, 1, 3, 5, and 7). Graft function was monitored by daily palpation. Regulatory T cell activity in the thymus and periphery was studied using an adoptive transfer model in which C3H hearts were transplanted to Scid-B6 mice. These mice were then reconstituted with lymphocytes by iv injection of either naïve B6 splenocytes ($5x10^5$) or a mixture of naïve B6 splenocytes with thymocytes or splenocytes at 1:1 ratio or the sorted CD4+CD25+ or CD25- T cells at 5:1 ratio from the tolerant mice. **Results:** Cardiac allografts were rejected acutely with a mean survival time (MST) of 8.3±1.9 days in control C57/B6 mice (n=9). In mice treated with anti-CD45RB, the majority (70%) of grafts (n=16) survived indefinitely (MST=106±47 days). However, thymectomized mice (n=6) were resistant to anti-CD45RB induced tolerance despite a modest prolongation of graft survival (MST=56±22 days) compared to survival (MST=9.3±1.3 days) in control thymectomized mice (n=4). In mice bearing a long-term functioning allograft, a second B6 cardiac allograft was universally accepted. In the transfer study, naïve B6 splenocytes alone rapidly rejected C3H hearts in Scid B6 mice (n=5, MST=8.4±1.7 days), but the survival of grafts (n=3) was significantly prolonged by addition of splenocytes and thymocytes from tolerant mice (MST=23±2.3 days and 31±6.1, respectively). In addition, our preliminary data reveal that both CD4+CD25+ and CD4+CD25- thymocytes from the tolerant mice were able to suppress rejection of cardiac allografts mediated by naïve B6 lymphocytes. **Conclusions:** Short-term administration of anti-CD45RB mAb effectively prevents allograft rejection and induces donor-specific tolerance. Our data demonstrate a requirement for the participation of recipient thymus and suggest that regulatory T cells generated in this compartment are responsible for anti-CD45RB induced transplantation tolerance. This represents an unusual example of antibody induced central tolerance.

Abstract# 1593

WHICH DONOR ANTIGENS STIMULATE REGULATORY T CELLS? Toshiro Ito,[1] Akira Yamada,[1] Mohamed H. Sayegh,[2] Hugh Auchincloss, Jr..[1] *[1]Transplantation Unit, Surgical Services, Massachusetts General Hospital, Boston, MA; [2]Laboratory of Immunogenetics and Transplantation, Brigham and Women's Hospital, Boston, MA.*

Some evidence suggests that co-stimulatory blockade generates regulatory T cells (Tregs) through the indirect pathway. The aim of present study was to investigate which donor antigens provide peptides that generate Tregs. Allogeneic hearts were transplanted into B10.D2 recipients treated with combination of anti-CD40L and CTLA-4 Ig. This led to prolonged survival. After 8-10 weeks, splenocytes from these recipients were transferred along with naive B10.D2 splenocytes into sublethally irradiated secondary B10.D2 recipients. Allogeneic heart transplants were performed. The experimental groups and results were as follows.

Group	1st transplant	2nd transplant (450cGy)	Survival time (days)
1	-	B6→B10.D2 (naive splenocytes only)	13,13,14,14,15,17
2	B6→B10.D2	B6→B10.D2	>100x6
3	B6→B10.D2	B10.BR→B10.D2	11,12,13,13,14,21
4	B6→B10.D2	(B6xB10.BR)F1→B10.D2	19,22,23,25,>100x2
5	BALB/c→B10.D2	BALB/c→B10.D2	78,>100x5
6	B6→B10.D2	BALB.B→B10.D2	16,16,17,18,18
7	BALB/c→B10.D2	BALB.B→B10.D2	10,11,11,16,16
8	B6.class II-deficient →B10.D2	B6→B10.D2	12,12,13,17,18,18,28,>100
9	B6.β2m-deficient →B10.D2	B6→B10.D2	16,24,>100x3

Tregs generated by co-stimulatory blockade can prevent graft rejection in the adoptive transfer recipients (group 2) in an antigen-specific manner (group 3) and with evidence of linked suppression (group 4). Tregs generated in the face of multiple minor antigen disparities could prevent rejection of grafts expressing the same antigens (group 5). However, Tregs generated by MHC antigen disparities were unable to prevent rejection of grafts that expressed these same MHC antigens with different minor antigens (group 6) and Tregs generated by minor antigen disparities were unable to prevent rejection of grafts that expressed new MHC antigen disparities (group 7). Tregs were also not generated efficiently when co-stimulatory blockade was used for class II-deficient grafts and tested with secondary grafts that expressed both class I and class II antigens. These results suggest that there is no single set of donor antigens that generates peptides for regulatory T cells through the indirect pathway and suggest that the outcome of secondary transplantation is determined by the relative numbers of regulatory cells and naive effector cells that are specific for new determinants.

Abstract# 1594

HUMAN INNATE REGULATORY NKT AND ALLOPEPTIDE-SPECIFIC CD4$^+$CD25$^+$ CELLS CONTROL BOTH DIRECT AND INDIRECT ALLORESPONSES *EX-VIVO*. Shuiping Jiang,[1] Robert I. Lechler.[1] *[1]Department of Immunology, Faculty of Medicine, Imperial College, Hammersmith Hospital, Du Cane Road., London, United Kingdom.*

Innate NKT and CD4$^+$CD25$^+$ T cells have been identified recently as spontaneously occurring regulatory cells in the control of autoimmunity. Although these regulatory cells appear to mediate transplantation tolerance, little is known concerning the regulatory role of human NKT cells in alloresponses and the antigen-specificity of CD4$^+$CD25$^+$ cells. Here we describe the characterisation of naturally occurring NKT cells from human peripheral blood lymphocytes and the induction of allopeptide (HLA-A2: 138-170) specific human CD4$^+$CD25$^+$ regulatory T cell lines by priming purified CD4$^+$CD25$^+$ cells *ex-vivo*. Results: TCR Valpha24$^+$Vbeta11$^+$ (NKT) cells represented a very tiny population of T cells ranging from 0.01% to 0.1%. Purified Valpha24$^+$Vbeta11$^+$ cells by FACS sorting suppressed IL-2 production by CD4$^+$ T cells stimulated by allogeneic DCs in the presence of alpha-galactosylceramide. The suppression was dose-dependent. More than 95% inhibition was seen at a ratio of Valpha24$^+$Vbeta11$^+$ cells to responder CD4$^+$ T cells of 1:50. The suppression was partly cell contact-dependent. When the two cell populations were stimulated separated by a semi-permeable membrane, minimal suppression was observed. Addition of anti-GITR antibody could partially reverse the suppression effected by Valpha24$^+$Vbeta11$^+$ cells. These results indicate that innate NKT cells are the most potent regulatory cells described so far and they may serve to the early phase of controlling alloresponses. We next compared NKT cells to the *in vitro*-generated allopeptide-specific CD4$^+$CD25$^+$ T cell lines. The CD4$^+$CD25$^+$ cells were anergic and showed sustained high CD25 expression. Most importantly, the CD4$^+$CD25$^+$ cells retained their ability to suppress antigen-driven responses of CD4$^+$CD25$^-$ cells. They inhibited not only IL-2 secretion by CD4$^+$CD25$^-$ T cells specific for the same allopeptide (suppression of indirect alloresponse), but also the direct alloresponse of naïve CD4$^+$CD25$^-$ T cells stimulated by semi-allogeneic DCs in the presence of the peptide ("linked suppression"). Interestingly, anti-GITR antibody had no effect on the suppression effected by the CD4$^+$CD25$^+$ cells. These findings suggest that peripheral CD4$^+$CD25$^+$ regulatory cells are a precommitted cell lineage from which cells with specificity for non-self-peptides can be selected. Taken together, these data may pave the way for using innate NKT cells and "customised" allopeptide-specific CD4$^+$CD25$^+$ regulatory T cells as potential therapeutic tools in manipulating both direct and indirect alloresponses *in vivo*.

Abstract# 1595

GRAFT SURVIVAL AND COST FOLLOWING GASTROINTESTINAL COMPLICATIONS IN RENAL TRANSPLANT RECIPIENTS TREATED WITH MYCOPHENOLATE MOFETIL. Karen L. Hardinger, Daniel C. Brennan, Nzisa Mutinga, Mark A. Schnitzler. *[1]Transplantation, Barnes-Jewish Hospital at Washington University, St. Louis, MO.*

Gastrointestinal (GI) complications are a common consequence of immunosuppressive medications. However, the long-term consequences are unknown. The purpose of this study was to estimate graft survival effects of GI complications among patients who were initially treated with mycophenolate mofetil (MMF) after transplantation. **Methods.** The USRDS database was analyzed for cadaveric renal transplant recipients between 1995-1998 with Medicare as the primary payer for transplantation. Patients were included who were treated with MMF at transplant discharge and had functioning grafts at 1 year post-transplant. GI complications (anorexia, diarrhea, ulcer, vomiting, nausea, hemorrhage, perforation or abdominal pain) were identified by ICD-9 codes during the first year after transplant. Reported costs are actual Medicare payments for medical services and supplies used during the second year post transplant. **Results.** GI complications were diagnosed in 25% of all patients receiving MMF. Of these patients, 73% continued MMF despite an association with GI complications. The best 4 year graft survival was seen in patients without GI complications who continued MMF (87.4%). In comparison for those who continued MMF, GI complications were associated with lower graft survival (87.4% vs 83.2%, P<0.001). Better graft survival was seen among patients that continued MMF (87.4%) when compared to those who discontinued MMF (81.7%, P<0.001) . The largest reduction in graft survival occurred when a GI complication was observed and MMF was discontinued (72.9%, P <0.001). Furthermore, GI complications are costly, adding $6,000-$8,000 to costs after transplantation.

Graft Survival and Cost

	Number	Graft Survival	P value	Costs	P value
No GI with MMF	4,392	87.4%	<0.001	$12,571	<0.001
GI with MMF	1,413	83.2%	<0.001	$18,241	<0.001
No GI, dc MMF	1,522	81.7%	<0.001	$12,312	<0.001
GI, dc MMF	521	72.9%	<0.001	$20,650	<0.001

GI - Gastrointestinal complications, MMF - mycophenolate mofetil, dc - discontinue

Conclusion. Discontinuing MMF by 1 year post transplant with a GI complication is associated with a significantly higher rate of graft failure. GI complications have detrimental effects on long-term graft survival and cost, especially when MMF is discontinued. Graft survival effects and costs of discontinuation of MMF should be considered when managing GI complications after renal transplantation.

Abstract# 1596

EFFECT OF TYPE OF IMMUNOSUPPRESSION REDUCTION IN PATIENTS WITH BK ALLOGRAFT NEPHROAPTHY (BKAN). Emilio Ramos,[1] Cinthia B. Drachenburg,[2] Miguel Portocarrero, Ravinder Wali,[1] David K. Klassen,[1] Jeffrey C. Fink,[1] John C. Papadimitriou,[2] Charles B. Cangro,[1] Matthew R. Weir.[1] *[1]Medicine, Division of Nephrology; [2]Pathology, University of Maryland School of Medicine, Baltimore, MD.*

Introduction: Over-immunosuppression is a risk factor for BKAN virus allograft nephropathy. An ideal protocol for reduction in immunosuppression is needed to minimize the effects the infection. **Design**: 93 patients (p) with BKAN were followed for a mean of 16.8 months after the reduction in IS. Maintenance IS consisted of FK506, MMF and pred in 86 p, CSA, MMF and pred in 5 p and azathioprine, pred and FK506 or CSA in 2 p. Protocols for reduction of IS: 37 patients received a calcineurin inhibitor (CI)FK506 n=35, CSA n=2) with MMF at 50% of the initial dose and pred. The targets levels for FK506 and CSA were 6-8 ng/ml and 75-100 mg/ml respectively. CI alone with prednis was given to 29 p (FK506 n=25 (18 low dose) and CSA n=4 (3 low dose). 27 p received sirolimus based IS 5 patients received sirolimus in combination with MMF (50% dose in 2 p) and pred. 2 p received sirolimus in combination with reduced dose of FK 506 and prednisone. The remaining 20 preceived only sirolimus and prednisone. 2 p received only MMF at 50% of initial dose and pred. 1 received only pred. After reduction of IS, 26% of p developed biopsy proved acute allograft rejection. During the observation period 26 p (28%) lost graft function and 21 p (22.5%) cleared the infection in subsequent biopsies and urine cytologies. There was no difference in the rate of acute allograft rejection between the IS reduction protocols. For patients in whom IS was reduced, those with pred and two immunosuppressant drugs (FK506, CSA or sirolimus and MMF) were compared with patients receiving pred and a single immunosuppressant drug (FK506, CSA, sirolimus or MMF). A significantly larger number of patients lost graft function in the first group (p=.01). More significant graft loss was identified when patients used a CI (FK506 or CSA) together with MMF and pred when compared to patients using a single drug (FK506, CSA, sirolimus or MMF) and pred (p=.0017). Interestingly, disappearance of BK viral cytopathic changes in subsequent biopsies and urines were significantly higher in patients receiving a single drug in addition to pred in comparison to patients receiving two drugs and pred (p=.009). **Conclusions**: Patients who were on a combination of pred and one other immunosuppressive agent had a much better graft survival, and a higher chance of clearing the BK viruria, compared to patients who were left on three immunosuppressive drugs, albeit at lower doses.

Abstract# 1597

DECREASED PREVALENCE OF OBESITY IN RENAL TRANSPLANT CHILDREN WITH TACROLIMUS. Aline Maria L. Pereira,[1,2] Ana Lucia C. S. Abreu,[1] Paulo K. Nogueira,[1,2] Paula G. P. Machado,[2] Jose Osmar M. Pestana.[2] *[1]Pediatrics, UNIFESP, Sao Paulo, SP, Brazil; [2]Hospital do Rim e Hipertensão - Nephrology Division, UNIFESP, Sao Paulo, SP, Brazil.*

The prevalence of obesity after TX is high, being a risk factor for graft survival and development of cardiovascular disease in adulthood. There are evidence in literature showing the role of the immunosuppressive drugs in the pathogenesis of obesity after TX. The aim of this study is to evaluate the anthropometric measurements in children and adolescents in different immunosuppressive protocols 6 months after TX. Eighty three children were prospectively studied during the first 6 months after TX. The patients were divided in 2 groups according to the immunosuppressive protocol: Group 1 = CyA + Prednisone + Azathioprine or Mycophenolate mofetil (n=60, age=11.3±3.2 years, time in dialysis=28.5±22.3 months); Group 2 = FK506 + Prednisone + Azathioprine or Mycophenolate mofetil (n=23, age=12.8±3.8 years, time in dialysis=29.1±22.0 months). Groups matched to age at TX and time on dialysis. The anthropometric evaluation [weight (W/A), height (H/A), weight for height (W/H %) and body mass index (BMI)] was taken monthly. To analyse the nutritional status, data were expressed in Z-score (SDS) of W/A and H/A. BMI values (W/H2) calculated were compared to reference ranges of Rosner et al (1998) according to height age. Patients with BMI >95th percentile were considered obese; overweight was defined as 85th percentile< BMI £95th percentile. Serum creatinine (SCr) was measured concomitantly with anthropometric evaluation. Main results (mean±SD) are shown in the table below.

Anthropometric evaluation before and after transplantation

	CyA		Tac		
	Tx	6 m after Tx	Tx	6m after Tx	One-way ANOVA
W/A (SDS)	-1.92±0.8	-0.99±1.08	-1.83±1.03	-1.23±1.00	p=0.053
H/A (SDS)	-2.30±1.29	-2.31±1.19	-2.24±1.17	-2.18±1.04	p=0.567
W/H (%)	96±9	116±15*	100±15	109±13	p=0.001*
BMI (kg/m2)	16.0±2.1	19.2±3.3*	17.0±3.1	18.6±2.9	p=0.006*
SCr (mg/dl)	7.2±3.3	1.2±0.7	8.4±3.8	1.3±0.4	p=0.433

Results demonstrate a greater percentage of overweight (18%) and obesity (7%) in the CyA group, in contrast to 7% and 0% respectively in the FK group. These findings suggest that the immunosuppressive protocol may contribute to a greater prevalence of overweight and obesity short term after TX in children.

Abstract# 1598

IS THERE AN EFFECT OF INDUCTION IMMUNOSUPPRESSION ON PTLD AND GRAFT/PATIENT SURVIVAL AFTER KIDNEY TRANSPLANTATION? Wida S. Cherikh,[1] H. M. Kauffman,[1] Maureen A. McBride,[1] Jude Maghirang,[1] Lode J. Swinnen,[2] Douglas W. Hanto.[3] *[1]Research Department, United Network for Organ Sharing, Richmond, VA; [2]Johns Hopkins Cancer Center, Baltimore, MD; [3]Division of Transplantation, Beth Israel Deaconess Medical Center, Boston, MA.*

Purpose. This study was conducted to determine the association of types of induction immunosuppression regimens with the incidence of PTLD and outcomes (graft and patient survival) after primary cadaveric and living kidney transplants. **Methods.** The study cohort included over 38,000 primary kidney transplant recipients from the UNOS/OPTN database during 1/1/97-12/31/00 with at least 7 days of survival, and who were reported to have one of the following induction immunosuppression therapies: monoclonal antilymphocyte, polyclonal antilymphocyte, interleukin-2 (IL-2) receptor antibody, or no induction. The study also examined recipient variables (age, gender, ethnicity, diagnosis, delayed graft function, and acute rejection at discharge), and other risk factors (discharge maintenance immunosuppression, donor type, and HLA mismatch level). To examine the effect of induction immunosuppression on PTLD and post-transplant outcomes, a multivariate Cox regression analysis was conducted. Records with follow-up >727 days were censored to ensure comparable follow-up among the induction groups. **Results.** Monoclonal was used in 2,714 (7%) of the recipients, polyclonal in 4,347 (11%) of the recipients, IL-2 in 7,809 (20%) of the recipients, and no induction in 62% (23,699) of the recipients. The actual incidence of PTLD was 0.88% with monoclonal, 0.83% with polyclonal, 0.50% with IL-2, and 0.51% with no induction (chi-square p=0.007). The results of the Cox analysis indicated that monoclonal induction was associated with an 87% increased risk of PTLD (p=0.01), as compared with no induction. The increased risk of PTLD associated with polyclonal induction was 39% (p=0.15), and with IL-2 induction was 14% (p=0.51). Among the three induction types, IL2-receptor antibody was associated with a 9% reduced risk of graft loss (p=0.07) and a13% reduced risk of mortality (p=0.05), as compared with no induction. Monoclonal and polyclonal induction therapies were not associated with a reduced risk of graft loss or mortality. MMF-based discharge maintenance immunosuppression was associated with a 36% decreased risk of PTLD (p=0.006), as compared with AZA-based. **Conclusion.** Among different induction therapies, IL-2 receptor antibody was associated with the smallest risk of PTLD, and a marginally improved survival. The benefit of various routine induction therapies for kidney transplant recipients should be weighed against the risk of developing PTLD.

Abstract# 1599

TRANSPLANT SUCCESS AND IMMUNOMODULATING DIETS. J. W. Alexander,[1] T. J. Metze,[1] H. R. Goodman,[1] M. J. McIntosh,[2] L. Zeng,[2] M. A. Cardi,[2] J. N. Austin,[2] S. Goel,[2] S. Safdar,[2] N. A. Greenburg,[3] E. S. Woodle.[1] *[1]Division of Transplantation, The University of Cincinnati, Cincinnati, OH; [2]The Emmes Corporation, Rockville, MD; [3]Department of Transplantation, The Christ Hospital, Cincinnati, OH; [4]Novartis Nutrition, Novartis Incorporated, St Louis Park, MN.*

This study was performed to determine whether dietary supplementation with immunonutrients can prolong rejection free survival and reduce complications in adult renal allograft transplantation patients receiving standard neoral (CsA), mycophenolate mofetil (MMF) and steroids as their initial immunosuppression. **Methods:** Patients were stratified for major risk factors and randomized prospectively to a control group (C=71) or a supplement group (S=76). All patients have been followed for > two years. Patients were censored at the time of their death (3), graft loss (5), patient initiated withdraw from study (22), or non-compliance (7), Patients in the supplement group received arginine (4.5g BID) and canola oil (15 g BID) daily. **Results:** Demographics were similar. Actual findings for 1 and 2 years are as follows. The supplements did not influence results in the first 30 days. The post 30 day rejection rate supplemented patients had decreased rejection 7 % vs 16 % at one year and 7 % vs 21 % at two years (P=0.02). The supplement group also showed a marked reduction in secondary complications, myocardial infarction 0 % vs 4 % at one year and 0 % vs 6 % at 2 years (P=0.05), CsA toxicity 6 % vs 12 % at one year and 8 % vs 19 % at 2 years (P=0.04), PTDM 3 % vs 7 % at one year and 3% vs 10 % at two years (P=0.05) and bacteremia 3 % vs 9 % at one year and 3 % vs 15 % at 2 years (P=0.02). **Conclusions:** Dietary supplementation with arginine and canola oil results in: 1) a reduction in rejection episodes, 2) improved graft survival and 3) reduction in CsA toxicity 4) significant reduction in the risk of myocardial infarction, post transplant diabetes, and decreased risk of bacteremia

Abstract# 1600

WITHDRAWAL OF SIROLIMUS LOADING ELIMINATES THE INCREASED INCIDENCE OF LYMPHOCELES FOLLOWING KIDNEY TRANSPLANTATION. John F. Valente,[1] Kelly A. Weigel,[2] Kenneth A. Bodziak,[2] Donald E. Hricik,[2] David S. Seaman,[1] Christopher T. Siegel,[1] Thomas C. Knauss,[2] James A. Schulak.[1] *[1]Department of Surgery, University Hospitals of Cleveland, Cleveland, OH; [2]Department of Medicine, University Hospitals of Cleveland, Cleveland, OH.*

In adult kidney recipients we found a greater incidence of wound complications and lymphoceles with sirolimus immunosuppression using a loading dose as compared to mycophenolate mofetil (MMF). We therefore sought to reduce the early sirlomus exposure. Between 1/1/2000 and 11/30/2002, 320 kidney transplants were performed at the University Hospitals of Cleveland. The 193 adult, kidney-only recipients treated with tacrolimus and prednisone were divided into three groups: Group 1 (n=84) 1 gm of MMF every 12 hours, Group 2 (n=74) 15 mg sirolimus load followed by 5 mg/day adjusted to trough levels of 10-20 ng/mL, and Group 3 (n=35) 5 mg sirolimus daily adjusted to levels of 10-20 ng/mL. All received tacrolimus, maintaining target levels of 8-12 ng/mL in Group 1 and 5-8 ng/mL in Groups 2 and 3. The incidence of wound complications, lymphoceles and superficial wound problems were compared.

Group	Total Population	Group 1	Group 2	Group 3
Regimen		MMF	Sirolimus load	Sirolimus
N	193	84	74	35
Age (years)	47.8±13.8	48.9±15.0	44.9±12.4	52±12.3
Female Gender	40.4%	41.7%	43.2%	34.3%
Diabetes	37.4%	38.1%	35.6%	40.0%
BMI*(kg/m2)	26.9±5.2	25.5±4.5	28.1±5.7	27.9±5.4
Cadaveric	63.7%	59.5%	68.9%	65.7%
Albumin (gm/dL)	3.8±0.6	3.8±0.5	3.7±0.6	4.0±0.5
Wound Comps**	44 (22.7%)	2(2.3%)	32(43.2%)	10(28.5%)
Lymphoceles	24(12.4%)	2(2.3%)	22(29.7%)	0
Superficial Comps**	19 (9.8%)	0	9(12.1%)	10(28.5%)
Rejection	11.9%	12.2%	8.3%	20.0%

*Body Mass Index, ** Complications

Wound complications (and lymphoceles in particular) were less frequent in Groups 1 and 3 vs. Group 2 ($p<0.05$, Analysis of Variance with Bonferroni post hoc test). Groups 2 and 3 showed more superficial wound problems than Group 1 ($p<0.05$). No difference was found in age, gender, BMI, diabetes, or rejection rates. Multivariate stepwise logistical regression showed sirolimus (p=0.002) and lower serum albumin (p=0.004) independently correlated with complications. Lymphocele occurrence correlated with sirolimus loading (p=0.0001). Superficial wound problems correlated with higher body mass index (p=0.003) and low albumin (p=0.05). Sirolimus promotes wound complications and lymphocele formation. Lymphoceles can be eliminated by initial dose reduction.

Abstract# 1601

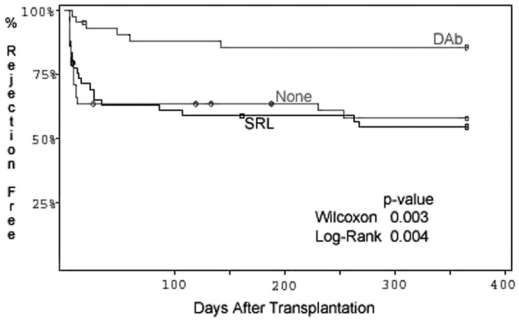
SIROLIMUS PROLONGS DELAYED GRAFT FUNCTION IN RECIPIENTS OF SUB-OPTIMAL CADAVERIC KIDNEY DONORS. G. Stallone,[1] B. Infante,[1] A. Schena,[1] S. Di Paolo,[1] L. Gesualdo,[1] P. Ditonno,[2] M. Battaglia,[2] G. Grandaliano,[1] F. P. Schena.[1] *[1]DETO, Div. of Nephrology, Univ. of Bari, Italy; [2]DETO, Div. of Urology, Univ.of Bari, Italy.*

Delayed graft function (DGF) has been identified as one of the main correlates of poor graft survival in renal transplantation. However, the factors influencing DGF have been poorly elucidated. In particular, the role of sirolimus (SRL) in the development and resolution of DGF is still debated. We investigated the risk factors for DGF, with a specific emphasis on the role of histological damage of donor kidney, and the impact of two different immunosuppressive regiments (SRL vs cyclosporine A, CsA) . Twenty consecutive sub-optimal cadaveric donors (Age 61.2±8.7 years, 16/20 with hypertension) were included in the study. Renal specimens were obtained by wedge biopsy performed after the perfusion (time-zero biopsy). The histologic lesions of the four renal compartments (glomeruli, tubules, interstitium and vessels) were scored by two pathologists blinded to the clinical history of the donor. The severity of chronic lesions was evaluated semiquantitatively using the criteria suggested by the '97 Banff classification. Kidneys were allocated to 40 caucasian recipients that received two different immunosuppressive regimens (CsA + corticosteroids + MMF (group A) and SRL + corticosteroids + MMF (group B). The recipients of each donor were randomly assigned to either group A or B. In 10 out of 20 donors both recipients presented with DGF. The organs displaying DGF (21) had a significantly higher degree of tubular damage ($p<0.01$). Whereas, we did not observe any significant difference in donor age and calculated creatinine clearance, cold ischemia time, number of HLA mismatches and the degree of other histological lesions between the groups of patients with and without DGF. Interestingly, the group B patients displayed a duration of DGF significantly longer than the patients of the group A (25±8 vs 15±4 days; $p= 0.02$). On the contrary, renal graft function at 1-year was significantly better in group B patients (1.9±0.3 vs 1.4±0.2 mg/dl; $p= 0.04$), although DGF was associated with a worse graft function at 1 year. Our data suggest that SRL prolongs the period of DGF in recipients of sub-optimal organs showing an higher degree of tubular lesions, most likely through its potent antiproliferative effects. On the contrary, SRL can delay the progression of chronic allograft injury, as clinically reflected by a better graft function at 1 year.

Abstract# 1602

PROLONGATION OF DELAYED GRAFT FUNCTION BY SIROLIMUS DOES NOT ADVERSELY AFFECT TRANSPLANT OUTCOMES. Ryan McTaggart,[1] Alan Bostrom,[1] Peter Bacchetti,[1] John Roberts,[1] Stephen Tomlanovitch,[1] Sandy Feng.[1] *[1]Surgery, Division of Transplantation, University of California - San Francisco, San Francisco, CA.*

Purpose: We have shown that sirolimus (SRL) prolongs delayed graft function (DGF). Previous studies suggest suboptimal transplants with longer DGF result in poorer outcomes. Our study aims to determine the effect of prolonged DGF mediated by SRL rather than suboptimal transplant quality on outcomes. **Methods:** Data were collected on 132 cases of DGF (dialysis within 1 wk of transplant) at UCSF (1/1/97-6/30/01). Three groups were defined by induction immunosuppression strategy: DAb: induction with a depleting antilymphocyte preparation (n=41; 31%); SRL (n=49; 37%); and None (n=42; 31%). All recipients received immediate steroids and mycophenolate mofetil; calcineurin inhibitors were initiated upon return of graft function. Patient survival, graft survival (± death-censored), and time to first rejection within 1 year of transplant was determined by Kaplan-Meier analysis. Graft function was also compared. **Results:** Groups were similar for donor, recipient, and transplant characteristics except DAb had more sensitized patients (non-primary transplant or PRA>30%) (50% vs.18% and 15%) and None had higher mean mismatches (4.5 vs. 3.9 and 4.0). The groups did not differ in patient survival ($p =0.15$), graft survival ($p =0.26$), or graft survival with death censored ($p=0.20$). Time to first rejection and incidence of rejection were similar for the SRL and None groups but significantly different than the DAb group ($p=0.003$).



There were no significant differences in 3mo and 1yr Cr ($p=0.86$; $p=0.57$).

12 Month Creatinine	Mean ±SD	Median
DAb	1.8±1.0	1.4
SRL	1.6±0.8	1.3
None	1.8±1.2	1.2

p-value = 0.57

Conclusions: In the DGF setting, induction immunosuppression with DAb reduced early rejection but had no effect on patient survival, graft survival, or graft function. SRL, in spite of prolonging DGF and increasing early rejection when compared to DAb, did not compromise transplant outcomes.

Abstract# 1603

MULTIPLE GESTATIONS IN FEMALE KIDNEY TRANSPLANT RECIPIENTS MAINTAINED ON CALCINEURIN INHIBITORS. Lisa A. Coscia,[1] Elyce H. Cardonick,[2] Michael J. Moritz,[3] Vincent T. Armenti.[1] *[1]Surgery, Thomas Jefferson University, Philadelphia, PA; [2]Obstetrics & Gynecology, Thomas Jefferson University, Philadelphia, PA; [3]Surgery, Drexel University College of Medicine, Philadelphia, PA.*

The purpose of this study was to analyze multiple gestations in female kidney recipients maintained on calcineurin inhibitors. Data were collected via questionnaires, telephone interviews and hospital records. Of 458 kidney recipients (681 pregnancy outcomes) reported to the National Transplantation Pregnancy Registry (NTPR), there were 13 kidney recipients who reported 14 multiple gestations with 32 pregnancy outcomes (10 sets of twins, 4 sets of triplets). There were 28 (88%) livebirths, 2 stillbirths (6%, a set of twins in the second trimester), 2 spontaneous abortions (6%, a set of twins in the first trimester). No reductions during pregnancy were reported. In 5 pregnancies there was some method of reproductive assistance. The mean age at conception was 28.9 ± 4.4 yrs (range 20-37 yrs) and the transplant to conception interval was 5.7 ± 5 yrs (range 0.5-19 yrs). Immunosuppression during pregnancy included cyclosporine-based (6 Sandimmune®, 7 Neoral®) and tacrolimus-based in 1. Maternal comorbid conditions during pregnancy included: hypertension 10/13 (77%), preeclampsia 4/14 (29%) and infection 3/12 (25%). There were no reports of gestational diabetes or rejection during pregnancy or postpartum. Mean serum creatinine (mg/dL) was 1.5 ± 0.6 pre-pregnancy, 1.5 ± 0.4 during pregnancy and 1.7 ± 0.6 postpartum (data available for 10 pregnancies). One recipient reported graft loss within 2 yrs postpartum. At last follow-up, 7 (54%) reported adequate graft function, 2 (15%) reduced, 1 (8%) dialysis dependent, and 3 (23%) were lost to follow-up. Mean gestational age (GA) was 33 ± 2.7 wks and mean birthweight (BW) was 1736 ± 616 gms. Nineteen infants (68%) had complications at birth, the majority secondary to prematurity. There was one neonatal death reported in a newborn secondary to cardiomyopathy (one surviving twin managed medically for cardiomyopathy), in the tacrolimus-treated recipient. At last follow-up, 21 children were reported healthy and developing well. Six children were lost to follow-up. CONCLUSIONS: Female kidney transplant recipients can successfully maintain a pregnancy with twins or triplets. There have been no multiples higher than triplets reported to the NTPR. As there has been an increase in the number of multiple gestations in the US with the use of fertility medications and in vitro fertilization, continued surveillance in the transplant recipient population is warranted.

Notes