Abstract# 534

HEPATIC ENDOTHELIAL CELL ACTIVATION IN XENOTRANSPLANTATION: DIFFERENTIAL UPREGULATION OF TWO TRANSCRIPTION FACTORS. Antonio Di Carlo,[1] Michael Tan,[1] Shuqing Liu,[1] Joseph Tector,[2] Peter Metrakos,[1] Jean I. Tchervenkov.[1] *[1]General Surgery, McGill University Health Centre, Montreal, QC, Canada; [2]Transplantation, University Of Indianapolis, Indianapolis, IN.*

Introduction: Type II endothelial cell activation as witnessed in xenotransplantation is dependent on transcription factors. Thus far the literature has focused on NF-κB. The pluripotent transcription factor Early Growth Response 1 (Egr-1) has been implicated in several models of endothelial pathobiology. The purpose of this study is to compare the up-regulation of Egr-1 and NF-κB in porcine hepatic sinusoidal endothelial cells (PHSEC) in a model of pig-to-human liver xenotransplantation. Materials and Methods: PHSEC were cultured in serum-free medium and incubated with 20% normal human serum (NHS). Complement was inactivated by heating human serum (HHS). Matrix comprised of silicon beads conjugated to aGal(1,3)bGal(1,4)Glc was used to remove the anti-α-gal XNA from human serum (XHS). Experimental conditions were: LPS 10μg/mL; dexamethasone 1μM; pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB, 100 μM. The up-regulation of Egr-1was assayed by semi-quantitative rt-PCR. The up-regulation of NF-κB was assayed by Western Blotting with antibodies to the p50 subunit (Santa Cruz). Results: The CH50 of HHS was 0 and 90-98% of anti-α-gal XNA were depleted from human serum. 20% NHS up-regulated both Egr-1 transcript and NF-κB protein. HHS (complement inactivated) attenuated the up-regulation of NF-κB by 30% but Egr-1 by 70%. The depletion of anti-α-gal XNA (XHS) similarly attenuated the up-regulation of both NF-κB and Egr-1 by 70%. XHS which was heat-inactivated (HXHS) did not further attenuate the up-regulation of these transcription factors. Exposure to LPS, a known stimulator of NF-κB, failed to up-regulate Egr-1 above basal levels. The transcription of Egr-1 was not attenuated by PDTC (inhibitor of NF-κB) or by dexamethasone. Conclusion: Both Egr-1and NF-κB are up-regulated in this *in vitro* model of pig-to-human liver xenotransplantation but with different responses. Egr-1, unlike NF-κB, is not a ubiquitous mediator of endothelial cell activation as it is up-regulated with xenogeneic stimuli (human serum) but not by septic conditions (LPS). Egr-1 is much more sensitive to complement inactivation than NF-κB. Low levels of anti-α-gal antibodies and other uncharacterized XNA are sufficient to up-regulate NF-κB and Egr-1. However, the up-regulation of Egr-1 is independent of NF-κB. Thus Egr-1 presents a novel target for the attenuation of endothelial cell activation in pig-to-human liver xenotransplantation.

Abstract# 535

IRF-1 ATTENUATES CYTOKINE-INDUCED IP-10 RELEASE IN MURINE ISLETS BY AN NO-INDEPENDENT MECHANISM. Marshall S. Baker,[1] Xiaojuan Chen,[1] Jeffrey J. Nelson,[1] Dixon B. Kaufman.[1] *[1]Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Background. Islet injury by pro-inflammatory cytokines is an important mechanism of islet dysfunction in models of islet allograft rejection. IFNγ acts synergistically in combination with IL-1β and TNFα on whole islets *in vitro* to induce iNOS gene expression, NO production, apoptosis, impair glucose stimulated insulin release (GSIR) and to induce gene expression for T-cell chemotaxins IP-10 and MCP-1. The effect of IFNγ may depend on the activation of transcription factor interferon regulatory factor 1 (IRF-1). In other cell types, IFNγ-receptor binding induces IRF-1 gene expression. IRF-1 subsequentlty acts as a transcription factor inducing down stream regulators of cell function and viability like iNOS. We have recently shown that blocking IRF-1 activation in islets abrogates the combined cytokine effect on iNOS gene expression, apoptosis and GSIR. We now test the hypothesis that IRF-1/NO signaling mechanisms mediate cytokine-induced IP-10/MCP-1 release. *Methods.* Whole islets isolated from wild type (WT), IRF-1$^{-/-}$ or iNOS$^{-/-}$ mice were cultured (24 hrs) in a mixture of cytokines (IL-1β, TNFα and IFNγ) plus/minus the iNOS inhibitor L-NMMA. RNase Protection Assay (RPA) was used to measure transcription for IP-10, MCP-1 and iNOS. ELISA was used to determine chemokine protein release. *Results.* Cytokine-treated WT islets demonstrated marked increases in iNOS (5x control), IP-10 (10x control) and MCP-1 (4x control) gene transcription and in chemokine protein release (100x control for IP-10, 10x for MCP-1). Cytokine stimulated iNOS$^{-/-}$ islets demonstrated no detectable expression of iNOS but the same degree of gene transcription for IP-10 and MCP-1 and the same amount of chemokine protein release as did WT. Cytokine stimulated IRF-1$^{-/-}$ islets demonstrated significantly less iNOS transcription (2x control) but more IP-10 and MCP-1 transcription (15x control for IP-10, 6x for MCP-1) and more IP-10 protein release (200x control) than WT. There was no detectable difference between stimulated WT and IRF-1$^{-/-}$ islets in MCP-1 protein release. L-NMMA had no detectable effect on gene expression or protein release in any islet type. *Conclusions.* IRF-1 activation attenuates cytokine-induced IP-10 release. This effect is not due to IRF-1-dependent NO production. Immunomodulatory methods interfering with IRF-1 signaling may augment graft infiltration and accelerate graft rejection following islet allotransplantation.

Abstract# 536

PREEXISTING PROSTATE CANCER IN THE SOLID ORGAN TRANSPLANT RECIPIENTS: DETERMINING THE RISK OF RECURRENCE. Manish Gupta, Todd D. Merchen, Jennifer Trofe, Thomas G. Gross, Thomas M. Beebe, Rita R. Alloway, Michael J. Hanaway, E. Steve Woodle, Joseph F. Buell. *[1]IPITTR, University of Cincinnati, Cincinnati, OH.*

Adenocarcinoma of the prostate (PCA) is the most common non-cutaneous malignant tumor in men as 30%-50% of 50-year-old men harbor microscopic adenocarcinoma, moreover, it is the second leading cause of cancer death of men in the Western world. Recent application of PSA testing has led to an increase in diagnosis of PCA in the general population. As a result, patients with history of PCA are being encountered with increasing frequency as transplant candidates. Finally the biological behavior of PCA in solid organ transplant recipients (SOTR) has not been defined. The purpose of this study was to evaluate clinical outcomes of SOTR with pre-existing PCA. **Methods**: A retrospective analysis of all SOTR with history of pre-transplant PCA was performed. **Results**: 90 males with pre-existing, treated PCA were identified. Transplant recipients included 77 renal, 10 heart and 3 liver transplants (Txp). Mean age at diagnosis and Txp was 61.3±6.3 years and 63.1±5.9 years respectively. The median interval between diagnosis/treatment and transplantation was 19.3 months (0-182.1 months). Maintenance immunosuppression included prednisone (n=85), calcinurin inhibitors (n=80), antiproliferative agent (n=55). Antibody therapy was utilized in 43 patients. Median follow-up was 20.5 months (33.8±42) after transplantation. Overall mortality was 28.8%, with death due to disease in 7/90 patients (7.8%) and recurrence rate of 17.7%. The most common cause of death was either cardiac or infectious (16/26). The median time to recurrence was 10.6 months after Txp and the median time of survival was 49.2 months after Txp for those who died of recurrent disease.

	N	# Recurrence (%)	# Dead of Disease (%)
THERAPY TYPE			
Surgery Alone	55	5 (9.0%)	1 (1.8%)
Surgery + Adjuvant Rx	13	2 (15.4%)	0
Hormonal	12	4 (33.3%)	2 (16.7%)
Radiation Alone	14	4 (28.6%)	1 (7.1%)
Unknown Rx	10	4 (40.0%)	3 (33.3%)
STAGE			
I	35	5 (14.3%)	1 (2.9%)
II	44	7 (15.9%)	3 (6.8%)
III	11	4 (36.4%)	3 (27.3%)
Wait Time (yrs)			
<2	54	7 (13.0%)	4 (7.4%)
2-5	24	7 (29.2%)	1 (4.2%)
>5	12	2 (16.7%)	2 (16.7%)

Rx = Therapy, Wait time is the interval between cancer therapy and transplantation

Conclusions: Preexisting PCA in SOTR has 1) a low but significant risk of recurrence and death due to recurrence, 2) associated risk of recurrence is related to stage at initial diagnosis, and 3) length of wait does not appear to be related to the risk of recurrence.

Abstract# 537

COLLECTIVE EXPERIENCE WITH RENAL TRANSPLANTATION FROM DONORS WITH A HISTORY OF BREAST CANCER. Amy L. Friedman,[1] Chandar Muthiah,[2] Thomas M. Beebe, E. Steve Woodle,[2] Joseph F. Buell.[2] *[1]Section of Organ Transplantation and Immunology, Dept of Surgery, Yale University School of Medicine; [2]Israel Penn International Transplant Tumor Registry, University of Cincinnati, OH.*

Long waiting times have led to marked increases in living kidney donation and use of higher risk cadaver donors. Though donors with known malignancies are usually excluded to prevent transmission of neoplastic cells with the transplanted organ, it is not clear that the history of a serious but apparently cured cancer should permanently preclude donation. For tumors that typically recur within a reasonable, defined time period, the minimal length of time to wait might be determined, but in the case of tumors known to exhibit long delayed recurrence, such as breast cancer, the decision may be less straightforward. We reviewed our experience with transplantation of kidneys obtained from donors with a history of breast cancer. **Results:** Overall, nine patients (3 women, 6 men) received organs from 8 female donors from December 1970 through November 1999. The mean follow-up was 40.1 months, with 6 survivors followed for a mean of 57.3 months. Three transplants from 2 cadaver donors occurred prior to 1980 (ERA I), with early deaths in all at 2, 2.5 and 12 months. One of these recipients developed breast cancer 4 months following txp that ultimately led to his demise. All six txp in Era II (after 1980) came from live donors. In 4/6 ERA II txp, early stage tumors were apparently cured prior to organ donation (53, 84, 96, unknown months). Two advanced tumors were identified in women at 0.3 (Stage IV) and 16 months (Stage III) following donation. ERA II recipients have experienced 100% survival, though the single patient with an organ from a donor with metastatic disease 1/6 (17%) also developed metastatic breast cancer at 12.6 months after transplant. The lesion disappeared with use of Tamoxifen and the cessation of immunosuppression, and the patient has experienced a disease free interval of 36 months. **Conclusions:** Txp of cadaver kidneys from donors with breast cancer is unlikely to permit adequate time either to accomplish a metastatic survey, or to assist an unprepared candidate to fully consider

the risk of cancer transmission, which is substantial, and may not be reasonable. However, kidney transplantation from live donors with a remote history (>48 months) of early stage breast cancer may be appropriate in the presence of informed consent and the absence of an alternate donor. Tumor transmission, though devastating, may be amenable to hormonal manipulation combined with cessation of immunosuppression.

Abstract# 538

A RANDOMIZED CONTROLLED TRIAL (RCT) OF GANCICLOVIR AND IMMUNE GLOBULIN PROPHYLAXIS FOR PREVENTION OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) AMONG HIGH RISK SOLID ORGAN TRANSPLANT RECIPIENTS. Atul Humar,[1] Diane Hebert,[1] Abhinav Humar,[2] Dele Davis,[3] Derek Stephens,[1] Brenda O'Doherty,[1] PICNIC, the EBV Prophylaxis Study Group, Upton Allen.[1] *[1]Univ of Toronto; [2]Univ of Minnesota; [3]Alberta Childrens Hosp.*

Background: Recipients who are seronegative for EBV pre-transplant and receive organs from a seropositive donor (D+/R-) are at high risk for PTLD and have been targeted for prophylaxis. This study evaluates two strategies aimed at preventing PTLD. The data herein presented represent outcome assessments for the first 6 months after transplant. Methods: This was a multicenter RCT in EBV D+/R- pediatric and adult transplant recipients. After randomization, patients received 3 months of antiviral therapy (ganciclovir IV or po) plus IV cytogam at regular intervals or ganciclovir plus IV placebo/no product. Additional oral antiviral therapy was given out to 1-year post transplant. After 3 months, patients were unblinded and those on cytogam received additional cytogam from month 3-6. EBV loads were done at one site and data were analyzed at 0, 1,2,3,4,5, and 6 months post-transplant. The ability of the regimens to suppress viral load was the primary outcome. Statistical analyses were done in a blinded manner. Results: Viral load data was analyzed from 28 (20 pediatric and 8 adult) patients (15 cytogam and 13 placebo/no product). Organ types were livers (n=11), kidneys (10), lungs (6), and kidney/pancreas (1). There was no difference in baseline demographic features between the two arms including age, sex, donor/recipient CMV status, and type of transplant (p= NS for all). Over the first 6 months post-transplant, detectable viremia occurred in 9/13 (69.2%) placebo/no product patients and 10/15 (66.7%) cytogam patients. Repeated measures ANOVA indicated no significant effect of randomization upon viral load over the 6-month period (p=0.8). Thus, we compared viral loads in adult versus pediatric patients irrespective of study group. Adults had lower viral loads than children (p=0.06; OR for lower viral load in adult = 2.9). PTLD developed in 2 patients (1 in cytogam arm, and 1 in placebo arm). Conclusions: Ganciclovir plus cytogam did not significantly improve EBV viral load suppression compared with ganciclovir alone over the first 6 months after transplantation. Follow-up out to one year will provide further definitive data. We documented differences in viral loads between adults and children. The implication of the latter finding on management requires further study.

Abstract# 539

RENAL RETRANPLANTATION AFTER POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE. Alexandre Karras,[1] Eric Thervet,[1] Yann Le Meur,[2] V. Baudet-Bonneville,[3] Michele Kessler,[4] Christophe Legendre.[1] *[1]Nephrology and Renal Transplantation, Hopital Saint Louis, Paris, France; [2]Nephrology, Hopital Dupuytren, Limoges, France; [3]Pediatric Nephrology, Hopital Trousseau, Paris, France; [4]Nephrology, Hopitaux de Brabois, Nancy, France.*

Introduction : Post-Transplant Lymphoproliferative Disease (PTLD) is a rare but severe complication after renal transplantation. Reduction or discontinuation of immunosuppression is crucial for controlling PTLD but can sometimes lead to graft loss. Retransplantation of these patients is considered to be dangerous, as re-introduction of immunosuppressive drugs might induce hematological relapse. Methods : We herein present 6 patients who underwent a second renal transplantation several years after a successfully treated PTLD. The aim of this retrospective, multicentric study was to evaluate the risk of lymphoproliferation reccurence. Results : Six patients (5 adults, 1 child) were studied, in 4 different centers. EBV serology was positive for all of them except the only child, before the first transplantation. All of them had received induction therapy with ATG and 4 had presented at least one acute rejection episode during their first transplantation. Lymphoma was detected early (mean delay from transplantation 190±53 days), and was monomorphic, B-cell, and EBV-related in all cases. PTLD was strictly limited in the renal allograft in 4 cases, but was multilocular in 1 and cerebral in 1. Tapering of immunosuppression (6/6) and surgical treatment (transplant nephrectomy in 5/6) led to hematological remission in all patients. The second transplantation was performed after a mean delay of 80±30 months after initial diagnosis of PTLD. Immunosuppressive therapy included steroids (n=6), ATG (n=2), anti-CD25 (n=2), CsA (n=4), tacrolimus (n=2), MMF (n=4), azathioprine (n=1). Two patients presented an acute rejection episode, treated with high-dose steroid therapy. After a mean follow-up of 17±8 months, none of these patients has shown reccurence of lymphoproliferative disease, despite a close monitoring (clinical and radiological assessment, EBV viral load). Conclusion : Renal retransplantation can be considered in patients who have former history of PTLD, with no major risk of reccurence of hematological malignancy, especially when lylmphoma was localized in renal allograft during the first transplantation.

Abstract# 540

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD) IN PEDIATRIC SMALL BOWEL AND MULTIVISCERAL RECIPIENTS. Regina G. Santos,[1] Rakesh Sindhi,[1] Michael Green,[2] George V. Mazariegos,[1] Jorge Reyes.[1] *[1]University of Pittsburgh, Thomas E. Starzl Transplantation Institute, Pittsburgh, PA; [2]Division of Infectious Disease, Children's Hospital of Pittsburgh, Pittsburgh, PA.*

BACKGROUND: Epstein-Barr virus (EBV)-associated PTLD is a critical complication of solid organ transplantation. **AIM:** Analyze a large cohort of pediatric small bowel (SB), liver/small bowel (LSB) and multivisceral (MVTx) transplant patients at a single center for incidence, effect of EBV-preemptive therapy begun in 1994, outcome and management of PTLD. **METHODS:** From 7/1990 to 10/2002, 93 children underwent 98 transplants: 28 SB, 58 LSB, 12 MVTx (3 without liver). Colon was included in 20 pts. Median age 3.2 yrs (0.5-18.3). Median follow-up was 29.4 mos (0.1-148 mos). **RESULTS:** Overall, 30.6% (30/98) pts developed PTLD, 16.6% (5/30) PTLD recurred. Median time to development of PTLD was 241 days (25-3090 days). Available data revealed that 30.3% (10/33) EBV-seronegative pts and 30.1% (19/63) EBV-seropositive developed PTLD (p=NS). Incidence of PTLD was 35.7% (10/28) in SB, 24.1% (14/58) in LSB, 55.5% (5/9) in MVTx , 33.3% (1/3) in MVTx without liver, and 45% (9/20) when the colon was also transplanted (p=NS). The introduction of preemptive therapy was associated with a decrease in PTLD incidence from 45.7% (16/35) to 23.8% (14/63) currently (p< 0.02). Incidence of PTLD by immunosuppressive regimen was 42.5% (17/40) with primary tacrolimus/prednisone induction; 41.6% (5/12) with additional azathioprine; 18.7% (3/16) with additional cyclophosphamide; 20.8% (5/24) with daclizumab induction; 0% (0/6) with Thymoglobulin induction; and 16/40 (40%) with OKT3 as rescue therapy for corticoid-resistant rejection (p=NS). All patients were treated with tacrolimus reduced dose and prednisone (2.5-10mg/d); 21 also received antiviral (Ganciclovir and/or Acyclovir) associated with CytoGam. Interferon was used in 2 patients with extensive PTLD, one recovered. Rituximab was given in 4 polymorphic PTLD, of who 2 recovered. One was successfully treated with cyclophosphamide after failure with Rituximab. PTLD required surgical resection in 25.8% (8/31) pts. Overall mortality was 56.6% (17/30) with 26.6% (8/30) PTLD-related deaths at a median interval of 3.3 months (range from 0.3 to 22 months) from diagnosis. Preemptive therapy did not change PTLD-related mortality significantly. Of 27 initial survivors 9 (33.3%) had acute cellular rejection. **CONCLUSIONS:** EBV-PTLD can be successfully treated with reduced immunosuppression and antiviral agents in most pts after SB, LSB and MVTx. Preemptive therapy reduces incidence of PTLD significantly but not PTLD-related mortality.

Abstract# 541

ALTERED INTENSITY AND EMERGENCE KINETICS OF LYMPHOPLASMACYTIC HYPERPLASIA (POSSIBLE PRE-PTLD) IN INTESTINAL TRANSPLANT RECIPIENTS RECEIVING CAMPATH IMMUNOSUPPRESSION. M. F. Soares,[1] M. Garcia,[1] M. Nicolas,[1] T. Kato,[2] N. Mittal,[2] S. Nishida,[2] D. Levi,[2] J. Nery,[2] J. Madariaga,[2] A. Tzakis,[2] P. Ruiz.[1,2] *[1]Pathology, U. of Miami, Miami, FL; [2]Surgery.*

Post transplant lymphoproliferative disorders (PTLD) are an important cause of morbidity and mortality in multivisceral and intestine transplant recipients. They are characterized by uncontrolled hyperplastic or neoplastic lymphoid growth with EBV infection and immune status as risk factors. Histological changes in PTLD ranges from expansile lymphoplasmacytic (LP) hyperplasia to frank lymphoma. We reviewed 1224 small bowel allograft biopsies obtained in the first 250 days post transplant from 52 patients who underwent isolated intestinal or multivisceral transplantation and received induction immunosuppression with either Campath (13) or Zenapax (Daclizumab) (39). The biopsies were analyzed as to the onset and intensity of lymphoplasmacytic infiltrates and associated EBV in situ hybridization (EBER) positivity. LP infiltrates was graded as: 1- baseline levels; 2- mild; 3- moderate; 4- severe; 5- lymphoma. Ten patients that received Campath (76.9%) and 23 patients that received Zenapax (58.9%) presented with lymphoplasmacytic infiltrates over the measured time period. Campath – treated patients developed earlier LP infiltrates, with average onset on day 49 post transplant (range 8-130); the intensity of the LP infiltrates was mild to moderate from day 1 to day 100 post transplant, decreasing to mild from day 101 to day 250 post transplant. No EBER positivity was detected in this group. Patients receiving Zenapax presented with LP infiltrates with average onset on day 82 post transplant (range 8-222), the intensity of which was mild to moderate from day 1 to day 100 post transplant. More persistent and intense LP infiltrates was observed after day 101 in this group. One case of lymphoma on day 115 post transplant and two cases of EBER positivity were observed in the Zenapax group. The appearance of any degree of lymphoplasmacytic infiltrate in intestine grafts is a relatively frequent occurrence and has the potential to be early PTLD. Campath induction immunosuppression results in an earlier appearance of LP lesions, but they are generally less intense than evident with Zenapax. These results may be secondary to more profound immunodeficiency following treatment with Campath as well as which immune cells are targeted. Long-term follow up of these patients will ultimately yield information as to malignant potential following these therapies.

Abstract# 542

RENAL TRANSPLANTATION FOLLOWING LUNG AND HEART-LUNG TRANSPLANTATION. Thomas Kaneko,[1] Areef Ishani,[1] Sehsuvar Erturk,[2] Marshall I. Hertz,[1] Arthur J. Matas,[1] Kay Savak,[1] Mark E. Rosenberg.[1] *[1]Medicine and Surgery, U of Minnesota, Minneapolis, MN; [2]Nephrology, Ankara University Medical School, Ankara, Turkey.*

The development of end-stage renal disease (ESRD) is an increasingly common complication following non-renal solid organ transplantation. The purpose of this study is to report the outcomes of patients receiving kidney transplants following lung or heart-lung transplantation. Between 1986 and 2002, 22 out of 219 patients developed ESRD an average of 50±46 months (range 3 to 138 months) after lung or heart-lung transplantation. The cause of ESRD was calcineurin inhibitor toxicity in all patients. 13 of these patients received a kidney transplant at our center, 3 were transplanted at other centers, and 6 were treated with dialysis alone. For those treated with dialysis alone, 5/6 have died a mean of 8.6±7.5 months after initiating dialysis. Of the 13 patients transplanted at our center (mean age 51.5±8.2; 7 men and 6 women) 5 received a preemptive transplant and 8 were on dialysis for a mean duration of 15±10 months. The donor sources were: 7 living related, 3 living unrelated, and 3 cadaveric. Antibody induction therapy was given in 10/13 patients (7 thymoglobulin; 2 ATG; 1 ALG). Infection was the most common complication with 10 patients requiring inpatient treatment on 19 occasions, with the respiratory tract being the most common site. Seven patients have died. The median survival was 21.4 months (1-68 months), with 1-year mortality of 54.5%. There were no episodes of acute rejection, and all graft loss was due to death with a functioning graft. Five of seven deaths were due to infectious causes, and all but three of these patients received antibody induction therapy. The mean estimated GFR at 1 year was 53±12.5 ml/min/1.73 m2. These results were compared to UNOS data for 119 kidney transplants following lung or heart-lung transplantation performed between October 1987 and June 2002. Induction therapy was used in 38% of patients. The acute rejection rate was 23.1% in the first year, but only 1.7% of grafts have been lost to acute rejection. Mortality was 17% at one year, with infectious causes accounting for 25.9% of deaths. The higher mortality and greater incidence of infection at our center may be due to more intensive immunosuppression particularly antibody induction therapy. In conclusion, renal transplantation is an effective renal replacement therapy for ESRD following lung or heart-lung transplantation. Antibody induction therapy should be avoided because of the risks of infectious complications and death, and the low risk of graft loss from rejection.

Lymphocyte Activation

Abstract# 543

CYTOKINES DIFFERENTIALLY REGULATE HOMING PROPERTIES AND ACTIVATION STATE OF CYTOTOXIC CYTOMEGALOVIRUS-SPECIFIC T CELLS. Ester M. M. van Leeuwen,[1,2] Laila E. Gamadia,[1,2] Rene A. W. van Lier,[2] Ineke J. M. ten Berge.[1] *[1]Div. of Nephrology and Clinical Immunology & Rheumatology, Dept. of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands; [2]Laboratory for Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands.*

Introduction: Cytomegalovirus (CMV) causes a lifelong latent infection which in immunocompromised individuals, like transplant recipients, can lead to serious disease. CD8+ T lymphocytes are important in controlling the viral infection. During latency, two prototypic types of CMV-specfic CD8+ T-cells can be found: CD45RA+CD27- and CD45RA-CD27+; both are CCR7-. These CMV-specific CD8+ T cells expand when stimulated with specific peptide and IL-2, IL-15 or IL-21. Additionally, CMV-antigen, which activates CD4+ T helper cells, supports proliferation. We now investigated whether the distinct cytokines differentially affect homing properties by analyzing expression of the chemokine receptor CCR7 and CD62L, which are both involved in migration of T cells to the secondary lymphoid organs. Furthermore we analyzed the expression of the co-stimulatory molecule CD27. **Methods:** PBMCs were isolated from CMV+, HLA-A2+ healthy individuals and cultured for 4-7 days in the presence of CMV-peptide plus either CMV-antigen, or IL-2, IL-15 or IL-21. After staining with HLA-A2 CMV-peptide tetramers and CD8, CCR7, CD62L and CD27 antibodies, cells were analyzed by FACS. **Results and Discussion:** Upon stimulation with peptide and IL-15 the T cells remained negative for both CCR7 and CD62L. Surprisingly, stimulation with peptide and antigen or IL-2 or IL-21 induced a strong upregulation of CCR7 and to a lesser extent CD62L. These data indicate that cytokines can differentially affect migratory properties of cytotoxic T cells upon (re)stimulation. For, helper cell derived cytokines (IL-2, IL-21) have opposite effects compared to tissue-produced cytokines (IL-15). Thus, the reactivation site of virus-specific cytotoxic T cells may determine their subsequent tissue distribution. Concerning the expression of CD27 on CMV-specific CD8+ T lymphocytes, we noticed that CD27- do not change their CD27-phenotype upon any of the stimulatory conditions. CD27+ CMV-specific CD8+ T cells partially downmodulate CD27 after stimulation with peptide plus antigen, IL-2 and IL-15 , whereas after stimulation with peptide and IL-21 all cells still express CD27. So, stimulation with different cytokines can result in different activation states of these cells.

Abstract# 544

LONG-TERM CARDIAC ALLOGRAFT SURVIVAL IN CD28$^{-/-}$4-1BB$^{-/-}$ DOUBLE KNOCKOUT MICE. Hong R. Cho,[1,2] Eun-A. Lee,[1] Byoung-Sam Kim,[1] Jae H. Seo,[3] Byoung S. Kwon,[1] Byungsuk Kwon.[1] *[1]The Immunomodulation Research Center, Univesity of Ulsan, Ulsan, Ulsan, Korea; [2]Deparment of Surgery, Ulsan University Hospital, College of Medicine, University of Ulsan, Ulsan, Ulsan, Korea; [3]Deparment of Pathology, Ulsan University Hospital, College of Medicine, University of Ulsan, Ulsan, Ulsan, Korea.*

4-1BB, a member of the tumor necrosis factor receptor superfamily, is a potent costimulator for T-cell activation. Our previous study has demonstrated that there was a significant delay of cardiac allograft rejection in 4-1BB$^{-/-}$ knockout mice, indicating that blockade of the 4-1BB costimulatory pathway is partially effective in the prolongation of cardiac allograft survival. In this study, we examined the effect of combined blockade of the 4-1BB and CD28 costimulatory pathways in mice of Balb/c background. A long-term cardiac allograft survival (C57BL/6 donor) was induced in CD28$^{-/-}$4-1BB$^{-/-}$ double knockout mice (>100 days survival in 5 of 6 mice). Blockade of the CD28 costimulatoy pathway had a partial effect in the long-term cardiac allograft survival (>100 days survival in 2 of 5 mice). Even though there was a significant delay in cardiac allograft survival in 4-1BB$^{-/-}$ knockout recipients compared to wild-type mice (mean survival time; 55 versus 9 days), all cardiac allografts were rejected within 91 days after transplant in 4-1BB$^{-/-}$ knockout recipients. In vitro mixed leukocyte reaction (MLR) assay showed that 4-1BB$^{-/-}$ knockout mouse T cells had the ability to respond to Balb/c antigen-presenting cells (APCs) to the same extent as wild-type T cells, whereas CD28$^{-/-}$ mouse T cells had proliferative defects in response to stimulation with allogeneic APCs. Double knockout mouse T cells essentially did not respond to stimulation with allogeneic APCs. This result indicates that CD28 signals may be more important to the early immune response and that 4-1BB may play a role in the immune response at the later stage. In sum, our results demonstrate that simultaneous blockade of the CD28 and 4-1BB costimulatory pathway is effective in the induction of a long-term cardiac allograft survival. Currently, we are investigating if combined treatment with blockers for CD28 (CTLA4-Ig) and for 4-1BB (anti-4-1BB mAb) can induce a long-term cardiac allograft survival.

Abstract# 545

ABI793, A NOVEL, FULLY HUMAN ANTI-CD154 MONOCLONAL ANTIBODY: EFFICACY IN CYNOMOLGUS MONKEY KIDNEY ALLO-TRANSPLANTATION. W. Schuler,[1] M. Bigaud,[1] F. Di Padova,[1] S. Geisse,[1] H. Gram,[1] V. Hungerford,[1] B. Kleuser,[1] C. Kristofic,[1] A. Kunkler,[1] K. Menninger,[1] S. Riesen,[1] R. Tees,[1] G. Wieczorek,[1] C. Wilt,[1] C. Wioland,[1] M. Zurini.[1] *[1]Novartis Pharma AG, Basel, Switzerland.*

Anti-CD154 (CD40 ligand) mAbs induce long-term graft survival in preclinical allo-transplantation (Tx) experiments. This is the first report on the efficacy and safety of ABI793, a novel fully human anti-human CD154 mAb, in cynomolgus renal Tx recipients. ABI793 [human IgG1:Cκ; hybridoma obtained following immunization of human Ig transgenic mice (Medarex)]: 1) binds selectively to human, cynomolgus and rhesus monkey CD154; 2) blocks in vitro CD154:CD40 binding, and inhibits the human MLR (IC50 = 0.3μg/ml); 3) is comparable to mouse anti-human CD154 mAbs 5c8 and 24-31 with respect to affinity, inhibitory capacity, and species specificity. However, ABI793 recognizes a different CD154 epitope than 5c8 and 24-31. Cynomolgus monkey recipients (Philippines, Mauritius; 3-8 kg) of MHC-mismatched life-suporting renal allografts were treated with ABI793 (20 mg/kg, i.v.) on post-Tx days 0, 1, 4, 11, 18, 28, 56, & 84. Graft function was monitored by sCrea; rejection was confirmed histologically. Vehicle-treated recipients rejected within 8-9 days post Tx. 5/9 recipients showed prolonged graft survival until 108, 148, 206, 266, & 328* days post Tx (*= no sign of graft rejection); 4/9 recipients were sacrificed because of high sCrea while being treated with ABI793 (23*, 30, 32, & 70 days post Tx). Within the first 2 months, 7/9 recipients experienced episodes of severe acute tubular necrosis (ATN; histologically confirmed) of unknown etiology without signs of histological rejection. ATN responded to fluid / diuretic treatment with full recovery of renal function. Thrombocytopenia, which responded to aspirin, was seen in some animals. Neither of these side effects was seen with ABI793 in auto- or in non-transplanted animals. Remarkably, our novel, non-complement-activating AFN746 human IgG4 construct of ABI793 prolongs graft survival in cynomolgus monkeys without causing thrombocytopenia. In conclusion, ABI793 treatment effectively prevents graft rejection in cynomolgus monkeys causing side effects not noted previously with anti-CD154 mAbs. Complement-mediated processes may be the etiology of these side effects [as suggested by our results with AFN746]. Absence of side effects with ABI793 in rhesus renal allo-Tx (see abstract by T. Kanmaz et al.) also suggests a species-specific mechanism and raises the question of the two species' predictive power for clinical Tx.

Abstract# 546

ICOS EXPRESSION IN MONKEY HEART ALLOGRAFTS UNDER CD154 COSTIMULATION BLOCKADE. Agnes M. Azimzadeh,[1,2] Guosheng S. Wu,[1,2] Engin Ozkaynak,[3] Geraldine G. Miller,[1] Steffen Pfeiffer,[1] Carsten Schroeder,[1,2] George L. Zorn,[1] Roland Newman,[4] James Atkinson,[1] Richard N. Pierson 3rd.[1,2] *[1]Vanderbilt University, Nashville, TN; [2]Cardiac Surgery, University of Maryland, Baltimore, MD; [3]Millenium Pharmaceuticals, Cambridge, MA; [4]IDEC Pharmaceuticals, San Diego, CA.*

Background. Selective blockade of CD154 prolongs cardiac allograft survival. Several lines of evidence (early acute cellular rejection, later class-switched anti-donor antibody) suggest that control of costimulation is incomplete despite intensive anti-CD154 monotherapy . Here we investigated whether expression of inducible costimulator (ICOS) in anti-CD154-treated grafts is temporally associated with various survival phenotypes. **Methods**. Serial biopsies and explants from 23 cynomolgus cardiac allografts were assessed by immunohistochemistry (IHC) and real-time RT-PCR. Recipients were treated with anti-CD154 monotherapy (n=13), anti-CD154 with ATG (n=6), or no antibody (controls, n=4). ICOS mRNA levels were measured using SYBRgreen and standardized to βactin. ICOS expression was localized by quantitative immunohistochemistry (IHC). **Results**. mRNA was only barely detectable (PCR: 0.6 +/- 0.4) in normal heart tissue (30 minutes after transplant or recipient native heart). In contrast, ICOS expression was prominent during rejection of untreated controls (PCR: 80+/-29; IHC: 14+/-5). Interestingly, ICOS expression was similarly elevated in all anti-CD154 monotherapy grafts at day 7 (PCR: 96+/-39; IHC: 17+/-11). ICOS remained detectable at reduced levels in surviving IDEC 131 monotherapy grafts at 2 and 4 weeks (PCR: 41+/-16 and 49+/-34 respectively). ATG induction, which prevented rejection before 3 weeks, also prevented early (day 7, 14) ICOS mRNA and protein (Day 7, PCR: 21+/-11; IHC: 2+/-3). However ICOS was expressed later (d 28) and consistently at rejection (IHC: 15+/-7). ICOS was localized primarily to graft infiltrating T cells. **Conclusions**. In contrast with rodent studies, CD154 blockade does not block ICOS expression in primate heart allografts. ICOS expression is associated with early and late heart allograft rejection. After perioperative ATG, late graft loss was associated with graft infiltration by ICOS+ T-cells. These findings support the hypothesis that incomplete control of T cell costimulation contributes to early and late graft loss with anti-CD154 monotherapy, and that ICOS is an attractive additional costimulation pathway target to prevent acute and perhaps also chronic rejection.

Abstract# 547

TARGETING THE ICOS-B7H COSTIMULATORY PATHWAY SYNERGIZES WITH RAPAMYCIN TO REGULATE ALLORESPONSES IN ISLET TRANSPLANTATION. Sulaiman A. Nanji,[1] Wayne W. Hancock,[2] Colin C. Anderson,[1] Andrew B. Adams,[3] Bin Luo,[1] Liqing Wong,[2] Anthony J. Coyle,[2] Christian P. Larsen,[3] A. M. James Shapiro.[1] *[1]Department of Surgery, University of Alberta, Edmonton, AB, Canada; [2]Department of Pathology, Abramson Research Center, Philadelphia, PA; [3]Department of Surgery, Emory University, Atlanta, GA.*

Blockade of costimulation is a promising strategy to prevent allograft rejection and induce tolerance. The recently identified inducible costimulatory molecule (ICOS) is important in providing costimulation to T cells. We investigated the role of the ICOS-B7h pathway using a specific mAb to ICOS in a fully allogeneic mouse model of islet transplantation. **Methods.** Balb/c islets were transplanted under the renal capsule of streptozotocin treated diabetic C57BL/6 mice. Recipients were treated with anti-ICOS (0.1mg/d) either alone, with cyclosporine (10mg/kg/d), or with rapamycin (rapa) (0.2mg/kg/d) i.p. for 14 days post-transplant. Mice with graft survival > 100 days underwent nephrectomy of their graft-bearing kidney to confirm graft function, followed by re-transplantation of donor-strain islets. Allo-specific T cell responses were evaluated for 1)in vivo proliferation of CFSE-labeled T cells, 2)IFN-γ production, 3)in vitro lymphocyte reactions, and 4)immunopathology. **Results.** Anti-ICOS treatment alone did not prolong graft survival (MST=13d). Cyclosporine and anti-ICOS togther resulted in marginal prolongation of graft survival (MST=16d). In contrast, rapa with anti-ICOS resulted in significantly prolonged graft survival, compared to mice treated with rapa alone (MST=47d and 24d, respectively, $p<0.01$). Fifty per cent of mice treated with anti-ICOS and rapa demonstrated normal graft function beyond 100 days. Re-challenge with donor islets in these mice resulted in delayed rejection of the second graft (MST=30d). Tolerated grafts demonstrated peri-islet infiltrates of $CD4^+$ T cells and macrophages, with a paucity of $CD8^+$ T cells. Anti-ICOS and rapa therapy significantly reduced proliferation frequencies of both $CD4^+$ and $CD8^+$ T cells (61% and 91% reduction, $p<0.05$) and production of IFN-γ by $CD4^+$ and $CD8^+$ T cells (89% and 90% reduction, $p<0.05$). Tolerant mice did not demonstrate donor-specific hyporesponsiveness in MLR or CTL assays, and co-culture of cells from naive and tolerant mice did not indicate T regulatory cell activity. **Conclusions.** These data demonstrate that combined treatment with anti-ICOS and rapa induces long-term graft acceptance in 50% of recipients, without donor-specific immunological tolerance. Blockade of the ICOS-B7h pathway has potential therapeutic benefit in light of its role in the regulation of alloresponses in vivo.

Abstract# 548

REGULATING TCR-INDUCED IL-2 RELEASE BY BLOCKING LAT EXPRESSION. Zheng Chang,[1] Stuart J. Knechtle,[1] Majed M. Hamawy.[1] *[1]Surgery, University of Wisconsin, Madison, WI.*

INTRODUCTION: Although current immunosuppressants have improved graft survival, they lack specificity and induce toxicities. T cells are central for the immune response to allografts; thus, developing T cell-specific immunosuppressive reagents might prevent graft rejection with minimal or no side effects. We employed an anti-sense strategy to examine if blocking the expression of the T cell signaling molecule LAT suppresses T cell activation and in turn prevents graft rejection. METHODS: Anti-sense LAT constructs were cloned into the replication-incompetent retroviral expression vectors pLNCX2 and then transfected by liposome-mediated transfection methods into the RetroPack PT67 packaging cell line. Supernatants containing virus titer of 10^5 CFU/ml were used to infect T cells. RESULTS: there was a marked decrease in the level of LAT in T cells infected with the anti-sense LAT-containing viruses compared to the level of LAT in the cells infected with the empty vector-containing viruses (Fig.1). TCR-induced IL-2 production was also markedly less in cells infected with the anti-sense LAT-containing virus compared to cells infected with an empty virus (the decrease in IL-2 release ranged from 25%-75%; example Fig. 2). CONCLUSION: These results show that regulating LAT expression in T cells is useful for controlling T cell activation. Future studies will examine if blocking LAT expression in T cells in vivo is sufficient for preventing graft rejection.

Figure 1

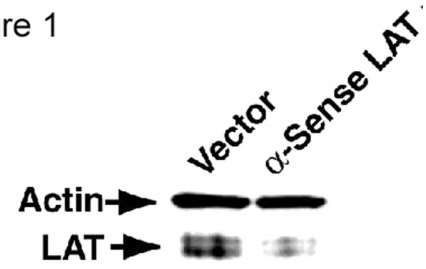
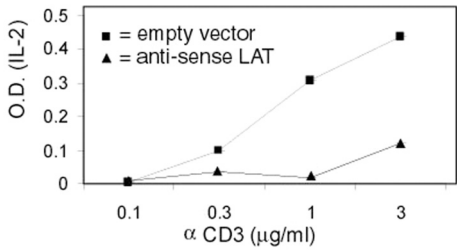


Figure 2



Abstract# 549

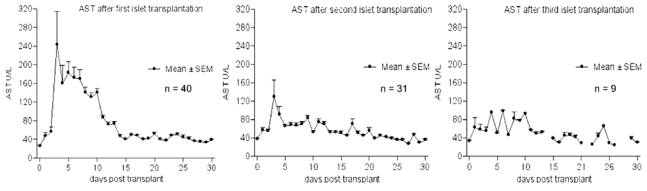
LFET-SIDED PANCREAS GRAFT PLACEMENT: SURGICAL RISK OR SURGICAL STANDARD? Rainer W. G. Gruessner,[1] David E. R. Sutherland,[1] Raja Kandaswamy,[1] Abhinav Humar,[1] Angelika C. Gruessner.[1] *[1]Department of Surgery, University of Minnesota, Minneapolis, MN.*

Introduction: About 80% of all pancreas (Pa) transplants (Txs) are drained via the systemic venous circulation. Most grafts are anastomosed to the right iliac vessels, but a previous kidney (Kd) or Pa graft on the right side may require Pa placement on the left side. Several studies showed that left-sided Pa placement increases surgical complications, in particular, postTx thrombosis. We investigated whether left-sided Pa placement increases surgical risk. **Patients and Materials**: Between 7/1986 and 6/2002, we performed 1156 Pa Txs: 1030 (89%) on the right and 105 (9%) on the left side; 21 (2%) were anastomosed to the aorta/cava. Of the 105 left Pa Txs, 89 were for Pa after Kd Tx [PAK] (20% of all PAKs); 15 (6%), Pa Txs alone [PTA]; and 1 (< 1%), sim. Pa and Kd Tx [SPK]. We compared surgical complications (graft thrombosis, infection, leak) and relaparotomy rates between left- and right-sided Txs within the first 100 days postTx using Fisher's exact test and Chi-^{2} tests. **Results**: For our entire series (n=1156), we found no significant differences in surgical complications and re-laparotomy rates for left (35%) vs. right (38%) Pa placement. For PAK recipients, left Pa placement was significantly more frequent in the cyclosporine [CSA] (35%) vs. tacrolimus [TAC] (16%) era. Rates of postTx graft thrombosis, infection, and leak in both immunosuppressive eras were not significantly different for left vs. right placement. The relaparotomy rate was significantly higher for left (28%) vs. right (18%) placement for re- (not primary) Txs. For PTA recipients, left Pa placement was 6% in both the CSA and TAC eras. Rates of graft thrombosis, infection, leak, and relaparotomy in both eras were not significantly different for left vs. right placement. Tx numbers (primary vs. reTxs) also had no effect. For SPK recipients, only 1 (out of 471) Pa grafts was placed on the left, so no formal comparison was made. **Conclusions**: (1) Pa Txs can be placed on the left (vs. right) side with similar technical success for PAK and PTA recipients. (2) The risk of postTx thrombosis is not higher for left (vs. right) graft placement for PAK or PTA recipients. (3) For PAK recipients only, the relaparotomy rate for left-sided reTxs (not primary Txs) significantly increased: thus, re PAKs on the left side should be avoided. (4) Surgical complications and relaparotomy rates were significantly lower for both left and right placement in the TAC (vs. CSA) era, for PAK and PTA recipients.

Abstract# 550

CHANGES IN LIVER ENZYMES AFTER CLINICAL ISLET TRANSPLANTATION. Ehab Rafael,[1] Edmond Ryan,[2] Sharleen Imes,[3] Jose Oberholzer,[4] David Bigam,[4] Norman Kneteman,[4] Breay Paty,[2] Jonathan Lakey,[4] James Shapiro.[4] *[1]Department of Transplantation Surgery, Huddinge Hospital, Karolinska Institute, Stockholm, Sweden; [2]Department of Medicine; [3]Capital Health Authority; [4]Department of Surgery, University of Alberta, Edmonton, AB, Canada.*

Background: We have previously reported our findings in clinical islet transplantation resulting in insulin independence with adequate metabolic control in patients with type I diabetes. This was achieved with a steroid-free protocol in combination with a mean islet mass of 13,000 IEQ/kg body weight. The aim of this study was to investigate whether elevations in liver enzymes observed after islet transplantation are related to graft characteristics, graft function and the safety of the procedure. ***Methods:*** 81 consecutive islet transplant procedures were performed in 41 recipients between March 1999 to November 2002. Liver function tests (LFT) were assessed three times weekly for the first four weeks post-transplant. Data are presented as mean±SEM. ANOVA was used for statistical analysis.



Results: The highest AST-levels were observed after the first islet transplant. After the second and third transplant, AST increase was less pronounced as shown in the figure. AST for all transplants peaked at 7±1 days at a value of 160±24 U/L (p<0.001). In 54% of the transplants the AST increased by more than 2.5 times. A 5-fold increase in AST was observed in 25% of the procedures. Changes in ALT were similar to AST. ALP increased more than 2-fold in 13% of the procedures. LFT normalized in all recipients within the first three weeks post transplant. Insulin requirements, graft characteristics and clinical outcome were not significantly different when comparing patients with >5- vs. <2.5- fold. Bilirubin remained within normal range for all recipients. ***Conclusions:*** After intraportal islet transplantation a significant increase in LFT levels was noticed. These levels normalized in all recipients within 3 weeks. In our series of human islet transplants, no correlation between the increase in LFT and clinical outcome in terms of risk or islet function was observed.

Abstract# 551

IS THE LIVER THE OPTIMAL SITE FOR ISLET TRANSPLANTATION? James F. Markmann,[1] Niraj M. Desai,[1] John A. Goss,[2] Shaoping Deng,[1] Evan Siegelman,[3] Mark Rosen,[3] Clyde F. Barker,[1] Ali Naji.[1] *[1]Surgery, University of Pennsylvania, Philadelphia, PA; [2]Surgery, Baylor University Medical Center, Houston, TX; [3]Radiology, University of Pennsylvania, Philadelphia, PA.*

Introduction: Islet transplantation is an emerging therapy for selected type I diabetics. Islet registry analysis correlated success with the use of the intraportal transplant site. Despite the success with this approach, a number of concerns exist regarding its application. First, there exists a low but significant risk of procedure complications, including bleeding and thrombosis. In addition, repeat islet doses may result in increased portal pressures. We analyzed our islet transplant experience with regard to the effect of intraportal islet infusion on the liver. **Methods:** We transplanted 15 islet preparations (12 infusions) in 9 patients under Edmonton protocol immunosuppression. Seven of 9 patients achieved insulin independence. Portal and systemic blood sampling for sirolimus and tacrolimus was performed immediately prior to islet infusion. Chemical shift MR imaging was performed in 4 patients to evaluate for hepatic steatosis. **Results:** No episodes of significant bleeding or thrombosis have been observed. Elevated LFT's (Ast/Alt) were observed in all patients in the post-transplant period but resolved spontaneously. Average portal vein sirolimus and tacrolimus levels were significantly higher than levels drawn from the peripheral circulation respectively (28.3 vs. 22.6 and 3.8 vs. 2.7). MR imaging in 4 patients at 9 to 14 months post transplant revealed 2 with well-functioning grafts who exhibited a unique pattern of branching periportal steatosis that has not been described previously. Of two patients that did not exhibit steatosis, one had lost the majority of graft function and one had temporarily resumed insulin at the time of imaging. **Conclusions:** Isolated islet transplantation to the liver via the portal vein is associated with exposure to an elevated level of immunosuppressive agents that may provide local immunosuppression to the graft while minimizing systemic exposure. During the immediate post transplant period however, high levels of potentially toxic agents may impair engraftment. We also determined that intrahepatic insulin secretion can result in localized hepatic steatosis that likely reflects abnormal local insulin utilization. Routine MR imaging may provide a non-invasive imaging modality to follow the fate of intrahepatic islets, and the presence of peri-graft steatosis may correlate with the degree of graft function. The current results provide additional cause to question whether the liver is the optimal site for islet engraftment.

Abstract# 552

A PROSPECTIVE, RANDOMIZED OF STEROID WITHDRAWAL WITH MYCOPHENOLATE (MMF) VS. SIROLIMUS (SRL) IN PANCREAS AFTER KIDNEY (PAK) TRANSPLANTS. R. Kandaswamy,[1] K. Khwaja,[1] A. Gruessner,[1] R. Gruessner,[1] A. Humar,[1] E. Glumac,[1] D. Sutherland.[1] *[1]Surgery, Univ. of MN, Mpls., MN.*

Intro: PAK recipients given Thymoglobulin (1.25 mg/kg X1) and Daclizumab (1mg/kg x2) for induction with tacrolimus and prednisone (withdrawn at 6 mos in those with no rejection episodes) for maintenance immunosuppression were randomized to also receive either MMF (n=26) or SRL (n=24). MMF dose was 2 g/day and SRL dose was 4 mg (levels 8-12 ng/ml). Donor demographics (age, race, and gender) were similar in the 2 groups. Actual 6 mo. and actuarial 1-year follow-up data is presented. **Results:** Patient and graft survival were not significantly different between the groups. There was 1 death (PTLD) in the SRL group. There was 1 rejection episode in each group, but no graft losses from rejn. There were 3 graft losses in the MMF group (1 thrombosis and 2 pancreatitis) and 2 in the SRL group (1 pancreatitis and 1 abdominal infection). Side effects (nausea, vomiting, diarrhea, arthralgias, tremors, headache) were not different between groups. BUN at 6 and 12 months was higher in the SRL group. Urine amylase was higher at 6 months in the MMF group. Cholesterol and TGL were higher at 6 months, but this difference became insignificant at 12 months. CMV, intraabdominal and wound infections were not different between groups.

OUTCOMES	**MMF**	**SRL**
Graft survival 1 yr	89%	92%
Patient survival 1 yr	100%	96%
Acute rejection	1	1
Intraabdominal infection	1	4
Wound infection	0	3
CMV infection	6	2
Thrombosis	1	0

	6 months			**1 year**		
	MMF	**Sirolimus**	**p**	**MMF**	**Sirolimus**	**p**
WBC	4.8 ± 1.2	4.8 ± 1		4.5 ± 0.7	4.4 ± 1.4	
Creatinine	1.5 ± 0.4	1.7 ± 0.5		1.5 ± 0.4	1.6 ± 0.4	
BUN	24 ± 0.8	31 ± 9	0.01	22 ± 6	28 ± 8	0.02
S amylase	77 ± 37	64 ± 31		81 ± 27	60 ± 32	0.09
S lipase	119 ± 152	68 ± 80	0.08	95 ± 103	60 ± 60	
Hb A_1C	5.2 ± 0.6	5.6 ± 0.8		5.5 ± 1.3	5.3 ± 0.4	
U amylase units/hr	6112±3881	3420±1953	0.04	4551±2886	3508±3543	0.35
Cholesterol	159 ± 33	194 ± 42	0.02	160 ± 40	158 ± 32	0.8
TGL	108 ± 41	192 ± 104	0.01	114 ± 51	190 ± 144	0.12

Conclusion: Our data shows no differences in primary endpoints (AR, graft survival, pt. survival) between the MMF and SRL groups. Urine amylase was higher in the MMF group, but rejection episodes were rare in both groups. The MMF had a better lipid profile at 6 months and better renal function (BUN) at both 6 and 12 months. Based on side effects, the MMF regimen was slightly better than SRL, but both were associated with excellent outcomes.

Abstract# 553

CMV INFECTIONS IN PRIMARY SIMULTANEOUS PANCREAS-KIDNEY (SPK) TRANSPLANTATION: RESULTS AT 1 YEAR OF A LARGE MULTICENTER TRIAL. Dirk Kuypers,[1] Jacques Malaise,[1] Frantisek Saudek,[1] Wolfgang Steurer,[1] Helmut Arbogast,[1] EUROSPK Study Group.[1] *[1]Eurospk Central Office, Brussels, Belgium.*

We present the 1-year analysis of an open, prospective, randomized, parallel-group study, in which 205 SPK transplant recipients from 11 centers in Europe and Israel were included. Following ATG induction, patients received either tacrolimus or cyclosporine-microemulsion along with mycophenolate mofetil and steroids. The donor (D) / recipient (R) serologic status was available for 196 patients who were divided in four groups according to their CMV match: group D+/R- (n = 48), group D+/R+ (n = 68), group D-/R+ (n = 35), group D-/R- (n = 45). CMV infection was defined as detection of CMV antibodies or positive antigenemia while CMV disease was also associated with fever and/or leucopenia, thrombocytopenia or invasive CMV. Prophylaxis with Acyclovir, Ganciclovir or both was given to 67.5% of the patients in the 4 groups. RESULTS: Incidence of CMV infection and disease is highest in the D+R- group (52%) followed by 40% in the D-R+ group, 36.8% in the D+R+ group and 8.9% in the D-R- group which is significantly lower than in the 3 other groups. According to the type of prophylaxis, the CMV infection rate is:

	D+R-	D+R+	D-R+	D-R-
No prophylaxis	70.6%	54.5%	58.3%	9.1%
Acyclovir	71.4%	54.5%	100%	5.5%
Ganciclovir or Ganciclovir + Acyclovir	33.3%	20.0%	11.1%	20%

The emergence of infection or disease is significantly lower when the patient received a prophylaxis with Ganciclovir or Ganciclovir + Acyclovir compared to no prophylaxis or Acyclovir alone: D+R- group: 33.3% vs. 71.0% (p = 0.0093), D+R+ group: 20.0% vs. 54.5% (p = 0.0032), D-R+ group: 11.1% vs. 79.2% (p = 0.003), D-R- group: 20% vs. 7.3% (ns). There are significantly less rejection episodes when the patient had a prophylaxis with Ganciclovir or Ganciclovir + Acyclovir compared to no prophylaxis or Acyclovir alone: 36.6% vs. 57% (p = 0.0048). CONCLUSIONS: Ganciclovir is efficient to prevent CMV infection or disease in the risks groups in kidney and pancreas transplantation. Effect of Ganciclovir as CMV prophylaxis on the incidence of rejection is possible.

Abstract# 554

KIDNEY/PANCREAS TRANSPLANTATION: HOW DURABLE IS THE CURE? Yolanda T. Becker,[1] Bryan N. Becker,[2] John D. Pirsch,[2] Jon S. Odorico,[1] Hans W. Sollinger.[1] *[1]Department of Surgery, University of Wisconsin, Madison, WI; [2]Department of Medicine, University of Wisconsin, Madison, WI.*

Simultaneous kidney pancreas (SPK) whole organ transplant is the only means to cure type I diabetes mellitus (DM) and its associated nephropathy. Yet, how durable is the cure? In 1998, the American Diabetes Association redefined impaired glycemia (IG) as 2 fasting glucose levels of ≥110mg/dl and DM as 2 fasting glucose levels≥126 mg/dl. We applied these new definitions to determine the incidence of IG or DM in long-term SPK recipients. We retrospectively examined SPK patient data from 1994-2001 at our center. Data was available for 430 individuals. Standard immunosuppressive treatment for these individuals consisted of antibody induction therapy, calcineurin inhibition, mycophenolate mofetil, and corticosteroids. Pt and graft survival were calculated using Kaplan-Meier. Risk for IG, DM, and graft loss was calculated using chi-square analysis. Statistical analysis was performed using SAS software and significance was defined as p ≤0.05. 40% of this cohort developed IG (111/430) or frank DM (87/430) by 8.5 years post-SPK transplant, although the demographics of these individuals were similar to the study population as a whole. Risk factors for post-SPK IG or DM included recipient BMI (relative risk (RR): 1.09; p ≤0.001) and tacrolimus (FK) use (IG RR: 1.8; p ≤0.004; DM RR: 1.9; p ≤0.005). Interestingly, FK levels *per se* (2 FK levels ≥15 within 1 year) were not a risk factor for IG or DM. Number of rejection episodes were associated with IG (RR: 1.4; p ≤0.02) and DM (RR: 1.4; p ≤0.04). The consequences of this post-SPK change in glucose metabolism were also significant as post-SPK IG or DM were at significant risk for kidney graft loss (IG RR: 2.6; p ≤0.02; DM RR: 5.4; p ≤0.0005). Donor BMI, type of pre-transplant dialysis, hypercholesterolemia, or degree of HLA mismatch did not increase the risk of IG or DM. Surprisingly, a significant number of SPK recipients in this study cohort developed IG or DM and the mere use of FK created a diabetogenic environment without an apparent level-response effect. Perhaps, most important, the SPK recipients who develped IG or DM were at significant risk for kidney graft loss. This may be due, in part, to aberrant glycemic control leading to diabetic changes in the renal allograft, changes in immunosuppression that potentiate chronic histologic damage in the allograft, or associated conditions. e.g. hypertensive injury, that are exacerbated in the diabetic milieu.

Abstract# 555

ASSESSING POTENTIAL TO INCREASE PANCREAS UTILIZATION FROM DECEASED ORGAN DONORS. Ellen Sheehy,[1] Paul Schwab,[1] Martin Mozes,[2] Marc Hurlbert,[3] Suzanne Truax,[4] Gene Osborne,[5] Dean Lichtenfeld,[2] Brenda Welsch,[6] Ann Roberson,[7] Kevin O'Connor.[8] *[1]Association of Organ Procurement Organizations, McLean, VA; [2]Gift of Hope Organ & Tissue Donor Network, Elmhurst, IL; [3]Research Department, Juvenile Diabetes Research Foundation International, New York, NY; [4]LifeCenter Northwest, Bellevue, WA; [5]California Transplant Donor Network, Oakland, CA; [6]LifeSource, St. Paul, MN; [7]Texas Organ Sharing Alliance, San Antnoio, TX; [8]New England Organ Bank, Newton, MA.*

Objectives: To describe reasons for nonrecovery and nontransplantation of pancreata in the US and to estimate potential for increase in pancreas recovery. Methods: 1893 organ donor charts for January-June 2001 were reviewed at 38 Organ Procurement Organizations (OPOs) by trained abstractors. Clinical data, including medical history, lab values, medications, and serologies, as well as data on request, placement and pancreas disposition for each donor was recorded. Reasons for nonrecovery were examined. An estimate for potential increase in pancreas recovery was derived by applying the top quartile OPO pancreas recovery rate to the sample average. Results: Pancreata were recovered from 633 donors (33%) and 405 (64%) were transplanted, 378 whole pancreas and 27 islet cell transplants. The most frequent reason for nonrecovery was not attempting to place the organ (638/1893, 34%). Next in frequency was inability to find a center to accept the pancreas (252/1893, 13%.) The family was either not asked to donate the pancreas or did not consent for pancreas recovery in 10% and 4% of cases respectively. 5% of pancreata were placed, but ruled out in the operating room. Donor age played a significant role at all stages (request, placement and acceptance for transplant). 66% (419/633) of recovered pancreata were from donors aged 11-40 years old. 20% were from donors aged 41-50 years, 10% from donors aged 51-60, and 2.5% from donors over age 60. Only 1 pancreas donor was under age10. OPO pancreas recovery rates ranged from 11% to 63% (mean=33%) and transplant rates ranged from 20% to 100% of recovered pancreata (mean=64%). The recovery rate of the top quartile of OPOs averaged 49% representing a 50% improvement over the study average of 33%. Many factors contributed to placement and recovery, including the presence and availability of an active, local pancreas program or research program and the effectiveness and availability of OPO and transplant center resources. Conclusions: The data provide a snapshot of pancreas recovery and transplant rates and reasons for non-recovery for 2001. Data suggest that recovery and transplant rates could be improved significantly. For OPOs, the process of conducting reviews combined with observing others' practices highlighted practical tactics for achieving higher rates of pancreas recovery.

Abstract# 556

AFRICAN-AMERICAN ETHNICITY IS NO LONGER A RISK FACTOR FOR EARLY ADVERSE OUTCOMES IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION WITH CONTEMPORARY IMMUNOSUPPRESSION. J. Rogers,[1] R. J. Stratta,[2] R. R. Alloway,[3] A. Lo,[4] E. E. Hodge.[5] *[1]Medical U of South Carolina; [2]Wake Forest U; [3]U of Cincinnati; [4]UT-Memphis; [5]Roche Research Labs, for the PIVOT Study Group.*

Background: The influence of ethnicity on outcome in simultaneous kidney-pancreas transplantation (SKPT) is controversial. **Aim:** To determine the impact of ethnicity on the major endpoints of a prospective, multi-center randomized study of two dosing regimens of daclizumab compared to no antibody induction in SKPT recipients. **Methods:** 297 SKPT patients were enrolled in the study and were randomized into 3 groups: daclizumab 1 mg/kg/dose every 14 days for 5 doses (Group I, n=107), daclizumab 2 mg/kg/dose every 14 days for 2 doses (Group II, n=112), and no antibody induction (Group III, n=78). All patients received TAC, MMF, and steroids as maintenance immunosuppresion. **Results:** 37 patients (12.5%) were African-American (AA) and 262 were non-African-American (NAA). A higher percentage of AA were randomized to Group II (18% AA) compared to Groups I and III (9% AA, p<0.05). Age, gender distribution, duration of diabetes, retransplants, graft ischemia times, and surgical techniques were similar between AA and NAA. The incidence of kidney delayed graft function was slightly higher in AA (13.5% AA vs. 7.7% NAA, p=0.23). At 6 months, there were no differences in patient survival (97% AA, 98% NAA), kidney graft survival (100% AA, 95% NAA) and pancreas graft survival (92% AA, 87% NAA) rates. The incidence of either kidney or pancreas allograft rejection at 6 months was comparable between AA and NAA (19% AA, 22% NAA, p=0.67). Median time to first rejection was also similar (53 days AA, 42 days NAA). TAC levels were similar at 6 months. Rejection rate did not differ across treatment groups among AA patients (Group I 22%, Group II 19%, Group III 14%). There were no differences in the incidences of serious adverse events, including infectious complications or readmissions between AA and NAA. Both AA and NAA had similarly excellent kidney function at 6 months as evidenced by mean serum creatinine (AA 1.56 vs. NAA 1.57 mg/dl). However, mean HgbA1C levels were slightly higher (6.4% AA vs. 5.5% NAA), mean C-peptide levels were slightly lower (3.8 ng/ml AA vs. 4.7 ng/ml NAA) and oral hypoglycemic use was more common (10.8% AA vs. 4.2% NAA, p=0.08) in AA at 6 months. **Conclusions:** In this prospective, multi-center study, AA ethnicity was not associated with an increased risk of early adverse outcomes in SKPT. Follow-up will be required to determine if long-term outcomes remain equivalent, particularly with regard to pancreas graft function.

Abstract# 557

HISTOLOGIC EVALUATION OF INTERFERON/RIBAVIRIN THERAPY IN LIVER TRANSPLANT RECIPIENTS WITH RECURRENT HEPATITIS C: EVIDENCE OF REJECTION DESPITE VIROLOGIC RESPONSE? R. T. Stravitz,[1] M. L. Shiffman,[1] A. J. Sanyal,[1] V. A. Luketic,[1] R. K. Sterling,[1] A. Ashworth,[1] M. P. Posner,[1] A. H. Cotterell,[1] D. Maluf,[1] A. S. Mills,[1] M. J. Contos,[1] R. A. Fisher.[1] *[1]Hepatology Section, Division of Transplant Surgery, and Department of Pathology, Virginia Commonwealth University, Richmond, VA.*

Recurrent hepatitis C virus (HCV) infection occurs universally after liver transplantation (LT), often with an accelerated course. Although some LT patients achieve virologic response (VR) and become HCV RNA (-), histologic response (HR) has not been evaluated. OBJECTIVE: To determine the frequency of HR in LT patients who received interferon (IFN)/ribavirin (RBV). METHODS: 20 LT patients with accelerated or advanced recurrent HCV were treated with standard IFN and/or PEG-IFN, with/without RBV, as tolerated. HCV RNA was monitored at 3-6 month intervals by quantitative PCR. Treatment was discontinued at 6 months in patients who remained HCV RNA + (NR). Patients with VR received 48 weeks of therapy and were then followed for > 6 months to monitor for sustained response (SVR). Responders unable to tolerate RBV (highest risk for relapse) were continued on maintenance PEG-IFN as long as HCV RNA remained undetectable. Liver biopsy was performed and scored according to Knodell in all patients at baseline, at time of discontinuation in patients with NR, at week 72 in patients with SVR, and after a mean of 26 months in patients with VR on maintenance PEG-IFN. HR was defined as a > 2 point reduction in inflammation. RESULTS: Only 3/20 patients tolerated RBV for 48 weeks. VR was achieved in 9/20 (45%), SVR in 5/20 (25%); 4/20 continued on maintenance PEG-IFN. Only 5/9 with VR/SVR achieved HR. Mean inflammation score in these 9 patients decreased from 8.8±1.8 to 7.0±1.7 (p=0.03). No change was observed in fibrosis (1.9±1.1 vs. 1.8±1.2). No significant change in inflammation or fibrosis was observed in patients with NR. 4/9 with VR had persistently elevated ALT during/after treatment, including 3/5 with SVR. 3 with VR developed acute cellular/ductopenic rejection on follow-up liver biopsy; all 3 had persistently abnormal ALT. 2/11 with NR also developed ductopenic rejection during/after treatment; 1 required re-LT. CONCLUSIONS: In LT patients with recurrent HCV, HR occurs in only half of patients with VR/SVR. In contrast to the non-LT population, significant histologic inflammation persisted in all patients despite VR/SVR. In addition, histologic changes consistent with rejection were observed in 25% of patients following treatment. Future studies must more thoroughly evaluate the histologic benefit of IFN/RBV in LT patients with recurrent HCV.

Abstract# 558

CORTICOSTEROID (CS) MAINTENANCE AND ITS IMPACT IN THE PROGRESSION OF HEPATITIS C HISTOLOGIC RECURRENCE (HHCVR) IN LIVER TRANSPLANT RECIPIENTS (OLT) AT ONE AND TWO YEARS (Y) POST TRANSPLANT (TX): A DOSE-DEPENDENT BENEFIT ? C. G. Fasola,[1] G. J. Netto,[1] M. Thomas,[1] N. Onaca,[1] E. Q. Sanchez,[1] S. Chinnakotla,[1] R. M. Goldstein,[1] M. F. Levy,[1] G. L. Davis,[1] G. B. Klintmalm.[1] *[1]Transplantation Services, Baylor University Medical Center, Dallas, TX.*

Purpose: Assess CS effect in HHCVR. **Methods:** Hep C 1994 -1998 OLT outcome (n=149), at Y1-Y2, analyzed according to CS amount (maintenance or bolus) received. Immunosuppresion (IS) based on calcineurin-inhibitors (CSA or FK) + Prednisolone. OLT divided by total amount of CS maintained in Y1 (range: 2,958 to 10,113 mg) and Y2 (range: 2,958 to 13,763 mg) as: Low (Y1<5,162; Y2<6,500), Medium (Y1:5,162-6,315; Y2:6,500-8500) or High doses (Y1>6,315; Y2>8,500). Selection criteria = percentiles 33 and 66 range. CS boluses were analyzed as 0, 1 or > 1. Laboratory data and liver histology evaluated by protocol at Y1-Y2 post TX. HHCVR assessed by degree of fibrosis (DF: stages 0 to 4), as per A.J.S. Path. 19(12):1409, 1995. HHCVR Progression was defined as none (Y1:S0 + Y2:S0); progressive (Y1:S0 or S1 + Y2:S1 or S2) or aggressive (Y1:S≥2 + Y2:S≥3); multiple parameters analyzed. X^2 tests* = tables. P ≤ 0.05 = significant. **Results:** Most parameters showed no statistical differences. OLT without CS boluses likely to have no HHCVR (stage 0) at Y2 (0 = 51%; 1= 31% and >1 = 18%), CS boluses were not associated with more severe HCVR (stages 3-4) at Y2 (0 = 16%; 1 = 28% and >1 = 18%, p= 0.10). In contrast, low dose OLT more likely to have severe DF at Y1 or Y2 than high dose OLT (table). Course of disease likely to be progressive or aggressive in low dose OLT. Graft-patient survivals over 90% at 1-5 years (p=0.12). **Conclusions**: OLT with low dose maintenance CS more likely to have severe and progressive HHCVR. Although CS boluses do not appear to increase the risk of severe disease, OLT with no boluses were less likely to have HHCVR. CS may be an important component of IS in HCV-OLT and minimize severe HHCVR. The current practice of early CS withdrawal should be reconsidered and examined in prospective studies.

CS Doses and HHCV Recurrence at Y2 post TX

DF	Low CS n (%)	Medium CS n (%)	High CS n (%)
Stage 0	9 (20%)	16 (42%)	24 (57%)
Stages 1 or 2	20 (46%)	16 (42%)	13 (31%)
Stages 3 or 4	15 (34%)	6 (16%)	5 (12%)
Total	44 (100%)	38 (100%)	42 (100%)

p = 0.006*

CS DOSES AND HHCVR PROGRESSION AT Y1-Y2 POST TX

HHCVR Pattern	Low CS n (%)	Medium CS n (%)	High CS n (%)
None	14 (30%)	18 (40%)	32 (68%)
Progressive	15 (32%)	16 (36%)	13 (28%)
Aggressive	18 (38%)	11 (24%)	2 (4%)
Total	47 (100%)	45 (100%)	47 (100%)

p = 0.0003*

Abstract# 559

LIVER TRANSPLANTATION FOR HCV CIRRHOSIS: RESULTS ARE NOT GETTING WORSE IN RECENT YEARS. Luca S. Belli,[1] Alberto B. Alberti,[1] Marcello Vangeli,[1] Aldo Airoldi,[1] Claudio Zavaglia,[1] Luciano De Carlis,[2] Gianfranco Rondinara,[2] Omar A. Slim,[2] Domenico Forti,[2] Giovambattista Pinzello.[1] *[1]Epatologia e Gastroenterologia, Ospedale Niguarda, Milan, Italy; [2]Dipartimento di Chirurgia Generale e Centro Trapianti Organi Addominali, Ospedale Niguarda, Milan, Italy.*

A worse outcome in HCV positive recipients with a faster progression of recurrent disease to overt cirrhosis has been reported in recent years and increased donor age and stronger immunosuppression have been indicated as major contributors to these worse results (M Berenguer, Hepatology 2002;36:202-210). We have reviewed our experience over the last 11 years with a particular aim at histologic disease progression. Methods: a retrospective analysis on 190 HCV positive recipients transplanted at our Center between January 1991 and December 2001 was performed. HBV and HDV coinfections were excluded. Protocol liver biopsies were avavilable at regular intervals for all patients. Immunosuppression consisted of a quadruple drug induction followed by either cyclosporine and corticosteroids or cyclosporin alone (steroids stopped between 1 and 5 months after transplant). Combined antiviral therapy (interferon + ribavirin) was used to treat 30 patients (transplanted after 1992) with HCV disease recurrence and a histologic grading score > 5 (Ishack). Results: Overall survival progressively improved over the last ten years mainly due to reduction of perioperative mortality (within 3 mo).

Survival in HCV infected recipients: effect of year of LT

	3 months	1 year	5 years
1991-92	70%	57%	45%
1993-94	74%	65%	50%
1995-96	77%	74%	69%
1997-98	84%	76%	NA
1999-01	93%	88%	NA

The histological outcome was assessed for a total of 150 patients with at least 6 months of follow up. In total 360 protocol liver biopsies were reviewed corresponding to a median of 2 biopsies per patient (range 1-10). The cumulative probability of developing HCV-related cirrhosis were 5% at 1 year, 9% at 3 years 15 % at 5 years and 27% at 7 years post-transplantation. No changes were noted in patients undergoing liver transplantation in recent years. In particular the cumulative probability of developing HCV related cirrhosis at 3 years after transplant has remained equal or less than 10% in all 3 periods (1991-94; 1994-98 and 1999-00) despite the progressive increase of donor age. We conclude that liver transplantation in HCV recipients still remains a clear indication for liver transplantation. Given the high rate of recurrent cirrhosis at 7 years after LT (27%) every effort has to be made to ameliorate antiviral strategies to prevent disease progression.

Abstract# 560

HISTOLOGIC GRADE OF INFLAMMATION IN HCV POSITIVE DONORS DOES NOT PREDICT RECURRENCE OF HCV ALTHOUGH FIBROSIS STAGE DOES. Richard K. Gilroy,[1] James Wisecarver,[2] Sandeep Mukherjee,[1] Jean Botha,[1] Wendy Grant,[1] Kym Watt,[1] Alan Langnas,[1] Byers Shaw.[1] *[1]Transplantation, University of Nebraska Medical Center, Omaha, NE; [2]Pathology, University of Nebraska Medical Center, Omaha, NE.*

Background: The use of HCV positive (+ve) donors may be associated with early histologic recurrence although use of HCV +ve organs does not appear to alter outcome in liver transplantation (OLT) for HCV. Some of these HCV +ve allografts have histologic abnormalities at the time of OLT and the impact of these abnormalities on outcome is unknown. **Aims:** Assess if HCV +ve donor histology alters the outcome of OLT for recipients. **Methods:** 26 patients (24 adults, 2 children) were identified who received HCV +ve donor livers between 11/90 and 11/2002. One experienced liver pathologist examined the histology and reports of all donor specimens and subsequent recipient transplant biopsies. Post-OLT biopsies were performed only where clinically indicated. Allograft histology was analyzed for diagnosis and, in cases of hepatitis, Scheuer's stage (S) and grade (G). For comparisons the students t-test was used. **Results:** Of the

26 recipients, 4 patients were excluded (2 early deaths (<14 days), one allograft used for a liver-intestinal allograft (pediatric) and one pediatric patient transplanted in 1991 and who had persistently normal liver enzymes and was HCV RNA negative.) Analysis of those remaining (n=22) demonstrated 11 with completely normal donor histology, 2 with Scheuer G1 and the remaining 9 with G2 inflammation. In addition 4 patients had S1 fibrosis and one S2 fibrosis with one of these having S1 fibrosis in the absence of inflammation. Comparing those with normal to abnormal histology, there was no difference in the frequency of rejection or recurrence of HCV. In those with fibrosis on original biopsy 2 patients died early from complications unrelated to HCV recurrence and the remaining 3 patients showed histologic recurrence at a mean of 36 days (range 12-69 days). One patient progressed from S1 to S3 in 21 days and following combination therapy cleared virus and demonstrated reductions in both S and G. In another patient (retransplanted with a HCV allograft G2 S1) recurrence was early (day 12) and within 6 months the patient was relisted for transplantation. In all 15 of the 22 had biopsy findings of HCV recurrence. **Conclusion:** Pre-OLT donor inflammation does not appear to influence time to recurrence when HCV +ve donor allografts are used. Donor fibrosis may predict early histologic recurrence of HCV.

Abstract# 561

ADVANCED DONOR AGE IS A SIGNIFICANT RISK FACTOR FOR EARLY GRAFT LOSS, EARLY SEVERE HEPATITIS ACTIVITY, AND EARLY SEVERE FIBROSIS AFTER LIVER TRANSPLANTATION FOR HEPATITIS C. Stephen Rayhill,[1] Patricia Kirby,[3] Daniel Katz,[1] Michael Voigt,[2] Douglas LaBrecque,[2] Warren Schmidt,[2] Charles Lutz,[3] Rachel Miller,[2] Alan Stolpin,[4] Lawrence Lu,[2] You Min Wu.[1] *[1]Surgery, University of Iowa, Iowa City, IA; [2]Medicine, University of Iowa, Iowa City, IA; [3]Pathology, University of Iowa, Iowa City, IA; [4]Radiology, University of Iowa, Iowa City, IA.*

The rapid recurrence of severe hepatitis C viral (HCV) hepatitis after liver transplantation remains a major problem. Evidence is accumulating that advanced donor age is a risk factor for early graft loss after liver transplantation for HCV. For renal transplantation, UNOS defines all organs from donors older than age 60 years old as expanded donors. Therefore, we have analyzed the effect of donor age on graft survival and histological outcome after liver transplantation for HCV. **Methods:** Using our longitudinal database (1991 onward), survival for all recipients of liver transplants for cirrhosis due to HCV was analyzed (n = 114 patients, n = 127 grafts). All liver transplant biopsies were analyzed (in a blinded fashion) and activity and fibrosis were graded according to the Ludwig scale. Severe activity was defined as moderate or worse activity and severe fibrosis was defined as bridging fibrosis or cirrhosis. Kaplan Meier survival analysis was performed for the entire population and after excluding patients who died or lost their grafts due to causes other than recurrent HCV. The log rank test was used to compare survival and Cox analysis was used to determine relative risks (RR per year of donor of age or age ≥ 60). **Results:**

	All Donor Ages					
	Graft	Survival	Severe	Activity	Severe	Fibrosis
Population	RR	p	RR	p	RR	p
All Recipients	1.02	0.009	1.02	0.02	1.03	0.001
NonHCV Losses Excluded	1.09	0.001	1.02	0.03	1.05	0.0001
	Donor Age ≥ 60					
	Graft	Survival	Severe	Activity	Severe	Fibrosis
Population	RR	p	RR	p	RR	p
All Recipients	1.5	0.4	2.0	0.08	2.6	0.02
NonHCV Losses Excluded	7.9	0.004	3.3	0.007	5.2	0.001

Inclusion of recipient age and sex, donor sex, cold ischemia time, and rejection in the multivariate model did not change the findings and did not reveal any other important variables. **Conclusions:** Liver transplant recipients with livers from older donors are at greater risk for early graft loss, early severe HCV activity, and early severe fibrosis. Rejection, cold ischemia time, and the other demographic variables did not appear to play a major role in early HCV recurrence.

Abstract# 562

ADULT AND PEDIATRIC LIVER TRANSPLANTATION FOR AUTOIMMUNE HEPATITIS. Thomas G. Heffron,[1] Gregory A. Smallwood,[2] Todd Pillen,[3] Brad Oakley,[3] David Welch,[3] Enrique Martinez,[4] Rene Romero,[4] Andrei C. Stieber.[1] *[1]Department of Surgery, Emory University School of Medicine, Atlanta, GA; [2]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [3]Department of Liver Transplantation, Children's Healthcare of Atlanta, Atlanta, GA; [4]Department of Medicine, Emory University School of Medicine, Atlanta, GA.*

Background: In the United States autoimmune hepatitis (AIH) affects between 100,00 and 200,000 persons and accounts for about 5.9% of transplant recipients. Recurrence of autoimmune hepatitis has been reported. **Aim**: The aim of this study was to evaluate pediatric patients being transplanted for AIH with adults being transplanted for the same disease. **Methods:** This is a retrospective review of both the adult and pediatric liver transplant programs consisting of 56 patients. Data collected included demographic, hospitalizations, steroid wean, recurrence of AIH, incidence of diabetes, colitis and survival. **Results**: Of the 56 AIH patients transplanted, 26.8% (15/56) were weaned off of steroids. When comparing the adult (n = 43) to the pediatric group (n = 13), adults were more likely to be weaned off of steroids (34.9%, p = 0.034). Diabetes did not improve with steroid weaning between groups [34.4% (11/32) vs. 30.7% (4/13); p = NS). Recurrence of AIH seemed to be more prevalent in the adult population [18.6% (8/43) vs. 7.7% (1/13), p = NS]. Fifty percent (50%) of the reviewed patients had rejections and indicated a 1.76 fold increase in relative risk to develop autoimmune recurrence [RR = 1.76; 95% CIRR (1.08, 2.86)]. The pediatric group had a 6.62 fold increase in relative risk to develop colitis following liver transplantation [RR = 6.62; 95% C.I.R.R. (1.36, 32.13); p = 0.02]. Mean days to recurrence were similar [1364 ± 1074 vs. 936; p = NS]. There was more hospitalized days in the pediatric group [20.5 (± 13.3) days vs. 51.7 (± 22.2) days, p = 0.039]. OKT-3 was rarely used (n = 5) in either group (9.3% vs. 7.7%, p = NS) and was not correlated to steroid weaning or recurrence. Of the 9 (16.1%) patients that had recurrence of AIH, only 3 patients had been weaned completely from steroids with a mean dose of 8.0 ± 6.4 mg/day. There were 23 (41.1 %) patients that received induction with daclizumab but only 2 of these patients were weaned from steroids. 3 year survival was similar (85.4% ± 0.05 vs. 92.3% ± 0.07). There was longer follow-up in the adult group [1469 (± 782) days vs. 865 (± 467) days, p = 0.02]. **Conclusions**: Based on this review, the pediatric patient was more likely to develop colitis following liver transplantation and tended to have less autoimmune recurrence. The adult group was more likely to be weaned from steroids. Additional studies are warranted to elucidate the differences between AIH populations.

Abstract# 563

RETRANSPLANTATION OF THE LIVER IN THE PEDIATRIC POPULATION: THE IMPACT OF EARLY VERSUS LATE GRAFT FAILURE ON OUTCOME. Gregory M. Tiao,[1] Maria Alonso,[1] John Bucuvalas,[1] Jorge Bezerra,[1] James Heubi,[1] Nada Yazigi,[1] William Balistreri,[1] Frederick Ryckman.[1] *[1]Pediatric Liver Care Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

The outcome of children who have undergone retransplantation (ReTxp) of the liver is worse than that of children who have undergone a single transplantation. Several studies have suggested that this difference may be due to a poor outcome in those children whose primary graft failed early and required ReTxp. We reviewed our experience in the management of children who have undergone ReTxp. **Methods:** A retrospective single center study was performed in which the charts of the thirty-eight patients who have required ReTxp were reviewed. Early graft failure was defined as ReTxp within four months of previous transplant. All others were considered late graft failure. Independent variables were primary diagnosis, age at retransplantation, number of acute rejections following primary transplant, type of graft used and type of immunosuppression. We performed univariate and multivariate analysis to identify independent predictors of mortality. **Results:** The incidence of retransplantation was 15% (38 of 247 patients). Three patients required a second ReTxp. The median age at ReTxp was 4.5yrs with a long-term follow-up of 5months-16 years. The cause of previous graft failure was chronic rejection 29%, hepatic artery thrombosis 27%, primary nonfunction 17%, bile duct complications 12%, acute rejection 5% and miscellaneous causes 10%. The one and five year patient survival was 73% and 45% respectively. Sepsis was the most common cause of patient mortality. Among the twenty-one who patients required early retransplantation, the mortality rate was 24%. In contrast, 53% of the patients who were retransplanted for late graft failure died ($p<0.03$). Despite the significant difference in outcome, no other independent variables that could predict mortality were identified. **Conclusion:** Patients who undergo retransplantation of the liver have a worse outcome than those who undergo a single liver transplant. Infectious complications were the most common cause of mortality in the ReTxp patient. In contrast to previous studies, we found patients whom experienced early graft failure had a better outcome than those patients who had late graft failure. Given the current shortage of organ donation, allocation of a second liver to patients with chronic graft loss requires careful consideration.

Abstract# 564

A COMPARATIVE and MULTIFACTORIAL ANALYSIS of RISK FACTORS in PEDIATRIC LIVER TRANSPLANTATION (PLTx) – PATIENT'S CONDITION MATTER, NOT GRAFT TYPE. Huda M. Noujaim,[1] Deirdre Kelly,[1] Sue V. Beath,[1] John A. Buckels,[2] David A. Mayer,[2] Darius F. Mirza,[2] Patrick J. McKieman,[1] Jean de Ville de Goyet.[1] *[1]Liver Unit, Birmingham Children's Hospital, Birmingham, United Kingdom; [2]Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Introduction: Controversy persists about that the use of variant liver graft types may be specifically associated with poorer outcome, independently of other factors. **Aims:** To review the outcome after PLTx using Full Size (FS), or Reduced (RL) and Split (SL) liver grafts, and analyze the role of various risk factors. **Patients and Methods:** 183 PLTx (169 patients) were reviewed retrospectively and 66 different parameters were used for -1- standard comparison between three groups (FS vs RL vs SL) and –2- multivariate analysis. **Results:** Significant differences between the three groups are detailed in Table. **Multifactorial analysis** indicates that independent risks factors are: Donor poor characteristics (hypotension and high AST levels), prolonged ischemic time, recipient condition at transplant (ITU bound and small age) and presence of complications after transplantation (primary non function, vascular complications, or reoperation for bleeding). Graft type was not an independent risk factor. **Conclusion** – RL and SL accounted for 2/3 of all PLTx, (72% after 1998) and were used for significantly smaller children than FS, with RS being used in recipient with worse clinical condition (higher PT and Bilirubin levels). Accordingly, outcome was lower with RL; however RL was not an independent and significant risk factor at multifactorial analysis. Overall, the analysis confirmed the important place of technical variant in PLTx. The results suggest that further progress might be expected with current approaches for improving recipient condition and increased technical expertise.

	Full Size	Reduce Graft	Split Graft	p
No PLTx / No Pts	66 / 60	72 / 67	45 / 42	
Period (1995-97)	40 (45%)	37 (42%)	12 (13%)	0.004
Period (1998-00)	26 (28%)	35 (37%)	33 (35%)	
Donor Weight (kg)	34.5±19.7	47.9±22.5	59.7±17.7	<0.0001
Recip pre PLTx - PT	26.9±20	30.2±28	20±11.6	0.03
Recip pre PLTx - Bilirubin (µmol/l)	170±229	228±200	186±196	0.06
Recipient Weight (kg)	31±17.8	16.2±13	13.6±9.2	<0.0001
Donor / Recipient Weight ratio	1.2±0.7	3.9±2.3	6.3±4.6	<0.0001
Waiting List (days)	84±102.9	90±129	81.5±116	0.97
Patient Survival (1 / 3 yrs)	93 % / 93 %	70 % / 68 %	88 % / 86 %	0.002
Graft Survival (1 / 3 yrs)	88 % / 88 %	67 % / 65 %	80 % / 75 %	0.008

CLINICAL ISLET TRANSPLANTATION

Abstract# 565

THREE-YEAR FOLLOW-UP IN CLINICAL ISLET-ALONE TRANSPLANT WITH THE EDMONTON PROTOCOL, AND PRELIMINARY IMPACT OF INFLIXIMAB AND CAMPATH-1H. James Shapiro, Edmond Ryan, Breay Paty, Charlotte McDonald, Peter Senior, Tara McCready, José Oberholzer, Norman Kneteman, Ray Rajotte, David Bigam, Kevin O'Kelly, Jonathan Lakey. *[1]Clinical Islet Transplant Program, University of Alberta, Edmonton, AB, Canada.*

43 C-peptide -ve T1DM patients (age 42±9, duration 25±11 years, weight 70 ± 9kg; pre-transplant insulin 0.6 ±0.2 U/kg/d) underwent islet alone transplants with 85 percutaneous transhepatic portal infusions at the University of Alberta. Treatments included Edmonton Protocol (EP) (n=31); infliximab (n=9); Campath-1H (n=3). Islets were prepared from cadaveric donor pancreata using controlled perfusion, Ricordi digestion and COBE purification (mean donor age 44 ± 13; cold ischemia 7.5 ± 4 hours; mean islet mass 369,940 ± 130,000 IE). More recently, islets were cultured in ITS-based supplemented media prior to transplant. Overall, insulin independence rate was maintained in 80% (one-year K-M survival, n=32 completed transplants), and C-peptide +ve rate was 89% (3-year K-M survival, n=43). Four patients lost C-peptide at 8.5, 9.0, 9.5 and 11.0 months respectively, from presumed autoimmune recurrence (2), acute rejection (1) and islet exhaustion (1). Mean HbA1C fell from 8.0% pre-transplant to 6.0% current ($p<0.001$). Mean C-peptide (fasting 2.3 ng/ml; stimulated 5.8ng/ml) remained stable across 3 years of follow-up. Insulin requirements fell by 1 U for every 16,400 IE transplanted islets. Recipient weight was reduced by a mean of 6% ($p<0.01$). Renal function remained unchanged from baseline in all but 2 cases with inadequate pre-transplant reserve, and rose despite replacement of tacrolimus with Cellcept. Serious complications included liver bleeds requiring transfusion (11%), hemobilia (2%), severe neutropenia <500 (5%), branch portal thrombus (2%), transient LFT rise (49%). There were no deaths, no malignancy, no PTLD and no CMV infections. Addition of infliximab (10mg/kg iv) reduced serum TNFα levels ($p<0.01$), lowered peak LFT rise and improved islet engraftment (AIRa 6.5 to 11.1, $p=0.05$) compared to concurrent EP controls. Campath-1H (20mg iv x 2) was given (n=3) without steroids, after pre-dosing with infliximab, with islets maintained in culture for 48 hours. Campath was well tolerated, and profound lympho depletion was maintained > 230 days (ALC 11%; CD3 6% of baseline). Islet graft function was substantially improved compared to controls. **Conclusion:** Three year follow up data and recent refinements with Campath and infliximab improved control and provided excellent outcomes in islet-alone recipients.

Abstract# 566

METABOLIC FUNCTION OF ISLET-ALONE TRANSPLANTS IS MAINTAINED BEYOND 2 YEARS POST-TRANSPLANT. Breay W. Paty,[1] Edmond A. Ryan,[1] Jonathan R. Lakey,[1] Sharleen Imes,[1] Charlotte MacDonald,[1] Peter A. Senior,[1] A. M. James Shapiro.[1] *[1]Departments of Medicine and Surgery, University of Alberta, Edmonton, AB, Canada.*

A total of 43 type 1 diabetes patients have received islet-alone transplantation at the University of Alberta. Of this group, complete 1-year post-transplantation metabolic data is available for 15 patients and 2-year data is available for 8 patients. Methods: Patients were transplanted using a standard immunosuppressive protocol including low dose sirolimus and tacrolimus between 1999 and 2001. Metabolic studies include intravenous and oral glucose tolerance tests and ensure-stimulated glucose and C-peptide tests. Data from 12 months (15 patients) and 24 months (8 patients) post-transplant are included for statistical analysis using one-way ANOVA testing. Results: Mean duration of follow-up for the group is 1.53 ± 0.13 years. At 12 months post-transplant (n = 15), mean acute insulin response to glucose (AIRg) was 12.3 ± 3.0 mU/l and fasting C-peptide was 0.60 ± 0.15 ng/ml. The glucose disposal constant (Kg) for the group was 0.90 ± 0.08 %. Basal and 90-minute ensure-stimulated glucose values were 6.4 ± 0.3 mM and 9.3 ± 0.8 mM respectively. Basal and 90-minute ensure stimulated C-peptide values were 1.69 ± 0.27 ng/ml and 3.14 ± 0.48 ng/ml respectively. After 1 year post-transplant, 2 patients had normal glucose tolerance. At 24 months post-transplant (n = 8), mean acute insulin response to glucose (AIRg) was 10.0 ± 3.7 mU/l and fasting C-peptide was 0.48 ± 0.15 ng/ml. The glucose disposal constant (Kg) for the group was 0.87 ± 0.07 %. Basal and 90-minute ensure-stimulated glucose values were 6.3 ± 0.5 mM and 9.2 ± 0.9 mM respectively. Basal and 90-minute ensure stimulated C-peptide values were 2.05 ± 0.54 ng/ml and 3.41 ± 0.79 ng/ml respectively. After 2 years post-transplant, one patient continued to have normal glucose tolerance. Two patients lost complete graft function (stimulated C-peptide negative) during the follow-up period. Reduction in AIRg was the earliest predictor of this loss. However, mean basal and stimulated glucose and C-peptide secretion was maintained in the islet transplant group over the entire testing period and at 1 and 2 years there were no statistical differences in graft function observed using the above tests. Conclusion: Islet-alone allotransplant recipients show evidence of persistent glucose intolerance but stable insulin secretory capacity sustained over 2 years post-transplant. A reduction in AIRg was the earliest predictor of islet graft dysfunction over time.

Abstract# 567

SUCCESSFUL SINGLE DONOR ISLET TRANSPLANTATION IN TYPE 1 DIABETES. Bernhard J. Hering,[1] Raja Kandaswamy,[1] Jeffrey D. Ansite,[1] Peter M. Eckman,[1] Masahiko Nakano,[1] Toshiya Sawada,[1] Ippei Matsumoto,[1] Sung-Hee Ihm,[1] Hui-Jian Zhang,[1] David E. R. Sutherland.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Islet transplantation is emerging as a viable treatment option for selected patients with type 1 diabetes. This pilot trial sought to determine whether optimization of pancreas preservation, islet processing, induction and maintenance immunosuppression would facilitate reversal of type 1 diabetes after single-donor islet transplantation. Pancreases were obtained from stable donors age <50 years and preserved for ≤8 hrs using the two-layer method. Islets were isolated using the automated method after perfusion of the pancreas with cold LiberaseÔ. Islets were purified on continuous iodixanol gradients, and cultured for 2 days. Islet preparations with viabilities >70%, total tissue volumes <10 cc, negative Gram stain, and endotoxin content <5 EU/kg were considered for transplant. 6,000 to 8,725 cultured islet equivalents/kg prepared from single donors were transplanted intraportally on day 0 following minilaparotomy or percutaneous transhepatic access into 8 C-peptide negative, non-uremic, type 1 diabetic patients with hypoglycemia unawareness. Immunosuppression was initiated 2 days before transplant and included rabbit antithymocyte globulin (through day +2, total dose 6 mg/kg), methylprednisolone (day –2 only), daclizumab, etanercept (through day +10), sirolimus, and reduced-dose tacrolimus (started on day +1). Tacrolimus was gradually replaced with mycophenolate mofetil starting one month posttransplant. All 8 transplant recipients achieved insulin independence with normal HbA1c levels and freedom from hypoglycemia. The time to insulin independence varied from 23 to 122 days. 5 of 8 patients have remained insulin-independent, with follow-up exceeding 1 yr in 2 patients. 3 of 8 patients have resumed exogenous insulin therapy preceded by subtherapeutic sirolimus trough levels (<10 ng/ml) in 3 and subtherapeutic MMF doses (1000 mg/day) in 2 of the 3 patients. Procedural complications, serious infections, or serious, unexpected, and islet- or immunosuppression-related adverse events were not encountered.The data suggests that a combination of maximized viable islet yield, islet culturing, preemptive induction immunosuppression including agents with anti-inflammatory properties, and nondiabetogenic maintenance immunosuppression can result in successful single-donor islet transplantation.

Abstract# 568

INSULIN INDEPENDENCE IN 13 PATIENTS FOLLOWING TRANSPLANTATION OF CULTURED HUMAN ISLETS. Rodolfo Alejandro,[1] Jacqueline V. Ferreira,[1] Tatiana Froud,[1] David A. Baidal,[1] Milene C. Geiger,[1] Muhammad Hafiz,[1] Lisa Rothenberg,[1] Ishmail Al-Abdullah,[1] Norma S. Kenyon,[1] Camillo Ricordi.[1] *[1]Diabetes Research Institute, University of Miami, Miami, FL.*

Introduction: Several clinical trials in islet transplantation are currently in progress utilizing fresh human islet allografts, including a multi-center trial. We proposed to assess the efficacy of in-vitro culture of human islets using an immunosuppression protocol similar to the one in Edmonton Methods & Results: 15 consecutive patients (7 males, 8 females) with type 1 DM received human islet allografts isolated using automated method with minor modifications, cultured in Miami defined media and administered via ultrasound guided percutaneous transhepatic intraportal infusion. Immunosuppression consisted of daclizumab induction and sirolimus/tacrolimus maintenance. 12 patients received 2 islet infusions; 3 patients received one infusion, 1 became insulin independent and 2 patients are awaiting second infusion. First transplant (Tx) averaged 7040±1962 IEQ/kg (n=13; range 4592-12013) and second Tx 7180±2113 IEQ/kg (n=12; range 3577-10306), with a total of 13667±3130 IEQ/kg (n=13; range 6250-18028). All patients achieved immediate insulin independence after second Tx and were free of insulin at 6 months. Glycosylated hemoglobin A1c decreased from 7±1% (n=13; range 6.2-9.4) to 6±0.5% (n=13; range 4.5-6.6). There were no episodes of hypoglycemia after discontinuation of insulin. 8 patients have remained insulin independent with stable islet allograft function and glycemic control and are now 526, 525, 420, 408, 374, 313, 256 and 179 days post first islet infusion. 5 patients had worsening in glycemic control and were restarted on insulin (34±11% of pre Tx dose; n=5; range 18-46%) on post Tx day 356, 399, 208, 430 and 243. At 1 year post-transplant, 69% of recipients were free of exogenous insulin, and the remainder 31% still had significant islet function but required insulin complementation. Conclusions: Human islet allografts cultured in Miami defined media resulted in insulin independence in 13 patients with normalization of glycemic control under steroid free immunosuppression regimen. However, there seems to be a partial loss of islet function or islet mass, similar to what is seen with fresh islets, which mechanisms need to be clarified.

Abstract# 569

FACTORS THAT CORRELATE WITH INSULIN INDEPENDENCE FOLLOWING SINGLE INFUSION ISLET TRANSPLANTATION. James F. Markmann,[1] Shaoping Deng,[1] Xiaolun Huang,[1] Adam Frank,[1] Ergun Velidedeoglu,[1] Niraj M. Desai,[1] Bryan Wolf,[3] Franz Matschinsky,[2] Nicolai Doliba,[2] Marko Vitamaniuk,[2] Clyde F. Barker,[1] Ali Naji.[1] *[1]Surgery, University of Pennsylvania, Philadelphia, PA; [2]Biochemistry, University of Pennsylvania, Philadelphia, PA; [3]Pathology, Children's Hospital of Philadelphia, Philadelphia, PA.*

Introduction: The Edmonton protocol demonstrated that consistent insulin independence was achievable with islet transplantation if an islet mass of >9000 IEq's/kg recipient is met. However the fact that success required multiple islet infusions procured from multiple cadaveric donors may limit application of this therapy. We detail our experience in 9 patients, of which 5 acheived insulin independence with a single infusion. **Methods:** Our center has performed 145 isolations since 2/00, of which 23 were performed with the intent to transplant. Fifteen preparations were transplanted to 9 patients by 12 infusions, and 8 preparations did not meet adequate transplant yield (n=6) or quality (n=2). Patients were treated with immunosuppression consisting of Daclizumab induction and maintenance sirolimus and tacrolimus. **Results:** Of 9 patients transplanted, 7 have completed the protocol and each has become insulin independent. Of the two patients not completing the protocol, one was transplanted recently and awaits a second dose and one was withdrawn from the study (after an unsuccessful first dose) due to a non-healing traumatic foot wound. Of the 7 completing treatment, 5 achieved insulin independence with a single infusion (3 from a single donor and 2 from 2 donors). Analysis comparing factors in those first doses resulting in insulin independence (n=5) with those that did not (n=4) indicated that the islet mass infused was equivalent (616,200 vs. 602,250 IEq's), and that success tended to be correlated with larger donors (101 kg vs. 90.9 kg) and smaller recipients (65.5 kg vs. 72.0 kg). Recipients of single infusion successes differed significantly ($p<0.05$) from patients not achieving insulin independence in that their average daily pretransplant insulin requirement was less (32.2 U/d vs. 47.5 U/d). Success was also correlated retrospectively with assessment of islet function by in vitro perifusion assays but did not correlate with static insulin release assays. **Conclusions:** Insulin independence following isolated islet transplantation can be achieved with a single infusion of an adequate islet mass in appropriate recipients with high quality islets. Additional improvements in the efficiency of the isolation procedure and engraftment post-transplant are required so islets isolated from each cadaveric donor can be used optimally.

Abstract# 570

COMPLICATIONS RELATED TO INTRAPORTAL ISLET INFUSION IN AUTOLOGOUS AND ALLOGENEIC ISLET TRANSPLANTATION. Pascal Bucher,[1] Zoltan Mathe,[1] Domenico Bosco,[1] Axel Andres,[1] Christoph Becker,[2] Laurence Kessler,[3] Michel Greget,[4] Jose Oberholzer,[1] Leo H. Bühler,[1] Philippe Morel,[1] Thierry Berney.[1] *[1]Cell Isolation and Transplantation Center, Geneva University Hospital, Geneva, Switzerland; [2]Radiology, Geneva University Hospital, Geneva, Switzerland; [3]Diabetes/Endocrinology, CHU, Strasbourg, France; [4]Radiology, CHU, Strasbourg, France.*

Aims: To investigate the morbidity rate of human intraportal islet infusion and their relation to the characteristics of the islet preparation and the infusion technique. Methods: Review of all intraportal islet infusions (from 1992 to 2002) for auto- or allo-transplantation (Tx) performed by our group in order to evaluate the morbidity rate of this procedure. Correlation of islet preparation characteristics to complications was analyzed. Results: During the study period, 16 islet auto-Tx were performed. No complication related to the islet infusion was recorded, eventhough the tissue volume injected was 13 ± 11ml. Basal and peak portal pressure were 13 ± 6 and 21 ± 6 mmHg. The rise in portal pressure was correlated to the volume of tissue injected ($p<0.05$). Sixty-eight intraportal allogeneic islet infusions were performed into 45 patients. 14 islet infusions were done as an open procedure during simultaneous islet/kidney transplantation, no complication being recorded in this group. Of 54 percutaneous intraportal infusions, 5 complications and no death were recorded (morbidity 7%). Complications were 2 portal branch thromboses and 3 intra-abdominal hemorrhages. One infusion was not completed because of an excessive increase in portal pressure. Basal and peak portal pressures were 14 ± 5 and 18 ± 6 mmHg in uncomplicated infusions, 15 ± 10 and 18 ± 9 mmHg in the portal thrombosis group, and 14 ± 4 and 18 ± 0 mmHg in the hemorrhage group. The rise in portal pressure was correlated to the volume of tissue injected ($p<0.05$). The mean tissue volume injected during uncomplicated infusion was 5.3 ± 4.4ml, as compared to 6.1 ± 2.6ml for the thrombosis group and 5.8 ± 2.6ml for the hemorrhage group. Complications occurred only after percutaneous infusion ($p=0.08$). Both portal thromboses resolved completely with anticoagulant therapy. Two of 3 patients with hemorrhage required blood transfusion, 1 of whom underwent coil embolization of an arterio-portal fistula and percutaneous drainage of the hemoperitoneum. Conclusions: Morbidity of intraportal islet infusion is low. Changes in portal pressure are related to the volume of the islet preparation infused, which does not seem to be associated with the occurrence of complication, as illustrated by the safety of islet auto-Tx in spite of large volumes of tissue infused.

Abstract# 571

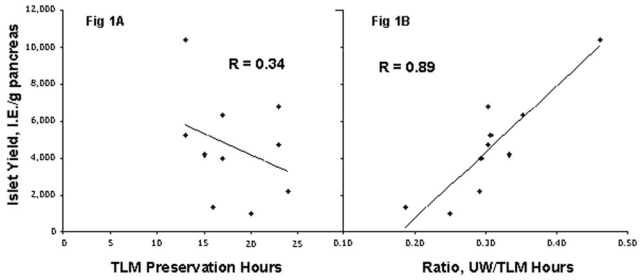
STANDARDIZATION OF HUMAN ISLET ISOLATION FOR A INTERNATIONAL MULTI-CENTER TRANSPLANT TRIAL. J. R. T. Lakey,[1] C. Ricordi,[2] B. Hering,[3] B. DiMercurio,[4] R. Lindblad,[5] J. O'Neil,[6] B. Olack,[7] J. A. Reems,[8] J. Ansite,[3] T. Kirlew,[2] H. Brandhorst,[9] F. Bertuzzi,[10] P. Bucher,[11] J. Oberholzer,[11] E. Ramos,[12] L. Viviano,[4] A. M. J. Shapiro.[1] *[1]Clinical Islet Program, University of Alberta, Edmonton, AB, Canada; [2]Diabetes Research Institute, University of Miami, Miami, FL; [3]University of Minnesota, Minneapolis, MN; [4]National Institute of Health, Bethesda, MD; [5]EMMES Corporation, Bethesda, MD; [6]Massachusetts General Hospital, Boston, MA; [7]Washington University, St Louis, MO; [8]Pacific Northwest Research Institute, Seattle, WA; [9]Justus-Leibig University, Giessen, Germany; [10], Milan, Italy;, [11]University of Geneva Hospital, Geneva, Switzerland; [12]Immune Tolerance Network, San Francisco, CA;*

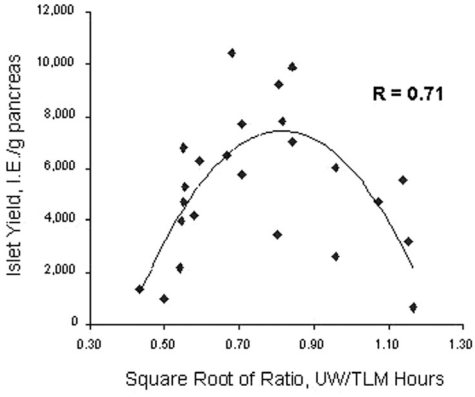
Consistency and reproducibility of human islet isolations have limited progress in the field of clinical islet transplantation. The Immune Tolerance Network (ITN) multi-center islet transplantation trial using the Edmonton Protocol, sought to define and validate islet isolation procedures amongst the nine participating groups. This was the first attempt at an international multi center trial for islet transplantation. The steps involved in defining and reproducing islet isolations involve developing a protocol, distribution of standard operating procedures including those used to enumerate islets, on-site inspection of facilities and isolation procedures, and review of qualifying data. A major challenge was the identification of suitable lots of Liberase enzyme for this trial. This involved pre-selection of large lots of enzyme, batch testing in investigational sites and distribution of selected/validated lots to each of the isolation groups. Each lot was required to meet qualifying preparation standards prior to inclusion in this trial. A total of 158 organs have been processed at the nine centers to date. Of the total isolations, 48 (27% of total) met the criteria for inclusion in the multicentre transplant trial. An additional 13 (9%) were transplanted under local protocols. Approximately 42% (97) of the isolations, failed to meet acceptable levels of islet recovery or viability. This includes the 30 preparations (22%) that were used for the qualifying stages of the trial. A total of 49 islet transplants have been performed in 32 patients to date as part of the ITN protocol. The mean mass of human islets isolated from those isolations that were transplanted is 377,256 +/- 82,256 (mean +/- SEM). Standardization of human islet isolation remains a difficult challenge. Each organ has unique donor characteristics and there are inconsistencies in enzyme lots, and variability of isolation techniques at each center. This trial has made great strides in standardizing islet enumeration, development and implementation of standard operating procedures and defining minimal site and staffing requirements to isolate human pancreatic islets for clinical transplantation.

Abstract# 572

RESUSCITATING UW-PRESERVED MARGINAL HUMAN PANCREATA WITH OPTIMIZED TWO-LAYER METHOD (TLM) PRESERVATION. R. Brian Stevens,[1] Theodore H. Rigley,[1] Jo Anna Reems.[2] [1]*Surgery, University of Nebraska Medical Center, Omaha, NE;* [2]*Puget Sound Blood Center, Seattle, WA.*

Background TLM resuscitation improves viable islet yield from UW-stored marginal human pancreata. Others have asserted the overall superiority of short (~5h) vs. longer (~14h) TLM preservation. We hypothesized that optimal pancreas resuscitation results from proportioning TLM resuscitation to the duration of UW storage. ***Methods*** Islets, isolated using Liberase and Pefabloc from human pancreata preserved in UW (n=11, 26±3h) or after conversion to TLM preservation (n=23, 29±5h), were assessed for viable yield and function. ATP content in preserved pancreata, and rate of ATP accumulation, were evaluated as markers of resuscitation. ***Results*** TLM preservation increased viable islet yield by 150 % (p=0.002) and correlated with ATP content (R=0.83,p=0.005) and accumulation rate (R=0.72, p=0.07). Duration of TLM preservation and islet yield did not correlate (Fig.1A), but for short UW times(<8h), a correlation existed between islet yield and the ratio of UW to TLM time (Fig.1B). For all pancreata, a polynomial correlates yield to the square root of the UW/TLM hours ratio (Fig 2). ***Conclusions*** TLM resuscitation time needs to be individualized against the duration of the cold ischemic injury of each pancreas, and the TLM interval should be between 1– 2X the time spent in UW storage. This relationship begins to fail when total preservation time (UW+TLM) exceeds 24 hours.

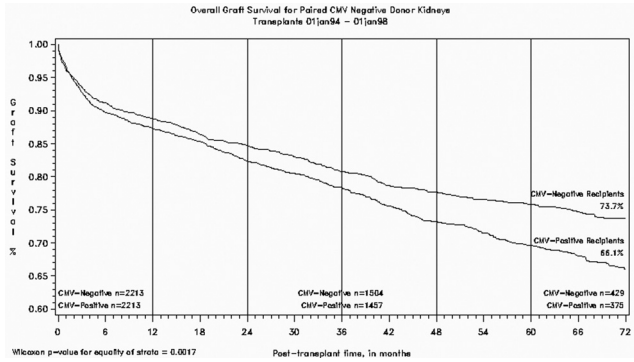




Abstract# 573

CMV MATCHING IS ASSOCIATED WITH SIGNIFICANTLY BETTER GRAFT SURVIVAL: A PAIRED KIDNEY ANALYSIS. Rajendra Baliga, Jesse Schold, Bruce Kaplan, Herwig-Ulf Meier-Kriesche. [1]*Department of Internal Medicine, University of Florida, Gainesville, FL.*

Cytomegalovirus (CMV) infection after renal transplantation is associated with increased risk for acute rejection, and may both directly and indirectly contribute to allograft dysfunction. CMV negative donor kidneys may have better outcomes when reserved for CMV negative recipients. To test this hypothesis we analyzed 4426 CMV negative paired donor kidneys of whom one went into a CMV negative recipient and the other went into a CMV positive recipient. We based this analysis on data provided by the USRDS on first cadaveric renal transplants executed between 1994 and 1998. Primary study endpoints were patient survival, death censored graft survival and overall graft survival. Univariate Kaplan Meier and multivariate Cox hazard regression models were utilized to investigate the impact of CMV serological status of the recipient on the CMV negative graft. We also analyzed pairs of CMV positive donors who distributed kidneys to CMV positive and CMV negative recipients. Kaplan- Meier survival curves showed that CMV negative kidneys had a significantly shorter 6-year graft survival when transplanted into CMV positive recipients as compared to CMV negative recipients (73.7% vs 66.1%, p<0.01). These results were confirmed by the multivariate analysis. The relative risk for death censored graft failure of a CMV negative donor kidney was 1.18 (CI=1.01-1.40), and for overall graft survival 1.13 (CI=1.00-1.28), when transplanted into a CMV positive recipient as compared to a CMV negative recipient. In summary CMV negative kidneys have better graft survival when transplanted into CMV negative recipients as compared to CMV positive recipients.



Abstract# 574

SAFETY AND EFFICACY OF VALGANCICLOVIR FOR CMV PROPHYLAXIS IN HIGH-RISK KIDNEY AND PANCREAS TRANSPLANT RECIPIENTS. A. K. Sundberg,[1] M. S. Rohr,[2] P. L. Adams,[3] R. J. Stratta.[2] [1]*Pharmacy;* [2]*General Surgery;* [3]*Medicine, Wake Forest Univ, Winston-Salem, NC.*

Background: Despite advances in serologic monitoring and anti-viral therapy, CMV remains an important source of morbidity in transplant recipients. Valganciclovir (VGC), the oral pro-drug of ganciclovir, has been shown to be safe and effective in the treatment of CMV retinitis. However, dosing guidelines for renal insufficiency have not been well established, and little data are available evaluating VGC for preventing CMV in kidney (KTX) and pancreas transplant (PTX) patients (pts). The purpose of this study is to evaluate the safety and efficacy of VGC for prevention of CMV infection in high-risk KTX and PTX recipients (R). **Methods:** Beginning 10/1/01, universal prophylaxis with VGC was used routinely in our high risk KTX and PTX pts. CMV seronegative Rs who received an allograft from CMV seropositive donors (D) were given VGC 900 mg daily for 6 months, with selective CMV-IGIV administration. D+/R+ and D-/R+ serologic combinations were given VGC 450 mg daily for 3 months. D-/R- KTX pts did not receive VGC, but D-/R- PTX patients received VGC 450 mg daily for 3 months. **Results:** During a 13 month study period, a total of 67 pts received VGC prophylaxis. The study group included 39 cadaveric KTXs, 14 living-donor KTXs, 10 K-PTXs, and 4 sequential PTX after KTXs. The distribution of CMV serology was as follows: 17 D+/R- (24%), 25 D+/R+ (38%), 19 D-/R+ (29%), and 6 D-R- (9%). A total of 52 pts (78%) received Thymoglobulin induction. All but 2 pts (3%) received maintenance therapy with FK, MMF, and prednisone. Patient, kidney, and pancreas graft survival rates are 98%, 92%, and 85%, respectively, with a mean follow-up of 5 months. The incidence of acute rejection was 4.5%. There were no cases of documented CMV infection in these pts. No major side effects of VGC were noted. Dosage adjustments for hematologic reasons occurred in 12 pts (18%), including 10 for leukopenia and 2 for thrombocytopenia. Mean white blood cell count nadir was 2400/ mm³, hemoglobin nadir was 9.8 g/dL, and platelet nadir was 176,000/ mm³. There were no reported cases of renal dysfunction directly attributed to VGC, but in pts with dosage adjustments for hematologic reasons, the mean serum creatinine was 2.0 mg/dL. **Conclusions:** VGC is a safe and effective agent for the prevention of CMV infection in high-risk KTX and PTX pts. More data are needed to assess the proper dosing of VGC in pts with renal insufficiency, particularly in the setting of potentially nephrotoxic immunosuppression.

Abstract# 575

VALGANCICLOVIR FOR PREVENTION OF CMV DISEASE: 12 MONTH FOLLOW UP OF A RANDOMIZED TRIAL OF 364 D+/ R- TRANSPLANT RECIPIENTS. M. D. Pescovitz,[1] C. Paya,[2] A. Humar,[3] E. Dominguez,[4] K. Washburn,[5] E. Blumberg,[6] B. Alexander,[7] R. Freeman,[8] N. Heaton,[9] C. Woodruffe-Peacock,[10] K. Macey,[10] R. Tansley,[10] Valganciclovir Study Group. *[1]Indiana Univ Med Cent; [2]Mayo Clinic, Rochester; [3]Univ of Toronto; [4]Univ of Nebraska Med Cent; [5]Univ of Texas Health Cent; [6]Univ of Pennsylvania; [7]Duke Univ Med Cent; [8]New England Med Cent; [9]King's College, London; [10]Roche Products Ltd.*

Purpose: Valganciclovir (VGCV) is a highly (60%) bioavailable prodrug of ganciclovir. Oral VGCV prophylaxis has been shown to be as efficacious as oral GCV in CMV disease prevention up to 6 mths in solid organ transplant recipients. The study's aim was to assess the 1 yr comparative efficacy/safety of these 2 regimens. **Methods:** Adult patients were stratified by organ type and randomized 2:1 to VGCV 900mg QD or GCV 1000mg TID adjusting for CrCl. Therapy started within 10 days post transplant and continued through day 100 with regular follow up to 12 mths. **Results:** 364 D+/R- SOT (177 liver, 120 kidney, 11 kidney-pancreas, 56 heart) patients were included in the ITT population. Incidence of Independent Endpoint Committee adjudicated CMV disease during the first 12 mths was 17.2% (41/239) with VGCV and 18.4% (23/125) with GCV (95% C.I. -0.07, 0.10). CMV disease incidence 6 mths post-transplant was 5% (12/239) in VGCV and 3.2% (4/125) with GCV. A recurrence of CMV following successful initial treatment occurred in 1 patient. While fewer patients developed viremia on VGCV compared with GCV, the proportions of patients with a measureable viral load (>400 copies/mL) by 6 and 12 mths were comparable (40% and 49% with VGCV vs 43% and 50%). Principle toxicity was neutropenia occurring later in the treatment period. In 8 patients where neutropenia/leucopenia resulted in discontinuation of study drug, cell counts returned to normal on cessation of therapy without sequelae. Neutropenia (<1000 cells/μL) was more common in patients receiving MMF (13.1 % concomitant MMF vs. 5.4% no MMF; p=0.028). 12 mths post-transplant, 6.1% of patients on VGCV died compared with 6.3% on GCV; no deaths were considered related to study drug. No related adverse events were reported in the second 6 mths post-transplant. **Conclusions:** The incidence of CMV disease in the first year post-transplant following VGCV prophylaxis was comparable to that observed with oral GCV. There was no significant increase in the incidence of late CMV disease (diagnosed between 6 and 12 mths) between the 2 arms. The overall safety profiles of the 2 regimens were comparable, with the most important event being neutropenia which was reversible on cessation of therapy. VGCV offers a well-tolerated, efficacious alternative to GCV with a more convenient dosing regimen.

Abstract# 576

HCV-RELATED KIDNEY DISEASE AFTER KIDNEY TRANSPLANTATION. John D. Pirsch,[1] Bryan N. Becker,[1] Yolanda T. Becker,[1] Jon S. Odorico,[1] L. Thomas Chin,[1] Stuart J. Knechtle,[1] Anthony M. D'Alessandro,[1] Hans W. Sollinger.[1] *[1]Departments of Medicine and Surgery, University of Wisconsin Medical School, Madison, WI.*

We examined the outcomes of HCV positive recipients receiving an HCV positive or negative kidney, HCV negative recipients receiving an HCV positive kidney and patients without HCV infection receiving a cadaver transplant at our center since 1990. The numbers of patients in each category is shown below.

D-/R-	D-/R+	D+/R-	D+/R+	Total
1139 (83%)	43 (3%)	115 (9%)	71 (5%)	1368

There were significant demographic differences in the four groups. Criteria developed for HCV+ donors into HCV- recipients included patients with a high risk for death on dialysis. These patients were older (average age 55 yrs) and 50% were diabetics (p=0.001). More retransplants were performed in the D-/R+ and D+/R+ groups (p=0.001). Blacks accounted for 21% of the D-/R+ and 45% of the D+/R+ groups compared to 8% of D-/R- and 6% of D+/R- groups (p=0.001). More HCV kidneys were transplanted before 1996 (p=0.001). Because of the differences in the groups, the analysis was adjusted for these covariates. Adjusted graft survival and patient survival were poorer in all of the groups with HCV infection prior to transplant or receiving a HCV positive kidney transplant (p=0.001). The 10 year survivals are shown below.

	D-/R-	D-/R+	D+/R-	D+/R+
Graft survival	50%	15%	23%	29%
Patient survival	72%	55%	50%	51%

Unadjusted patient death rate was similar for all groups except for the D+/R- cohort (66% died during follow-up). Only six patients died of liver failure during follow-up: 4 D+/R-, 1 D-/R+, 1 D-/R-. There was no difference in death from sepsis or malignancy. There were no statistical differences in infection or acute rejection between the four groups. There was a significant difference in the incidence of CAN between the groups (p=0.001). At 10 years, there was a 23% incidence of biopsy-proven CAN in the D-/R- group. This compared to an incidence of 41% in the D-/R+, 44% in the D+/R-, and 43% in the D+/R+ groups. The incidence of de novo glomerulopathy, recurrent disease, and MPGN was similar in all four groups. It would appear from this data that there is a significant risk for graft loss in the late transplant period due to CAN. There is also a significant mortality risk in patients with HCV infection or in recipients who are HCV negative and receive a HCV positive kidney transplant.

Abstract# 577

A PROSPECTIVE STUDY OF HEPATITIS C NEGATIVE RECIPIENTS OF HEPATITIS C POSITIVE KIDNEYS - TEN-YEAR FOLLOW-UP. Ronald P. Pelletier,[1] Baris Akin,[1] Mitchell L. Henry.[1] *[1]General Surgery, The Ohio State University College of Medcine, Columbus, OH.*

The use of renal allografts from cadaveric donors with serologic evidence of previous infection with the hepatitis C virus continues to be debated. Our transplant program previously reported a prospective study involving 42 hepatitis C negative cadaveric kidney recipients transplanted between 2/15/90 and 2/15/93 that had received a kidney from a donor with positive hepatitis C serology (Study group) (Transplantation 1994; 57(6): 826-31). This group was compared to a cohort of 104 hepatitis C negative control patients transplanted during the same period of time who received a kidney from a donor with negative hepatitis C serology (Control group). This study found that while the rate of hepatitis C transmission was high (56% by PCR) in the study group, the incidence of abnormal LFTs (22%) and acute hepatitis (6.5%) was no different than that seen in the Control group. At the time of the analysis in 1993, there were 89 patients continuing to be followed in the control group and 34 in the study group. From that time through 12/02 there has been a similar incidence in the Study and Control groups in regards to patient death (20.6% and 21.6% respectively) and graft loss (11.8% and 8% respectively). In addition, the incidence of persistently elevated AST was similar (11% and 3% in the Study and Control groups respectively, p=ns), as was the incidence of a persistently elevated ALT (8.7% and 3% in the Study and Control groups respectively, p=ns), and hyperbilirubinemia (3.7% and 3% in the Study and Control groups respectively). Importantly, no patient in the Study group has died due to complications related to hepatitis C infection. We conclude that with 10 years of follow-up, there is no evidence of an increased hepatitis C-related or unrelated morbidity or mortality following transplantation with a kidney obtained from a cadaveric donor with serologic evidence of hepatitis C infection.

Abstract# 578

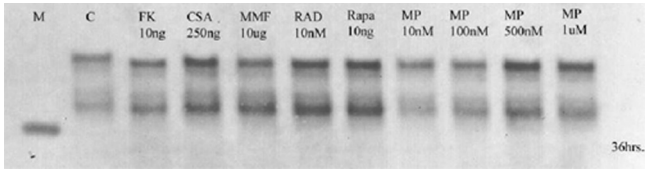
EVIDENCE THAT RECOVERY OF HEPATITIS C VIRUS INFECTION AFTER α-INTERFERON THERAPY IN DIALYSIS PATIENTS DO NOT RELAPSE AFTER RENAL TRANSPLANTATION. Nassim Kamar,[1] Olivier Toupance,[2] Mathias Buchler,[3] Karine Saune,[4] Jacques Izopet,[4] Dominique Durand,[1] Lionel Rostaing.[1] *[1]Nephrology, Dialysis and Transplantation, CHU Toulouse-Rangueil, Toulouse, France; [2]Department of Nephrology, CHU, Bretonneau hospital, Reims, France; [3]Nephrology, CHU, Maison Blanche Hospital, Tours, France; [4]Department of Virology, CHU Toulouse-Purpan, Toulouse, France.*

To date, there is no available treatment of HCV infection after renal transplantation (RT). Fifteen HCV-positive / RNA-positive hemodialysis patients (M/F :12/3 ; median age 46 years) received α -interferon (α -IFN) (9MU/week) during 6 or 12 months (3 and 12 patients respectively). Thirty-eight (2—57) months after the end of α-IFN therapy they underwent a renal transplantation (RT). At RT, HCV serology was positive in 14 patients and HCV viremia was negative in all patients. Immunosuppression relied on anticalcineurin agents with or without steroids and/or antimetabolites ; in addition eleven of them received induction therapy with antithymocyte globulins. At the last follow-up after RT (23 (2—88) months), HCV serology was found to be still positive in only 12 patients and HCV viremia remained negative in all patients. Moreover, HCV RNA was not present in monocluear cells when assessed in 8 patients. Serum AST, ALT and γ -GT levels were respectively 23 (10—46) IU/L, 18 (11—74) IU/L and 28 (11—107) IU/L at last follow-up as compared to 14 (7—41) IU/L, 17 (6—77) IU/L and 17 (13—79) IU/L at RT ($p > 0.05$). Three patients presented an acute rejection, one presented a suppurative lymphocele, one died from a sepsis and four presented a cytomegalovirus infection. In conclusion, hemodialysis patients waiting for a RT have to be treated by α-IFN since when HCV RNA clearance occurred, they do not relapse after transplantation despite chronic immunosuppressive treatment.

Abstract# 579

MODULATION OF HEPATITIS B VIRAL REPLICATION BY DIFFERENT IMMUNOSUPPRESSIVE DRUGS IN HEPATOCYTE CULTURE. Chiu-Ching Huang, Chau-Ting Yeh. *[1]Department of Nephrology, Chang-Gung University, Taipei, Taiwan; [2]Department of Hepatology, Chang-Gung University, Taipei, Taiwan.*

A1MS: Our previous study revealed 75% of HBV(hepatitis B virus) carriers developed HBV reactivation on a cyclosporine based immunosuppression after renal transplantation,some even developed life-threatening fibrosing cholestatic hepatitis. Understanding how immunosuppressive drugs influence HBV replication may be of considerable clinical relevance in the selection of immunosuppressive drugs for these patients. Our aim is to determine how these drugs influence intrahepatic HBV replication in HepG2(HR8) hepatocyte culture. **METHODS :** HepG2(HR8) is a hepatoma cell line transfected with HBV ,stably producing HBV DNA and all HBV antigens. HepG2(HR8) cells were subcultured for 4 days, then incubated for 36 hours with various concentrations of methylprednisolone (10^{-6}-10^{-10}M), cyclosporin-A (10-1000ng/ml), tanolimus(1-1000ng/ml), mycophenolate mofetil (10^{-4}-10^{-7}M), sirolimus (10^{-6}-10^{-10}M) and SDZ RAD (10^{-6}-10^{-10}M) individually. Intracellular HBV DNA were assessed using Southern blot hybridization analysis. The amount of intracellular HBV DNA were quantified using a BAS 2000 image analyzer. . **RESULTS :** Tacrolimus had no effect on intracellular HBV DNA production. Methylprednisolone, cyclosporin-A, mycophenolate mofetil, sirolimus and SDZ RAD all increased intracellular HBV DNA production in a dose-dependent fashion(Fig1). **CONCLUSION :** Tacrolimus had no effect on intracellular HBV DNA production in HepG2(HR8) hepatocute culture. Renal transplant recipients with history of HBV infection may select tacrolimus as the first priority for immunosuppression.



Abstract# 580

WEST NILE VIRUS ENCEPHALITIS IN ORGAN TRANSPLANT RECIPIENTS: ANOTHER HIGH RISK GROUP FOR MENINGOENCEPHALITIS AND DEATH? D. DeSalvo,[1] P. Roy-Chaudhury,[1] T. Merchen,[1] K. Konijetti,[1] M. Gupta,[1] R. Boardman,[1] C. Rogers,[1] M. Hanaway,[1] J. Buell,[1] K. Konoplev,[2] R. Smith,[2] E. S. Woodle,[1] R. Peddi.[1] *[1]Division of Transplantation, University of Cincinnati, Cincinnati, OH; [2]Dept. of Pathology, University of Cincinnati, Cincinnati, OH.*

West Nile Virus (WNV) is a mosquito borne flavivirus that is a human, equine and avian neuropathogen. Over the last three years WNV infection has been spreading westward across the continental United States with transmission to humans thought to be predominantly from mosquito bites (although blood transfusions and organ transplants have also been implicated). Patient populations at increased risk for meningoencephalitis include the elderly and possibly, immunosuppressed patients. We report herein on two very similar neurologic presentations of WNV encephalitis in immunosuppressed renal transplant patients, albeit with very different clinical outcomes. Both patients were Caucasian males in their forties, on standard immunosuppressive therapy who presented within nine months of transplantation with a viral prodrome of fever, gastrointestinal symptoms and headache. This rapidly progressed into an aggressive encephalitis with marked obtundation and impressive MRI findings of mid brain and thalamic edema. Indeed in our second patient we considered the MRI findings to be diagnostic of WNV encephalitis, even before serological confirmation. In both patients the first definitive confirmation of WNV infection was made by an IgM ELISA analysis of the CSF. Our first patient underwent a rapid neurological decline and died two weeks following admission, despite aggressive supportive care and discontinuation of immunosuppression. Our second patient (who presented two weeks later), was treated with a complete cessation of immunosuppression, and also enrolled into an experimental alpha interferon trial based on the typical MRI findings (prior to serological confirmation). This patient had a marked improvement in mental status and currently has a near normal lifestyle. He did not lose his kidney to rejection and his serum creatinine is at his pre-WNV baseline level. **Conclusions:** (1) WNV infection can cause an aggressive encephalitis in immunosuppressed transplant recipients (2) WNV encephalitis has diagnostic MRI findings of mid-brain and thalamic edema (3) All immunosuppression should be discontinued and patients should be aggressively supported (4) Initial serological confirmation should be made with an IgM ELISA on the CSF or blood (5) Strong consideration should be given to enrollment into a recently initiated clinical trial of alpha interferon.

Abstract# 581

INFLUENZA VACCINATION PRACTICES IN THE TRANSPLANT COMMUNITY. Rajiv D. Poduval,[1] Michelle A. Josephson.[1] *[1]Department of Medicine, Section of Nephrology, University of Chicago, Chicago, IL.*

Introduction: Despite the availability of the influenza vaccine, and recommendation to vaccinate transplant (TXP) recipients, immunization practices are not standardized in the TXP community. Influenza, a common infection among TXP recipients, can result in fatal pneumonitis in this population. **Aim:** To study current influenza vaccination practices prevalent in the TXP community. **Methods:** A survey questionnaire that dealt with influenza vaccination practices was administered to caregivers in 301 UNOS certified TXP centers in the United States. Participation in the survey was voluntary, and no financial remuneration was offered for completing the survey. **Results:** Out of 301 TXP centers contacted, 170 (56%) returned the completed survey. Of the returned surveys, 78/170 (46%) were completed by surgeons, 54/170 (32%) by TXP nephrologists, 30/170 (18%) by nurse coordinators, and the remaining by administrators and other specialists. The majority of programs provided long-term post-TXP primary care for their patients. 118/170 (69%) centers provided post-TXP care for > 6months, and 111/170 (65%) provided care for > 1 year. Of the 14 (8%)centers that did not routinely vaccinate patients, 7/14 (50%) attributed it to general safety concerns, 5/14 (36%) to adverse effects, and 13/14 (93%) to the poor response to vaccination seen in the TXP population. Adverse effects to the vaccine were reported by 32/170 (19%) centers. These included flu like illness reported by 26 centers, and acute rejection, by 10 centers. The results are given below:

Influenza vaccination Practices in 170 Transplant Centers

Vaccination Practice	Yes	No
1. Program recommends vaccination	156 (92%)	14 (8%)
2. Program routinely vaccinates kidney TXP recipients	143 (84%)	27 (16%)
3. Program vaccinates family members of patients	37 (22%)	133 (78%)
4. Program vaccinates TXP clinic staff	71 (42%)	99 (58%)
5. Person completing survey takes influenza vaccine	103/160 (64%)	57/160 (36%)

Conclusion: 1. The influenza vaccine is not universally administered to all TXP recipients in the United States. **2.** Moreover, the vaccine is not routinely offered to the immediate family of TXP patients, or to medical personnel working in TXP clinics. **3.** Interestingly, a large number of medical personnel do not use the influenza vaccine themselves, despite the potential risk of transmitting infection to their immunocompromised patients. **Clinical Relevance:** Given the potentially life-threatening manifestations of influenza in the TXP population, the administration of the influenza vaccine should be universally implemented by the TXP community.

KIDNEY: ALLOCATION AND PUBLIC POLICY

Abstract# 582

EQUALIZATION OF DISPARATE WAITING TIMES FOR NON-CAUCASIAN CADAVERIC KIDNEY TRANSPLANT RECIPIENTS IN AN ALLOCATION SCHEME WHICH ELIMINATES HLA MATCHING. Kenzo Hirose,[1] Miriam Silva-Torres,[2] Wida S. Cherikh,[3] Barry Levin,[4] Phyllis Weber,[2] Gerald S. Lipshutz,[1] Stephen Tomlanovich,[1] Nancy L. Ascher,[1] John P. Roberts,[1] Peter G. Stock.[1] *[1]Department of Surgery, University of California, San Francisco, SF, CA; [2]California Donor Network, Oakland, CA; [3]United Network for Organ Sharing, Richmond, VA; [4]Division of Transplantation, California Pacific Medical Center, SF, CA.*

BACKGROUND: Current US allocation systems include HLA matching for prioritizing cadaveric renal tx (CRT). This scheme is disadvantageous to ethnic minorities in terms of prolonging waiting times due to increased HLA disparity. In 1993, CADN (California Donor Network), which serves an ethnically diverse region, obtained a UNOS approved variance to eliminate HLA matching from allocation schemes. This study compares mean waiting times (MWT) to first cadaver (CAD) tx, acute rejection (AR) rates, and tx success from CRTs allocated by CADN between 12/93 and 12/99 to the rest of the US. METHODS: This is a retrospective review of primary CRTs allocated by CADN since the beginning of the variance in 1993. MWT to first tx, one-, three- and five-year pt and graft survival, and one-year incidence of AR were compared to US results using the UNOS database. SRTR data was used to compare current one-year tx success and AR rates at UCSF (allocation per CADN) to the rest of the US. RESULTS: MWT for ethnic minorities relative to Caucasians were made significantly more equitable by eliminating HLA matching from the allocation system. SRTR data in the current era (1/1/99-6/30/01) demonstrate a 94% one year graft survival at UCSF (n=536) versus 91% in the rest of the US (n=30,909). Current AR at UCSF is 15%. (see tables).

Abstract #582 Figure

CADN

Ethnicity	n =	MWT ethnicity (days)/ MWT Caucasian (days)	% graft survival/% patient survival 1 yr	3 yr	5 yr	1-yr AR (%)
Caucasian	2821	1815/1815 = 1.0	88/93	79/88	70/85	29
African American	842	2170/1815 = 1.2	85/95	72/91	56/85	30
Hispanic	1423	2078/1815 = 1.1	89/95	80/90	72/86	27
Asian	1147	1799/1815 = 1.0	91/96	84/92	76/91	19

REST OF USA

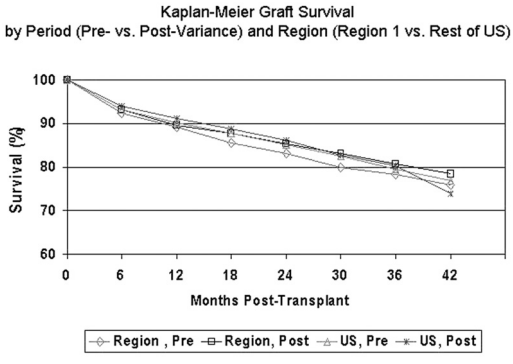
Ethnicity	n =	MWT ethnicity (days)/ MWT Caucasian (days)	% graft survival/% patient survival 1 yr	3 yr	5 yr	1-yr AR (%)
Caucasian	48268	703/703 = 1.0	88/94	79/86	68/82	34
African American	26208	1245/703 = 1.8	86/95	71/89	57/82	39
Hispanic	9905	1112/703 = 1.6	90/96	82/92	71/86	28
Asian	3309	1156/703 = 1.6	91/96	84/93	76/89	29

CONCLUSIONS: These data support the hypothesis that elimination of HLA matching in organ allocation provides a more equitable distribution of organs among different ethnicities based on equalization of MWT. Graft and pt survival, and incidence of AR have not been adversely effected by adoption of this allocation scheme.

Abstract# 583

A REGION ALLOCATION PLAN BALANCING EQUITY WITH UTILITY ENHANCES MINORITY TRANSPLANTATION WITH COMPARABLE OUTCOMES FOR ALL PATIENTS. Francis L. Delmonico,[1] Maureen A. McBride,[2] Paul E. Morrissey,[1] Richard J. Rohrer,[1] William E. Harmon,[1] Wida S. Cherikh,[2] Jonathan Himmelfarb,[1] Jeffrey Stoff,[1] Giacomo Basadonna,[1] Martha Pavlakis,[1] Jane Goguen,[1] Helen Mah,[1] Edgar Milford,[1] Michael Chobanian,[1] Kevin O'Connor,[1] James Whiting,[1] Richard Luskin,[1] Marc Lorber,[1] George S. Lipkowitz.[3] *[1]Medical Director, New England Organ Bank, Newton, MA; [2]United Network for Organ Sharing, Richmond, VA; [3]Medical Director, LifeChoice Donor Services, Windsor, CT.*

This allocation plan distributes kidneys by a single Region list for 14 transplant centers {TxC}. It balances opportunity for patients listed with long waiting times with promoting local donor availability (population distance points PDP). A 5 year experience has accrued. **Allocation Method:** Wait time points accumulate up to 3 years (then they are capped). PDP (calculated by the distance between donor hospital {DH} and candidate's TxC), decrease as the distance between DH to TxC increases. PDP are capped as a circle around DH encompasses 5 million population. Points are awarded for pediatric and sensitized patients, and only a 0 BDr MM. **Study Method:** The relative risk (RR) of receiving a transplant in Region vs the rest of US was computed using a time-dependent Cox model during the pre-(9/1/95-11/30/97) and post-variance (12/1/97-6/30/00) periods. A multivariate Cox analysis estimated the adjusted survival rates for each ethnic group. **Results:** Region Blacks (and other minorities) had a greater likelihood of transplantation during the post-variance period as compared to the rest of the US (RR = 0.73 vs. 0.67, p<0.001). Despite the increase in proportion of less well-matched kidneys in Region during the post-variance period, there were no significant differences in graft and patient survival rates between the Region and rest of the US.



Conclusion: A region wide allocation system that prioritizes time waiting (and de-emphasizes HLA matching) can preserve an excellent outcome for all Region patients (comparable to US), and increase the likelihood of transplantation for minority candidates.

Abstract# 584

HLA MATCHING IN CADAVERIC RENAL TRANSPLANTATION REVISITED: MAJOR IMPACT OF THE FULL HLA-DR COMPATIBILITY ALLOWING SIMPLER AND EQUITABLE ALLOCATION OF ORGANS. Ilias I. N. Doxiadis,[1] Johan W. de Fijter,[2] Marko J. K. Malat,[2] Peter de Lange,[1] Lendert C. Paul,[2] Guido G. Persijn,[3] Frans H. J. Claas.[1] *[1]Immunohaematology and Bloodtransfusion / ETRL, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [3]Eurotransplant International Foundation, Leiden, Netherlands.*

Even with the most potent immunosuppressive drugs matching for the HLA antigens remains a well-accepted, reliable, and effective parameter for allocation of cadaveric kidneys. On one side well-matched transplants are associated with a significantly longer patient / graft survival and a lower chance of returning to the waiting list. On the other hand, due to the imperative of matching, patients with rare HLA phenotypes wait longer for an organ accumulating on the waiting list. Allocation procedures demand a fair policy offering every patient the best possible chance for an organ, with the highest chances for graft survival, irrespectively of age, sex, and race. The present studies show that such an allocation procedure is indeed feasible. For study 1, we selected from the Eurotransplant data base all single kidney transplants performed between 1985 and 2000, in which the recipients were >18 years and the donors > 5 years old (N=38,272), excluding special programs. End point was graft loss censored for death with functioning graft. For study 2 we selected from the Leiden data base all transplants matching the above criteria (N=638). In study 1 we divided the cohort into a group of 0 and 1 HLA-DR mismatches (DRMM) and analyzed the additional influence of HLA-A, B compatibility on graft loss. In the 0 DRMM group a highly significant ($p<0.005$) effect of HLA-A, B matching for both primary and retransplants is seen, whereas a HLA-A,B matching effect is virtually absent in the 1 DRMM group. Similar results were observed in study 2. Here biopsy proven acute rejection within the first 180 days was taken as the end point. The cohort of 1 DRMM had a significant higher incidence of acute rejection episodes. The matching degree of HLA-A, B did not influence the relative risk. Within the group of 0 DRMM the relative risk (RR) increased with the number of HLA-A, B mismatches. However, the RR in all these groups was lower compared to the RR of the 1 DRMM group. These results clearly show that aiming for full HLA-DR compatibility confers more benefit than the current standard matching procedure. As a fully HLA-DR matched donor can also be found for patients with rare HLA phenotypes, such allocation will result in shorter waiting times for this subgroup of patients and an increase of graft survival for the whole patient population.

Abstract# 585

PROPOSAL FOR AN EFFICIENT AND EQUITABLE KIDNEY TRANSPLANT ALLOCATION SYSTEM IN THE U.S. Robert A. Wolfe,[1,2] Friedrich K. Port,[1] Sarah H. Rush,[1] Philip J. Held,[1] Akinlolu O. Ojo,[1,2] Robert M. Merion,[1,2] Alan B. Leichtman.[1,2] *[1]SRTR/URREA, Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI.*

It is assumed that allocation of organs to candidates with fewer HLA mismatches (MM) can only be achieved by allocating fewer organs to minorities, especially African-Americans (AA), whose HLA antigens are less commonly represented in the donor pool. Recent allocation rules for non-zero MM kidneys have ordered candidates by assigning points based primarily on waiting time and number of HLA MMs. Approved new rules emphasize matching at the DR locus, which strongly affects graft survival. In this analysis, we examined a general class of rules that, among candidates with 1 DR-MM, gives more points to HLA-DR phenotypes that are rare in the donor pool. Such rules recognize that candidates with common antigens are more likely to receive a 0 DR-MM organ in the future if they are not allocated the currently offered 1 DR-MM organ. To approximate the experience of a typical organ procurement organization, we simulated a set of 2,255 candidates (28% AA) on the waitlist (450 at start and accruing 1,805 over 8 years) and 1,200 donor organs (15% AA) in a region allocating 150 kidneys per year. The table shows the number of kidneys transplanted and the number of DR-MMs, by race, using this newly approved system versus (1) a recently approved algorithm that assigns 1 and 2 points for a 1 and 0 DR-MM transplant, and (2) the recent rule giving 7, 5, and 2 points for a 0, 1, and 2 BDR-MM. This simulation suggests that by considering potential matches between the recipient and donor HLA pools, matching at DR can be improved for AA and White candidates without further disadvantaging minorities. The recent rule, based on matching at the B and DR loci, led to much greater inequities by race than do either the approved or the proposed rules. The concept behind the new system is general and can give more emphasis to either equity or matching. This simulation does not account for 0 ABDR-MM priority, but the new system will likely lead to more 0MM organs, more equity by race, and better matching than do recent or approved rules.

Outcome	Proposed New System		Approved U.S. System (2 and 1 DR points)		Recent U.S. System (7,5, and 2 points for 0, 1, and 2 MM at B and DR)	
	All	AA / White	All	AA / White	All	AA / White
# Transplants	1,200	310/890	1,200	309/891	1,200	270/930
% DR MM = 0	77.0	71.6/78.9	56.6	45.6/60.4	49.8	44.4/51.4
% DR MM = 1	22.8	28.1/21.0	42.9	53.1/39.4	47.1	52.2/45.6
% DR MM = 2	0.2	0.3/0.1	0.5	1.3/0.2	3.1	3.3/3.0

Abstract# 586

THE IMBALANCE OF NATIONALLY-SHARED HLA-MATCHED KIDNEYS BETWEEN BLOOD GROUP A AND B PATIENTS COULD BE PARTIALLY RESOLVED BY ALLOCATING ZERO MISMATCHED A_2 KIDNEYS TO B PATIENTS. Christopher F. Bryan,[1] Wida S. Cherikh,[2] Yulin Cheng,[2] Mark I. Aeder,[3] Nicolas A. Muruve,[4] Paul W. Nelson,[5] Charles F. Shield III,[6] Bradley A. Warady,[7] Franz T. Winklhofer.[8] *[1]Midwest Transplant Network, Westwood, KS; [2]Biostatistics Department, United Network for Organ Sharing, Richmond, VA; [3]Research Medical Center, Kansas City, MO; [4]University Hospital, Columbia, MO; [5]Saint Luke's Hospital, Kansas City, MO; [6]Via Christi-St. Francis Regional Medical Center, Wichita, KS; [7]The Children's Mercy Hospital, Kansas City, MO; [8]University of Kansas Medical Center, Kansas City, KS.*

Sharing of HLA-matched kidneys results in superior long-term graft survival, and provides almost half of the kidneys transplanted into highly sensitized (≥80%) patients. The following table shows the distribution of HLA-matched kidneys (n=8,162) by blood group, transplanted in the United States from January 1, 1988 to March 31, 2002, as compared to the blood group and minority representation on the wait list.

ABO	% HLA-matched Kidneys	% on Wait List *(on 6/30/02)*	% Difference *(0 mm vs. Wait List)*	% Minority *(8/7/00)*	% Total Txs
B	10.4%	17.4%	40%↓	65.9%	13%
A	40.1%	26.5%	51%↑	46.0%	38.2%
O	46.7%	53.7%	13%↓	55.4%	43.1%
AB	2.6%	2.4%	8%↑	52.8%	5.8%

Blood group B candidates received 40% fewer HLA-matched kidneys than their representation on the waiting list, whereas blood group A patients received 51% more HLA-matched kidneys than their waiting list representation. One way to help increase the access of B candidates to HLA-matched kidneys would be to allocate HLA-matched A_2 kidneys to B candidates. One of the requisites for successful $A_2 \rightarrow B$ transplantation is to have a low anti-A titer history. The data in the following table show that at least 89.6% (78/87) of blood group B candidates tested in our OPO consistently have a low anti-A titer history, which is not influenced by HLA sensitization.

	Peak PRA Level 0 - 9%	11 - 79%	≥80%	Total
Low Anti-A Titer History *(n=78)*	88.3% *(n=53)*	100% *(n=14)*	84.6% *(n=11)*	89.6% *(n=78)*

Conclusion: Allocating HLA-matched blood group A_2 kidneys to blood group B recipients would increase access of blood group B candidates and would result in a more equitable distribution ofthe blood group A and B HLA-matched kidneys than are currently nationally shared. There is a precedent for such an allocation scheme ($A_2 \rightarrow B$), since UNOS recently implemented a national voluntary variance that allows OPOs to locally allocate A_2 kidneys to their B list. Finally, one additional benefit to sharing HLA-matched $A_2 \rightarrow B$ kidneys may be an increased transplantation rate for highly sensitized B candidates, a group that is disadvantaged in the current allocation scheme due to a combination of their blood group frequency and ethnic makeup.

Abstract# 587

THE RELATIVE ABILITY OF ALLOCATION FACTORS TO PREDICT INDIVIDUALS' TIME TO ALLOGRAFT FAILURE. Kevin C. Mange,[1] Jude Maghirang,[2] Wida S. Cherikh.[2] *[1]Center for Clinical Epidemiology/ Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Research, United Network for Organ Sharing, Richmond, VA.*

Background: National allocation of cadaveric renal allografts (CRT) has been guided by factors repeatedly demonstrated to have significant associations with allograft failure. However, the relative values of these factors to predict allograft failure between individuals, which would inform prioritization or weighting of factors in allocation policy, remains unaddressed. Methods: Using data collected from recipients of CRT between 3/6/95 through 12/30/00 (N=37,399) with follow-up through 8/31/02, a baseline multivariable Cox proportional hazards model was fit retaining donor-related factors that had a P value <0.05 with allograft failure (death, dialyisis or retxp), and recipient ethnicity. The V-statistic (range 0.0-1.0, Schemper,Biometrics, 2000), which is analogous to R^2 in linear regression, but accounts for censoring events, was utilized as an indication of the proportion of variation explained (pve) by predictors and of the relative value of the predictors. The baseline model was compared to models separately fit with recipient-related factors known at the time of potential allocation. Results: All recipient-related factors in the Table had highly statistically significant associations with the primary outcome, yet very low absolute ability to predict allograft survival between individuals (Table). Zero HLA mimatches had the largest pve of 6.04%. Conclusions: 1) The absolute pve was low for all models; and 2)Given the low absolute pve's, allocation schemes should primarily consider factors given priority by society and/or provide for the equal opportunity of persons to undergo CRT.

Comparison of Predicted Variation of Allograft Survival Explained

Model	P Value for Addition of Variable	V-Statistic	Absolute Difference	%Change Relative to Baseline Model
Baseline Model	———	0.0520	———	———
+Recipient Age	<0.0001	0.0585	0.0065	12.5
+Cause of ESRD	<0.0001	0.0588	0.0068	13.1
+Peak PRA	<0.0001	0.0538	0.0018	3.5
+Most Recent PRA	<0.0001	0.0548	0.0028	5.4
+0 HLA Mismatches	<0.0001	0.0604	0.0084	16.2
+Peak PRA + Above Factors	<0.0001	0.0781	0.0261	50.2
+ Most Rec PRA + Above Factors	<0.0001	0.0785	0.0265	51.0

Abstract# 588

EFFECT OF UNOS ZERO MISMATCH (0MM) KIDNEY ALLOCATION ON MINORITY PATIENTS AT A SINGLE CENTER, 1995-2002. Steven Fineman, Sharon Hudson, Clifton Kew, Arun Chandrakantan, Bruce Julian, John Curtis, Carlton Young, Michael Gallichio, Mark Deierhoi, Robert Gaston. *[1]Transplant Center, University of Alabama at Birmingham, Birmingham, AL.*

Mandatory sharing of 0 MM kidneys originated in the 1980s, before modern immunosuppression. To ascertain its impact on our center in the current era, we analyzed 1156 consecutive cadaveric renal transplants performed between 8/95 and 6/02. All received quadruple therapy with antibody, calcineurin inhibitor, MMF, and steroids. During this period, blacks (B) made up 65-68% of the waiting list.

Cadaveric Transplants 8/95 until 6/02

	Total	Black (%)	White (%)
n	1156	597 (52)	559 (48)
zero mm	**182**	**42 (23)**	**140 (77)**
median wait mo	11.1	23.1*	10.3
median dialysis mo	21.2	38.3*	17.6
acute rej 3 mo %	14	17	13
acute rej all %	21	29	19
graft surv 1 y %year	96†	91*	97†
graft surv 3 y %	91†	87†	92†
Half life y	17.2	10.2	20.9†
any mm	**974**	**555 (57)**	**419 (43)**
ABDR mm	3.4±1.3	3.7±1.2*	3.0±1.2
median wait mo	20.9	26.7*	13.9
median dialysis mo	32.6	39.2*	22.9
acute rej 3 mo %	24	21	25
acute rej all %	36	40*	30
graft surv 1 year %	91	91	91
graft surv 3 year %	80	77*	84
half life y	9.2	7.7*	12

p<0.05 * black vs white † zero vs any mismatch

7% of transplanted blacks and 25% of whites received a 0 MM kidney. More blacks had DGF (13 vs 7%, p=0.002) despite similar cold ischemia times. Graft survival in 0 MM blacks was no better than MM whites (p=0.77). Early transplantation improved graft survival (p=0.04), an effect more pronounced in blacks than whites. Multivariate analysis revealed black ethnicity and donor age to be the strongest risk factors for graft loss, with no independent effect of ABDR matching. Conclusion: At this center, with a predominantly black waiting list, Caucasian recipients remain the primary beneficiaries of 0 MM kidneys. Black patients wait approximately twice as long as whites regardless of match, with little discernable improvement in matching or outcomes. Strategies to promote transplantation earlier in the course of ESRD may be of particular benefit among black patients.

Abstract# 589

EFFECT OF ORGAN ALLOCATION ON CADAVERIC KIDNEY TRANSPLANT OUTCOMES: A SINGLE CENTER EXPERIENCE. Brian D. Shames,[1] David P. Foley,[1] Luis A. Fernendez,[1] Thomas Chin,[1] Yolanda T. Becker,[1] John Pirsch,[1] Stuart J. Knechtle,[1] John S. Odorico,[1] Hans W. Sollinger,[1] Munci Kaloyoglu,[1] Anthony M. D'Alessandro.[1] *[1]Transplant Surgery, University of Wisconsin, Madison, Madison, WI.*

Current UNOS policy mandates sharing of 0-antigen mismatch (0-MM) kidneys with required "payback" of a kidney to a common national pool. Further, kidneys are allocated based on a "points" system and are distributed nationally. Points kidneys tend to be kidneys that the local OPO/transplant center did not want to utilize secondary to percieved poor quality. The purpose of this study was to examine our results with points kidneys. Methods: We analyzed our results with kidney donors from 1997 to 2000 based on mode of allocation: 0-MM/payback (n=130), points (n=67), and routine heart beating cadaveric kidneys from our local OPO (CAD, n=644). Delayed graft function (DGF-need for dialysis in the first week post transplant), cold time, and calculated GFR were analyzed by ANOVA. Patient and graft survival, and rejection rates were analyzed using Kaplan-Meier methods. Results: 24% of the donors for the points kidneys group were considered expanded donors. Shipped in organs (0-MM and points) had significantly prolonged cold ischemic times (p<0.05) and a higher incidence of DGF (p<0.05). However, GFR at 1 year was not significantly different for any of the groups. The incidence of rejection was significantly decreased in 0-MM recipients; however, there was no difference in immunological graft loss among groups. Patient and graft survival were similar among groups at 1 and 5 years. Conclusions: Patient and graft survival were similar among groups despite a significant increase in delayed graft function in both points kidneys and 0-MM. In most cases these points kidneys could be used locally rather than sent to other centers.

Group	N	Cold time (hrs)	DGF	GFR 1yr (ml/min)	Graft Survival 1/5 yr	Patient Survival 1/5 yr
CAD	644	21±6*	19%*	65	92%/78%	96%/87%
0-MM	130	24 ±6*	21.5%*	66	96%/76%	99%/87%
Points	67	27±7*	31.3%*	69	92%/74%	99/87%

*p<0.05 vs. other groups

Abstract# 590

THE EFFECT OF AGE MATCHING ON FIRST CADAVERIC RENAL TRANSPLANT RECIPIENT SURVIVAL. Douglas S. Keith,[1] Jonathan C. Prather,[1] Douglas J. Norman,[1] Murali S. Golconda,[1] Angelo M. de Mattos.[1] *[1]Nephrology and Transplant Medicine, Oregon Health and Science University, Portland, OR.*

Background: The growing organ shortage in transplantation is making it more imperative to maximize utility of available cadaveric organs. Donor-recipient age matching (i.e. matching younger donor kidneys to younger recipients and vice versa) has been proposed as a means of improving overall utility in renal transplantation. The purpose of this study was to determine the impact of donor-recipient age matching on patient survival in young and old first cadaveric renal transplants. Methods: Using data from the United Network of Organ Sharing Standard Transplant and Analysis and Research Files, 50,320 patients with a first cadaveric renal transplant between January 1990 and December 1997 were identified. Patients were divided into to three groups based on recipient age, 0-40 years old, 41-54 years old and 55 years or older. Using Cox proportional hazard modeling the adjusted ten-year survivals in each group were determined based on donor age groups. Survivals were adjusted for year of transplantation, recipient age, sex, race, donor sex, donor race, hla mismatch, renal diagnosis, cmv status, cold ischemia time and prior dialysis. Also percentage of deaths with graft function were calculated for each recipient age group. Results: Table 1 shows the ten-year survivals for all three recipient groups. Increasing donor age was associated with poorer patient survivals in all recipient age groups. The percentage of deaths with graft function for each recipient age group was 60%, 69% and 76% for recipient age groups 0-40, 41-54 and 55 and older respectively. The trend in poorer patient survival persisted even in the 55 and older group in whom three quarters died with graft function. Conclusions: Donor-recipient age matching would improve survivals in younger recipients but would worsen survivals in older recipients by decreasing the number of young donor kidneys available to older recipients. Because of the significant effect of donor age on recipient survival, consensus regarding donor-recipient age matching needs to be reached among the public and transplant professionals.

Table 1: Ten-year Patient Survivals

Recipient Age Group -> Donor Age Group	0-40 Years Old	41-54 Years Old	55 Years or Older
0-17 Years Old	84%	69%	48%
18-29 Years Old	83%	67%	46%
30-41 Years Old	82%	66%	45%
42-54 Years Old	78%	64%	37%
55 Years or Older	76%	58%	35%

Abstract# 591

DONOR-RECIPIENT AGE MATCHING IN CADAVERIC RENAL TRANSPLANTATION. Douglas S. Keith,[1] Jonathan C. Prather,[1] Douglas J. Norman,[1] Murali S. Golconda,[1] Angelo M. de Mattos.[1] *[1]Nephrology and Transplantation Medicine, Oregon Health and Science University, Portland, OR.*

Background: The current UNOS allocation scheme for cadaveric renal transplants is heavily weighted to HLA matching and waiting time with no provisions for donor and recipient age matching. Donor age has a significant effect on graft function and longevity. The purpose of this study was to determine if age matching is occurring among first cadaveric renal transplants in spite of no provisions for it in the current allocation scheme. Methods: Using data from the United Network of Organ Sharing Standard Transplant and Analysis Research Files, 50,320 patients with first cadaveric renal transplants between January 1997 and December 1997 were identified. Patients were grouped by donor and recipient age groups. The percentage difference between the the actual frequency of allocation based on age and that assuming random allocation was determined. Results: The table below shows the frequency distribution of cadaveric kidneys based on donor and recipient age groupings. As recipient age increased, a higher percentage of older donor kidneys were allocated than would be expected if random allocation of organs by age were occurring. Simularly, as recipient age decreased, a higher percentage of younger donor kidneys were allocated to younger recipients than would be expected if random allocation were occurring. Conclusions: Significant age matching of cadaver kidneys is occurring in spite of no provisions in the current UNOS allocation scheme. Although this may make sense from a utilitarian perspective, consensus among the public and transplant professionals regarding age matching organs should be arrived at to insure a just system of allocation.

Distribution of Cadaveric Kidneys by Donor and Recipient Age

Recipient Age -> Donor Age	0-17 yrs	18-39 yrs	40-54 yrs	55+ years
0-6 yrs	203 (146.1)	778 (17.9)	718 (-14.0)	442 (-21.6)
7-17 yrs	511 (58.1)	2741 (6.0)	3221 (-1.5)	1916 (-13.3)
18-29 yrs	537 (10.2)	4109 (5.4)	4926 (-0.1)	3070 (-7.8)
30-41 yrs	355 (-5.3)	3067 (2.3)	3858 (1.7)	2449 (39.7)
42-54 yrs	268 (-35.4)	3276 (-1.2)	4338 (3.4)	2879 (1.6)
55+ yrs	65 (-74.7)	1541 (-24.9)	2558 (-1.5)	2494 (42.3)

Number of Transplants (% Different from Expected for Random Age Allocation) for Recipient Age

Abstract# 592

SIGNIFICANCE AND THERAPEUTIC POTENTIAL OF TARGETING TNF-α SIGNALING IN ALLOIMMUNE RESPONSE. Masayuki Sho,[1] Marlies E. J. Reinders,[1] Hiroshi Harada,[1] Ingrid H. C. Vos,[1] Dmitry V. Samsonov,[1] Sharon R. Nahill,[2] Bradford H. Hirth,[2] Edward R. Lee,[2] Jill Gregory,[2] John M. Williams,[2] David M. Briscoe,[1] Mohamed H. Sayegh.[1] *[1]Medicine, Children's Hospital, Boston, MA; [2]Genzyme Corporation, Cambridge, MA.*

TNF-α is a proinflammatory cytokine that mediates physiological as well as pathological responses in a variety of diseases. Previous attempts at targeting TNF-α (anti-TNF-α mAb treatment) resulted in only a marginal effect on allograft survival. Genz-29155 (29155) is a novel small molecule that inhibits TNF-α signaling. We investigated the significance of TNF-α signaling and the efficacy of this inhibitor in vivo using a murine cardiac transplantation model. Treatment with 29155 prolonged MHC mismatched C57BL/6 allograft survival in BALB/c recipients (MST = 18d, P = 0.0059 vs. control). As confirmed by ELISPOT and flow cytometric analysis, 29155 treatment significantly inhibited T cell responses and alloantibody production. RNase Protection assay showed that this treatment suppressed local immune activation as demonstrated by significantly decreased expression of chemokines, such as RANTES and IP-10, as well as adhesion molecules, such as VCAM-1 and ICAM-1, in transplanted grafts. Furthermore, an in vitro transmigration assay revealed that 29155 treatment inhibited migration of human PBMC across endothelium (HUVEC). Taken together, these results suggest that 29155 functions via inhibition of immune cell activation and downregulation of recruitment and infiltration of alloreactive cells into vascularized grafts. For future clinical application, we explored the potential of 29155 in combination with conventional immunosuppressants or CD154 blockade. Strikingly, the combination of 29155 with rapamycin resulted in indefinite allograft survival and induced donor-specific tolerance, while cyclosporine failed to synergize with 29155. Furthermore, all mice accepted allografts after the combined therapy of 29155 with CD154 blockade indefinitely. These combination therapies significantly inhibited the development of chronic rejection as confirmed by histological analysis on long-term surviving grafts. In addition, using an MHC class II disparate model that exhibits spontaneous development of chronic vasculopathy (Bm12 into C57BL/6), 29155 monotherapy significantly inhibited the development of arteriosclerosis in transplanted cardiac grafts (P = 0.0002 vs. control). Our data highlight the therapeutic potential of TNF-α signal transduction inhibition as well as significant pathogenic and regulatory role for TNF-α signaling in both acute and chronic allograft rejection.

Abstract# 593

LF15-0195 PROMOTES TOLEROGENIC DENDRITIC CELLS BY INHIBITING IKK ACTIVITY. Mu Li,[1] Thomas E. Ichim,[1] Dejun Zhou,[1] Xuyan Huang,[1,4] Xiaotao Yan,[1] Robert Zhong,[1-4] Wei-Ping Min.[1-3] *[1]Department of Surgery, University of Western Ontario, London, ON, Canada; [2]Multi-Organ Transplant Program, London Health Sciences Centre, London, ON, Canada; [3]Transplantation and Immunology, Lawson Health Research Institute, London, ON, Canada; [4]Robarts Research Institute, London, ON, Canada.*

BACKGROUND: LF 15-0195 (LF), an analogue of 15-deoxyspergualine (DSG) is a novel immunosuppressant that prevents allograft rejection and induce donor-specific tolerance. The purpose of this study was to study mechanisms responsible for LF-induced tolerance by characterizing its effects on dendritic cell (DC) function. METHODS: DCs were cultured from bone marrow progenitors and treated with LF *in vitro* at various concentrations. Expression of MHC class II, costimulatory molecules, IL-10 and IL-12 were measured by flow cytometry or RT-PCR. Activation of IKK and NF-kB was assessed by Western blot and gel shift assays. LF-treated DC, or DC isolated from LF treated mice after allogeneic heart transplant, were cultured with allogeneic T cells. T helper cell polarization was assessed by cytokine specific RT-PCR. RESULTS: LF-treated DC showed immature and DC2-like phenotype as evidenced by reduced MHC class II and CD40 expression, increased IL-10 production, and a lack of IL-12. LF-treated DC failed to evoke an allogeneic MLR. LF inhibited IKK and subsequently NF-kB activity after DC were stimulated with TNF-a/LPS. DC treated with LF *in vitro* promoted Th1 to Th2 polarization, showing decreased IL-2 and IFN-gamma but increased IL-4 and IL-10. Similar to *in vitro* experiments, DC isolated from tolerant LF-treated cardiac allograft recipients showed: a) Impaired NF-kB activity; b) DC2-like phenotype; c) Decreased ability to stimulate MLR and d) induced polarization of naïve T cells into Th2. CONCLUSION: Immunosuppression and induction of tolerance by LF 15-0195 is mediated through blockade of NF-kB signaling and generation of tolerance-promoting DC.

Abstract# 594

IMMUNOTOXINS DT390-IL-2 AND DT390-RANTES SELECTIVELY INHIBIT ALLOREACTIVE T CELL PROLIFERATION IN MIXED LYMPHOCYTE REACTION. Ping Feng,[1] Stuart J. Knechtle,[1] Huaizhong Hu.[1] *[1]Division of Transplantation, Department of Surgery, University of Wisconsin Medical School, Madison, WI.*

Most of the currently used strategies of T cell depletion for tolerance induction in transplantation indiscriminately targeted on whole T cell population, and the consequent immunocompromised condition usually put the recipients at high risk of bacterial and virus infections. In this study, two diphtheria toxin (DT)-based immunotoxins were designed to selectively target on activated alloreactive mouse T cells by binding to CCR5 and IL-2 receptor, which are expressed upon activation during graft rejection. Constructed under the control of SV40 promotor, plasmids encoding DT390-RANTES and DT390-IL-2 were transfected into eukaryotic fibroblast cell lines NIH 3T3 separately, and 4 ng/ml of protein expression level was achieved for both immunotoxins as detected by ELISA. The IC50 was 2×10^{-11} M for DT390-RANTES and 1×10^{-11}M for DT390-IL-2, determined by protein synthesis inhibition assay. Inhibitory effects on alloreactive T cell proliferation were examined in a 6-day mixed lymphocytes reaction (MLR) with CFSE-labeling technique. 10×10^6 CFSE-labeled C57BL/6J (H-2b) splenocytes were employed as responder cells, and 10×10^6 irradiated Balb/c (H-2d) splenocytes as stimulator cells. In the presence of the immunotoxins (2ng/ml), proliferation of alloreactive T cells was significantly inhibited up to 60% by DT390-RANTES and up to 88% by DT390-IL-2 compared to the controls. While both immunotoxins exhibited similar inhibitory effects on CD8+ T cells, DT390-IL-2 seemed to be more potent on CD4+ T cells than DT390-RANTES. Meanwhile, B cells and non-alloreactive T cells, which remained non-proliferative in MLR, were not affected by the immunotoxins. The results showed that both DT390-IL-2 and DT390-RANTS could be successfully expressed, and they selectively inhibited the proliferation of the activated alloreactive T cells in MLR, suggesting a promising potential of the immunotoxins in induction of graft tolerance without impairing anti-infection immunity.

Abstract# 595

FARNESYLTRANSFERASE INHIBITION: A NOVEL METHOD OF IMMUNOMODULATION. Ming-Sing Si,[1] Ping Ji,[1] Mike Lee,[1] Jennifer Kwok,[1] Shi-Chung Ng,[2] David K. Imagawa.[1] *[1]UCI Transplantation Laboratory, Department of Surgery, University of California Irvine College of Medicine, Orange, CA; [2]Cancer Research, Abbott Laboratories, Abbott Park, IL.*

Background: Farnesyltransferase inhibitors (FTI's) are anticancer compounds that inhibit Ras GTPases by preventing farnesylation, a post-translational modification that allows these oncogenic signal transduction proteins to insert into the cell membrane where they become activated and activate their effector molecules. Since Ras GTPases play key roles in T cell activation and effector function, we hypothesized that FTI's have immunomodulatory properties and are potential antirejection agents. An investigation was performed on the potent FTI A-228839 to evaluate this hypothesis in the *in vitro* setting. **Methods:** The *in vitro* effects of A-228839 on lymphocyte activation and function were evaluated. Lectin or antigen presenting cell (APC) induced lymphocyte proliferation in the presence of varying concentrations of A-228839 was measured. The effects of A-228839 on the morphology of the murine 1E5 T cells were assessed by microscopy. Intracellular calcium ($[Ca^{2+}]_i$) kinetics of lectin-activated lymphocytes were monitored by flow cytometry. The effects of A-228839 on lectin induced peripheral blood mononuclear cells (PBMC) cytokine production were assessed by a cytometric bead array method. Activation-induced apoptosis was measured by annexin V staining and flow cytometry. **Results:** A-228839 inhibited lectin induced proliferation ($IC_{50} = 0.24 \pm 0.11$ μM). The inhibitory effects of A-228839 on lectin induced lymphocyte proliferation were additive to those of CsA. A-228839 was more effective in inhibiting APC induced T cell proliferation ($IC_{50} = 0.10 \pm 0.09$ μM). A-228839 significantly disrupted the polarized shape of 1E5 T cells at physiologic concentrations. A-228839 altered PBMC baseline $[Ca^{2+}]_i$ but did not affect $[Ca^{2+}]_i$ kinetics during lectin induced lymphocyte activation. A-228839 inhibited lymphocyte Th1 cytokine production ($IC_{50} = 0.37 \pm 0.08$ μM, 0.78 ± 0.25 μM and 0.60 ± 0.22 μM for IL-2, TNF-α and IFN-γ, respectively) and promoted apoptosis in lectin-activated lymphocytes. **Conclusions:** A-228839 potently inhibits lymphocyte activation and function. Our results are the first to demonstrate that selective farnesyltransferase inhibition is a novel target for immunomodulation and that FTI's represent a novel class of immunomodulatory agents. Studies are currently being performed to evaluate the potential of FTI's to prevent allograft rejection in animal transplant models.

Abstract# 596

DEVELOPMENT OF A JAK3 INHIBITOR TO PREVENT ORGAN TRANSPLANTATION. Paul S. Changelian,[1] Craig R. Kent,[1] Kelly S. Magnuson,[1] Perry S. Sawyer,[1] Bret D. Perry,[1] William B. Brissette,[1] Sandra McCurdy,[1] Timothy Strelevitz,[1] Kwansik Yoon,[1] Michael Fisher,[1] Eileen A. Elliott,[1] Elizabeth M. Kudlacz,[1] John J. O'Shea,[2] Todd Blumenkopf,[1] Michael Hines,[1] Michael Munchhof,[1] John Doty,[1] Jeffrey Casavant,[1] David Whipple,[1] Jiamin Sun,[1] Johnson Kim,[1] Mary Saltarelli,[1] Mark E. Flanagan.[1] *[1]Antibacterials, Inflammation and Immunology, Pfizer, Inc., Groton, CT; [2]Lymphocyte Cell Biology Section, NIH, Bethesda, MD.*

Background: Congenital deficiency of JAK3 leads to Severe Combined Immunodeficiency (SCID), suggesting that inhibitors of JAK3 could provide potent immune suppression to prevent transplant rejection. **Methods**: The catalytic domain of human JAK3 was used to establish an enzymatic screening assay for identification of small molecular weight inhibitors. Parallel assays were developed for JAK2 and JAK1. Enzymatic inhibitors were profiled in human and murine cellular assays. Compounds with adequate potency and specificity were profiled in a stringent model of heterotopic heart transplantation (DBA2 heart into C57/BL6 host). In some cases, mice were euthanized and RNA extracted from allogeneic hearts after 7 days, for profiling by Taqman analysis. **Results**: Initial screening identified a 300 nM (IC50) inhibitor of JAK3 kinase. Chemical modifications resulted in CP-690,550, a 1 nM inhibitor of JAK3. This compound was 20 and 100-fold less potent vs. JAK2 and JAK1, respectively. When tested against a panel of >40 other protein kinases, CP-690,550 was > 3,000-fold specific for JAK3. The IC90 for CP-690,550 in JAK3 and JAK2-dependent cell based assays were 30 and 870 ng/ml, respectively. In heterotopic heart transplant studies, control allografts rejected within 9 days. Exposures of approximately 60 ng/ml in this model resulted in 50% of grafts surviving >28 days, with 25% surviving >100 days. Differences in heart RNA expression from control or drug-treated animals will be presented. **Conclusions**: We have developed a potent inhibitor of JAK3. CP-690,550 has excellent selectivity against the majority of other kinases tested, and modest selectivity vs. other members of the JAK kinase family. When used as monotherapy, CP-690,550 is able to significantly extend survival in a rigorous murine model of heart transplantation. More recently, this compound has demonstrated monotherapy efficacy in a non-human primate model of renal transplantation. These data suggest that JAK3 blockade is a potential therapeutic approach for prevention of rejection in human organ transplantation.

Abstract# 597

SIGNALING OF INTERLEUKIN-1β IN HUMAN ENDOTHELIAL CELLS IS INFLUENCED BY MYCOPHENOLIC ACID THROUGH ENHANCED BINDING OF MYD88 TO IRAK-1. Peter Bertalanffy,[1] Ernst Wolner,[1] Guenter Weigel.[1] *[1]Cardiothoracic Surgery, University of Vienna, Vienna, Vienna, Austria.*

We have recently demonstrated that during incubation with mycophenolic acid (MPA) interleukin (IL)-1β-induced nuclear translocation of NF-κB and binding to the intercellular adhesion molecule (ICAM)-1 promoter is enhanced in human endothelial cells which results in an up-regulated expression of ICAM-1 on the cell surface. In further experiments we have shown that the phosphorylation of inhibitor of κB (IκB) is amplified during treatment with MPA. The signaling cascade initiated by IL-1β involves a trimeric protein complex of IL-1β, the transmembrane IL-1 receptor type I (IL-1RI), and IL-1R accessory protein (IL-1RAcP). Activation of the IL-1RI leads to recruitment of IL-1R associated kinase-(IRAK) to the receptor complex via its association with IL-1RAcP and the adaptor protein MyD88. Upon recuitment, IRAK is highly phosphorylated and subsequently dissociates from the receptor complex to interact with tumor necrosis factor receptor-associated factor 6 (TRAF6) which in turn is involved in IκB inducing kinase (IKK) and NF-κB activation. In an attempt to study the upstream signaling events that are responsible for the enhanced activation of NF-κB observed during treatment with MPA human umbilical vein endothelial cells (HUVEC) were incubated with 15 μM MPA or vehicle for 24 h and then activated with 10 [nano]g/ml IL-1β for 10, 20, and 30 min. The cells were then harvested, lysed, and centrifuged. The supernatant was immunoprecipitated with polyclonal antibody against MyD88. The samples were then fractionated on 10% SDS-PAGE, transferred to a nitrocellulose membrane, and subjected to immunoblot analysis with IRAK-1 antibody. The signals obtained in immunoblots were quantitated with a densitometer. The formation of MyD88/IRAK-1 complexes reached the maximum after 10 min. and was enhanced in cells that had been pretreated with MPA (14.3± 0.9; mean± SD of densitometric values; $p<0.01$) compared to IL-1β alone (9.9± 1.2; mean± SD of densitometric values). Binding of MyD88 to IRAK-1 preceded IκB phosphorylation and the percentual increase of complex formation followed that of transcription and surface expression of ICAM-1. This is the first demonstration that the immunosuppressant MPA influences IL-1β signaling through MyD88/IRAK-1 and provides the rational to further investigate the role of MPA in the complexity of signaling networks in regulating NF-κB activation pathways.

Abstract# 598

***IN VIVO* IMMUNOSUPPRESSIVE EFFECTS OF NC1153, A NOVEL SELECTIVE JANUS TYROSINE KINASE 3 (JAK 3) ANTAGONIST.** Mo-Er Wang,[1] Stanislaw M. Stepkowski,[1] Robert Kirken,[2] Jonathan Dimmock,[3] Barry D. Kahan.[1] *[1]Surgery, The University of Texas Medical School, Houston, TX; [2]Integrative Biology, The University of Texas Medical School, Houston, TX; [3]College of Pharmacy, University of Saskatchewan, Canada.*

Objective: To develop a proliferation signal antagonist selective for transduction of cytokine signal 3 in T and B lymphocytes, exploiting Janus Kinase 3 (Jak 3) as a specific target, to achieve synergistic effects with cyclosporine (CsA). Methods: The Mannich base NC1153 was injected either intravenously (i.v.) for 7 days at doses of 2.5, 5, 10, or 20 mg/kg/day or administered by oral gavage (p.o.) for 7 or 14 days at doses of 20, 40, 80, 160, 240, or 320 mg/kg/day to ACI ($RT1A^a$) recipients of Lewis ($RT1^l$) heterotopic kidney allografts. In addition, to assess drug interaction, some recipients of kidney allografts were treated with cyclosporine (CsA; 2.5, 5, 10, or 20mg/kg x 3 days, p.o.) alone or in combination with NC1153 therapy. Significance in differences of mean graft survival time (MST) was assessed by the Wilcoxon method. In addition, the drug interaction was evaluated using the median effect model to calculate the concentrations of each drug alone or in combination necessary to achieve a 100-day graft survival; the quality of drug interaction was measured by the combination index (CI) values: CI=1 shows additive, CI<1, synergistic, and CI>1, antagonistic, interactions. Results: NC1153 showed dose-dependent prolongation of graft survival: doses of 20 mg/kg/day NC1153 delivered i.v. for 7 days produced a MST of 24.8±6.6 days (p=0.00003 vs. untreated control; MST=8.8±0.5 days) and 240 mg/kg/day NC1153 delivered p.o. for 7 or 14 days, 47.8±19.59 or >60 days (both $p < 0.00001$). Treatment with CsA alone (2.5, 5, 10, or 20mg/kg/d for 3 days) produced dose-dependent effects achieving at the highest dose a MST of 24.50±4.58 days ($p<0.0001$). The combined treatments revealed a potent synergistic interaction in graft survival in comparison with monotherapy with each agent. For example, although a 7-day i.v. administration of 2.5 mg/kg/day NC1153 alone produced a MST of 9.5±1.4 days, and a 3-day 10 mg/kg/day CsA alone of 21.2±5.3 days, two-drug combination prolonged survival to 41.4±9.8 days (p=0.00002). The best results were observed at NC1153:CsA dose ratios of 4:1 and 2:1, yielding the CI values of 0.11 and 0.27, respectively. Conclusion: A new and selective Jak3 inhibitor, NC1153, is immunosuppressive in vivo in a kidney transplant model, and exerts marked synergistic effects in combination with CsA.

Abstract# 599

MONOTHERAPY WITH THE HUMAN ANTI-CD154 MONOCLONAL ANTIBODY ABI793 PROLONGS ALLOGRAFT SURVIVAL IN RHESUS MONKEYS. Turan Kanmaz,[1] John H. Fechner,[1] Jose Torrealba,[2] Hyoung Tae Kim,[1] Yinchen Dong,[1] Terry D. Oberley,[2] Jacqueline M. Schultz,[2] Walter Schuler,[3] Huaizhong Hu,[1] Majed M. Hamawy,[1] Stuart J. Knechtle.[1] *[1]Surgery, University of Wisconsin, Madison, WI; [2]Pathology, University of Wisconsin, Madison, WI; [3]Novartis.*

ABI793, a novel fully human anti-human CD154 monoclonal antibody (Novartis Pharma AG) provides for excellent graft survival in cynomolgus monkey renal allotransplantation while causing thrombocytopenia and reversible acute tubular necrosis unrelated to rejection (see abstract by W. Schuler et al.). To address the question whether these side effects are species specific we studied ABI793 in rhesus monkeys. Outbred rhesus monkeys underwent renal allotransplantation from MHC-mismatched donors. Donor-recipient pairs were selected based on DNA typing of DR-B alleles which were at least one haplotype mismatches. Seven recipient monkeys were treated intravenously with ABI793 on posttransplant days 0, 1, 4, 11, 18, 28, 56, and 84 at a dose of 20 mg/kg. Mixed lymphocyte reactions (MLR) and peripheral blood lymphocyte phenotype analysis were performed on all monkeys prior to transplantation and periodically. Graft function was monitored by daily urine output, weekly serum creatinine, and monthly renal biopsy. Outcomes were compared to six untreated control monkeys undergoing renal allotransplantation under the same conditions. Untreated recipients rejected their allografts at days 5, 7, 7, 7, 8, and 9. Graft survival time for the seven ABI793-treated monkeys were as follows: 13,44,139,149,154,158, and 221 days. One of the monkeys was sacrificed because of urine leak on postoperative day 13 and there was no evidence of rejection of the allograft at autopsy. Three monkeys were sacrificed because of clinically and histopathologically confirmed acute rejection (days 44, 149, and 158). Two monkeys were sacrificed because of both acute and chronic rejection (days 154 and 221). One monkey was sacrificed on day 139 without clinical signs of rejection to observe the effects of ABI793 on the monkey in the absence of rejection at autopsy. Compared to untreated recipients, all seven monkeys had markedly prolonged allograft survival. CD3, CD4, and CD8 lymphocyte subsets remained unaltered during and after treatment. MLR analysis following the treatment showed suppression that was not donor-specific. There was no clinical or autopsy evidence of thromboembolic or any other obvious side effects. Monotherapy with ABI793 provides long-term renal allograft survival in MHC-mismatched rhesus monkeys in the absence of thromboembolic or other detectable side effects.

Abstract# 600

PROLONGED REJECTION-FREE RENAL ALLOGRAFT SURVIVAL WITH THE ANTI-CD154 ANTIBODY IDEC-131 COMBINED WITH DONOR-SPECIFIC TRANSFUSION (DST) AND SIROLIMUS IN NON-HUMAN PRIMATES (NHPs). Edwin H. Preston,[1,2] He Xu,[1] Jonathan P. Pearl,[1] Frank V. Leopardi,[1] Douglas A. Hale,[1] Lynt B. Johnson,[2] David M. Harlan,[1] Allan D. Kirk.[1] *[1]Transplantation and Autoimmunity, NIDDK, NIH, Bethesda, MD; [2]Surgery, Georgetown University Hospital, Washington, DC.*

Therapies targeting key costimulatory signals such as CD154 have shown great promise towards achieving transplantation tolerance. This pre-clinical study was conducted in rhesus monkeys to test the efficacy of the anti-CD154 antibody IDEC-131 alone or in combination with pre-transplant DST, and/or sirolimus in preventing renal allograft rejection. Heterotopic renal transplantation (rTx) and bilateral native nephrectomies were performed in MHC-mismatched NHPs (n=20). IDEC-131 therapy consisted of 20 mg/kg on days –1, 0, 3, 7, and once weekly for 8 weeks. DST consisted of 7mL/kg whole blood given on day –1. Sirolimus therapy was started day 0 and was given orally at 1mg/kg/day for three months.

Therapy	Survival	Rejection-Free Survival
No therapy	7, 7, 7, 6, 5	7, 6, 5, 5, 5
Sirolimus, DST	7, 7	6, 5
Sirolimus alone	29, >20	21, >20
IDEC-131, DST	57, >43	7, 42
IDEC-131 alone	>172, 44, 8	>172, 44, 7
IDEC-131, sirolimus	240, >198, 86	9, 9, 42
IDEC-131, sirolimus, DST	>394, >375, 173	>394, >375, 168

All primates treated with the combination of IDEC-131, sirolimus, and DST (n=3) remained free of intervening acute rejection during their 3-month course of therapy. The absence of rejection while undergoing therapy was not observed consistently in any other groups although select NHPs maintained normal allograft function beyond the completion of therapy with IDEC-131 alone (n=1) and IDEC-131 and sirolimus (n=1). Animals treated with the combination of IDEC and sirolimus all had early rejection episodes that resolved with ongoing therapy, though 2 of 3 NHPs subsequently lost their allografts to rejection. One-year after rTx, long-surviving animals treated with IDEC-131, sirolimus, and DST (n=2) underwent donor, autologous, and 3rd party skin graft challenge. Rejection-free skin-graft survival was 55 and >22 days for donor allografts while 3rd party grafts were rejected at 12 and 11 days. To date, rejection of donor skin allografts has not precipitated kidney rejection suggesting that these animals remain tolerant to renal derived donor antigens. This pattern of delayed donor skin rejection and normal 3rd party rejection appears to be consistent with hyporesponsiveness to donor tissues except skin specific antigens. These data confirm the utility of combining IDEC-131, sirolimus and DST to prevent allograft rejection. This is a promising regimen for human transplant trials.

Pediatric Liver Transplantation

Abstract# 601

OUTCOMES OF WHOLE DECEASED, SPLIT DECEASED, AND LIVING DONOR LIVER TRANSPLANTS IN PEDIATRIC RECIPIENTS. John P. Roberts,[1] Tempie E. Hulbert-Shearon,[2,3] Robert M. Merion,[2,3] Friedrich K. Port,[2] Robert A. Wolfe.[2,3] *[1]University of California San Francisco, San Francisco, CA; [2]SRTR/URREA, Ann Arbor, MI; [3]University of Michigan, Ann Arbor, MI.*

Purpose: For pediatric patients, the lateral segment from an adult liver provides adequate mass to allow survival following transplantation. There are two sources for the lateral segment, living donors (LD) and deceased donors (DD), in addition to the availability of whole donor organs from pediatric donors. Which source provides the best patient and graft survival? **Methods:** We compared outcomes by graft source for pediatric and young adult age groups (<30 years) with Cox regression models for 6,483 (5,155 full deceased [DD-F], 748 split or partial deceased [DD-S], and 580 LD) recipients of first single-organ liver transplants during 1989-2000. Recipients were followed for up to 1 year or until death for patient survival analyses and until death or graft failure for graft survival analyses. Risk of mortality and graft failure was assessed by age group (<2, 2-10, 11-16, 17-29) and organ type (LD, DD-F, DD-S) and was adjusted for recipient race, ethnicity, sex, diagnosis, life support, medical urgency status at transplant, ABO compatibility, and year of transplant. **Results:** Of the recipients under 17 years, half were <2 years. Recipients aged <2 years had higher risk of graft failure (RR=1.48, p<0.0001) and mortality (RR=1.32, p=0.03) with DD-S livers than DD-F livers. Patients <2 years who received DD-S livers also had higher risk of graft failure (RR=1.93, p<0.001) and mortality (RR=1.43, p=0.07) than LD livers. The risk of graft failure appeared to be higher for DD-F than for LD (RR=1.31, p=0.06). For patients aged 2-10, the risk of graft loss (RR=1.64, p=0.01) and mortality (RR=1.82, p=0.01) was higher for LD than DD-F. For ages 11-16, there was a significantly higher risk of graft failure with LD than DD-F livers (RR=3.72, p<0.0001) and for LD than DD-S (RR=3.02, p=0.01), while the mortality risk was not significantly different for the three graft types. For patients aged 17-29, the risk of graft failure was significantly higher in this age group for DD-S than for DD-F livers (RR=2.39, p=0.02) while the mortality risk showed similar non-significant trends. **Conclusion:** In the age group <2 years, LD or whole organ transplantation provides superior graft survival as compared to DD-S and trends toward better patient survival. LD loses this advantage in the older children. Because of the relative unavailability of DD-F livers, LD transplantation should be the preferred graft in the most common pediatric age group (<2) undergoing transplantation.

Abstract# 602

PEDIATRIC LIVER TRANSPLANTATION (LT) WITH LIVING-RELATED DONORS (LRD) PROVIDES SIGNIFICANTLY BETTER GRAFT SURVIVAL THAN LT WITH POST-MORTEM DONORS (PMD): A COMPARATIVE STUDY IN 236 CHILDREN. Raymond Reding, Tran Thanh Tri, Christophe Bourdeaux, Jérémie Gras, Etienne Sokal, Magda Janssen, Jean-Bernard Otte. *[1]Pediatric Liver Transplant Program, Université Catholique de Louvain, Brussels, Belgium.*

When compared to PMD, pediatric LT with LRD was shown to result in a significantly lower *pre*-LT mortality (1). We hypothesized that the use of LRD may also improve *post*-LT results, because of the appropriate preparation of the recipients, of the avoidance of uncontrolled waiting time, and of the better quality of LRD grafts. All consecutive primary pediatric LT performed since the initiation of our LRD program were reviewed. Patients and methods: Between July 1993 and April 2002, a total of 236 children received a primary LT from a LRD (n=100) or a PMD (n=136). The median recipient age (range) at LT was 1.0 year (0.4-13.1) in the LRD group, versus 2.4 years (0.2-14.9) in the PMD group ($p<0.001$). The two main pre-LT diagnoses in the LRD group were biliary atresia (n=69, 69%) and hepatic malignancy (n=10, 10%), versus biliary atresia (n=72, 53%) and fulminant hepatitis (n=14, 10%) in the PMD group ($p<0.01$). Median donor age (range) was 33.3 years (19.0-54.6) in the LRD group, versus 10.3 years (0.4-67.2) in the PMD group ($p<0.001$). All children in LRD group received a left liver graft, whereas the PMD group included whole (n=62, 46%), reduced-size (n=55, 40%), and split (n=19, 14%) liver grafts. Results: Actuarial patient survival rates at 1, 3, and 5 years were 94%, 92%, and 92% in the LRD group, versus 89%, 87%, and 85% in the PMD group (NS). The corresponding actuarial graft survival rates were 92%, 89%, and 89% in the LRD group, versus 80%, 79%, and 77% in the PMD group ($p=0.027$). The retransplantation rate was 3% in the LRD group, versus 11% in the PMD group ($p=0.022$). The rate of vascular complications (hepatic artery or portal vein thrombosis) leading to graft or patient loss was 2% in the LRD group, versus 7% in the PMD group ($p=0.078$). Conclusions: (1) Since the initiation of our LRD program in 1993, LT using living donors represented 42% of our total activity, versus 8% for split liver grafts; (2) despite the significantly younger age of the recipients and increased technical difficulty, graft survival was significantly higher and retransplantation rate lower in the LRD group, with a trend towards a lower incidence of vascular complications leading to graft loss; (3) the detailed analysis of the factors involved in these differences between the LRD and PMD groups is currently ongoing by means of a multivariate analysis. (1) J Pediatr 1999; 134: 280-6.

Abstract# 603

OUTCOME OF TECHNICAL VARIANT VERSUS WHOLE ORGAN PEDIATRIC LIVER TRANSPLANTATION. Ivan R. Diamond,[1] Ravinder Anand,[2] Annie Fecteau,[1] Lan Zeng,[2] the SPLIT Research Group. *[1]Pediatric Academic Multi-Organ Transplant Program, The Hospital for Sick Children, Toronto, ON, Canada; [2]The EMMES Corporation, Rockville, MD.*

Introduction: Technical Variant Liver Transplantation comprising reduced, split, and live-donor liver transplantation evolved to address the need for size appropriate grafts for pediatric recipients. The aim of this paper is to examine the outcome and morbidity of Technical Variant (Tv) techniques relative to Whole Organ (Wo) transplants in the Studies of Pediatric Liver Transplantation (SPLIT) database, a database of pediatric liver transplant recipients at 38 North American transplant centers. **Method:** Data was analyzed for all patients undergoing their first transplant between the inception of SPLIT in 1995 and June 2001. The primary analyses focused on the results of Wo versus Tv grafts, with subgroup analysis planned to compare each of the Tv techniques to Wo transplant. Major factors analyzed included demographics, surgical, post-transplant complications including rejection episodes, and patient and graft survival. **Results:** 811 patients (420 Wo & 391 Tv) were transplanted during the index time period. Mean age at transplant was 6.6 years Wo and 2.7 years Tv (*$p<0.0001$*). Mean time from listing to transplant was 5.9 months Wo and 2.9 months Tv (*$p<0.0001$*). One-year patient survival was 90.6% Wo and 80.5% Tv (*log rank $p=0.0001$*), and graft-survival was 84.1% Wo and 72.9% Tv (*log rank $p=0.001$*). The incidence of biliary (leaks and strictures) complications was 15.7% Wo and 28.4% Tv (*$p<0.0001$*). The incidence of vascular complications (Hepatic Artery Thrombosis, Portal Vein Thrombosis and Hepatic Vein Thrombosis) was not increased in the Tv groups 21.7% versus 20% Wo (*p-value=0.54*). Hepatic Artery Thrombosis was the most common indication for retransplant; 61% of the Wo children who were retransplanted and 25% in the Tv group (*$p<0.0001$*). Primary graft non-function was a more common indication for retransplantation in the Tv group 33.9% vs 12.2% in Wo group (*$p=0.0244$*). No difference was noted with respect to time to first rejection episode (12-month probability of 53.3% Tv and 57.8% Wo). **Conclusions:** Technical Variant Liver Transplantation expands the pediatric donor pool, and is associated with a decreased waiting time from listing to transplant. However, technical variant techniques are associated with lower overall graft and patient survival. Early deaths however may have lead to bias in comparison of post transplant events. Centre specific effects are not accounted for in our results, and in interpreting these results the effect of the learning curve needs to be explored further.

Abstract# 604

SURVIVAL AMONG PEDIATRIC LIVER TRANSPLANT RECIPIENTS: IMPACT OF SEGMENTAL GRAFTS. Peter Abt,[1] Rachel Rapaport-Kelz,[1] Adam Frank,[1] Luis Campos,[1] James Markmann,[1] Abraham Shaked,[1] Kim Olthoff.[1] *[1]Department of Surgery, University of Pennsylvania, Philadelphia, PA.*

Background: Living donor (LD), as well as cadaverc segmental and in situ split liver allografts have become an important alternative to whole cadaveric organs in the pediatric population, successfully reducing death on the waiting list for small children. The aim of this study was to determine comparative survival based on graft type in the pediatric population, and to identify variables associated with outcome in partial liver grafts. **Patients and Methods:** We performed a retrospective cohort study (1991-2001) among children 12 years or less using the UNOS scientific registry. Kaplan-Meier analysis was used to compute patient and graft survival, with the log-rank statistic for comparison between groups. Cox proportional hazards analysis was used to identify factors associated with graft survival. **Results:** During the 10 year study period, 3631 children received their first liver transplant, the majority receiving whole cadaveric grafts. Children under the age of one were more likely to receive a LD or segmental cadaveric graft (16.7% and 24.1%) than children older than 1 year of age (8.2% LD and 17.4% segmental grafts). After controlling for multiple donor and recipient variables including recipient age, medical condition of the recipient, laboratory values, and donor age, living donor grafts appeared to have an improved survival compared to cadaveric segmental grafts (Table). Poorer grafts survival among recipients of cadaveric segmental grafts was reflected by a higher rate of retransplantation and inferior patient survival. **Conclusions:** Recipients of LD grafts appear to have a survival advantage when compared to those transplanted with segmental grafts from cadaveric donors, but similar to full-size cadaveric grafts. Less favorable graft function in the recipients of cadaveric segmental grafts is reflected by an increased need for retransplantation and worse patient survival. Controlling for donor and recipient variables in this study suggests that better outcome in the LD transplant setting may be related to use of high quality organs that are taken from stable donors and transplanted within a short cold ischemic time.

Graft and patient survival based on type of graft

Graft type	N (%)	3 yr graft survival	3 yr pt survival	Retxp
Whole cadaveric	2513 (69.2)	69.6%	76.9%	13.2%
Segmental cadaveric	715 (19.7)	64.3%†	73.6%†	16.9%‡
Living donor	403 (11.1)	73.9%	79.7%	13.2%

†P<0.05 compared to LD and whole cadaveric ‡P<0.05 compared to LD

Abstract# 605

COMPLETE IMMUNOSUPPRESSIVE DRUG WITHDRAWAL AS A UNIFORM APPROACH TO POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN PEDIATRIC LIVER TRANSPLANTATION. Melissa Hurwitz,[1] Dev M. Desai,[2] Kenneth L. Cox,[1] Carlos O. Esquivel,[2] Maria T. Millan.[2] *[1]Department of Pediatrics, Stanford University School of Medicine, Palo Alto, CA; [2]Department of Surgery, Stanford University School of Medicine, Palo Alto, CA.*

Purpose: Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) in pediatric liver transplant recipients is associated with high mortality (up to 60%) especially in the high risk, predominantly EBV-naïve, younger age groups. This study assesses patient outcome and graft loss to rejection when, in addition to anti-viral medications and/or chemotherapy, complete withdrawal of immunosuppressive agents (IMS) is instituted as the mainstay of therapy. Methods: A retrospective analysis of 335 pediatric patients whose liver transplants were performed by our team from September 1988 to September 2002 was performed through review of computer records, database and patient charts. Results: Fifty-one patients developed either EBV or PTLD; 82% were ≤ 2 years of age. Of the 51 patients, 20 had a positive tissue diagnosis for PTLD and 31 were diagnosed with EBV infection, 14 of whom had positive tissue for EBV. Sixty percent of patients who developed PTLD and 51.6% of patients with EBV received antibody for induction or treatment of rejection prior to onset of disease. Forty-five patients (88%) received post-transplant antiviral prophylaxis with DHPG. Antiviral treatment included DHPG in 85% and Acyclovir in 13% (Cytogam was used in 41%). In those with PTLD, treatment included chemotherapy (n=1), Rituximab (n=2), and ocular radiation (n=1). IMS was stopped in all patients with PTLD and in 19 with EBV; it was held as long as there was no allograft rejection. Nine patients have remained off IMS with excellent allograft function for 94.4 ± 25.3 months. Of the 21 patients who restarted IMS for acute rejection, 18 responded to steroids and/or reinstitution of low-dose calcineurin inhibitors. The mean time to rejection was 123.6 ± 157 days (range, 7 to 484 days). Two patients were re-transplanted for chronic rejection; one of these died from sepsis. The mortality rate in our series was 30% in those with PTLD and 6% in those with EBV. The cause of death was related to PTLD or sepsis in all cases; no deaths were due to graft loss from acute or chronic rejection. Conclusions: PTLD is associated with high mortality in the pediatric population. Aggressive management is warranted including early cessation of IMS, anti-viral therapy, and chemotherapy for advanced disease. Episodes of rejection occurring after ceasing IMS can be successfully treated with standard therapy without graft loss due to acute rejection.

Abstract# 606

PREDICTORS OF LENGTH OF STAY FOR PEDIATRIC LIVER TRANSPLANT RECIPIENTS. John C. Bucuvalas,[1] Ravinder Anand,[2] Lan Zeng,[2] Split Research Group.[2] *[1]Pediatric Liver Care Center, Cincinnati Childrens Hospital, Cincinnati, OH; [2]The EMMES Corporation, Rockville, MD.*

Approximately 100 million dollars are spent on the hospitalization for the 400-500 children who undergo liver transplantation (LT) each year. The trends of resource use for children who have received LT have not been defined. Using length of stay as a surrogate marker for hospital resource use, we sought to identify factors which impacted on length of stay and assess the trends of hospitalization after LT for a representative population of pediatric liver transplant recipients. **Methods:** The study population included 953 patients who underwent primary LT between 1995 and 2002 and survived at least 90 days. Data were retrieved from the Studies of Pediatric Liver Transplantation (SPLIT) data registry which is comprised of 38 pediatric LT centers in North America. The primary outcome was the number of hospital days in the first 30 days after LT. Independent variables were age, sex, race, pediatric end-stage liver disease score (PELD) at LT, year of LT, organ type (whole vs. cadaveric technical variant vs. living), primary disease, length of operation, insurance status (Medicaid vs. private), and site of transplantation (US or Canada). Results are expressed as mean ± SE. We performed univariate analysis to identify potential predictors of length of hospitalization using Student's t-test or Wilcoxon test, as appropriate. **Results:** The mean number of hospital days in the first 30 post LT days was 19.9 ± 0.3. The number of hospital days was less for children who underwent LT after age 1 year (18.8 ± 0.4 vs. 21.8 ± 0.4), were White (19.2 ± 0.3 vs. 20.9 ± 0.4), had private insurance (18.5 ± 0.3 vs. 20.4 ± 0.5), received whole organs (18 ± 0.4 whole vs. 20.2 ± 0.6 living vs 23.6 ± 0.5 cadaveric technical variant), or were transplanted in the US (19.2 ± 0.3 vs. 24.6 ± 0.6). PELD score at LT ($r=0.25$, $p<0.0001$) and the length of operation ($r=0.09$, $p<0.0001$) correlated directly with the number of hospital days in the first 30 post LT days. We observed no trend in the number of hospital days in the first 30 days after LT from 1995-2002. Sex and primary diagnosis did not predict hospital days. **Summary:** Demographic factors, clinical status, and organ type correlate with resource use in the first 30 days after LT. Further analyses are necessary to identify independent predictors of hospital days after transplantation. Athough no trend in hospital days was observed in the period from 1995-2002, the primary outcome measure does not identify the proportion of hospital days due to readmission.

Abstract# 607

A MULITVARIATE ANALYSIS OF FACTORS EFFECTING PATIENT AND GRAFT SURVIVAL AFTER PEDIATRIC LIVER TRANSPLANTATION. Sue V. McDiarmid,[1] Ravinder Anand,[2] SPLIT Research Group. *[1]Pediatrics and Surgery, University of California, Los Angeles, Los Angeles, CA; [2]Emmes Corp, Rockville, MA.*

Studies of Pediatric Liver Transplantation (SPLIT) collects data from 38 US and Canadian centers for children undergoing liver transplant (tx). In this study previously reported univariate analyses of baseline and post tx factors effecting pt and graft survival were updated with 2002 data, and used in multivariate models to determine independent predictors of pt and graft survival 6 months after a first liver tx. Results: 1092 children were 1st tx recipients. Baseline factors in the univarate analyses were: age, sex, race, diagnosis,organ type,donor age,status and PELD score at tx, cyclosporine vs tacrolimus 1° therapy, growth failure, warm and cold ischemia times, introp. blood use, initial hospital and ICU days. Post tx factors were: reoperations, septicemia, HAT, PVT, biliary leaks and strictures, rejection, retransplantation. All post tx events were modeled using time varying variables. Factors sig at the univariate level ($p<0.05$) were entered into Cox multivariate regression models. Model 1 examined baseline variables alone and showed that the sig factors for pt death were diagnosis, organ type, warm ischemia time and blood use.Only blood use was sig for graft failure. Model 2 combined baseline and post tx factors.Table 1. Conclusions: Model 2 shows that post tx factors have the strongest effect on pt and graft survival: reoperation, retransplant, septicemia and vascular thromboses.The only baseline factor of sig for pt survival was status at tx. PELD at tx did not effect pt and graft survival in any multivariate analysis.

p values (hazard ratio)	Patient Survival 751	Graft Survival 664
Growth Failure Ht±Wt Z >-2 vs <-2	0.44 (1.25)	0.64 (1.12)
Status at Tx Cont Hosp vs at home	0.03 (2.17)	0.09 (1.67)
Donor age	ND	0.58
Organ Type	0.60	0.38
Diagnosis	0.54	0.25
CsA vs Tac	0.68 (1.12)	0.56 (1.15)
Blood Use	0.19 (1.0)	0.14 (1.00)
Warm Ischemia Time	0.47 (1.00)	0.42 (1.00)
Cold Ischemia Time	ND	0.87 (0.99)
#Rejections:1 vs 0	0.057 (0.516)	0.78 (0.92)
Reoperation	<0.0001 (9.45)	<0.0001 (15.75)
HAT	0.14 (1.67)	<0.0001 (4.45)
PVT	0.47 (1.32)	0.001 (2.65)
Septicemia	<0.0001 (4.51) ,	0.016 (2.05)
Retransplant	<0.0001 (7.88)	ND
Biliary Leak	0.56 (0.81)	0.46 (1.25)
Anastomotic Sricture	0.56 (1.44)	0.84 (0.24)

Abstract# 608

MORBIDITY TEN YEARS AFTER PEDIATRIC LIVER TRANSPLANTATION - IT IS NOT ONLY ABOUT THE LIVER. Yaron Avitzur,[1] Mae Cantos,[1] Carolina Jimenez-Rivera,[1] Enza De Luca,[1] Annie Fecteau,[1] Nicola L. Jones,[1] David Grant,[1] Vicky L. Ng.[1] *[1]Pediatric Academic Multiorgan Transplantation Program, Hospital for Sick Children, Toronto, Canada.*

Improvements in short-term outcome after pediatric liver transplantation have resulted in a growing population of long-term survivors. However, the long-term morbidity of these children is less well characterized. The aim of this study was to characterize the outcome and morbidity of patients ten years after pediatric liver transplantation. Methods: A retrospective chart review of all (n=74) children who underwent liver transplantation before October 1992 at our center. Exclusion criteria included transfer of follow up to other centers (n=10) and death before the ten-year anniversary (n=32). For the 32 remaining patients outcome measures included: laboratory tests and growth percentiles at ten years after transplantation. Medical complications and rejection episodes developing during the ten-year period were also recorded. Results: Actuarial graft survival for the ten-year survivors at 1,5, and 10 years was 91%, 84%, and 75% respectively. Six (18%) and 2 (6%) patients required a second and third transplant respectively, with hepatic artery thrombosis (n=4), and chronic rejection (n=2) being the most common indications. Synthetic liver function was preserved in all ten-year survivors. The cummulative annual rate of acute liver rejections declined from 1.44 rejections/patient/year in the first year to 0.32 by year 10. Eight (25%) patients developed chronic liver rejection. At ten years after transplantation, medical complications included chronic renal failure (78%), mild chronic anemia (60%), and hypertension (31%). Low compliance was reported in 7 (22%) adolescents, which contributed to development of acute rejection in five. At ten years mean percentile for weight was 43±33%, and for height 38±30%. No long-term diabetes was noted and mild hyperlipidemia was detected in 6 (18%) patients. Twenty-six (81%) children experienced a major infection during the ten-year period. Varicella-zoster virus (n=20), manifesting as either chicken pox or herpes zoster, was the most common. Twenty patients had an elevated EBV DNA PCR on the ten-year anniversary and EBV related malignancies were diagnosed in 7 (22%) patients. Conclusions: Despite good graft function in children 10 years after liver transplantation, co-morbidities place them at increased risk of developing end-organ damage. Future treatment and follow up regimens, as well as awareness of transplant physicians to these co-morbidities, will be instrumental to further improve outcome in these children.

Abstract# 609

RENAL FUNCTION IN LONG-TERM PEDIATRIC LIVER TRANSPLANT SURVIVORS. K. Campbell,[1] N. Yazigi,[1] F. Ryckman,[1] M. Alonso,[1] G. Tiao,[1] W. Balistreri,[1] H. Atherton,[1] J. Bucuvalas.[1] *[1]Pediatric Liver Care Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: While an acute decrease in renal function during the first post-transplant year is a recognized complication of calcineurin inhibitor (CI) use following liver transplantation (LT), the effect of long-term administration of CI on renal function in children following LT is unknown. Given the potential for their longer survival, it is critical to identify those children at increased risk for progressive renal damage. **Purpose**: To determine the prevalence of renal insufficiency (defined as GFR < 70 cc/min/1.73m²) and to identify the predictors of renal dysfunction in children who are long-term survivors of LT. **Methods**: We conducted a single center retrospective cohort study of 117 children ≥3 years post-LT. Demographic and clinical data were obtained from chart review and from a prospectively maintained LT database. The primary outcome was measured GFR at ≥3 years post-LT. Independent variables were primary diagnosis, age at LT, race, gender, primary immunosuppression, time since LT, blood pressure at one year post-LT and measured GFR at one year post-LT. We performed univariate and multivariate analyses to identify independent predictors of GFR. **Results**: All patients received either cyclosporin (before 1996, 62%) or tacrolimus (after 1996, 38%). The median age at LT was 2 years. Average time since LT was 7.6 ± 3.4 years (range 3-14.6 years). When the last available GFR for all patients was analyzed, renal insufficiency was present in 32%. In univariate analysis, CSA immunosuppression ($p<0.01$) and increased time since LT ($r = -0.52$; $p<0.01$) correlated with lower long-term GFR, while GFR at one year post-LT correlated directly with long-term GFR ($r=0.54$; $p<0.01$). GFR did not correlate with age at LT, race, gender, primary diagnosis or blood pressure at one year post-LT. When primary immunosuppression was excluded, multiple linear regression revealed that lower GFR at one year post-LT ($p<0.01$) and increased time since LT were associated with lower long-term GFR ($p<0.01$; $r^2=0.45$). **Conclusions**: Renal dysfunction is a common complication in children who are long-term survivors of LT. Although serial measurements of GFR are necessary to adequately define the relationship between renal function and time since LT, our data support the concept of a continuing decline in renal function post-LT. Our observations are of critical importance since children may live long enough to move from a stage of renal insufficiency characterized by asymptomatic decreased GFR to severe renal disease.

IMMUNOSUPPRESSION: EFFECTS ON GENE EXPRESSION, CYTOKINES, AND LYMPHOCYTES

Abstract# 610

INDUCTION THERAPY WITH CAMPATH-1H COMPLETELY DEPLETES PERIPHERAL BLOOD DENDRITIC CELLS BUT NOT THOSE IN THE BONE MARROW. Manuel R. Carreno,[1] Yide Jin,[1] Laphalle Fuller,[1] James M. Mathew,[1] Rolando O. Garcia-Morales,[1] Gaetano Ciancio,[1] George W. Burke,[1] Violet Esquenazi,[1] Joshua Miller.[1] *[1]Dept of Surgery-Div of Transplantation, Univ Miami Med School & Miami VA Med Center, Miami, FL.*

Direct and indirect presentation of alloantigens in solid organ transplantation are thought to contribute to rejection, and both mechanisms are believed to involve (myeloid) dendritic cells (DC1), while a tolerogenic effect has been proposed for certain (lymphoid) dendritic cells (DC2) that lack co-stimulatory molecules and occur in a higher concentration in bone marrow (BM). In this study we examined in flow cytometry the effect of Campath-1H induction therapy on these cell subsets in peripheral blood of kidney allograft recipients and *in vitro* on BM and peripheral blood cell preparations. Additionally, we looked at the effect of *in vitro* treatment with Campath-1H on various progenitor cells that express CD34, CD38, and CD33. In peripheral blood we found that DC1 expressed 10-fold more CD52 surface antigen than DC2 (dim); however, both were removed by Campath-1H treatment *in vivo*. The pre-treatment ratio of DC1/DC2 (>7.0) returns slowly over a period of 3-4 weeks, but the DC2 level increase appeared at least twice as fast as DC1 (DC1/DC2 <3.2 in the immediate post-treatment period). By contrast, although 60-80% CD34 peripheral blood cells were removed by Campath-1H treatment, they returned to pre-treatment levels by 1 week. *In vitro* peripheral blood cells and BM cells from vertebral bodies and iliac crest were treated with 20 ug/ml of Campath-1H in autologous fresh human or rabbit serum (complement source) for 3, 6, 18, and 24 hours. The *in vitro* treatment of peripheral blood completely removed all cell lineages as *in vivo*. However, in BM preparations, the CD34, DC2, and DC1 were reduced in different proportions, i.e., by ~50%, ~10%, and ~40%, respectively. Conclusion: Subsets of peripheral blood cells and BM cells exhibit different sensitivities to Campath-1H treatment. Additionally, the *in vivo* return of DC2 cells was more rapid than DC1, which supports the notion that DC1 alloactivation (myeloid) cells developing from residual monocytes returns more slowly in peripheral blood after Campath-1H treatment, while the (more resistant) DC2 'tolerogenic' (lymphoid) cells are mobilized from the marrow more rapidly and may allow for graft acceptance.

Abstract# 611

THYMOGLOBULIN INDUCES PROFOUND AND PROLONGED SUPPRESSION OF CYTOKINE EXPRESSION IN HUMAN KIDNEY TRANSPLANT RECIPIENTS. John P. Vella,[1] Shay Harris, Jonathan Himmelfarb. *[1]Division of Nephrology & Transplantation, Maine Medical Center, Portland, ME.*

Thymoglobulin® is a rabbit polyclonal anti-thymocyte globulin that minimizes the risk of allograft rejection. It is our hypothesis that Thymoglobulin® induced immunosuppression is due to a functional effect on T cells in addition to lympholysis. Blood was obtained from 47 transplant recipients pre- and defined times post-transplantation. Four immunosuppressive protocols were studied: Group 1: Thymoglobulin®+triple therapy, (G1, n=13); Group 2: MMF+Pred (G2, 6 Ag match live donor protocol, n=9); Group 3: Triple therapy (G3, n=16) and Group 4: Daclizumab, Sirolimus, MMF+Pred (G4, n=9).The capacity for mitogen stimulated PBL intracellular cytokine production by CD3+, CD4+ and CD8+ T lymphocytes was measured by flow cytometry. Thymoglobulin® induction therapy resulted in profound and prolonged lymphocyte depletion ($p<0.05$ compared with groups 2-4). The effect was seen to a much greater extent in the CD4>CD8 subset ($p<0.01$). The extent and duration of lymphopenia differed between groups with the most severe depletion seen in the Thymogobulin® group and least in MMF/Pred group (P1>P4>P3>P2, $p<0.005$). Cytokine production was profoundly effected by immunosuppressive protocol. CD3, CD4 &CD8 production of IL-2, TNF-α and INF-γ was significantly reduced in group 1 compared with groups 2-4 ($p<0.05$-0.005) by day 30. The rate of recovery therafter differed between groups. For example, INF-γ production recovered in all groups by day 60 while TNF-α recovery was variable between groups. Interestingly, IL-2 production was significatly more suppressed in G1>G4 out to day 90 in spite of the fact that patients in group 4 recieved an anti-CD25 monoclonal antibody (6% versus 22%, $p=0.05$). This late and profound cytokine suppression was seen in spite of CD3 T cell recovery to 30% of pre-transplant levels. By way of another example, CD3+ T cell production of IL-2 remained at 8% of pretransplant levels in spite of overal CD3 recovery to 30% by day 60 ($P<0.05$). Conclusion: These data indicate that induction immunosuppression with Thymoglobulin® causes more *profound and prolonged lymphopenia than that provided by conventional double or triple therapy including newer anti-CD25 regimens*. There is in addition, a functional effect on T cell cytokine production that is greater than the effect on cell count alone. These observations explain the differential efficacy of the above regimens in preventing allorejection.

Abstract# 612

ASSOCIATIONS BETWEEN FTY720 BLOOD LEVELS VS LYMPHOPENIA AND ACUTE REJECTION EPISODES IN DE NOVO KIDNEY TRANSPLANTATION. J. M. Kovarik,[1] H. Tedesco-Silva,[2] R. Schmouder,[1] P. Pellet,[1] S. Fornairon.[1] *[1]Novartis Pharmaceuticals, Basel, Switzerland and East Hanover, NJ; [2]Hospital do Rim e Hipertensao, Sao Paulo, Brazil.*

Associations between blood concentrations of the lymphocyte homing agent FTY720 vs the magnitude of lymphopenia and the occurrence of acute rejections were explored in kidney transplantation. **Study design and data:** 163 de novo kidney recipients were randomized to receive a 3-month FTY maintenance regimen of 0.25 mg/day (n = 41), 0.5 mg/day (n = 41), 1 mg/day (n = 40), or 2.5 mg/day (n = 41) with Neoral and corticosteroids. Trough FTY blood concentrations (Cmin) and lymphocyte counts were obtained weekly during treatment and for one month after the last dose. Biopsy-confirmed acute rejection episodes up to month 3 were included in the evaluation. **FTY pharmacokinetics and lymphopenia:** FTY Cmin reached steady state by week 5 and exhibited dose-proportionality: 1.3 ± 1.1, 2.2 ± 1.3, 4.3 ± 2.6, 9.3 ± 5.2 ng/ml at the four dose levels. The elimination half-life was 11.2 ± 7.1 days. The nadir lymphocyte count (see below) was reached on day 2 or 3 and remained stable to month 3. Lymphocyte counts recovered to ≥ 1 x 10^9/L within 2 weeks (0.25, 0.5 mg), 4 weeks (1 mg) or 6 weeks (2.5 mg) after FTY treatment. **Exposure-response associations:** The lymphocyte nadir and the majority of acute rejection episodes occurred in the first 5 weeks. The average FTY Cmin for each patient was determined over this period and divided into quintiles (32 patients/quintile). As tabulated below, the lymphocyte nadir was concentration-dependent. An inhibitory Emax model estimated the FTY Cmin for a half-maximal reduction in lymphocytes was 0.6 ± 0.4 ng/ml. At Cmins ≥ 4 ng/ml, a near maximal reduction was observed which approached an estimated minimal lymphocyte count of 0.15 ± 0.08 x 10^9/L. An average FTY Cmin of 3.7 ng/ml in the first month posttransplant served as an informative level of drug exposure separating acute rejection rates of about 30% from a rate of 6%.

Parameter	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
FTY Cmin (ng/ml)	0.2 - 0.6	0.7 - 1.1	1.2 - 2.0	2.1 - 3.6	3.7 - 10.5
Lymphocytes x 10^9/L	0.85 ± 0.38	0.60 ± 0.40	0.43 ± 0.22	0.34 ± 0.19	0.28 ± 0.15
Acute rejection rate	28%	33%	22%	39%	6%

Conclusions: Average FTY trough blood levels ≥ 4 ng/ml in the early weeks posttransplant were associated with a near maximal lymphocyte decrease and with evidence for rejection prophylaxis in conjunction with full-dose Neoral. These FTY concentrations are attainable with regimens ≥ 2.5 mg/day.

Abstract# 613

ALTERATION IN THE INTRAGRAFT ENVIRONMENT SECONDARY TO IMMUNOSUPPRESSION: A COMPARISON OF GENE EXPRESSION IN HUMAN RENAL ALLOGRAFTS TREATED WITH SIROLIMUS VERSUS TACROLIMUS. Walter D. Park,[1] Dora I. Ninova,[1] Timothy S. Larson,[1] Mikel Prieto,[1] Mark D. Stegall.[1] *[1]Transplant Center, Mayo Clinic and Foundation, Rochester, MN.*

Introduction. Chronic allograft nephropathy is a major cause of late graft loss after kidney transplantation. We hypothesize that alterations in the intragraft environment secondary to nephrotoxic immunosuppressive regimens contribute to chronic allograft nephropathy. In this study, we compared intragraft gene expression differences in human renal allografts treated with either sirolimus or tacrolimus. **Methods.** Under an IRB-approved protocol, two kidney biopsies were obtained from 5 Tacrolimus/MMF/Prednisone-treated and 6 Sirolimus/MMF/Prednisone-treated kidney transplant recipients 4 months after transplantation. The first biopsy was formalin fixed, paraffin embedded and a section stained for pathological consideration. Each slide was scored by two pathologists as histologically normal, with no acute cellular or humoral rejection and minimal fibrosis (cg1, ci1, or less). The second biopsy was used for RNA extraction and the extract was hybridized on U95Av2 Affymetrix high-density microarrays. **Results.** Using a t test we identified 179 transcripts with significant changes ($p<0.05$) in gene expression. We monitored the magnitude of specific changes in gene expression using the following equation: $\Delta HI = HI_{Tacrolimus} - HI_{Sirolimus}$. A total of 102 transcripts were significantly downregulated and 77 were significantly upregulated. The most significantly downregulated transcripts were K+ voltage-gated channel 4 (ΔHI -1.41 ± 0.33, p 0.0015,), Budding uninhibited by benz. 1β (ΔHI -1.16 ± 0.27, p 0.0015) and BMP-2 inducible kinase (ΔHI -1.10 ± 0.27, p 0.0018). The most upregulated transcripts were Claudin 5 (ΔHI 0.71 ± 0.17, p 0.0014), Echinoderm microtubule assoc. protein (ΔHI 1.21 ± 0.33, p 0.0045) and Coagulation factor II receptor (ΔHI 0.54 ± 0.16, p 0.0053). **Conclusions.** Significant changes in gene expression were identified between the tacrolimus-treated and sirolimus-treated grafts, even though by pathology all eleven grafts appeared histologically normal. As expected, the patients on calcineurin-inhibitor therapy had higher gene expression related to leukocyte trafficking (TCCR and S100-A9), adhesion (VLA-4 and ESM1) and fibrosis (Collagen VI and XV). Other molecules previously unknown in transplantation such as Claudin 5 and BMP-2 inducible kinase were also altered (confirmed by RT-PCR) and further investigation may yield new insights into the mechanisms by which these drugs interact with the graft.

Abstract# 614

TGF-β1 EXPRESSION IN PROTOCOL BIOPSY AFTER RENAL TRANSPLANTATION: THE EFFECT OF DIFFERENT IMUNOSUPPRESSANTS. Ondrej Viklicky,[1] Ivo Matl,[1] Ludek Voska,[1] Alena Lodererova,[1] Radka Bohmova,[1] Ilja Striz,[1] Stefan Vitko.[1] *[1]Institute for Clinical and Experimental Medicine, Prague, Czech Republic.*

Background: Transforming growth factor β1 (TGF-β1) is a key profibrogenic cytokine associated with the pathogenesis of the chronic allograft nephropathy (CAN). The aim of the study was to compare morphological findings and tissue TGF-β1 expression in protocol kidney graft biopsy, and plasma TGF-β1 levels, in patients treated with different immunosuppression. *Methods:* The protocol kidney graft biopsies were carried out in 79 patients with stable renal function (serum creatinine < 250 μmol/L), treated with mycophenolate mofetil, steroids and either cyclosporine (CsA; n=49) or tacrolimus (TAC; n=30) at twelve months after transplantation. Morphological findings were evaluated using Banff-´97 classification. TGF-β1 expression was analysed using immunohistochemistry and semiquantitatively scored in different renal structures (total score 0-18). TGF-β1 plasma concentration was evaluated using ELISA TGF-β1 kit. *Results:* TGF-β1 expression within the graft tissue was significantly higher in patients treated with CsA as compare to TAC (9.9±4.1 vs. 5.0±3.2; p<0.001); however, the TGF-β1 plasma concentration was lower in CsA group than in TAC group (5369±4505 vs. 7339±4774 pg/mL; p<0.05). There was higher plasma total cholesterol level (6.21±1.19 vs. 5.33±1.05 mmol/L; p<0.01) and higher diastolic blood pressure (84.3±9.6 vs. 79.3±8.8 mmHg; p<0.05) in CsA group as compare to TAC group. CAN occurred in 68% of all biopsies and there were no differences in the CAN prevalence and the CAN grade between CsA and TAC groups. There were no relations between the TGF-β1 tissue expression, TGF-β1 plasma concentration, HLA mismatches, PRA, donor age and CAN grade in both groups. *Conclusion:* The decrease of TGF-β1 expression in one-year protocol kidney graft biosy in TAC-treated patients with stable renal function suggests a possible benefit for long-term graft acceptance. *Supported by the grant No 6373/2000.*

Abstract# 615

CHRONIC TYPE 1 ANGIOTENSIN II RECEPTOR BLOCKADE ATTENUATES LYMPHOCYTE RESPONSIVENESS IN RENAL ALLOGRAFT RECIPIENTS. Bryan N. Becker,[1] Lynn M. Jacobson,[2] Rebecca J. Muehrer,[1] Debra A. Hullett.[2] *[1]Medicine, University of Wisconsin, Madison, WI; [2]Surgery, University of Wisconsin, Madison, WI.*

Animal studies suggest that angiotensin II (Ang II) is an immunomodulatory hormone and a cytokine. Ang II receptors have been identified on lymphocytes, monocytes and macrophages. Therefore, Ang II signaling through its ligand-receptor interaction in different types of immune cells serves as a potential target for immunomodulatory therapy. The advent of Ang II receptor blockers (ARBs) for the clinical management of high blood pressure has provided us with an opportunity to examine whether ARBs also have immunomodulatory effects *in vivo* in human transplantation. We obtained serial (every 3 months) peripheral blood lymphocyte (PBL) samples from a cohort of patients with biopsy-proven early chronic allograft nephropathy (CAN). Patients (n = 11) were randomized upon biopsy to ARB treatment (losartan 25-50 mg daily without ACE inhibition) or no ARB or ACE-inhibitor as part of their blood pressure management. The majority of patients were on low dose or no calcineurin inhibition with corticosteroids (5-10 mg daily) and mycophenolate mofetil (500-1000 mg twice daily). Average glomerular filtration rates (GFR) were not significantly different between the groups. PBL stimulation assays were performed by culturing PBLs from ARB and non-ARB-treated individuals for 72 hours in CD3/CD28-coated plates, then incubating 1 x 10^5 cells per well in 96-well plates with [^{3}H]-thymidine, to measure lymphocyte proliferation. ARB-treated individuals demonstrated a significant reduction in the Δ proliferation index (change in proliferation from initiation of treatment to measurement at 3 months; p = 0.025). There was a trend towards decreased proliferation at 3 months comparing ARB versus non-ARB-treated patients. By 6 months however, ARB-treated patients demonstrated significantly less lymphocyte proliferation with daily ARB treatment (p = 0.044). Notably, the proliferation indices from ARB-treated patients were not significantly different from control, non-transplant patients. These data suggest that ARBs are truly immunomodulatory therapy and may have a role as adjunct treatment following kidney transplantation beyond just their anti-hypertensive and anti-proteinuric effects.

Abstract# 616

CAN ATP LEVELS MONITOR IMMUNOSUPPRESION? Pam M. Kimball,[1] Anne King,[1] Richard Stirling.[1] *[1]Surgery, Medical College of Virginia, Richmond, VA.*

Immunosuppression is an inexact science. Development of biomarkers as indices of T-cell activation may allow greater precision in tailoring immunosuppression to the individual patient. It has been suggested that intracellular ATP levels may be a suitable biomarker. We evaluated the impact of changes in immunosuppression upon intracellular ATP levels in CD4 cells from10 patients during the first 30 days after renal transplantation (RTX). All recipients received cyclosporine or Prograf, mycophenolate mofetil and prednisone. Heparinized whole blood was collected biweekly, treated with PHA for 15 hrs and ATP levels in CD4 cells measured by luminescence. Immunosuppression was either decreased (protocol steroid taper from 60 to 20 mg/day by POD 25) or increased (due to low trough levels of calcinurin inhibitors). Clinically, 7 patients had immediate graft function and 3 had DGF. No rejections occurred during the study period. First, ATP levels were compared between healthy volunteers (Controls) and RTX recipients. RTX patients with functioning grafts had lower ATP levels than Controls although significance was not attained (385 ± 51 vs 498 ± 33 ng/ml, p=.07). Patients with DGF had significantly lower ATP levels than Controls (298 ±36 pg/ml, p=.0003). Since it seems unlikely that a specific ATP value will indicate immune activation, our subsequent analysis focused on the trend of ATP changes during transitions in drug dose or regimen. Reducing the level of immunosuppression resulted in a significant increase (p=.0001) in ATP levels. At the end of the 3-fold reduction in steroid dose, ATP levels increased in 6/7 patients by 67 ± 7 % (range 50-80%). The remaining patient showed no change in ATP (<10%). Conversely, increasing the level of immunosuppression significantly decreased (p=.001) ATP levels. The dose of calcinurin inhibitor was doubled in 2 patients. Corresponding ATP levels decreased by 49 ±6%. In addition, l patient received 125 mg thymoglogulin for 4 days. ATP levels were less than 100 ng/ml during therapy and doubled when thymoglobulin was withdrawn. ATP levels did not correlate with sCr levels in patients with functioning grafts (p=ns) or DGF (p=ns). SUMMARY. Intracellular ATP levels in CD4 cells were inversely responsive to changes in immunosuppression. The results suggest that this easy and inexpensive test may be a useful indicator of T-cell activation status after RTX. As such, the test may permit immunosuppression to be tailored to meet the needs of the individual patient and may help discriminate immunologic from non-immunologic events after transplantation.

Abstract# 617

MAINTENANCE THERAPY WITH CYCLOSPORIN A, NOT FK506, INDUCES CYP 3A4 AND INHIBITS PGP ACTIVITY IN STABLE RENAL TRANSPLANT RECIPIENTS. Wim Lemahieu,[1] Bart Maes,[1] Kristin Verbeke,[2] Yves Vanrenterghem.[1] *[1]Internal Medicine, Division of Nephrology, UZ Gasthuisberg KULeuven, Belgium; [2]Laboratory of Radiopharmaceutical Chemistry, UZ Gasthuisberg KULeuven, Belgium.*

Background: Cyclosporin A (CsA) and tacrolimus (FK 506) are substrates and - at high concentrations - inhibitors of cytochrome P450 3A4 (cyp 3A4) and P-glycoprotein (Pgp). This study aimed to determine the effects of maintenance therapy with CsA or FK 506 on the in vivo activity of intestinal and hepatic cyp 3A4 and Pgp systems. **Methods:** ^{14}C Erythromycin was used as a probe for cyp 3A4 and Pgp after intravenous and oral administration on 2 consecutive days, in order to estimate hepatic and intestinal enzyme activity respectively. Catabolism by cyp 3A4 generates $^{14}CO_2$ that is exhaled. The fraction of tracer that escapes demethylation by cyp 3A4 and re-excretion in the gut lumen by Pgp is excreted in the urine. Recovery of ^{14}C at time infinite (*m*) in breath reflects activity of cyp 3A4 directly and *m* in urine corresponds inversely with Pgp activity. Two groups of 9 stable renal transplant (tx) recipients taking CsA or FK 506 performed the test at 1 year after tx. Other maintenance immunosuppressive therapy consisted of low dose steroids (4 mg of methylprednisone) and mycophenolate mofetil (n=17) or azathioprine (n=1). The test results of the two groups were compared with Wilcoxon's Rank Sum Test . The results were also compared with those obtained in 27 healthy controls (hc). **Results:** Intestinal and hepatic cyp 3A4 activity was significantly increased in patients taking CsA in comparison with those on FK 506 and hc (p<0.01 and <0.001 respectively). Intestinal and hepatic Pgp activity was significantly diminished in patients taking CsA in comparison with those on FK 506 and hc (p=0.014 and <0.001 respectively). There was no significant difference between patients taking FK506 and hc for any of the measured elimination pathways. **Conclusions:**In contrast with patients taking FK 506, cyp 3A4 and Pgp activity was significantly altered in patients taking CsA compared with healthy controls. This suggests that patients taking CsA are more prone to disregulation of bio availability of concomitantly given drugs.

cyp 3A4 and Pgp activity in patients on CsA vs FK 506 vs healthy controls (hc)

	IV : hepatic		PO : intestinal	
	breath test: m	*urine test: m*	*breath test: m*	*urine test: m*
	cyp 3A4	**1-Pgp**	**cyp 3A4**	**1-Pgp**
FK 506	6.12	14.72	0.88	2.36
CsA	***8.06***	***27.14***	***3.95***	***13.1***
hc	6.44	13.26	0.63	3

Abstract# 618

CALCINEURIN INHIBITOR WITHDRAWAL IN STABLE KIDNEY TRANSPLANT PATIENTS LEADS TO A DECREASE IN DONOR-SPECIFIC CTL FREQUENCY. Barbara J. van der Mast,[1] Jacqueline Rischen-Vos,[1] Petronella de Kuiper,[1] Lenard M. B. Vaessen,[1] Willem Weimar.[1] *[1]Internal Medicine, Transplantation Laboratory, Erasmus Medical Center, Rotterdam, ZH, Netherlands.*

Recently we have shown that a low frequency of donor-specific cytotoxic T-lymphocyte precursors (CTLpf) identifies kidney transplant patients in whom the immunosuppressive load can be safely tapered. In a prospective study, calcineurin inhibitors (CNI, i.e. CsA or Tacrolimus) are withdrawn in our kidney transplant patients two years after transplantation. They are converted and tapered to 50% of the original dose of MMF or AZA based on the donor-specific CTLpf (CTLpf < $10/10^6$ PBMC). We questioned whether stopping CNI has an effect on the donor-specific CTLpf, as CNI might also hinder immune responses leading to graft acceptance. We measured the frequency of donor and third-party reactive CTL's in 40 patients before and after withdrawal of CNI (mean time between samples: 4.4 months). In addition, the T-cell reactivity of PBMC to donor and third-party antigens was tested in MLR, and to tetanus toxoid (TET) as nominal antigen to test the general immune response. Donor-specific CTLpf significantly decreased after CNI withdrawal (median 17 vs. $9/10^6$ PBMC, p=0.0004). In contrast, no difference was observed in third-party reactive CTLpf, donor and third-party reactive MLR, and responsiveness to TET. The decrease in CTLpf correlated with the time between the two blood samples (before and after stopping CNI, p=0.04, r=-0.33). This decrease was caused by stopping CNI, since there was no correlation between CTLpf and the duration of the CNI treatment after transplantation. In conclusion, after withdrawal of CNI the donor-specific CTLpf decreases. We hypothesize that CNI suppress regulatory mechanisms that have the potential to down-regulate donor-specific CTL responses.

Abstract# 619

INTRAGRAFT RENAL ALLOGRAFT TRANSCRIPTIONAL ANALYSIS DURING RABBIT ANTI-THYMOCYTE GLOBULIN (RATG) INDUCTION AND SIROLIMUS MONOTHERAPY COMPARED TO STANDARD OF CARE TRIPLE IMMUNOSUPPRESSION. Robert L. Kampen,[1] Steve C. Hoffmann,[1] Douglas A. Hale,[1] Roslyn B. Mannon,[1] Linda C. Cendales,[1] S. John Swanson,[2] Allan D. Kirk.[1] *[1]Transplant Section, NIDDK, National Institutes of Health, Bethesda, MD; [2]Organ Transplant Service, Walter Reed Army Medical Center, Washington, DC.*

We have recently shown that aggressive induction with RATG (total dose 15-20mg/kg) allows for monotherapy sirolimus maintenance immunosuppression. Analysis of differences between this maintenance minimization approach compared to standard triple immunosuppression (STI) has yet to be performed. We therefore studied RNA levels in allografts from patients treated with high dose RATG induction and sirolimus monotherapy using Real Time Quantitative PCR compared to biopsies from STI patients. Transcript profiles were analyzed under various clinical conditions: post-reperfusion, stable allograft function, and during rejection, and standardized against non-transplanted kidney. STI kidneys immediately after graft reperfusion showed elevated levels of transcripts for IL6-8, IL10, IL12, Bcl2, Bax, Bcl2, TNFα, TGFβ, CD54, gCSF, mCSF, ECE1, IkB and NFkB compared to normal kidney. Grafts treated with RATG prior to reperfusion showed no significant differences in the magnitude or character of this profile. Thus, the salutary effect of RATG was not attributable to immediate differences in gene transcription. STI grafts after stabilization (>30 days post transplant) were characterized by increased levels relative to normal kidney of transcripts for CD3, HLADR, CD86, CD154, IFNγ, perforin, TNFα, IkB, bcl2 and mCSF. Again, grafts on monotherapy sirolimus were similar. Thus, these grafts had no evidence of elevated intragraft inflammation despite lower maintenance therapy. During acute rejection, STI grafts were characterized by increased transcripts for IL10, TNFα/β, TGFβ, CD3, IL2, HLADR, perforin, granzyme, fasL, IFNγ, CD28, CD40, CD80, CD86, CD152, and CD154. Conversely, rejection occurring on monotherapy sirolimus was characterized by comparatively less CD3, CD28, CD154, IL2 and IL12 relative to STI rejections – generally a picture of reduced T cell activation. All rejections were steroid sensitive. Thus, the reperfusion and maintenance phases appeared similar. Rejections, when they occurred, appeared less vigorous from the standpoint of T cell activation. This was not due to a histologically reduced infiltrate. These data suggest that this reduced maintenance approach raises the threshold for rejection perhaps as a result of the initial aggressive induction, rather than by innately influencing the allograft.

Abstract# 620

ALEMTUZUMAB (CAMPATH 1-H) FACILITATES PREDNISONE-FREE IMMUNOSUPPRESSION (IP) IN KIDNEY TRANSPLANT RECIPIENTS. Joseph R. Leventhal,[1] Lorenzo Gallon,[2] Dixon B. Kaufman,[1] Joan Stuart,[1] Alan J. Koffron,[1] Jonathan P. Fryer,[1] Michael Abecassis,[1] Frank P. Stuart.[1] *[1]Dept. of Surgery, Northwestern University, Chicago, IL; [2]Dept. of Nephrology, Northwestern University, Chicago, IL.*

Campath 1H is a humanized anti-CD52 monoclonal antibody, which rapidly depletes peripheral blood B and T cells, without affecting stem cells. Effective for lymphoproliferative disorders, Campath 1H has also shown promise as an induction agent in renal transplant (Tx) recipients (Calne et al Transplantation 1999. From 10/01 until 8/02, 127 renal tx recipients were treated with 1 intraoperative 30mg dose of Campath 1H. Intravenous methylprednisolone was administered for 3 days (500mg, 250mg, 125 mg). Maintenance IP consisted of mycophenolate mofetil (MMF) and tacrolimus (Tac) without oral steroids. In 37 pts (Group 1), Tac was started Day 7-10 post-Tx, while in 90 pts (Group 2), Tac was initiated on POD#1. 1 dose of Campath 1H resulted in profound and long-lasting reduction in circulating lymphocyte counts. In Group 1, pt survival (PS), graft survival (GS), and incidence of acute rejection (AR) is 97%, 89%, and 24% respectively at 1 yr. Biopsy-proven AR episodes have occurred in 9 pts, with mean onset at 125 days post-Tx. 6/9 AR attempts have been mild to moderate (Banff IA, IB x 5). 3 AR occurred within 2 weeks post-Tx; 2 were antibody mediated. 8/9 AR attempts in Group 1 required anti-lymphocyte Rx. In Group 2, PS, GS, and AR are 100%, 100%, and 7.7%. Biopsy-proven AR occurred in 7 pts, with mean onset at 107 days post-Tx. No AR was observed in the first post-Tx month. 6/7 AR were mild to moderate (Banff IAx3, IBx3). 5/7 AR attempts in Group 2 required anti-lymphocyte therapy. Mean AR-free serum creatinine is 1.34 mg/dL for Groups 1 & 2 post-Tx @ 1 yr. All pts received CMV prophylaxis with valganciclovir 450mg p.o. daily; 2 pts experienced symptomatic CMV disease. Post-Tx complications have consisted of 1 bacterial urinary tract infection, 2 superficial wound infections, and 2 recurrences of FSGS. 56% of pts experienced leukopenia (WBC < 3000cells/uL). The combination of Campath 1H, Tac, MMF, and short course of i.v. steroids permit effective early post-Tx IP, without the need for oral prednisone. Unlike our previous experience with steroid avoidance using IL2-R antagonists for induction, rejection in pts receiving Campath 1H with early Tac initiation occurs later (> 30 days) post-Tx. Delayed introduction of Tac was associated with poorer outcomes. Leukopenia complicated pt management, and likely reflects additive marrow suppression by MMF and valganciclovir, not an effect of Campath.

Abstract# 621

RESULTS FROM A HUMAN TOLERANCE TRIAL USING ALEMTUZUMAB (CAMPATH-1H) WITH DEOXYSPERGUALIN (DSG). Allan D. Kirk, Douglas A. Hale, Linda C. Cendales, Steve C. Hoffmann, Robert L. Kampen, David E. Kleiner, Roslyn B. Mannon, Jonathan P. Pearl, Terri H. Wakefield, S. John Swanson. *[1]Transplant Section, NIDDK, National Institutes of Health, Bethesda, MD.*

We have previously investigated depletion with the CD52-specific antibody Alemtuzumab (Amab) as a potentially tolerogenic intervention for human renal transplantation (RTx). Although depletion has been shown to permit RTx with reduced maintenance immunosuppression, it has not induced tolerance. Early rejections associated with monocyte mobilization and infiltration have occurred in all patients treated solely with Amab despite several dosing strategies. Non-human primate (NHP) studies have suggested that the addition of the monocyte inhibitory drug DSG to T cell depletion induces tolerance. We therefore performed a tolerance trial in 5 patients adding DSG to Amab induction modeled after prior NHP studies. All patients underwent perioperative Amab therapy (0.3mg/kg on days –1, 1, 3 and 5) followed by live donor RTx. Two patients received DSG (4mg/kg x 1, 2.5 mg/kg x 13) from day –1 to 12 after which no immunosuppression was given. Lymphocyte and monocyte depletion was rapid and complete. However, both had rejection episodes on days 24 and 29 requiring the addition of a steroid taper to monotherapy immunosuppression (sirolimus x1, tacrolimus x1). Both rejections were characterized by monocyte mobilization with histology similar to 7 prior patients that received only Amab induction. Reasoning that DSG would be more effective if present during monocyte mobilization, the next 3 patients received the same dose of DSG from day 10 to 21. DSG prevented lymphocyte and monocyte resurgence and led to lymphocyte and monocyte counts <1% of baseline. Nevertheless, all 3 patients had rejection episodes on days 24-32, each despite profound T cell and monocyte depletion. Analysis of the peripheral cell phenotype at rejection showed that the sparse lymphoid cells were 75-90% memory phenotype T cells. Rare NK cells and monocytes were also detected. Biopsy analysis showed a predominantly monocytic infiltrate that was indistinguishable histologically or by transcription profile from patients receiving Amab alone or Amab and perioperative DSG. These data demonstrate that human allograft rejection can occur despite profound depletion of lymphocytes and monocytes. The addition of DSG does not induce tolerance despite a clear therapeutic effect of the DSG. Following depletion and despite rejection, patients can be maintained on monotherapy immunosuppression without late rejection even after lymphocyte repopulation.

Abstract# 622

CHARACTERISTICS OF REPOPULATING T CELLS IN RENAL TRANSPLANT RECIPIENTS TREATED WITH CAMPATH-1H.

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Campath-1H is a humanized anti-CD52 monoclonal antibody that depletes leukocytes within the peripheral blood and has been used in clinical trials to prevent renal transplant rejection. We hypothesized that the peripheral T cell population of renal transplant recipients would be significantly altered following treatment with Campath-1H and rapamycin. METHODS: Campath-1H (20mg IV) and methylprednisolone (500mg IV) were administered on days 0 and 1 of transplant. Patients were started on rapamycin monotherapy (2 mg po qd) within 24h of transplant, with a target level of 10-15 ug/ml. Peripheral T cell Vβ expression was analyzed pretransplant (n=6), and at 3 (n=3), 6 (n=6), and 12 (n=6) mos, by TCR Vβ CDR3 length analysis using PCR and fluorescent spectratype analysis. Cell surface markers for CD3, CD4, CD8, CD25, CD28, CD52, CD45RA, CD45RO, and CD69 were analyzed by flow cytometry. RESULTS: CD3, CD4 and CD8 rapidly depleted. CD4 and CD8 gradually returned to 20% and 40% pretransplant values by 12 mo. CD25 and CD69 had a similar decreased expression, but remained depressed by 12 mo. At time of T cell repopulation, there was no unusual expression of CD28, CD52, CD45RA or CD45RO. Following transplantation, all recipients had deletion of at least 5 TCR Vβ families, with an average deletion of 36% (24-46%) of pretransplant families. Following transplant, all patients had an increase in the percentage of Vβ families with a skewed distribution of VDJ length (ave=48%) at 3 to 6 mos. In addition, 2 recipients demonstrated oligoclonality in 2 separate Vβ families. By 12 mos post-transplant, in most patients, Vβ families reverted to a normal distribution, and in some patients additional Vβ families were expressed compared to pretransplantation. CONCLUSION: The T-cell population in peripheral blood of renal transplant recipients treated with Campath-1H showed significant changes in cell surface markers and in TCR β usage following transplant. By 12 mos, although the Vβ families reverted to a normal distribution, the CD3, CD4, and CD8 expression remained moderately depressed. There were no overall differences in CD28, CD52, CD45RA, CD45RO expression in the repopulating T cells. Longer-term follow-up is needed.

Abstract# 623

EXCELLENT LONG-TERM OUTCOME OF ABO-INCOMPATIBLE LIVING-RELATED KIDNEY TRANSPLANTATION IN JAPAN.

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Background We have been performing ABO-incompatible kidney transplants since 1989 to expand the opportunities for kidney transplantation from living donors because of a shortage of cadaveric graft in Japan. The goal of this study is to determine whether short-term and long-term outcomes of ABO-incompatible transplantation are any different than ABO-compatible cases. **Patients and methods** This survey focused on 441 patients in whom the follow-up survey could be completed. Patients (65% males and 35% females) were monitored in this study from January 1989 through December 2001 in 55 institutions. **Results** The overall patient survival rates at 1,3,5,7 and 9 years after transplantation were 93%, 89%, 87%, 85%, and 84%, respectively, with overall graft survival rates of 84%, 80%, 71%, 65%, and 59%. The past 3 year outcomes demonstrates that the graft survival rate at 1 year has increased 81%, 83% and 84%. The graft survival rate was significantly higher in patients aged 29 and younger compared with those aged 30 and older. In particular, very high graft survival rates were observed in children aged 15 years or younger. The patients with anticoagulation therapy (n = 223) show higher graft survival rates than those without anticoagulation therapy (n = 218). There were no significant differences between A-incompatible and B-incompatible recipients with respect to clinical outcomes. Overall graft loss occurred in 130 patients, and was caused by chronic rejection (37 patients), death (33), acute rejection (28), hyperacute rejection (9), and accelerated acute rejection (4). These outcomes demonstrate low potential of chronic rejection compared to ABO-compatible cases. Biopsy-confirmed acute rejection at 3 month post-transplant occurred in 256 (58%) of the study patients. Acute rejection during induction period was developed in 174 out of 291 in cyclosporine group (60%) and in 82 out of 150 in FK group (55%). Biopsy-confirmed chronic rejection occurred in 47 out of 301(16%). The complication of splenectomy including infection did not differ between the ABO-incompatible and compatible patients. **Conclusions** This study confirms that long-term outcome in ABO-incompatible living kidney transplant patients are excellent, and are similar to outcome observed in ABO-compatible kidney transplantation. Therefore, use of ABO-incompatible living donor kidneys is a radical, but effective, treatment for end-stage renal disease.

Abstract# 624

HIGH EFFICACY OF FTY720 WITH REDUCED CYCLOSPORINE DOSE IN PREVENTING REJECTION IN RENAL TRANSPLANTATION: 12-MONTH PRELIMINARY RESULTS.

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FTY720 is a novel therapeutic agent with a unique effect on the homing of lymphocytes without affecting their functions or properties. The potency of FTY720 in preventing rejection has been shown with a conventional dose of cyclosporine (CsA) after renal transplantation (RTx) **Purpose:** This ongoing trial is a 12-month, multicenter study evaluating the efficacy and safety of FTY720 with a reduced dose of CsA **Methods:** Adult patients undergoing a primary cadaver or a living donor transplant were randomized and received the first dose of study medication prior to RTx. The 4 treatment groups are: I FTY720 5 mg/d+ reduced CsA exposure, II FTY720 2.5 mg/d+reduced CsA exposure, III FTY720 2.5 mg/d+full CsA exposure, and IV MMF+full CsA exposure. All patients received corticosteroids, but no antibody induction was allowed. The Neoral dose was adjusted according to the C-2h levels with C-2h targets in gps I -II maintained at 50% or less than C-2h targets in gps III-IV **Results:** The intent-to-treat population consists of 258 patients who received a transplant and at least one maintenance dose of study drug. No relevant differences in race (79% caucasians) or donor source (65% cadaveric Tx) of recipients were noticed. The difference in C-2h levels achieved over the 12 months in reduced vs full CsA groups was > 50%. Preliminary efficacy results are presented below.

Endpoint:	Gp I N=72	Gp II N=72	Gp III N=76	Gp IV N=38
First biopsy-confirmed acute rejection	19.4%	37.5%	15.8%	21.1%
Graft loss	1.4%	12.5%	7.9%	7.9%
Death	1.4%	2.8%	2.6%	2.6%

There was no difference in Banff grading of rejection with FTY720 vs MMF. Patients in Gp II were switched to standard of care of therapy due to the risk of underimmunosuppression beyond month 6. The median GFR at the last study visit was 67, 61, 65, and 62 ml/mn in gp I, II, III and IV respectively. The notable safety difference was a higher incidence of bradycardia with FTY720 than with MMF (25% vs 5%, respectively). All of these events were reversible and without any sequela **Conclusion:** FTY720 combined with CsA is as effective as MMF in prevention of acute rejection in RTx. A FTY720 5 mg dose allows a significant reduction in CsA therapy and may offer a better safety/efficacy profile than conventional immunosuppressive regimen.

Abstract# 625

EXCELLENT GRAFT FUNCTION IN *DE NOVO* KIDNEY TRANSPLANT RECIPIENTS TREATED WITH EVEROLIMUS, REDUCED DOSE NEORAL® AND SIMULECT®: 6 MONTHS ANALYSIS.

Stefan Vitko,[1] John Whelchel,[2] Jossette Eris,[3] Scott Campbell,[4] Bernard Bourbigot,[5] Tomas Haas,[6] Annette Jappe for the 2307 Study Group.[6] *[1]Transplant Center, Institute of Clinical and Experimental Medicine, Praha, Czech Republic; [2]Organ Transplant Services, Piedmont Hospital, Atlanta, GA; [3]Renal Unit, Royal Prince Alfred Hospital, Camperdown, Australia; [4]Department of Renal Medicine, Princess Alexandra Hospital, Woollongabba, Australia; [5]Unite de Transplantation Renale, Hopital de la Cavale Blanche, Brest, France; [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* renal and heart transplants. This class of compounds have 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 episodes, graft loss, death or lost to follow-up in this 1-year multicenter, randomized, open label, trial. **Methods:** 133 patients were randomized to everolimus 1.5 mg/day (N=65) or 3 mg/day (N=68) in combination with Simulect®, corticosteroids and reduced administration of Neoral®. Neoral was tapered using C_2 monitoring: 500-700ng/ml (week 0-8) and 350-450 ng/ml (after week 8). 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 (15.4% and 17.6% respectively, p=NS), and the incidence of biopsy-proven acute rejection was lower in the 3mg everolimus group (8.8% and 13.8% in the 1.5 mg group, p=NS). Renal function at 6 months was comparable between both treatment arms with a mean (median) serum creatinine of 142 (135) µmol/L in everolimus 1.5mg and 137 (127) µmol/L in everolimus 3 mg. Mean creatinine clearance (Cockcroft-Gault) was 64.3 and 59.8 mL/min respectively. CMV infection were reported in 3.1% and 1.5% in 1.5 and 3 mg groups respectively. Thrombocytopenia was lower in the 1.5 mg everolimus group (3.1% and 8.8%). The incidence of hyperlipidemia reported as adverse event was comparable between the groups (30.8% and 27.9% in the 1.5 mg and 3 mg groups, respectively). **Conclusions:** This preliminary results suggest that de novo renal transplant patients treated with everolimus in combination with Simulect®, steroids and reduced dose of Neoral® maintain good efficacy results as well as excellent renal function. Additional long term results are required, however this type of regimen may be considered as a new treatment protocol to individualize patient care for kidney transplant recipients.

Abstract# 626

COMPARISON OF TWO STEROID-FREE REGIMENS - BASILIXIMAB/TACROLIMUS AND TACROLIMUS/MMF - WITH TACROLIMUS/MMF/STEROID THERAPY AFTER RENAL TRANSPLANTATION. Stefan Vitko,[1] Marian Klinger, Kaija Salmela, Zbigniew Wlodarczyk, Gunnar Tydén, the ATLAS Study Group. *[1]Transplantcenter, IKEM, Prague, Czech Republic.*

Aim: This 3-arm, 6-month, open, prospective study evaluated two steroid-free regimens - basiliximab/tacrolimus (B/Tac) and tacrolimus/MMF (Tac/MMF) - in comparison with triple tacrolimus/MMF/steroid therapy (control). **Methods**: Patients were randomized to receive either basiliximab and tacrolimus (B/Tac), tacrolimus and MMF (Tac/MMF); or tacrolimus, MMF, and steroids (control). The initial oral tacrolimus dose was 0.2 mg/kg/day, subsequent doses were adjusted to trough levels of 10-20 ng/mL (days 0-28) and 5-15 ng/mL (days 29-183). The MMF dose was 2 g/day for days 0-14, and 1 g/day thereafter. In the steroid-free arms, 500 mg i.v. prednisolone on day 0, but no maintenance steroids were given. In the control arm, steroids were as follows: 500 mg (day 0), 125 mg (day 1), then tapered from 20 mg/day (days 2-14) to 5 mg/day (>day 43). **Results**: In total 457 patients were randomized to B/Tac (n=152), Tac/MMF (n=151), and control (n=147), 7 patients were excluded. The study was completed by 82.9% (B/Tac), 94.7% (Tac/MMF), and 91.2% (control) of patients. Patient baseline characteristics were similar in all groups. The incidences of biopsy-proven acute rejection were 26.3% (B/Tac), 30.5% (Tac/MMF), and 8.2% (control), p<0.001(multiple test for comparison with control); B/Tac vs. Tac/MMF, p=ns. The incidences of corticosteroid-resistant acute rejection were 5.3%, 4.0%, and 2.0%, p=ns. Graft survival (94.7%, 96.7%, and 95.9%, p=ns) and patient survival (99.3%, 99.3%, and 100%, p=ns.) were similar in all groups. Median serum creatinine at month 6 was 135.1 µmol/L (B/Tac), 135.0 µmol/L (Tac/MMF) and 122.5 µmol/L (control), median calculated creatinine clearance was 55.4 mL/min, 59.1 mL/min, and 65.3 mL/min, respectively. The overall safety profile was similar in all groups; significant differences were reported for anemia (14.5% vs. 12.6% vs. 24.5%), diarrhea (5.9% vs. 17.9% vs. 12.9%), leukopenia (5.9% vs. 18.5% vs. 7.5%), and tremor (4.6% vs. 7.3% vs. 0.7%), for the B/Tac, Tac/MMF and control groups, respectively. **Conclusion**: Steroid-free immunosuppression using basiliximab induction and tacrolimus monotherapy or tacrolimus and MMF is feasible, however, the incidence of acute rejection was higher in the steroid-free arms compared with a tacrolimus/MMF/steroid combination therapy. The efficacy of B/Tac and Tac/MMF was similar.

Abstract# 627

STEROID-FREE IMMUNOSUPPRESSION WITH A COMBINATION OF DACLIZUMAB, TACROLIMUS AND MMF IS EFFICACIOUS AND SAFE: RESULTS OF A LARGE MULTICENTER TRIAL IN RENAL TRANSPLANTATION. Lionel Rostaing,[1] Diego Catarovich, Georges Mourad, Hans-Helmut Neumayer, Paolo Rigotti, the CARMEN Study Group. *[1]Service de Transplantation d'Organes, CHU Rangueil, Toulouse, France.*

Aim: This open, randomized, multicenter, 6-month study evaluated the efficacy and safety of a steroid-free regimen based on daclizumab / tacrolimus / MMF (Dac/Tac/MMF) in comparison with a standard tacrolimus / MMF / steroid (Tac/MMF/S) regimen in renal transplant recipients. **Methods**: In both treatment groups, patients received 0.1 mg/kg Tac pre-operatively. The initial oral Tac dose was 0.2 mg/kg/day; subsequent doses were adjusted to trough levels of 10-20 ng/mL (days 1-21), 10-15 ng/mL (days 22-41), and 5-10 ng/mL (days 43-183). The MMF dose was 2 g/day for the first 14 days and 1g/day thereafter in both arms. In the Tac/MMF/S arm, patients received 500 mg i.v. steroids on day 0, 125 mg on day 1, and then 20 mg (days 2-14) tapered to 5 mg (day 43 onwards). In the Dac/Tac/MMF arm a single dose of 500 mg i.v. steroids was administered on day 0; no maintenance steroids were given. A dose of 1 mg/kg daclizumab was given pre-operatively (day 0) and on day 14. **Results**: In total 551 patients were randomized, 13 patients were not transplanted leaving 278 patients in the Tac/MMF/S arm and 260 patients in the Dac/Tac/MMF arm (ITT). Patient baseline characteristics were similar in the two groups. In the Tac/MMF/S group 90.6% (Tac/MMF/S) and 82.3% of (Dac/Tac/MMF) patients completed the study; 8.6% (Tac/MMF/S) and 16.5% (Dac/Tac/MMF) of patients were withdrawn. The incidence of biopsy-proven acute rejection was 16.5% in both treatment arms, the incidence of corticosteroid-resistant acute rejection was 4.3% (Tac/MMF/S) and 5.0% (Dac/Tac/MMF), p=ns. In total 8 patients died, 3 in the Tac/MMF/S group and 5 in the Dac/Tac/MMF group. In the Tac/MMF/S group 12 grafts (4.3%) were lost compared to 21 graft losses (8.1%) in the Dac/Tac/MMF arm, p=ns. Median serum creatinine at month 6 was 125.0 µmol/L (Tac/MMF/S) and 131.0 µmol/L (Dac/Tac/MMF), median calculated creatinine clearance was 51.2 mL/min (Tac/MMF/S) vs. 48.6 mL/min (Dac/Tac/MMF), respectively. The most frequent adverse events were UTI (30.2% vs. 25.4%), anemia (20.9% vs. 23.5%), and leukopenia (18.0% vs. 21.5%). The treatment groups differed in the incidences of pneumonia (1.1% vs. 4.2%, p=0.028) and new onset diabetes mellitus (5.4% vs. 0.4%, p=0.001). **Conclusion**: The steroid-free regimen of daclizumab / tacrolimus / MMF was as efficacious as a standard Tac / MMF / S regimen. The safety profiles of both regimens were comparable.

Abstract# 628

A COMPARISON OF ACCESS TO AND OUTCOME OF KIDNEY TRANSPLANTATION (KTx) BETWEEN PATIENTS TREATED WITH HEMODIALYSIS (HD) OR PERITONEAL DIALYSIS (PD). Denis Glotz,[1,2] Ylana Chalem,[3] Jean-Philippe Ryckelynck,[4] Christian Verger,[5] Philippe Tuppin,[3] French Study Group. *[1]Nephrology, Hopital Georges Pompidou, Paris, France; [2]INSERM U430, Paris, France; [3]Statistiques et Evaluation, Etablissement Français des Greffes, Paris, France; [4]Nephrology, C.H.U., Caen, France; [5]Nephrology, C.H. Dubos, Pontoise, France.*

Although peritoneal dialysis is recognized as one of the methods of treatment of End Stage Renal Disease (ESRD), there have been recurrent concerns about the access of patients treated by this method to KTx as well as reports showing increased complications of transplantation in such patients, such as graft thrombosis and infections.Thus, a number of transplant centers seem reluctant to perform KTx in PD patients, raising concerns about the accessibility of transplantation for those patients. The aim of this study was, using a multivariate analysis of the French Transplant database, to provide a comprehensive view of the impact on transplantation of pre-transplant modality of treatment of ESRD. From 1997 to 2000, 9406 patients were registered on the waiting list, and 5071have received a KTx in France. After exclusion of paediatric patients, double transplantations and living donors, study populations consisted of 7717 patients on the waiting list and 3811transplanted patients. The variables analysed for the waiting time were age of recipient, degree of HLA immunization, existence of diabetes mellitus, year of registration on the waiting list, HLA alleles frequency, length of dialysis duration, pre-existing transplantation, blood group, sex and obviously type of dialysis (HD, PD). The variables analysed for the outcome of transplantation (recipient death or graft failure) included all of the above plus waiting time, donor age, donor-recipient age difference, donor blood group, donor recipient blood group identity/compatibility, donor sex,donor-recipient sex identity/mismatch, HLA mismatches, type of kidney (left/right), local harvest or shipped kidney and cold ischemia time. PD patients were younger, had less anti-HLA antibodies are were more frequently waiting for a first transplant. Multivariate analysis showed a shorter waiting time for PD patients, which became equivalent to HD patients when taking into account the mean waiting time of each transplant center as a variable. Outcome of KTx was equivalent for HD and PD patients. Strikingly, pre-emptive KTx was associated with a 50% decrease of graft loss in the first two years post-transplant. Thus, modality of treatment of End Stage Renal Disease has no impact on access to, or outcome of KTx, except for pre-emptive transplantation.

Abstract# 629

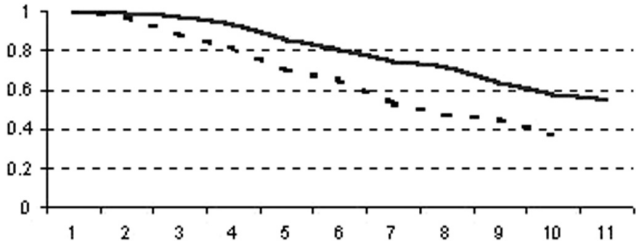
DURATION OF DIALYSIS EFFECTS CADAVERIC KIDNEY GRAFT SURVIVAL ONLY IN OLDER RECIPIENTS. Mark A. Schnitzler,[1] James F. Whiting,[2] Karen Hardinger,[3] Zoltan Kalo,[4] Stuart B. Boxerman,[1] Jeffrey A. Lowell,[1] William Chapman,[1] Daniel C. Brennan.[1] *[1]Wasington University School of Medicine, St. Louis, MO; [2]Maine Medical Center, Portland, ME; [3]St. Louis College of Pharmacy, St. Louis, MO; [4]Semmelweis University, Budapest, Hungary.*

Existing estimates of the association of dialysis duration with reduced graft survival are problematic given differences in recipient charcteristics by duration. Multivariate methods will not properly "control" for non-random sampling or selection bias effects in retrospective data. Given the difficult statistical problems associated with selection effects, we assessed the impact of dialysis duration in subsets of recipients of cadaveric renal transplants who had received dialysis prior to transplantation. **Methods:** Data for 28,401 first cadvaeric kidney alone transplants between 1995 and 1999 with recorded evidence of dialysis prior to transplantation were drawn from the USRDS data base. Associations with dialysis duration were compared with multivariate controls for patients waiting between 1 day and 1 year with patients waiting 1-3 years, 3-5 years, and 5-10 years on dialysis. Longer dialysis duration was strongly related to age over 45, recipient race, PRA > 0, and cause of ESRD. Therefore, patients were subset into groups by these factors. **Results:** Overall, dialysis lasting more than one-year prior to transplantation was associated with increases in risk of graft loss of 9% at 1-3 years ($P = 0.03$),21% at 3-5 years ($P < 0.001$), and 32% at 5-10 years ($P < 0.001$). No results were statistically significant, nor were there clinically important trends, in patients under the age of 45 at transplant. However, in patients over the age of 45 waiting on dialysis was associated with increases in risk of graft loss of 12% at 1-3 years ($P = 0.02$), 35% at 3-5 years ($P < 0.001$), and 49% at 5-10 years ($P < 0.001$). This effect was relatively small but significant at ages 45 – 55 increasing in size and significance with age. The effect was smaller in African Americans. Sub-setting patients by PRA and cause of ESRD did not alter these conclusions. **Conclusion:** There is considearble evidence that existing estimates of the graft survival effects of dialysis duration are not relevant for younger patients and underestimated for older patients. Older patients are also known to be at heightened risk of death while awaiting transplant on dialysis. Together this suggests that early transplant, perhaps through the use of expanded criteria donors, may be of particular importance in older patients.

Abstract# 630

RETURN TO DIALYSIS AFTER GRAFT LOSS IS NOT ASSOCIATED WITH DISMAL SURVIVAL RATES. Sarbjit V. Jassal,[1] Derek Yung,[1] Naisu Zhu,[2] Kim Badovinac,[2] Lilyanna Trpeski,[2] Stanley S. A. Fenton.[1] *[1]Dept of Medicine, University Health Network, Toronto, ON, Canada; [2]Canadian Institute for Health Information, Ottawa, ON, Canada.*

Preliminary reports suggest that only 48% of those returning to dialysis after allograft failure survive longer than 5 years. We reviewed the Canadian experience and found the survival in our population to be significantly better than previously predicted. Methods: Using administrative data, collected prospectively over the period 1991-2000, by the Canadian Organ Replacement Registry, we compared patient survival from the time of transplant failure to that of a cohort of patients starting dialysis for the first time. Patients were matched for age, gender, diabetes and treatment period (1991-5 and 1996-2000). To limit the effect of selection bias, dialysis patients with any comorbid conditions were excluded. Subsequent models comparing survival in these 2 populations using the time of first renal replacement therapy and the time of transplantation were derived to adjust for the effect of RRT vintage. Results: As shown in the graph, 55% of patients who returned to dialysis after graft loss (solid line) were still alive at 10 years. Survival was better than those patients who were not offered transplantation despite having no comorbid conditions (hatched line). In the secondary models, survival of those who had a transplant but then returned to dialysis after graft loss remained superior to those who remained on dialysis regardless of whether they were matched at the time of initial renal replacement therapy or at the time of transplant surgery. Conclusions We conclude that the Canadian experience is significantly different from the US experience, with higher survival rates in patients who receive a transplant, even after graft loss.



Abstract# 631

RELATIONSHIP BETWEEN PRE-TRANSPLANT OBESITY AND POST-TRANSPLANT CARDIOVASCULAR EVENTS, DIABETES MELLITUS, GRAFT AND PATIENT SURVIVAL IN RENAL TRANSPLANT RECIPIENTS. Yugo Shibagaki,[1] Katy Russo,[1] Ann Rivinus,[1] Jonathan Prather,[1] Aaron Markovich,[1] Murali Golconda,[1] Douglas Norman,[1] Douglas Keith,[1] Jae Chung,[1] Amira Al-Uzri,[1] Angelo de Mattos.[1] *[1]Transplant Medicine Program, Oregon Health and Science University, Portland, OR.*

Background: Obesity has been associated with increased surgical complications, overall and cardiovascular mortality in renal transplant (tx) recipients. Data regarding outcomes in patients who are obese pre-tx are contradictory. In this report we describe the association between pre-tx obesity and the incidence of non-fatal cardiovascular events (CE), post-tx diabetes mellitus (PTDM), patient and graft survival. **Methods:** A cohort of 637 adult, kidney-only recipients transplanted at our center between 1991 and 1997 was analyzed. Height and weight were used to calculate Body Mass Index (BMI). CE was defined by any new-onset angina, myocardial infarction, bypass surgery, PTCA, peripheral arterial ischemic events, TIA or CVA, endarterectomy or bypass surgery after the date of transplantation. We applied the WHO definition to diagnose PTDM. Multivariate analysis was used to adjust for factors commonly associated with CE, PTDM, graft and patient survival. **Results:** 608 patients were followed up to at least 5 years. Mean BMI at transplantation was 24.98 ± 0.41. There was no significant difference in the BMI between diabetics vs. non-diabetics. 55.6 % of recipients had a BMI of 25 or lower, 29.8% between 25 and 30, and 14.6% had a BMI greater than 30 (obese). At 3 months post tx, 32.2% of the patients were obese. 82 pts (13.5%) experienced at least 1 CE during the study period. After adjusting for the presence of diabetes pre-tx, donor type, and recipient age, the risk of developing of a CE within 5 years post-tx increased by 7.2% per 1 point increase in the BMI at the time of transplant (p=0.006). Similarly, the adjusted risk of PTDM in the same period increased by 9.6% per unit increase in the BMI (p=0.002). Patient survival was significantly worse in obese patients (p=0.005), however graft survival did not reach statistical significance (p=0.07). The adjusted risk (by presence of DM, donor type, re-tx status, and age) for patient death increased by 5.6% per unit increase in BMI (p=0.016). Likewise, the adjusted risk for graft loss increased by 1.8% (p=0.3). **Conclusions:** Obesity at the time of renal transplantation is a significant risk factor for non-fatal cardiac events, post-tx diabetes, and patient death. A longer followup period may be necessary to determine if pre-tx obesity is associated with worse graft survival.

Abstract# 632

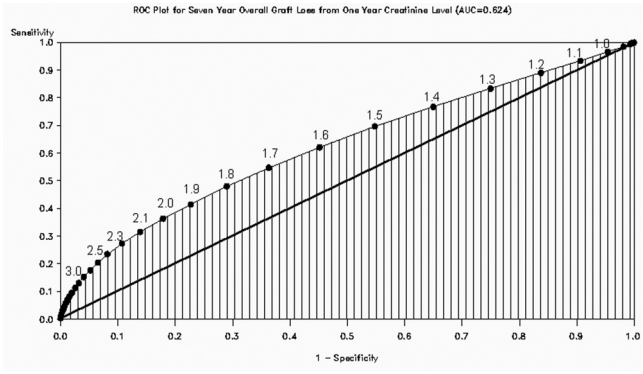
WHAT HAPPENS TO PATIENTS WITH CHF DUE LEFT VENTRICULAR SYSTOLIC DYSFUNCTION DEFINED AS LEFT VENTRICULAR EJECTION FRACTION (LVEF) OF LESS THAN 40% (CONGESTIVE CARDIOMYOPATHY IN UREMIA) IN DIALYSIS-DEPENDENT PATIENTS AFTER SUCCESSFUL KIDNEY TRANSPLANTATION? Ravinder K. Wali,[1] Greg Wong,[2] Lavanya Bellumokunda,[1] Steve Blahut,[1] Riple Hansalia,[2] Cinthia Drachenberg,[3] Meredith Brisco,[2] Steve Bartlett,[4] Michael Fisher,[5] Matthew Weir,[1] Simu Thomas.[4] *[1]Department of Medicine, Division of Nephrology; [2]Medicine; [3]Pathology; [4]Department of Medicine, Division of Transplantation; [5]Department of Medicine, Division of Cardiology, University of Maryland School of Medicine, Baltimore, MD.*

Congestive heart failure in patients with ESRD on maintenance dialysis is a difficult clinical diagnosis unless accompanied by an objective assessment of left ventricular function. Methods. 478 (36.2%) of 1320 patients who received kidney transplant here between June 1998 to December 2001 had history of congestive heart failure at the time of initial transplant evaluation. 114 (53%) had LVEF % of ≤ 40%. 79 patients with more than 3 months of functioning renal transplant were reevaluated at 6-9 months and again at 12-24 months after kidney tranaplantation. At baseline, mean (± SD) LVEF% was 31.8 ± 6.7 and 98% patients had New York Heart Association Class III & IV. Based on a priori definition, 54/79 (68.4%) achieved LVEF ≥ 50% (group A) as compared to 25 patients in whom LVEF persisted below 50% (group B). Increase in the mean left ventricular ejection fraction achieved by month sixth was maintained in the group as a whole and actually increased further on the repeat measurement to 52.8 ± 12.8 (p=0.02) at the mean (± SD) of 20.0 ± 14.2 (months). 19 of 79 patients died during the follow-up and persistence of LVEF of <50% was associated with more than 6-fold increase in the rate of death. 14 of 25 (56%) patients died in group B vs 5 of 49 (9.3%) in group A (p<0.001). LVEF of >50% was associated with a significant decrease in the rate of dying of progressive heart failure and also in the risk of sudden cardiac death, p<0.001. The reduction in mortality was not related to any of the pre or post-transplant covariates except the post-transplant LVEF% as assessed by cox regression analysis. Improvement in LVEF resulted in a significant drop in the rate of hospitalizations for symptomatic congestive heart failure in patients in group A, 11% vs 56% in group B patients (p<0.001). Conclusion: Successful kidney transplantation is associated with reversal of systolic dysfunction. The LVEF of ≥ 50% is accompanied by significant reduction in the rate of death, hospitalization for CHF and improvement of NYHA functional class. The most important risk factor for the lack of improvement in LVEF was in those patients with longer duration of dialysis prior to transplantation.

Abstract# 633

IS RENAL FUNCTION PREDICTIVE OF GRAFT SURVIVAL? GOOD CORRELATION BUT POOR PREDICTION. Herwig-Ulf Meier-Kriesche, Jesse Schold, Bruce Kaplan. [1]*Department of Internal Medicine, University of Florida, Gainesville, FL.*

With the improving results in kidney transplantation (Tx) outcomes become increasingly difficult to measure. Graft and patient loss rates are low enough to make it almost impossible to power clinical trials for these endpoints. For this reason intermediate term endpoints (surrogate endpoints) are being proposed to assess outcomes in kidney Tx. Renal function one year after Tx and changes in renal function have been shown to have a strong correlation with graft and patient survival, but despite of claims that these measures predict graft loss no studies have assessed the predictive value of these tests. We conducted a retrospective study of all first Tx recipients age 18 and above between 1988 and 1999 recorded in the USRDS registry that had at least two years of follow up information (data available through December 2001), to determine the predictive nature of renal function measures a serum creatinine (Scr), calculated GFR and changes in Scr over time. Endpoints addressed were patient death, death-censored graft loss, and overall graft loss. Logistic regression was used to analyze the likelihood of particular outcomes for a given follow up period. Output from the logistic regression was further utilized to generate predictive diagnostics and ROC plots. Based on a Scr level at one year the area under the ROC curve for a minimum of 2 and 7 years were 0.627 and 0.624 respectively. As displayed in the graph there is no good cut off available. When a Scr of 1.8 was used as cutoff to predict a graft loss at 7 years the test yielded a 48% sensitivity and 71.0% specificity. Similar marginal predictive values were obtained for renal function change and calculated GFR. The predictive value of these tests did not improve significantly in the multivariate analysis. In summary even though renal function measures at one year after Tx show very significant statistical correlations to patient and graft survival they are by themselves very poor predictors of renal transplant outcomes.



Abstract# 634

PREDICTING PATIENT SURVIVAL IN THE KIDNEY TRANSPLANT ASSESSMENT CLINIC. A PRACTICAL CLINICAL APPLICATION. Gabriel C. Oniscu,[1] Helen Brown,[2] John L. R. Forsythe.[1] [1]*Transplant Unit, The Royal Infirmary of Edinburgh, Edinburgh, United Kingdom;* [2]*Information and Statistics Division, Scottish National Health Service, Edinburgh, United Kingdom.*

AIM: Currently, there is no method to estimate individual survival once a patient is listed for a kidney transplant. This study set out to devise a method to predict individual survival in the assessment clinic under different treatment assumptions, based on a patient's own sociodemographic and comorbidity data. **METHODS:** Extensive sociodemographic and comorbidity data were collected from the case-notes and the Scottish Renal Registry and United Kingdom Transplant databases for 1022 patients listed for transplantation in Scotland between 1.01.1989 and 31.12.1999. A baseline step-wise time-dependant Cox regression analysis (model 1) identified the factors with a significant impact on the risk of death, independent of the treatment modality. Three further models were built to predict survival at 1, 3, 5 and 10 years after listing, on continuous dialysis (model 2), assuming immediate transplantation after listing (model 3) and assuming transplantation after 17 months on the waiting list the average waiting time (model 4). **RESULTS:** Sociodemographic factors, diabetes, cardiovascular, respiratory, cerebrovascular, gastrointestinal diseases and neoplasia impact on survival independent of the treatment modality. These factors were linked with the treatment modality (models 2-4) and were quantified in a risk score estimating survival on dialysis or transplantation from the listing moment. For easier clinical use, a spreadsheet was designed (figure). Survival for any patient (X) is instantaneously calculated on the computer as each risk factor present is ticked (see figure). **CONCLUSION:** Individual survival can be predicted in the assessment clinic. This allows patients to make an informed decision regarding the risks and the benefits associated with either form of treatment and may contribute to a better allocation of the treatment resources.

Survival prediction for a female patient from centre 2 aged 40 with comorbidity as shown

Risk factor	**Patient X**
Centre 2	√
Centre 3	
Female gender	√
35-49 y	√
50-59 yr	
60-64 yr	
>65 yr	
Diabetes	√
Hypertension	√
IHD	√
Valvular disease	
PE	
Arrhthymias	
Other heart disease	
Respiratory disease	
CVD	√
Neoplasia	
GI disorders	
Blood group A	
Malnourished	
Smoker	

Survival estimate	**1yr**	**3yr**	**5yr**	**10yr**
Baseline model	0.91	0..68	0.44	0.08
Continuous dialysis	0.86	0.43	0.08	0.00
Immediate transplant	0.94	0.84	0.74	0.28
Transplantation after 17 months	0.86	0.66	0.58	0.24

Abstract# 635

THE ERA EFFECT IN KIDNEY TRANSPLANTATION: IS GRAFT SURVIVAL STILL IMPROVING? Herwig-Ulf Meier-Kriesche, Jesse Schold, Bruce Kaplan. [1]*Department of Internal Medicine, University of Florida, Gainesville, FL.*

Results in renal transplantation have been improving over the last decades to the point of conferring a consistent survival advantage over maintenance dialysis. Graft survival has been reported to be improving both in the short and long term. With the growing indication for kidney transplantation as therapy of choice in patients with end stage renal disease and the resulting shortage in transplant organs more high risk transplants are likely being performed. To investigate if despite of this trend outcomes in renal transplantation keep improving we analyzed 70,000 first renal transplants performed between 1995 and 2000 based on data provided by the SRTR. Primary study endpoints analyzed by year of transplant were, graft loss (GL), death censored graft loss, patient death, patient death with a functioning transplant (DWFG) and patient death after graft loss (DAGL). Univariate Kaplan Meier and multivariate Cox models were used to investigate potential changes by year of transplant for the whole population and for living and cadaveric transplants separately. There was no significant era effect detected in the univariate and multivariate models for living or cadaveric transplants (Table). In all models there was a trend towards worse results in more recent era particularly for death after graft loss but this difference did not reach statistical significance. When we analyzed the same endpoints for patients beyond one year of transplantation there was a statistically significant trend towards worse outcomes in more recent era for both graft and death censored graft survival and particularly strongly for death after graft loss in both uni- and multivariate analysis. In summary over the last 5 years despite important advances in immunosuppressive therapies which have improved intermediate endpoints and short term outcomes, graft and patient survival rates have not followed that trend. Long term results show actually a concerning trend towards worse long term outcomes.

Relative Risk (from Cox model) for Graft and Patient Loss by Transplant Year

Outcome	1995	1996	1997	1998	1999	2000
Graft loss (GL)	1 (Ref)	0.99	1.07	1.02	1.08	1.09
Death	1 (Ref)	0.99	1.11	0.99	1.03	1.05
Death censored GL	1 (Ref)	1.00	1.06	1.06	1.08	1.10
DWFG	1 (Ref)	0.97	1.07	0.95	1.06	1.05
DAGL	1 (Ref)	1.06	1.16	1.03	0.93	1.27

Abstract# 636

PATIENT SURVIVAL FOLLOWING RENAL TRANSPLANT FAILURE IN CANADA. Greg Knoll,[1] Norman Muirhead,[2] Lilyanna Trpeski,[3] Kim Badovinac.[3] [1]*Division of Nephrology Kidney Research Centre, The Ottawa Hospital, Ottawa, ON, Canada;* [2]*Division of Nephrology, University of Western Ontario, London Health Sciences Centre, London, ON, Canada;* [3]*Canadian Organ Replacement Register, Canadian Institute for Health Information, Toronto, ON, Canada.*

Studies in the United States have shown that allograft loss is associated with an increased risk of death. It is postulated that the loss of the protective effect of the kidney transplant results in the excess death rate. It is not known whether other factors, such

a dialysis delivery, patient demographics or the health care system contribute to the excess risk. The purpose of this study was to determine if renal allograft loss was a predictor of mortality in a recent cohort of Canadian patients. Primary adult renal transplants (n=4714) from 1994-1999 were included in the analysis. The start of the observation period was the date of transplantation and patients were followed until death or December 31, 2000. A Cox model was created with allograft failure entered as a time-dependent variable to determine independent risk factors for death. Patients died (n=394) due to the following: cardiac (28%), infection (16%), other vascular disease (11%), malignancy (11%) and other (34%). There were 255 patient deaths with graft function and 139 patients died after allograft failure. One and five-year patient survival was 99.6% and 95.0% for those without graft failure and 99.2% and 87.8 % for those with graft failure. Patients with renal allograft failure had a threefold increase risk of death compared to those who maintained allograft function. Predictors from the of the Cox model are shown in the table. We conclude that renal allograft failure is an independent predictor of mortality in a Canadian population. It is likely that the increased death is due to the loss of transplant function rather than health care system factors or patient demographics.

Variable	Hazard Ratio	95% CI
Allograft failure	3.4	2.7 - 4.2
Age (per yr)	1.05	1.03 - 1.07
Charlson co-morbidity score (ref=2)		
- score=3-4	1.5	1.1 - 1.9
- score=5-10	1.2	0.99 - 1.5
ESRD diagnosis (ref=GN)		
- diabetes	1.7	1.3 - 2.3
- renal-vascular/hypertension	0.9	0.6 - 1.4
- polycystic	0.7	0.5 - 0.9
Pre-transplant ESRD time (ref>2yrs dialysis)		
- dialysis 0-2 yrs	0.9	0.8 - 1.2
- pre-emptive	0.7	0.6 - 0.9
Living donor	0.6	0.5 - 0.9

Tolerance in Human and Nonhuman Primates

Abstract# 637

THE FIRST HUMAN DEMONSTRATION OF ACQUIRED TRANSPLANTATION TOLERANCE: B CELL CLONAL ELIMINATION IN ABO-INCOMPATIBLE INFANT HEART TRANSPLANT RECIPIENTS. Frank Fan,[1] Andrew Ang,[1] Stacey M. Pollock-BarZiv,[1] Anne I. Dipchand,[1] Lori J. West.[1] *[1]Cardiology and Immunology, Hospital for Sick Children/Univ of Toronto, Toronto, ON, Canada.*

Background: We previously reported that the apparent requirement for ABO-compatibility between heart graft donors and recipients is not applicable in infants. Here, we investigated the underlying mechanism of this phenomenon by studying the immunobiology of B-lymphocytes from infant recipients of ABO-incompatible heart grafts. Methods: The ontogeny of isohemagglutinins was studied with standard agglutination and ELISA assays in samples from patients followed for up to 6 years after infant heart transplantation. Patients' peripheral blood mononuclear cells were cultured; the existence and function of donor-type blood group antigen specific B-lymphocytes were investigated by ELISPOT and FACS analysis. Results: Production of isohemagglutinins specific to the donor ABH antigens appears to be abrogated. No serum factors that might suppress red cell agglutination were found. Further, patients' B cells remained unable to produce anti-donor type isohemagglutinins in long-term cultures despite either 1.) potent non-specific cytokine stimulation or 2.) specific stimulation via co-culture with donor-type human erythrocytes. These findings demonstrate that donor-specific B cell non-responsiveness has been established in these patients. In ELISPOT assays, antibody-producing cells specific for donor blood group were not detected even under conditions of stimulation, whereas abundant anti-A and anti-B antibody-producing cells were detected in age-matched blood group O recipients of group O grafts. In group O recipients of group A grafts, the sub-population of B-lymphocytes bearing B cell receptors (BCR) specific for blood group A antigen, analyzed by FACS, was almost undetectable, similar to samples from blood group A controls. Immunohistochemical study of graft biopsies showed long-term persistence of donor-type ABH antigens. Moreover, C4d, IgM and IgG deposition was absent. Conclusions: We conclude that our infant ABO-incompatible heart transplant recipients have developed B cell tolerance to donor blood group antigens, most likely by clonal deletion. Persistence of donor antigen is likely important to this phenomenon, which is not prevented by systemic immunosuppressive medications. We propose that the development of B cell tolerance to ABH antigens in these young children mirrors the normal development of self-tolerance by B cells. We believe this to be the first demonstration of neonatally-induced transplantation tolerance in the human setting.

Abstract# 638

DONOR SKIN GRAFT ACCEPTANCE AFTER HEMATOPOIETIC RECONSTITUTION USING MOBILIZED PERIPHERAL BLOOD AND A SUBLETHAL PRECONDITIONING REGIMEN. Kimberly L. Gandy,[1] Nelson J. Chao,[2] Gwynn D. Long,[2] Virginia Sartain,[1] Hilliard F. Seigler,[1] Dave A. Rizzieri.[2] *[1]Surgery and Immunology, Duke University Medical Center, Durham, NC; [2]Medicine and Immunology, Duke University Medical Center, Durham, NC.*

Though the potential for hematopoietic reconstitution in tolerance induction is well accepted, design of a successful and widely applicable clinical regimen has yet to be achieved. Our institution has experience in treatment of malignancy with an outpatient regimen involving sublethal preconditioning followed by infusion of cells from mobilized peripheral blood. This protocol results in high degrees of hematopoietic reconstitution, rejection having been observed in only 2 of 34 patients to date. The candidacy of this protocol for solid organ tolerance induction was evaluated using donor-specific skin transplantation. Patients were given a five-day preconditioning protocol consisting of Campath-1H X 5 days, cyclophosphamide (500mg/m² X 4 days), fludarabine (30mg/m² X 4 D), followed by the infusion of Campath-1H treated mobilized peripheral blood cells. Non-HLA identical recipients received a short course of mycophenolate for GVHD prophylaxis, but HLA identical patients received no immunosuppression after hematopoietic transplantation except when needed to treat GVHD. Seven HLA identical patients and two 4/6 matched patients were studied. All patients received 4-6mm skin grafts 0-9 months after hematopoietic transplantation. All but one patient accepted the skin graft, this patient having rejected the hematopoietic graft. RFLP analysis at 4 weeks showed donor engraftment of 99, 17, 46, 83, 94, 90, 1 and 96% (data last patient pending). One patient showed signs of early graft rejection when the percentage of donor-derived cells detected in peripheral blood was low, but accepted the remainder of the graft as the percentage of donor-derived cells increased. Only 2 of 9 patients, however, have remained off all immunosuppression throughout evaluation, the remainder requiring some degree of immunosuppression for low grade GVHD. Two patients expired from progression of their disease, and one patient expired from severe GVHD after DLI. A new one-day preconditioning regimen has recently been tested which appears to have less GVHD. This regimen has been successful in the previously mentioned patient that rejected the hematopoietic graft and appears to be equally successful for tolerance induction. In conclusion, these data verify in a human model that high degrees of hematopoietic reconstitution result in donor-specific graft acceptance and have been used to design our current trial for tolerance induction in renal transplantation.

Abstract# 639

DRUG-FREE "TOLERANT" KIDNEY RECIPIENTS AND PATIENTS UNDER MINIMAL IMMUNOSUPPRESSION PRESENT A STRONG AND ALTERED BLOOD T CELL CLONAL REGULATION. Sophie Brouard,[1] Alexandre Dupont,[1] Magali Giral,[1] Stephanie Louis,[1] Frederique Moizant,[1] Catherine Ruiz,[1] Marina Guillet,[1] Jean-Paul Soulillou.[1] *[1]ITERT-INSERM U437, CHU Hotel Dieu, Nantes, France.*

Immunosuppression exposure in transplantation is linked to an increased incidence of malignancies, infectius diseases and metabolic disorders. Therefore, immunosuppression withdrawal would represent a major advance. Whereas most of the patients who discontinue immunosuppression reject their graft, a small cohort do not, suggesting that immune non-responsiveness can be achieved in the clinic. Given that "operational tolerance", may result from an active regulation, this study aims at identifying T cell selection/activation in the blood of "tolerant" patients. **Methods**: The Vβ transcriptome was analysed (CDR3 length distribution (LD), Vβ/HPRT transcript ratio) in the blood of "operationally tolerant" (Tol) patients (drug-free or under low dose of steroid monotherapy (≤10mg/kg)) for more than 3 years and compared to normal individuals (N^L), immunosuppressed patients with stable graft function (Sta) and those with chronic rejection (CR). Cytokine transcript accumulation was measured on sorted selected T cell populations from the different groups by real time PCR. **Results:** Blood of Tol recipients exhibited an unusual TCR pattern combining strong Vβ transcript accumulation (>30% Vβ families with a transcript ratio>10, *$p<0.001$*) and altered CDR3-LD (37+/-14%, *$p<0.001$*). This result contrasted with the moderately altered CDR3-LD and low Vβ transcript accumulation in the Sta and CR groups (>80% Vβ families with a ratio<5, 24+/-3% and 25+/-5% CDR3-LD alterations). T cells from families with a high Vβ/HPRT ratio and skewed CDR3-LD sorted from Tol patients using anti-Vβ antibodies were characterized by a lack of Th1/Th2 cytokine transcripts (IL3 and IL2, $p<0.05$ vs CR or N^L), suggesting a state of hyporesponsiveness. However, level of the IL2α chain receptor transcript was increased ($p<0.05$ vs CR), a finding also supported by a significant increase in $CD4^+CD25^+$ T cells in the blood of Tol patients. These results suggest that this biased TCR usage, which may be a "surrogate marker" of tolerance in the blood of "tolerant" patients, might reflect selected regulatory populations. They also corroborate pattern of blood compartimentation of transcriptome alteration seen in experimental models of tolerance. Such a finding provide a basis for the exploration of the effect of immunosuppression withdrawal in patients with stable graft function and for the understanding of the mechanisms of tolerance in humans.

Abstract# 640

ALLORESPONSES IN TOLERANT RECIPIENTS OF COMBINED KIDNEY/BONE MARROW TRANSPLANTATION WITH NON-MYELOABLATIVE CONDITIONING. Yasuhiro Fudaba,[1] Juanita M. Shaffer,[1] Annette Kraus,[1] A. Benedict Cosimi,[1] Francis L. Delmonico,[1] Bimalangshu Dey,[1] Tatsuo Kawai,[1] Steven McAfee,[1] Fredric Preffer,[1] Nina Tolkoff-Rubin,[1] David H. Sachs,[1] Susan L. Saidman,[1] Thomas R. Spitzer,[1] Megan Sykes.[1] *[1]Massachusetts General Hospital/Harvard Medical School, Boston, MA.*

Introduction: We analyzed in vitro responses in 4 patients who received combined non-myeloablative MHC-matched sibling bone marrow (BM) and kidney transplantation (Tx) for the treatment of multiple myeloma (MM) with renal failure. Patients 3 and 4 were participants in an Immune Tolerance Network study. Methods: Mixed lymphocyte reaction (MLR), cell-mediated lymphocytotoxicity (CML), and limiting dilution assays (LDA) were performed. Donor chimerism was monitored by microsatellite analysis. T cell recovery was followed by flow cytometry. Results: In Patient 1, initial multilineage mixed chimerism declined to undetectable levels after Day 105. CyA was discontinued on Day 73 and the transplanted kidney has shown no evidence of rejection > 4 years. Early CD8 T cell recovery and strong anti-donor cytotoxic T lymphocyte (CTL) responses were seen in CML and LDA, without anti-donor MLR responses. The myeloma is in complete remission (CR) > 4 years. In Patient 2, initial mixed chimerism became undetectable after Day 123. CyA was discontinued on Day 77 and the kidney has shown no rejection > 2 years. Early recovery of anti-3rd party responses but not anti-donor responses were seen in MLR and CTL and helper T lymphocyte (HTL) LDA. Urinary light chain was reduced by 90% after Tx. In Patient 3, multilineage mixed chimerism converted to donor full chimerism by Day 62. MMF was added to control mild chronic GvHD and CyA was discontinued on Day 379. The patient showed transient anti-donor responses in MLR and CTL-LDA prior to conversion to donor full chimerism, and high anti-host CTLp frequencies following conversion to donor full chimerism. The kidney has shown no rejection. The patient is in CR > 15 months. In Patient 4, initial mixed chimerism declined to undetectable levels by Day 71. CyA was discontinued on Day 60. The kidney has shown no rejection > 5 months. Direct CTL assay showed no anti-donor response (Day 35). Urinary light chain persisted 100 days post-Tx. Conclusions: Combined kidney/BMTx with non-myeloablative conditioning for MM patients is a promising strategy for successful induction of renal allograft tolerance and also provides potent anti-myeloma effects. Donor marrow rejection and anti-donor alloresponses can appear in patients despite maintenance of tolerance to donor kidneys.

Abstract# 641

THYMIC DEPENDENT LONG-TERM ACCEPTANCE OF RENAL ALLOGRAFTS IN JUVENILLE BABOONS TREATED WITH A SHORT COURSE OF FK506. Parsia A. Vagefi, Rolf N. Barth, Shin Yamamoto, John C. LaMatinna, Chisako Kamano, David H. Sachs, Kazuhiko Yamada. *[1]Department of Surgery, Transplantation Biology Research Center, Massachusetts General Hospital/Harvard Medical School, Boston, MA.*

Background: Previous studies from our laboratory demonstrated induction of tolerance to fully mismatched renal allografts in miniature swine when transplanted with a 12-day course of tacrolimus (FK506). In the present study we have extended our short course FK506 tolerance induction protocol to a pre-clinical non-human primate model of renal transplantation, and have investigated the role of the thymus in allograft acceptance. **Methods:** Baboon recipients were divided into two groups: Groups A and B. Group A renal allograft recipients (n=4) consisted of juvenile baboons, where as Group B renal allograft recipients (n=3) consisted of an old baboon, and two juvenile thymectomized baboons. All renal allograft recipients received a 28-day continuous course of FK506 (target blood levels 40-80ng/ml). **Results:** All but one Group A recipient demonstrated long term allograft survival (PODs 572, 117, 208/48, 52): one animal maintained its original donor renal graft for 572 days, and rejected a third party renal allograft in 5 days; the second recipient eventually rejected its graft on POD 117; the third recipient maintained its original donor kidney rejection-free for 208 days at which point a second kidney graft from the original kidney donor was transplanted without immunosupression, although the second kidney remained rejection free, the animal was sacrificed on POD 48 due to a rise in creatinine secondary to stenosis at the site of vesicoureteral anastomosis which could not be surgically repaired; the final Group A recipient died from a systemic infection on POD 52. In contrast, Group B juvenile thymectomized recipients rejected their grafts on PODs 13 and 78. The old baboon in Group B rejected its graft on POD 50. **Conclusions:** These data suggest that an intact thymus was required for long-term renal allograft survival, as old and juvenile thymectomized baboons failed to show long-term renal allograft acceptance. Understanding the role of the thymus in establishing renal allograft transplantation tolerance may allow for the development of tolerance induction strategies that would obviate the need for chronic immunosuppression in pediatric transplant recipients.

Abstract# 642

CD4+CD25+ REGULATORY T CELLS DO NOT SIGNIFICANTLY REGULATE THE DIRECT PATHWAY OF ALLORECOGNITION IN STABLE RENAL TRANSPLANT PATIENTS. David S. Game,[1] Maria P. Hernandez-Fuentes,[1] Afzal N. Chaudhry,[2] Robert I. Lechler.[1] *[1]Department of Immunology, Imperial College Faculty of Medicine, Hammersmith Hospital, Du Cane Road, London, United Kingdom; [2]Department of Renal Medicine, Imperial College Faculty of Medicine, Hammersmith Hospital, Du Cane Road, London, United Kingdom.*

CD4+CD25+ Regulatory T cells have been shown to regulate a variety of autoimmune and allogeneic responses in mice and humans. Mouse models have shown that adoptive transfer of this subset of T cells can prevent rejection of cardiac allografts in a donor-specific manner. In vitro studies in humans have shown that CD4+CD25+ cells are hyporesponsive to polyclonal stimuli and that they can suppress the responses of CD4+CD25- cells. To date there is no published data on the role of CD4+CD25+ cells in regulating alloresponses in human transplant recipients. The technique of limiting dilution analysis (LDA) allows derivation of the frequency of alloreactive T cells in peripheral blood of renal transplant patients; the influence of a regulatory population can also be assessed. Using this technique, it has previously been shown that there is a reduced frequency of direct pathway donor-specific T cells in renal transplant recipients when compared to the frequency of T cells reactive to an HLA matched third party. We hypothesised that if CD4+CD25+ cells are important in maintaining hyporesponsiveness in the direct pathway then depletion of these cells would influence the alloreactive cell frequency and/or other parameters of regulation obtained by LDA. We performed LDA for cell proliferation and IL2 secretion and ELISPOT for IFNg in 10 living-related renal transplant patients with stable renal function and one HLA-DR mismatch to donor. In no case did depletion of CD25+ cells significantly increase the frequency of donor-specific T cells nor indicate regulation against donor when compared to an equally HLA-DR mismatched third party. We conclude that the action of CD4+CD25+ regulatory cells is not the main mechanism of donor-specific hyporesponsiveness in the direct pathway of allorecogniton. Our interpretation is that T cell anergy is driven by overwhelming co-stimulation deficient direct pathway presentation and the additional contribution of the CD4+CD25+ cells is not significant/detectable. We would predict that the low frequency indirect pathway would be more amenable to suppression by these cells.

Abstract# 643

CHRONIC HUMORAL REJECTION IN RENAL ALLOGRAFTS IN CYNOMOLGUS MONKEYS ON MIXED CHIMERISM: CORRELATION OF C4D, ALLOANTIBODY, AND CHRONIC ALLOGRAFT ARTERIOPATHY AND GLOMERULOPATHY. Rex N. Smith,[1] Tatsuo Kawai,[2] Svetlan Boskovic,[2] Ognjenka Nadazdin,[2] Ichiro Koyama,[2] Patricia Della Pelle,[1] Anthony B. Cosimi,[2] Robert B. Colvin.[1] *[1]Department of Pathology, Massachusetts General Hospital, Boston, MA; [2]Department of Surgery, Massachusetts General Hospital, Boston, MA.*

Tolerance regimens are sometimes complicated by late graft failure of undetermined cause. Here we seek evidence that late renal allograft failure in monkeys treated on mixed chimerism protocols can be due to chronic humoral rejection (CHR). METHODS: Cynomolgus monkeys received allografts with donor bone marrow, CsA (1 month), total body and thymic irradiation, and transient ATG or anti-CD40L and variations of this regimen (Kawai et al, Transplant, 68:1767, 1999). Renal biopsies and necropsy kidneys were scored for chronic allograft glomerulopathy (CAG, GBM duplication) and chronic allograft arteriopathy (CAA, intimal fibrosis) and stained with a polyclonal antibody to C4d. DSA were determined by flow cytometry. RESULTS: Analysis of 34 autopsies and 30 biopsies revealed that 22 had peritubular capillary (PTC) C4d, which was associated with DSA in 20 (91%); in contrast, 9/42 (21%) of the C4d neg animals had DSA ($p< 0.001$). 4 of the 9 DSA+C4d neg animals turned C4d positive on biopsies 1-5 months later. Normal Cynomolgus kidneys did not stain for C4d. DSA and C4d staining correlated independently with CAG ($p<0.001$ for both). Among the 15 allografts with CAG, C4d deposition was present in 13 (87%) and DSA was detected in 13 (100%). In contrast, among those 43 without CAG, C4d was present in 9 (21%) and DSA was detected in 9 (21%). 2 C4d+ animals without CAG developed it, 56-325 days later. Alloantibody without C4d staining did not correlate with CAG ($P=0.8$). We restricted our evaluation of CAA to necropsy specimens for their better sampling of arteries. CAA was highly correlated with C4d ($p=004$) and DSA ($p=. 007$). Among the 7 allografts with CAA, C4d deposition was present in 6 (86%) and DSA was detected in 7 (100%). In contrast, among those 27 without CAA, C4d was present in 7 26(%) and DSA was detected in all 7. CONCLUSIONS: CHR in monkeys is manifested by C4d deposition in peritubular capillaries and is associated with CAG, CAA and DSA. CHR can arise despite vigorous treatments to promote immunological tolerance, suggesting that B cells are not easily tolerized. All tolerance protocols should collect data on alloantibody mediated chronic rejection.

Abstract# 644

DENDRITIC CELL SUBSETS IN PERIPHERAL BLOOD OF PATIENTS SUCCESSFULLY WITHDRAWN FROM IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION (LTx). George V. Mazariegos,[1] Alan Zahorchak,[1] Adriana Zeevi,[1] Bridget Flynn,[1] Alison J. Logar,[1] Jorge Reyes,[1] Angus Thomson.[1] *[1]University of Pittsburgh Medical Center, Thomas E. Starzl Transplantation Institute, Pittsburgh, PA.*

AIM: To examine whether predominance of the putatively tolerogenic dendritic cell (DC) 2 subset correlates with clinical tolerance following liver transplantation (LTx). **METHODS:** PMBC from patients (pts.) who have been successfully withdrawn from immunosuppression (IS) following LTx (Group A), those who are undergoing propsective drug weaning (Group B), and those in whom drug withdrawal failed or has not been attempted (Group C) were isolated from peripheral blood by Ficoll density centrifugation. Total DC were identified as HLA-DR+ and lineage marker (CD3, CD14, CD19, CD20) negative on four-color flow cytometric analysis. Subpopulations of IL-3R alpha HLA-DR+ lin-/{precursor (p)DC2} and CD4+ CD11c+HLA-DR+lin-(pDC1) were further quantified. 13 adults served as healthy controls. **RESULTS:** 40 clinically stable pts with normal liver function were eligible for study. Group A pts have been off IS for a mean of 3.3 yrs. Group B pts are undergoing uninterrupted drug weaning for a mean of 1.3 yrs. Group C pts failed drug withdrawal due to rejection or recurrent autoimmune disease (n=6) or never had drug withdrawal attempted (n=5). Mean patient demographic data, % DC, and DC subsets are listed in Table 1. Statistical analysis was by Mann-Whitney U test. **CONCLUSION:** This investigation demonstrates that the plasmacytoid DC lineage (pDC2) is more prevalent in patients successfully withdrawn from IS as well as those on low levels of IS and may prospectively allow identification of clinically tolerant patients. **ACKNOWLEDGEMENT:** This research was performed as a project of the Immune Tolerance Network, a 7 year clinical reasearch project headquartered at UCSF and supported by NIAID, NIDDK and the Juvenile Diabetes Research Foundation.

TABLE 1

Group	A. Off IS	B. Prospective Weaning	C. Drug Withdrawal Failed or Not Attempted	Normal Adult Controls
# of pts	6	23	11	13
Age @ LTx (yr)	6.5 (0.4-16.3)	2.4 (0.2 - 9)	29.1 (1.6-61)	N/A
Current age (yr)	16.2 (3-25.8)	11.6 (2-24)	34.8 (2.9-65.2)	34.3 (25-53)
Time Off Drugs/ Weaning (yr)	3.3 (0.8-7.9)	1.3 (0.1-9.7)	N/A	N/A
% pDC	1.9 (.86-3.3)	2.4 (.7-10.5)	2.9 (0.5-8.2)	2.1 (.6-4.5)
% pDC2	21.8 (7.1-43.6)#	17.1 (6.9-37)	4.7 (0.9-13.5)	13 (5.1-37.9)
%pDC1	53.8 (43-74.5)*	61.3 (34.7-81.8)	73.1 (50-91.4)	64.4 (35.2-80.8)
%pDC2/%pDC1	.49 (.1-1.1)^	0.31 (.1-.86)	0.07 (0.014-0.18)	.26 (.07-1.1)

Group A vs. Group C: #p<0.003; *p<.016, ^p<.001

Abstract# 645

COMBINED BLOCKADE OF THE CD40 AND CD28 PATHWAYS SYNERGIZES TO PROMOTE ISLET ALLOGRAFT SURVIVAL IN A NON-HUMAN PRIMATE MODEL. Andrew B. Adams,[1] Nozomu Shirasugi,[1] Elizabeth A. Strobert,[2] Shannon R. Cowan,[1] Phyllis A. Rees,[1] Rose Hendrix,[1] Norma S. Kenyon,[3] Thomas C. Pearson,[1] Christian P. Larsen.[1] *[1]Emory Transplant Center and Department of Surgery, Emory University School of Medicine, Atlanta, GA; [2]Yerkes National Research Primate Center, Emory University School of Medicine, Atlanta, GA; [3]Diabetes Research Institute and Department of Surgery, Miami University School of Medicine, Miami, FL.*

Recent reports describing the use of a steroid-free immunosuppressive regimen ("Edmonton Protocol") have renewed enthusiasm for the use of islet transplantation to cure diabetes. Clinical application of this approach is limited by the toxic side effects of the immunosuppressive agents. Blockade of costimulatory signals is an effective therapy to prolong allograft survival with minimal toxicity. Anti-CD154 alone or in combination with CTLA4-Ig has been reported to dramatically prolong islet and renal allograft survival in non-human primate models. Unfortunately due to reported thromboembolic complications in clinical trials, the future use of anti-CD154 in patients is uncertain. In previous studies we have reported a successful calcineurin inhibitor-free regimen using the novel costimulatory reagent LEA29Y to block the B7 pathway in combination with sirolimus. Replacement of sirolimus, which has, among other unwanted effects, hypertriglyceridemia, thrombocytopenia, wound healing defects, and unknown effects on islet function, would be beneficial. In the current study, we evaluate the effectiveness of Chi220, a chimeric anti-human CD40 mAb, alone and in combination with LEA29Y in a non-human primate islet transplant model. Total pancreatectomy was used as a model of insulin dependent diabetes in Rhesus macaques. Donor/recipient pairs were selected from divergent colonies and class I/II disparity documented by molecular typing. Islets were prepared and >10,000 IEQ/kg were transplanted via intra-portal infusion. Animals received either LEA29Y alone (0,4,7,14, then every 2 wks), anti-CD40 alone (2wk course), or LEA29Y and anti-CD40 (same regimens). Recipients receiving the combined treatment had dramatically prolonged islet allograft survival (>47, >40 days). In contrast, islet allograft survival following treatment with either agent alone was 15 days for Chi220 alone and >10 days for LEA29Y. Rodent data has demonstrated immunosuppressive synergy when both the CD40 and CD28 pathways are targeted. Primate studies have failed to demonstrate a survival benefit when combined treatment is compared to anti-CD154 treatment alone. Here we report for the first time the suggestion of synergistic activity when anti-CD40 mAb is combined with LEA29Y. Anti-CD40 may avoid potential thromboembolic complications and thus could be a viable option for clinical application in transplantation.

Abstract# 646

CD4 HELP IN ALLOREACTIVE CD8+ T CELL ACTIVATION, MEMORY FORMATION AND GRAFT REJECTION. Yuan Zhai,[1] Linzhong Meng,[1] Ronald W. Busuttil,[1] Jerzy W. Kupiec-Weglinski.[1] *[1]Dumont-UCLA Transplant Ctr., University of California-Los Angeles, Los Angeles, CA.*

Background: We have shown that alloreactive CD8+ T cell activation may proceed in CD4-dependent and CD4-independent pathways. CD154 costimulation plays an essential role in both of these pathways. Moreover, naive alloreactive CD8+ T cells are sensitive to CD154 blockade, whereas allo-Ag primed alloreactive CD8+ T cells (effector and memory T cells) are CD154-blockade-resistant. Although CD4 help is not essential for initiation/activation of alloreactive CD8+ T cells, the question remains as to whether or not CD4 help is required for generation/maintanence of alloreactive memory CD8+ T cells. In this study, we focused on the role of CD4 help in alloreactive memory CD8 formation, and contrasted the mechanism of allograft (Tx) rejection in primed WT vs. CD4 KO mice. **Methods:** WT or CD4 KO mice (B6) were first primed with B/c skin, followed 40 days later by a B/c heart. CD154 blockade (MR1 mAb; 0.5mg/mouse) at the time of skin priming (day 0) or cardiac engraftment (day +40) was applied. Activation of alloreactive CD8+ T cells was measured by FACS by detecting the expression of CD62L and CD44 markers. **Results:** Skin-primed WT mice rejected cardiac Tx within 4 days, and CD154 blockade failed to affect cardiac Tx survival (MST±SD=4±2 days). Allogeneic, but not syngeneic, skin grafts induced a significant increase in the frequency of peripheral $CD8^{+}CD44^{high}CD62L^{low}$ cells, representing activated alloreactive CD8+ T cells (6% vs. 45% of CD8+ T cells at day 10). Their numbers waned down to 10% by day 40. After secondary cardiac Tx, effector cells in the PBL increased to 30% despite the presence of MR1 mAb. In the absence of CD4 help, the primed CD4 KO recipients rejected cardiac Tx in <10 days. Interestingly, unlike in WT mice, CD154 blockade significantly prolonged cardiac Tx survival in primed CD4 KO hosts (MST>40 days). Although allogeneic skin also induced a significant increase in the frequency of $CD8^{+}CD44^{high}CD62L^{low}$ cells (from 3% in naive to 20% at day 10 post-Tx), and their numbers waned down to 10% by day 40, the secondary cardiac Tx failed to trigger a significant increase of effector cells in PBL of primed CD4 KO mice. **Conclusion:** Although CD4 help is not necessary for alloreactive CD8 activation, our data indicate that CD4 help is required for alloreactive memory cell generation, particularly central memory type in PBLs. These cells are resistant to CD154 blockade in primed WT hosts, and are absent in primed CD4 KO recipients, which might explain why sensitized CD4 KO mice remain sensitive to the CD154 blockade.

Abstract# 647

T-INDEPENDENT XENOANTIBODY PRODUCTION OCCURS PREDOMINANTLY IN THE SPLENIC PERIARTERIOLAR LYMPHOCYTE SHEATH (PALS) AND IS DEPENDANT ON FDC-M1 POSITIVE CELLS THAT IS DEFECTIVE IN BALB/C NUDE MICE. Yehong Yan, Josef Goebels, Chris Peeter, Mark Waer. *[1]Lab for Experimental Transplantation, Leuven University, Belgium.*

Rationale and aims: In T- independent immune responses, the spleen plays a pivotal role in both antigen presentation and antibody production, but the precise mechanisms, especially as regarding T-independent xenoantibody production are not very clear yet. Materials and methods: Hamster hearts were transplanted in nude C57BL/6 and nude Balb/c mice. NK or NK1.1 depletion, splenectomy, splenocyte or splenic stromal cell transfer, splenic tissue transplantation were performed in nude mice before or at the same time of transplantation of hamster heart. Xeno-antibody production, complement activity, expression of cytokines and chemokines were examined. Different components of the spleen such as red pulp, marginal zone, lymphoid follicules, PALS were extensively explored by immunohistochemistry. Results: Hamster hearts were rejected in C57BL/6 nude mice about 5 days after transplantation, but could survive in Balb/c nude mice and splenectomized C57BL/6 nude mice. Splenocyte transfer to splenectomized C57BL/6 nude mice could not restore the rejection. Transplantation of C57BL/6 nude mice splenic tissue, or injection of C57BL/6 nude mouse stromal cells under Balb/c nude mouse renal capsules could result in rejection of hamster heart. Rejection was IgM and complement dependent and NK cell independent. Macrophages played an important role in graft destruction, as macrophage infiltration could be seen in the rejected grafts as well as IL-1, IL-6, iNOS, MIP expression in rejected grafts. Histology revealed that the most significant differences between the rejecting C57BL/6 nude mouse spleen and non-rejecting Balb/c nude mouse spleen was in the PALS. These differences committed as: 1° expansion of plasma cells and IgM positive cells, 2° upregulation of dendritic cell markers (CD21/CD35, FDC-M1), and some macrophage markers (CD169, MOMA-1) in the PALS when rejection happened, 3° FDC-M1 positive cells could be found in the PALS of irradiated C57BL/6 nude mice spleens, but were rare in the spleens of irradiated Balb/c nude mice. Conclusions: The C57BL/6 nude mice can reject hamster heart grafts, whereas eusplenic Balb/c nude mice and splenectomized C57BL/6 nude mice can not. Transplantation of C57BL/6 nude splenic tissue or injection of its splenic stromal cells under Balb/c nude mouse renal capsules can restore rejection and IgM production. The defect in the Balb/c nude mice seems to be the interdigitating dendritic cells in the splenic PALS.

Abstract# 648

TRAF6 KO MICE HAVE DEFECTS IN LYMPHOID MATURATION AND SURVIVAL AND DEVELOP TH2-TYPE INFLAMMATORY AUTOIMMUNITY. Elise Chiffoleau,[1] Takashi Kobayashi,[2] Matthew C. Walsh,[2] Patrick Walsh,[1] Carolyn King,[2] Wayne W. Hancock,[3] Yongwon Choi,[2] Laurence A. Turka.[1] *[1]Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, PA; [3]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.*

The tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) participates in signal transduction of both TNFR superfamily members (RANK, CD40) and of IL-1R/ Toll like receptor (TLR) superfamily members (IL-1R, IL-18R, TLR1-9) leading to NF-kB actvation in diverse cell types. Here we have utilized TRAF6 knockout mice to examine the role of TRAF6 in T cell function and homeostasis. TRAF6 deficient mice have severe osteopetrosis due to a defect in RANK signaling in osteoclasts and die at age 2 weeks. Thymic cellularity is reduced, but composition is normal except for a 50% reduction in the percentage of CD4+CD25+ regulatory T cells. In the periphery, lymph nodes are absent, and splenic T cells are remarkable for a 2 fold increase in effector (CD25+, CD69+) or memory (CD44+highCD62L+low) phenotype cells compared with age-matched heterozygote littermates. In vitro, these T cells stimulated with anti-CD3/ anti-CD28 express high levels of Th2 cytokines (IL-4 and IL-10) and TGFb. In contrast, the naïve TRAF6 knockout T cells have impaired survival in the absence of activation, but when stimulated proliferate and survive normally. To assess the physiological function of TRAF6 in vivo, lethally irradiated syngeneic mice were reconstituted with TRAF6 knockout fetal liver cells. These mice lose weight as soon as 4 weeks after reconstitution, become progressively cachexic and have a high mortality rate. Detailed histologic studies reveal marked mononuclear cell infiltration of liver and lungs, with vasculitis and necrosis. Spleen have markedly elevated numbers of CD11b+/Gr1+ monocytes/neutrophils and memory T cells which express also Th2-type cytokines and TGFb. We postulate that the development of autoimmunity is due to a defect in regulatory T cell generation and that the predominance of Th2-type T cell differentiation is attributable to the requirement of TRAF6 signaling for APC support of Th1 responses. The relative roles of TRAF6 in T cells versus APCs is being assessed in ongoing studies using mice with a floxed TRAF6 allele to generate a T cell specific knockout. In conclusion, TRAF6 represent an important signaling molecule involved in lymphoid development and homeostasis, and in the avoidance of autoimmunity. Selective manipulation of this pathway may prove useful for immunomodulatory therapy.

Abstract# 649

THE MATRICELLULAR PROTEIN, THROMBOSPONDIN, IS NECESSARY FOR THE INDICTION AND EXPRESSION OF TGFB-MEDIATED IMMUNE REGULATION. Alice A. Bickerstaff,[1] Pravin T. P. Kaumaya,[1] Nicholas A. Flavahan,[1] Charles G. Orosz.[1] *[1]Surgery/Transplant, The Ohio State University, Columbus, OH.*

C57Bl/6 recipients of DBA/2 cardiac allografts that are temporarily treated with gallium nitrate or anti-CD4 mAb will accept their allografts for >100 days, and exhibit donor-reactive regulatory T cell activity that blocks donor-reactive DTH responses. TGFβ is a critical component of regulatory T cell function in these mice (*Transplantation* 69: 1517, 2000). However, TGFβ must be activated to function biologically, and one of the primary agents of TGFβ activation in vivo is the matricellular protein, thrombospondin (TSP). We investigated whether TSP contributes to the immune regulation displayed in cardiac allograft acceptors. In assays of donor alloantigen-induced DTH reactivity, we observed that delivery of either anti-TSP mAb or anti-TGFβ mAb to the DTH challenge site could rescue donor-reactive DTH responses in cardiac allograft acceptor mice. When FVBN TSP KO hearts were transplanted into normal C57Bl/6 mice, non-suppressed mice rapidly rejected the TSP KO allografts, while mice temporarily treated with GN accepted the TSP KO allografts. However, splenocytes from these TSP KO allograft acceptors failed to display TGFβ-mediated regulation of donor-reactive DTH responses. This demonstrates that TSP expression within the allograft is required either for the development of regulatory T cells or for their sequestration in the spleens of allograft acceptor mice. Further, this data suggests that immune mechanisms other than TGFβ-mediated immune regulation contribute to allograft acceptance, since the mice accepted the allografts in the apparent absence of TGFβ-mediated regulatory T cell activity. It has been reported that LSKL, a tetrapeptide derived from the latency associated peptide of inactive TGFβ, can block the activation of TGFβ by TSP. We observed that LSKL, like anti-TGFβ and anti-TSP mAbs, can restore donor-reactive DTH responses when placed in the DTH challenge sites of allograft acceptor mice. Together, these data demonstrate that TSP plays an integral role in the expression of TGFβ-mediated immune regulation in allograft recipients.

Abstract# 650

PROLONGATION OF CARDIAC ALLOGRAFT SURVIVAL BY ANTI-DONOR MHC CLASS II DNA VACCINATION IS CARACTERIZED BY A DECREASE IN TH1-TYPE RESPONSES. Helene Peche,[1] Bryce van Denderen,[2] Jean Christian Roussel,[1] Benjamin Trinite,[1] Jean Paul Soulillou,[1] Maria Cristina Cuturi.[1] *[1]ITERT-INSERM U437, Nantes, Loire Atlantique, France, Metropolitan; [2]St Vincent's Institute of Medical Research, Fitzroy, VIC, Australia.*

Introduction: Donor MHC class I and II antigens play an important role in both allograft rejection and tolerance, whilst DNA vaccination enables a highly efficient immune response to be induced. In this study we took advantage of this protocol to determine the role of indirect presentation of donor antigens in the modulation of allograft responses and to study the mechanisms involved. **Material and Methods:** DNA vaccination was performed with a plasmid (pcDNA3.1zeocin) coding for donor MHC class II or class I molecules. Rat recipients of full class I and II histoincompatible heart allografts, were treated prior to transplantation with 300 mg of DNA delivered either intraperitonealy or intravenously. Graft survival was evaluated daily by monitoring palpation through the abdominal wall, and anti-donor responses were analyzed 5 days after transplantation. **Results:** No significant prolongation of allograft survival was observed following vaccination with class I antigen-encoding plasmid DNA (MST = 6.8 ± 1 days, n=7). Vaccination with plasmid encoding for class II antigens significantly prolonged ($p<0.01$) allograft survival when administrated intravenously or intraperitonealy (iv: MST = 41.8 ± 36.6 , n = 9 or ip: MST = 30.9 ± 27, n= 13) compared to untreated rats (MST = 6.8 ± 2, n=8). In order to define the mechanisms involved in allograft prolongation, anti-donor responses were examined 5 days after grafting in vaccinated recipients. Compared to untreated recipients, a lower proliferation of splenocytes from vaccinated recipients against donor antigens was observed. Moreover, the anti donor MHC class I humoral response was characterized by a decrease in Th1 type alloantibodies and an increase in Th2 type alloantibodies. Furthermore, as reflected in the decrease of the anti donor proliferative response, we also observed a substantial decrease in the total amount of graft infiltrating cells and a significant reduction in IFNg mRNA expression. **Conclusion:** Indirect presentation of donor MHC class II antigen by DNA vaccination prevents acute allograft rejection but is not sufficient to induce allograft tolerance. This effect was mediated by the decrease in anti-donor Th1 responses which could be due to a deletion or anergy of the alloreactive CD4+ T cells.

Abstract# 651

THE CYCLIN-DEPENDENT KINASE INHIBITOR p21 INHIBITS ALLOIMMUNE RESPONSES IN VITRO AND IN CARDIAC TRANSPLANTATION. Theodore H. Welling,[1] Sherri C. Wood,[1] Guanyi Lu,[1] Keri L. Csencsits,[1] D. Keith Bishop.[1] *[1]General Surgery, University of Michigan, Ann Arbor, MI.*

Introduction: The cyclin-dependent kinase inhibitor p21 is an important regulator of T cell proliferation. p21 deficiency results in increased T cell activation and systemic autoimmunity. However, the role of p21 in allospecific T cell activation and allograft rejection has not been defined. We hypothesized that p21 deficiency results in increased allospecific T cell activation and that p21 overexpression inhibits the alloimmune response. Methods: This hypothesis was tested in vitro using allogeneic mixed lymphocyte cultures and in vivo using a vascularized cardiac allograft model. T cell responses of wild type (WT) and p21 knockout (-/-) mice were evaluated using ^{3}H-thymidine uptake and ELISPOT assays to assess Th1 (IFN-gamma) and Th2 (IL-4) priming. To assess the effect of p21 overexpression, WT allografts were transfected with adenovirus encoding p21. In Vitro Results: Allospecific in vitro proliferation in p21-/- T cells was increased by greater than 200% over WT. In vitro priming to alloantigen showed no difference in IFN-gamma production between WT and p21-/- T cells, whereas IL-4 production was 40% less in p21-/- suggesting a Th1 polarized response in p21-/- recipients. In Vivo Results: Following cardiac allograft transplantation, proliferative responses of lymphocytes from p21-/- recipients were 100% greater than that of cells from WT recipients. Priming of both Th1 and Th2 in p21-/- recipients was elevated with a 50% increase in IFN-gamma and a 75% increase in IL-4 producing cells compared to WT recipients. Since p21 deficiency causes greater CD4 T cell activation in models of autoimmunity, p21-/- and WT allograft recipients were depleted of CD8+ cells. p21-/- recipients had elevated Th1 and Th2 responses with 200% greater number of IFN-gamma producing cells and 50% greater IL-4 producing cells than WT recipients. Finally, when WT recipients received WT allografts transfected with p21, graft survival was prolonged (range 12-30+ days) compared to control transfection (range 7-8 days). Conclusions: p21 deficiency enhances alloimmune responses during transplantation with increased T cell proliferation and cytokine production. Additionally, p21 over expression by gene transfer results in prolonged allograft survival. These findings suggest that p21 manipulation may prove beneficial in controlling alloimmune responses during transplantation.

Abstract# 652

NEW INSIGHTS INTO TH1/TH2 PARADIGM ON IMMUNE REGULATION IN ALLOIMMUNE RESPONSE. Masayuki Sho,[1] Alberto Sanchez-Fueyo,[2] Terry B. Strom,[2] Mohamed H. Sayegh,[1] Xin Xiao Zheng.[2] *[1]Medicine, Children's Hospital, Boston, MA; [2]Medicine, Beth Israel Deaconess Medical Center, Boston, MA.*

To redefine the Th1/Th2 paradigm on immunoregulatory networks in transplantation tolerance, we utilized STAT4$^{-/-}$ (impaired Th1 response), STAT6$^{-/-}$ (impaired Th2 response) and wild type (WT) BALB/c recipients, that were rendered tolerant to C57BL6 allografts by CTLA4Ig treatment. We employed an adoptive transfer system in which irradiated BALB/c cardiac allograft recipients were injected with syngeneic lymphocytes harvested from naïve and/or tolerant hosts. While adoptive transfer of naïve 50x10^6 WT cells induced prompt rejection, the transfer of 50x10^6 cells from either STAT4$^{-/-}$, STAT6$^{-/-}$ or WT tolerant mice preserved allograft function. When co-transferred together with naïve cells at 1:1 ratio, all tolerant cells could prevent naïve cells from rejecting allografts, indicating that immunoregulatory cells could be generated in both Th1 and Th2 environments. Interestingly, when co-transferred at 1:2 ratios (25x10^6 tolerant plus 50x10^6 naïve), Stat4$^{-/-}$ tolerant cells exhibited a greater capacity to regulate rejection than WT or Stat6$^{-/-}$ (Stat4$^{-/-}$ vs. WT; P=0.003, Stat4$^{-/-}$ vs. Stat6$^{-/-}$; P=0.001), suggesting that Th2-polarized tolerant hosts exhibited more potent regulatory networks than Th1 or WT tolerant hosts. To determine responsible T cell compartment, we inoculated a mixture of naïve WT cells with CD4$^+$CD25$^+$ depleted cells from tolerant Stat4$^{-/-}$ or Stat6$^{-/-}$ into BALB/c Rag$^{-/-}$ cardiac graft recipients. Depletion of the CD4$^+$CD25$^+$ subset resulted in the loss of the regulatory function regardless of cell phenotype, indicating that regulatory Stat4$^{-/-}$ and Stat6$^{-/-}$ CD4$^+$CD25$^+$ cells, and not their effector counterparts, were responsible for the differences between Th1- and Th2-polarized states. To prove that regulatory Th2-polarized CD4$^+$CD25$^+$ cells have an intrinsically higher immunosuppressive capacity, we collected cells from naïve Stat4$^{-/-}$ and Stat6$^{-/-}$. Adoptive transfer of 2x10^5 naïve BALB/c CD25$^-$ cells resulted in prompt rejection of C57BL/6 skin grafts. The co-transfer of 4x10^5 CD4$^+$CD25$^+$ T cells from naïve STAT4$^{-/-}$ with 2x10^5 naïve WT CD4$^+$CD25$^-$ T cells elicited a longer survival than that of co-transfer of Stat6$^{-/-}$ CD4$^+$CD25$^+$ T cells (P=0.0096). In summary, we have determined that: 1) CD4$^+$CD25$^+$ T cell dependent immunoregulatory networks can be identified in both Th1 and Th2-polarized tolerant recipients; 2) Th2-polarized tolerant state is more robust than Th1; and 3) these differences can be attributed to an intrinsically higher immunoregulatory functions of Th2-polarized CD4$^+$CD25$^+$ regulatory T cells.

Abstract# 653

ALLOGRAFT TOLERANCE, IMMUNE REGULATION, AND CHRONIC REJECTION. Charles G. Orosz,[1] Alice A. Bickerstaff,[1] Jun Wang,[2] Ying Dong,[2] Christine Guo,[2] Marvin Newton-West,[2] Nozomu Sirasugi,[2] Christian P. Larsen,[2] Kenneth A. Newell.[2] *[1]Surgery/Transplant, The Ohio State University, Columbus, OH; [2]Surgery, Emory University, Atlanta, GA.*

C57BL/6 mice given Balb/c cardiac allografts and gallium nitrate accept the allografts for >100 days, generate donor-reactive regulatory T cells within 60 days, and the grafts develop interstitial fibrosis and neointimal hyperplasia (Group A mice). In contrast, C57BL/6 mice given Balb/c cardiac allografts and mAb to CD40L (MR1), CTLA-4Ig, busulfan, and donor bone marrow, accept the allografts for >100 days, have no detectable regulatory T cells at 60 days, develop hematopoietic macrochimerism (beginning at day 20- 30), delete donor-reactive T cells (beginning at day 30 -40), and develop allograft tisue remodeling (Group B mice). This suggests that different mechanisms promote allograft acceptance in these two situations. While Group A mice displays immune regulation, Group B mice display clonal deletion. Further, these observations suggest that chronically active regulatory T cells are related to the development of pathologic tissue remodeling within the accepted allografts. Clearly, regulatory T cells produce TGFβ, which is a potent histogenic agent. To further study this, mice from Groups A and B were tested in DTH assays for donor-reactive regulatory T cells at various times post-transplant. Splenocytes from Group A mice exhibited little TGFβ-mediated immune regulation at day 30, but displayed strong regulation at day 60 and 100, and then lost regulation by day 150. In contrast, splenocytes from the Group B mice displayed TGFβ-mediated regulation day 30, but lost most of this by day 60. Thus, Group B mice do, indeed, develop regulatory T cells, which develop more rapidly and disappear much more quickly than those of Group A mice. Interestingly, the loss of regulatory T cells coincides roughly with the development of clonal deletion within the same mice. In general, this means that the allografts from Group B mice may experience regulatory T cell activity, including the production of the immunoregulatory and histogenic cytokine, TGFβ, for a much shorter time than those in Group A mice. Based on these observations, we propose the following hypothesis: therapy involving MR1, CTLA-4Ig, busulfan and the infusion of donor bone marrow permits the development of short-lived donor-reactive regulatory T cells, and the clonal deletion of graft-reactive T cells. This clonal deletion includes the graft-reactive regulatory T cells, and thus limits the TGFβ-promoted remodeling of allograft tissues.

Abstract# 654

HISTOPATHOLOGICAL EVALUATION IN DONOR KIDNEY PROCURED BY LAPAROSCOPIC DONOR NEPHRECTOMY. Tomokazu Shimizu,[2] Kazunari Tanabe,[1] Hideki Ishida,[1] Tadahiko Tokumoto,[1] Hiroaki Shimmura,[1] Nobuo Ishikawa,[1] Hiroshi Kawaguchi,[2] Michio Tokiwa,[2] Yutaka Yamaguchi,[3] Hiroshi Toma.[1] *[1]Department of Urology, Tokyo Women's Medical University, Tokyo, Tokyo, Japan; [2]Department of Urology, Iwaki Hinyoukika Clinic, Iwaki, Fukushima, Japan; [3]Department of Pathology, Jikei University, Kashiwa Hospital, Kashiwa, Chiba, Japan.*

(Aim)We performed histopathological evaluation in donor kidneys procured by laparoscopic donor nephrectomy (LDN) using allografts biopsies done immmediately after donor nephrectomy at the time of kidney transplantation(0-hour biopsies).(Materials and methods);Sixty-four donors underwent LDN between December, 1999 and November, 2002 at our institute. The mean age of subjects was 54.4 years, 17 males and 47 females. Among 64 cases, hand-assisted LDN was used in 10 cases and retroperitoneal LDN was in 54 cases. Allograft biopsies were performed immediately after LDN (0-hour biopsies) in all 64 cases and those materials were enrolled in this study. All specimens were examined by routine light and immunofluorescent microscopy.(Results);Arterio-arteriolosclerosis was seen in 49 (77%), arteriolar hyalinosis was in 38 (59%), and tubular atrophy was in 32 (50%) specimens respectively. The mean glomerular sclerosis rate was 12%. Mild acute tubular necrosis (ATN), which supposed to resulted from ischemic change due to vascular injury during LDN was shown in 15 (23%) materials. Notable and characteristic changes which could not be usually seen in 0 hour biopsy specimens of traditional open donor nephrectomy were 'subcapsular cortical damage' including hemorrhage and fibrin deposits in the renal capsule, and degeneration of tubular cells and tubular necrosis, glomerular congestion and interstitial hemorrhage beneath the capsule. In this study such characteristic 'subcapsular cortical damage' was recognized in 22 materials (34%).(Conclusion); ATN and subcapsular cortical damage were major histopathological alterations in donor kidneys procured by LDN.

Abstract# 655

RISK OF HOSPITALIZATION DUE TO INFECTION NOW EXCEEDS RISK DUE TO ACUTE REJECTION AT EARLY AND LATE TIME POINTS IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS. Vikas Dharnidharka,[1] Donald Stablein,[2] William Harmon.[3] *[1]University of Florida, Gainesville, FL; [2]Emmes Corporation, Rockville, MD; [3]Children's Hospital, Boston, MA.*

Newer immunosuppressive agents have dramatically reduced the rates of acute graft rejection (AR) over the last decade. However, their greater potency may have exacerbated the problem of post transplant infections (PTI). We analyzed a large multi-centered registry of pediatric renal transplant recipients to determine the relative risk of hospitalization from PTI versus AR in the years 1987 to 2000. We also determined whether the relative risks for PTI hospitalization overall or its subsets (bacterial, viral or fungal) were trending significantly upwards in recent years. In 1987, in the first 6 months post transplant, the AR associated hospitalization risk exceeded the equivalent hospitalization risk of PTI (32.8% versus 27.9%), whereas by the year 2000 the PTI associated hospitalization risk at this early time point is double that of AR associated hospitalization risk (Table). An identical trend is seen in the 6-24 months post transplant period. Risks of AR hospitalization have trended significantly downwards while risks of PTI associated hospitalization have trended significantly upwards ($P < .001$). In the highest risk period of viral infections (6-24 months), the risk of viral infection related hospitalization has risen from 11.1% to 16.6% from 1987 to 2000 ($p < 0.001$ for trend by transplant year by logistic regression). When examining prognostic value of these variables for subsequent graft survival, hospitalization for a fungal infection at any post transplant time period was an independent risk factor ($RR = 1.64$, $p = 0.02$), even after adjustment for other variables in a Cox proportional hazards model, while hospitalization for bacterial or viral infections were not significantly associated with poorer graft survival. We conclude that the causes of hospitalization at all times up to 24 months post transplant, including the critical early 6 months, have shifted away from AR to PTI. Viral infections are increasing in the period 6-24 months. Hospitalization for fungal infections represents an independent risk for poor graft outcome.

Hospitalization Experience (%) for selected causes and follow-up period in early and late transplant cohorts

Transplant Year	Time Period	Viral Infections	All Infections	Acute Rejection
1987	1-6 months	15.7	27.9	32.8
2000	1-6 months	14.2	24.0	12.0
1987	6-24 months	11.1	20.4	23.8
2000	6-24 months	16.6	30.8	14.6

Abstract# 656

ANEMIA IN RENAL TRANSPLANT RECIPIENTS: AN EMERGING CONCERN. Reshma Kewalramani,[1] Wolfgang C. Winkelmayer,[2] Mark Rutstein,[1] Steven Gabardi,[1] Jodi Greenfield,[1] Tania T. VonVisger,[1] Ajay K. Singh,[1] Anil Chandraker.[1] *[1]Renal Unit, Brigham and Women's Hospital, Boston, MA; [2]Division of Pharmacoepidemiology and Pharmacoeconomics, Brigham and Women's Hospital, Boston, MA.*

Purpose: Although anemia has been known to be a short term complication after kidney transplantation, it has not been well studied in the longer term. Studies to date have been small in size and have not fully evaluated the effects of medications, particularly ACE inhibitors and ARBs, on the development of anemia. The current study was undertaken to address these issues. Methods: Retrospective cross-sectional study of 376 consecutive patients regularly followed-up between June 2001-June 2002. Several patient characteristics were abstracted from medical records. Serum hematocrit (Hct), the main outcome variable, was found to be normally distributed. Thus, univariate and multivariate linear regression was used to test for associations between Hct and other covariates. Results: The mean time since transplantation was 7.7 years (+/- 6.7). The mean Hct was 36% (+/-5.4). Hct was >36% in 48.1% of patients, between 33-36% in 28.6% and <33% in 14.2% of patients. Overall, 10% of patients were on Erythropoietin (Epo) therapy. However, of those patients with Hct <30%, only 41.5% patients received Epo. From univariate analyses, statistically significant associations with Hct were found for female gender, higher BUN, higher Cr (all P<0.0001), concurrent Rapamycin therapy (P=0.02), and concurrent ACE-I therapy (P=0.002). Multivariate analyses simultaneously controlling for all other covariates revealed that female gender was associated with a lower Hct (-2.9%; P<0.0001), as was increasing renal insufficiency (compared to Cr<1.5mg/dL: Cr 1.5-2.0: -1.5%; Cr2.0-3.0: -4.0%; Cr >3.0: -7.2%; P<0.0001). Patients on ACE inhibitors had a Hct that was -1.6% lower (P=0.005) compared to patients without such treatment. A similar effect was found for ARB therapy, but the numbers were too small to obtain a significant effect (-1.2%; P=0.24). Among the immunosuppressant drugs, there was an indication for a lower Hct among patients on either MMF (-1.3%; P=0.06) or FK (-2.4%; P=0.02). Conclusion: Anemia is highly prevalent and under treated in patients with functioning renal transplant. When controlling for known determinants of Hct (gender, renal function) as well as other potential confounders, we found that concurrent treatment with ACE inhibitors was independently associated with a lower Hct. The effects of anemia on patient/graft survival is unknown but deserves firther investigation as anemia is known to adversely impact the health of other patient goups.

Abstract# 657

SLEEP-APNOEA SYNDROME: A COMMON AND UNDERDIAGNOSED COMPLICATION IN OBESE AND NON-OBESE PATIENTS AFTER RENAL TRANSPLANTATION. María J. Díaz de Atauri,[1] P. Ausin,[1] I. Martínez,[1] Beatriz Espejo,[2] Blanca Bueno,[2] María Valentín,[2] Esther González,[2] Beatriz Domínguez-Gil,[2] Amado Andrés,[2] Jose M. Morales.[2] *[1]Neumology Department, 12 de Octubre Hospital, Madrid, Spain; [2]Nephrology Department, 12 de Octubre Hospital, Madrid, Spain.*

Obstructive sleep apnoea syndrome (OSAS) has been associated with obesity and end-stage renal disease with improvement after renal transplantation. However, there is no information about the frequency and severity of OSAS in renal transplant patients. The aim of the study was to analyze if OSAS is more frequent and severe in obese versus non obese patients after renal transplantation. A series of patients who received a kidney transplant in our unit between June 1989-March 2001 were selected: Group A (N=27): Obese patients, [body mass index (BMI)>30] and Group B (N=20): Non obese patients (BMI< 30 at transplantation). All patients filled a questionnaire about OSAS related symptons at a similar time after transplantation. BMI and neck circunference were measured and a cardio-respiratory polygraphy (ApnoeScreen, Jaeger) was performed. OSAS was diagnosed when apnea hypopnea index (AHI) was ≥10 and it was considered severe when AHI was ≥30. Demographic data were similar between both groups (age, gender, immunosuppression, renal function and blood pressure) except for mean BMI at the moment of the study (39±7.5 and 25±3 in group A and B respectively).

RESULTS

	Group A (Obese; N=27)	Group B (Non-obese; N=20)	p
OSAS	25 (93%)	19 (95%)	NS
AHI	41±21	21±10	0.001
Severe OSAS	18 (66.6%)	6 (30 %)	<0.0025
CPAP therapy	20 (74%)	12 (60%)	NS

Remarkably, the frequency of OSAS was almost universal in both groups. In the multivariable analysis, obesity was the only independent significant risk factor for severe OSAS [OR 5.7(1.32-24.7) CI 95%]. Therapy with continuous positive airway pressure (CPAP) was instaured according OSAS and comorbidity in both groups. In conclusion, OSAS seems to be an extraordinary frequent complication after renal transplantation. Surprisingly, it occurs both in obese and in non-obese patients. As expected, severe OSAS was associated with obesity. Until more information is available, our results suggest that OSAS should be suspected in all patients after renal trasplantation, specially in obese patients. An early diagnosis and therapy could have important clinical implications.

Abstract# 658

RHABDOMYOLYSIS AND MYOGLOBINURIA FOLLOWING LAPAROSCOPIC DONOR NEPHRECTOMY. Aiping Sui,[1] Yolanda Becker,[2] Jimmy Light,[1] Frederick Finelli.[1] *[1]Department of Surgery, Washington Hospital Ceter, Washington, DC; [2]Department of Surgery, University of Wisconsin, Madison, WI.*

Purpose: Rhabdomyolysis with myoglobinuria and acute renal failure after a surgical procedure is a rare but serious complication. Herein we describe four cases in whom this syndrome developed in various degrees following laparoscopic donor nephrectomy. Methods: Case study. Results: In our report, all four patients underwent prolonged laparoscopic donor nephrectomy (5-8.5 hrs) in the right lateral decubitus position. Case 1 and case 2 showed clinical evidence of rhabdomyolysis, including severe muscle pain, fever, myoglobinuria, increased CPK and creatinine. Case 3 and case 4 did not show typical symptoms and signs of rhabdomyolysis, but each had a short period of myoglobinuria. Conclusion: Rhabdomyolysis following surgery can result from vascular damage due to prolonged pressure on immobilized muscle. Laparoscopy is generally considered to be a safe procedure, but long operative times and decubitus positioning may predispose to this condition. Prompt diagnosis and aggressive hydration and alkalization will usually prevent permanent kidney failure. The injured area must be observed carefully for the development of a compartment syndrome requiring fasciotomy. Careful positioning, shortened operative times and heightened awareness can limit the occurrence and severity of this complication.

Abstract# 659

INFLUENCE OF CALCINEURIN-INHIBITOR FREE IMMUNOSUPPRESSION ON CAROTID INTIMA MEDIA THICKNESS IN CHRONIC ALLOGRAFT NEPHROPATHY. Barbara M. Suwelack,[1] Ulf W. Gerhardt,[1] Helge Hohage.[1] *[1]Medizinische Klinik und Poliklinik D, Universitaetsklinikum Muenster, Muenster, NRW, Germany.*

Cardiovascular (CV) morbidity and mortality influences longterm patient and allograft survival after kidney transplantation. The intima media thickness of the A. Carotis communis (IMT-CC) is severely impaired in renal transplant recipients (RTX). This parameter of vascular structure is an accepted predictor of cardiovascular complications and may be influenced adversely by calcineurin-inhibitors (CNI). However, it is not known whether withdrawal of CNI and addition of MMF in RTX may improve structural properties of the large arteries. Therefore we studied the evolution of the carotid IMT after CNI withrawal and replacement with mycophenolate mofetil (MMF) over a period of 9 months. In a prospective study longterm RTX (n=32, age: 48±2 years, 7±0.5years after TX!) with histologically proven chronic allograft nephropathy were randomized to either withdrawal or continuation of CNI therapy after addition of MMF in both groups. Prednisolone was continued in both groups. Using a high resolution B-mode ultrasound-system (Biosound 2000 II s.a., Biosound Inc., Indianapolis, USA) with a 8 MHz transducer) the IMT-CC of the far wall was measured by a standardised protocol. Enddiastolic (ECG triggered) vessel diameter and the atherosclerotic plaquescore were measured at baseline and about 9 months later. The results are shown in table 1. Data are mean ± SEM. * p<0.05 A vs B. The changes of 1/creatinine and in pulse pressure (DPP) were significant between the groups. Course of IMT was significantly influenced by DPP only (stepwise multiple regression). Even in longterm RTX with high CV-risk, CNI-withdrawal in combination with additional antiproliferative immunosuppressive agents like MMF significantly improve carotid IMT, plaquescore and vessel wall diameter compared to CNI continuation This finding may be associated with a possible reduction of cardiovascular risk. This effect is partly attributable to pulse pressure changes.

Table 1

Parameter	A-CNI-withdrawal N=16		B-CNI-continuation N=16	
	start	end	start	end
PP(mmhg)	61±4	57±4*	50±3	60±4
SBP(mmHg)	142±4	137±4*	133±3	147±3
Diast-Dia-CC(mm)	7.33±0.26	6.83±0.29*	7.53±0.25	7.60±0.32
IMT-CC (mm)	0.88±0.03	0.86±0.03*	0.90+0.02	0.92±0.01

Abstract# 660

PRESERVING RENAL EPITHELIAL CELL POLARITY AS A MARKER OF THERAPEUTIC EFFICACY IN THE SETTING OF DELAYED GRAFT FUNCTION. Bryan N. Becker,[1] Yolanda T. Becker,[2] R. Michael Hofmann,[1] Andreas Friedl.[3] *[1]Medicine, University of Wisconsin, Madison, WI; [2]Surgery, University of Wisconsin, Madison, WI; [3]Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI.*

The contribution of delayed graft function (DGF) to chronic allograft nephropathy (CAN) and late graft loss is significant. We previously noted that induction treatment with rabbit anti-thymocyte polyclonal antisera (Thymoglobulin®, Thymo) led to improved 12-month kidney transplant function compared to treatment with monoclonal anti-CD25 antibodies in the setting of DGF (Am J Transplant 2002; 273). Thymo contains multiple antibodies; some of which may bind to molecules on renal epithelial cells (REC) as well as circulating lymphocytes. We hypothesized that this extra antibody effect in the context of early treatment with Thymo (intra-operative or day 0) could increase the number of intact renal epithelial cells and reduce tubular injury in kidneys at risk for DGF, e.g. extended donor or non-heart-beating donors, extended cold ischemia time (CIT). We used immunohistochemistry to assess time 0 biopsy samples from 13 patients who received kidneys at risk for DGF. We stained for markers of REC polarity (β-catenin, E-cadherin, and Na-K ATPase distribution) as a surrogate for REC integrity. Biopsies were scored 0-staining absent to 4+-staining diffusely present at the basolateral (BL) cell surface. 7 patients initially received a monoclonal anti-CD25 antibody. 6 received peri-operative Thymo (avg. dose 1.5 mg/kg). Patients also received bolus corticosteroids and mycophenolate mofetil. Week 1 biopsies were also assessed when available (anti-CD25 n = 5; Thymo n = 4). There were no significant differences in CIT, degree of HLA mismatch, or donor age. Time 0 biopsies from each cohort demonstrated 2+ to 3+ β-catenin and E-cadherin protein BL distribution. β-catenin and E-cadherin protein were more likely to maintain their BL distribution in Thymo-treated patients at week 1 ($p < 0.04$) and there was a trend towards more normal Na-K ATPase distribution in Thymo-treated patients (N.S., $p = 0.068$). These data demonstrate an association between Thymo treatment and preservation of REC polarity in the at-risk DGF setting. While subject to β-error, this study suggests that Thymo may help conserve functional renal mass during DGF as a mechanism for improving overall transplant function following this type of injury.

Abstract# 661

GENDER, RACE, TACROLIMUS LEVEL, AND BK POLYOMAVIRUS NEPHRITIS. Paulo N. Rocha,[1] Sara E. Miller,[2] David N. Howell,[2] Stephen R. Smith.[1] *[1]Medicine, Duke University Medical Center, Durham, NC; [2]Pathology, Duke University Medical Center, Durham, NC.*

Although the risk factors for BK polyomavirus nephritis (BKN) have not been well defined, the disease has been linked to the newer immunosuppressants tacrolimus (TAC) and mycophenolate mofetil (MMF). A recent study, however, suggested that BKN occurs as a complication of the treatment of acute rejection (AR) with methylprednisolone. To examine the relationship between the treatment of AR and BKN, we analyzed all kidney transplants performed at our center between January 1999 and August 2001 (n = 286). After a mean follow-up of 622 ± 63 days, we identified 9 cases of BKN (3.1%) using urine electron microscopy (EM). Of these, 8 also underwent allograft biopsy, which confirmed the diagnosis. The mean time to diagnosis of BKN was 328 ± 59 days. No patient with BKN had a prior history of AR. During the same period, 59 patients were diagnosed with AR (21%) and treated with methylprednisolone. The mean time to diagnosis of AR was 164 ± 37 days ($p = 0.004$ vs time to diagnosis of BKN). None of these patients went on to develop BKN. We contrasted the characteristics of patients that developed BKN with those of patients that developed AR and found that BKN patients were more likely to be Caucasian (78% vs 46%, $p = 0.04$) and male (89% vs 51%, $p = 0.03$). Moreover, the mean TAC levels from the time of transplant to the time of diagnosis were higher in the BKN group compared to the AR group (12 ± 0.4 vs 9 ± 0.3 ng/ml, $p < 0.001$). The treatment of BKN consisted mainly of a reduction in immunosuppression. MMF was discontinued in 7/9 patients, all of whom experienced disappearance of viruria (median time to negative urine EM = 147 days, range 29 to 257) and remained independent of dialysis at last follow up. In 2/9 BKN patients, MMF doses were only reduced. Both have persistent viruria more than 1 year after the initial diagnosis, and although one still has good graft function, the other ultimately lost the graft due to the viral infection. In summary, our study does not show an association between the treatment of AR and the development of BKN. Our findings suggest that Caucasian males exposed to higher TAC levels are at greater risk of developing BKN. Discontinuation of MMF was associated with clearance of viruria and preservation of renal function. We conclude that transplant physicians should be aware of the potential relationship between high TAC levels and BKN, especially in Caucasian males. Substantial reduction in immunosuppression should be considered in patients with BKN. Urine EM is a helpful tool for the diagnosis and follow-up of these patients.

Abstract# 662

ONE YEAR OUTCOMES IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANT RECIPIENTS RECEIVING AN ALTERNATIVE DOSING REGIMEN OF DACLIZUMAB. R. J. Stratta,[1] R. R. Alloway,[2] A. Lo,[3] E. E. Hodge,[4] PIVOT Investigators. *[1]Surgery, Wake Forest University, Winston-Salem, NC; [2]Nephrology, University of Cincinnati, Cincinnati, OH; [3]Pharmacy, University of Tennessee, Memphis, TN; [4]Roche Research Laboratories, Nutley, NJ.*

Background: We previously reported that an alternative dosing regimen of daclizumab was associated with a low incidence of acute rejection, graft loss, and death in simultaneous kidney-pancreas transplant (SKPT) recipients receiving tacrolimus (TAC), mycophenolate mofetil (MMF), and steroids. This is a report of the one year data. **Methods:** A total of 298 SKPT patients were enrolled into this prospective, multicenter, randomized, open-label study. The patients were randomized into three groups: daclizumab 1mg/kg/dose every 14 days for 5 doses (Group I, n=107), daclizumab 2mg/kg/dose every 14 days for 2 doses (Group II, n=113), and no antibody induction (Group III, n=78). All patients received TAC, MMF, and steroids as maintenance immunosuppression. **Results:** There were no differences in baseline characteristics among the three groups, except for a higher proportion of African-Americans in Group II. Group II had the lowest incidence of composite events (acute rejection, graft loss, or death) at 1 year.

Clinical Outcomes	Group I (n=107)	Group II (n=113)	Group III (n=78)	p value (I vs III)	p value (II Vs III)
Acute rejection	22.4%	22.1%	34.6%	0.047	0.040
Median time to acute rejection	23 days	96 days	23 days	NS	<0.05
Kidney graft loss	5.6%	1.7%	3.8%	NS	NS
Pancreas graft loss	14.0%	11.6%	12.8%	NS	NS
Acute rejection, graft loss, or death	36.4%	32.7%	48.7%	NS	0.016

The adverse event profiles were comparable among the three groups, except for a higher incidence of infection and readmissions in Group III.

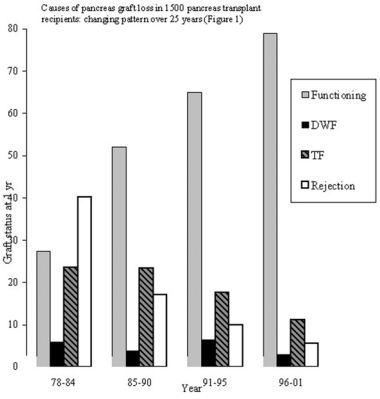
Adverse Events	Group I	Group II	Group III	p value
Hospitalization	66%	66%	82%	0.03
Infection	54%	65%	78%	<0.01
Cytomegalovirus infection	8%	13%	8%	NS
Serious Adverse Events	5%	8%	3%	NS

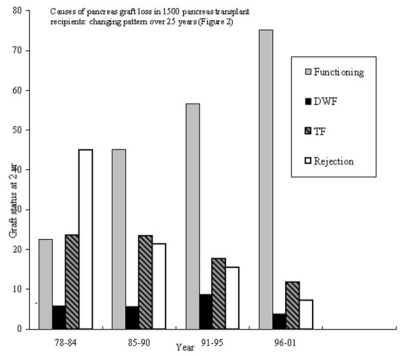
Renal allograft function was excellent and comparable among the three groups (serum creatinine 1.5 ± 0.8mg/dL in Group I, 1.4 ± 0.6mg/dL in Group II, and 1.8 ± 1.7mg/dL in Group III). At one year, the median C-peptide levels were 3.0 ng/ml, 3.5 ng/ml, and 2.7 ng/ml, and the hemoglobin A1C levels were 5.4%, 5.7%, and 5.7%, in Groups I, II, and III, respectively. **Conclusions:** Induction with daclizumab was safe and effective in reducing the incidence of acute rejection when compared to no induction. The alternative 2 dose regimen of daclizumab was as effective as the conventional 5 dose regimen; this has important clinical implications since the 2 dose regimen is logistically more desirable.

Abstract# 663

CAUSES OF PANCREAS GRAFT LOSS IN 1500 PANCREAS TRANSPLANT (PTx) RECIPIENTS: CHANGING PATTERN OVER 25 YEARS. Abhinav Humar,[1] Massimo Asolati,[1] James V. Harmon,[1] Raja Kandaswamy,[1] Rainer W. G. Gruessner,[1] Angelika C. Gruessner,[1] David E. R. Sutherland.[1] [1]*Surgery, University of MN, Minneapolis, MN.*

Background: Results with PTx have improved significantly over the last 25 years. We looked at patterns of graft loss in a large group of patients transplanted at a single center over 25 years. **Results:** Between 1978-2001, 1549 PTx were performed at our center (585 SPK, 561 PAK, and 403 PTA). Of these, 795 (51.3%) continue to function, and 18 (1.2%) have partial function (minimal insulin use). The remaining 736 (47.5%) have failed for the following reasons: acute or chronic rejection (309, 20%), technical failure (255, 16.5%), death with function (140, 9.0%), primary non-function (11, 0.7%), and other (21, 1.3%). The highest proportion of currently functioning grafts are in SPK recipients (57.6%), followed by PAK (51.3%), and PTA (42.2%) ($p<0.01$). This is largely due to a higher risk of graft loss due to rejection after PTA (35.0%) vs PAK (21.0%) vs SPK (8.6%) ($p<0.01$). Causes of graft loss were looked at in 4 different time periods: 1978-84, 1985-90, 1991-95, and 1996-01. Percentage of functioning grafts and causes of graft loss at 1 yr (Fig1) and 2 yrs (Fig2) posttransplant are shown . The greatest improvements have been in early immunologic graft loss rate and technical failure rates. **Conclusions:** Results continue to improve after pancreas transplant with fewer grafts being lost to immunologic and technical causes.





664

CAN ACUTE REJECTION BE PREVENTED IN SPK TRANSPLANTATION? A RANDOMIZED, PROSPECTIVE STUDY WITH THYMOGLOBULIN/ZENAPAX INDUCTION, TACROLIMUS AND STEROID MAINTENANCE, COMPARING RAPAMYCIN WITH MYCOPHENOLATE MOFETIL. G. W. Burke,[1] G. Ciancio,[1] A. Mattiazzi,[1] C. Gomez,[1] A. Rosen,[1] K. Suzart,[1] J. Miller.[1] [1]*Surgery, University of Miami, Miami, FL.*

INTRODUCTION. The availability of new immunosuppressive agents offers hope that acute rejection (AR) may be entirely eliminated. **MATERIALS AND METHODS.** From 9/2000 thru 12/2002, 67 SPK have been performed in patients with type 1 insulin dependent diabetes mellitus and end stage renal disease. These patients have been part of a prospective, randomized trial in which they received thymoglobulin (1.0 -1.5 mg/kg intraoperative, and next 4 days) and zenapax (1 mg/kg x 2 doses, intraoperative, and two weeks), tacrolimus and steroids as baseline immunosuppression. They were randomized to receive either mycophenolate mofetil (MMF) (1 gm BID), or rapamycin (Rapa) (4 mg/day – target trough level 8 – 10 ng/ml) in addition to baseline immunosuppression. Thirty-three patients received MMF, and 34 received Rapa. All patients received ganciclovir/cytovene and bactrim prophylaxis for CMV and PCP respectively. **RESULTS.** There have been 5 episodes of acute rejection (AR) in the MMF group – all except one in patients who were either off MMF (wound infection, pneumonia), or steroids. The AR occurred at 1, 3, 5, 6 and 8 months post SPK. Each of these episodes was steroid resistant but responsive to antibody therapy (OKT3 or Thymoglobulin). There has been one episode of AR in the Rapa group at 3 weeks ($p=.12$ vs MMF). There was one instance of CMV hepatitis (Rapa) which responded to ganciclovir. There were two cases of wound dehiscence requiring surgery (one in each group). There have been three deaths (2 MMF, one Rapa), two kidney graft losses (MMF group), three pancreas losses (1 MMF/2 Rapa). The lipid profile (cholesterol, triglycerides, HDL, LDL) is essentially normal in both groups, however, a large proportion of Rapa patients are receiving lipid lowering agents. The overall actuarial survival with one year mean follow-up for patient, kidney and pancreas is 95%, 92% and 91%. **CONCLUSION**. In a randomized, prospective study with a mean one-year follow-up, the incidence of AR has been limited with one exception to only those instances where recipient's immunosuppression was significantly reduced. There was a smaller percentage of AR in the Rapa vs MMF group, although this was not statistically significant. There were no graft losses associated with AR. In those patients tolerating protocol immunosuppression, there was only one (1%) episode of AR.

Abstract# 665

PANCREAS TRANSPLANTATION IN THE PREDNISONE-FREE ERA. Dixon B. Kaufman,[1] Joseph R. Leventhal,[1] Lorenzo G. Gallon,[2] Michele A. Parker,[3] Alan J. Koffron,[1] Jonathan P. Fryer,[1] Michael M. Abecassis,[1] Frank P. Stuart.[1] [1]*Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL;* [2]*Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL;* [3]*Medicine, Duke University Medical Center, Durham, NC.*

This is a single center, retrospective study of prednisone-free immunosuppression in 103 pancreas transplant (tx) recipients (90 SPK, 13 pancreas-alone (PA)). Induction therapy consisted of either rabbit ATG (n=61; tx dates 2/00-10/01) or Campath (n=42; tx dates 11/01-12/02). Maintenance therapy included tacrolimus (level 9-11 ng/ml) and either MMF (2-3 gm/d) (n=20) or sirolimus (4 mg/d, level 4-6 ng/ml) (n=83). Corticosteroids (as Solumedrol) were given post-operatively for 3-6 doses: 500, 250, 125, (60, 40, 20 mg), and then eliminated. The 48 historical controls (36 SPK, 12 pancreas-alone) received IL-2RA induction, tacrolimus, MMF and steroids. Donor/recipient demographic profiles and surgical technique were similar in all groups. Two year actuarial patient, graft survival, and rejection rates were calculated according to Kaplan-Meier analysis. Graft loss was defined as death or return to dialysis (kidney) or need for insulin therapy (pancreas). Rejection included all biopsy-proven and/or treated cases.

Two Year Actuarial Survival/Rejection Rates

Group	N	mean F/U	Patient	Kidney	Pancreas	Rejection
SPK Control	36	41±10 mo.	94.4%	86.1%	86.1%*	13.9%
SPK Pred-free	90	18±11 mo.	98.6%	93.2%	97.1%*	12.5%
PA Control	12	40±14 mo.	100%	————	91.7%	0%
PA Pred-free	13	13±10 mo.	100%	————	86.7%	15.4%

*$p<0.012$ (Cox-Mantel test)

For SPK recipients, patient and kidney survival, and rejection rates in the control and pred-free cohorts were similar. However, pancreas graft survival was statistically significantly better in the pred-free group. Specifically, in the control group there were 2 deaths (both sepsis), 4 kidney losses (death x 2, acute cortical necrosis, rejection), and 4 pancreas losses (death x 2, thrombosis, rejection). In the pred-free group there was 1 death (sepsis), 3 kidney losses (death, chronic rejection x 2), and 2 pancreas losses (death, thrombosis). Stratification of the pred-free group according to induction (RATG vs Campath) or maintenance (MMF vs sirolimus) therapy showed similar outcomes. In the smaller pancreas-alone tx groups, patient and pancreas survival rates, and rejection rates were not statistically or clinically significantly different. There was a trend toward higher rejection rates in the pred-free group. We conclude that rapidly eliminating corticosteroids in SPK and PA recipients can be safely acheived and will not compromise outcomes.

Abstract# 666

LONG-TERM PANCREAS GRAFT SURVIVAL EXCEEDS LONG-TERM KIDNEY GRAFT SURVIVAL IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION: DIFFERENTIAL EFFECT OF CHRONIC CALCINEURIN INHIBITOR THERAPY? Jeffrey Rogers,[1] Prabhakar K. Baliga,[1] Kenneth D. Chavin,[1] Angello Lin,[1] Rana C. Pullatt,[1] Osemwegie Emovon,[1] Fuad Afzal,[1] David J. Taber,[2] G. Mark Baillie,[2] Elizabeth E. Ashcraft,[2] P. R. Rajagopalan.[1] [1]*Departments of Surgery;* [2]*Pharmacy Services, Medical University of South Carolina, Charleston, SC.*

Background/Aim: Comparison of long-term pancreas graft and kidney graft survival in simultaneous kidney-pancreas transplantation (SKPT) has not yet been described. **Methods:** A retrospective review of 10,363 SKPT entered in the Scientific Registry of Transplant Recipients between 10/5/87 and 9/30/02 was performed. Technical failures (pancreas or kidney graft loss < 7 days post-transplant) were excluded from analysis. All patients received immunosuppression with calcineurin inhibitor (CNI),

antimetabolite, and steroids. Graft survival was computed using the Kaplan-Meier method and comparisons made using the Log-Rank test. **Results:** Mean follow-up was 5.1 years (range 1 month-15 years). Kidney graft and pancreas graft actuarial survival are depicted below.

Kaplan-Meier Kidney and Pancreas Graft Survival

	1 Year	3 Years	6 Years	9 Years	12 Years	15 Years
Kidney Graft	90%	83%	72%	59%	51%	43%
Pancreas Graft	86%	81%	71%	62%	56%	50%

p=0.0001

Conclusions: Long-term pancreas graft survival exceeds long-term kidney graft survival in SKPT, with actuarial pancreas graft survival surpassing actuarial kidney graft survival beyond 6 years post-transplant. This difference may be due to the effect of chronic CNI nephrotoxity. The significant incidence of (native) renal failure in long-surviving heart transplant recipients lends credence to this contention. These findings provide rationale for a shift towards CNI-minimizing immunosuppression regimens in pancreas transplantation. Such regimens should aim to preserve long-term kidney graft function (or native renal function in solitary pancreas transplantation) without compromising long-term pancreas graft survival.

Abstract# 667

PATIENT SURVIVAL FOR VARYING SEQUENCES OF KIDNEY AND PANCREAS TRANSPLANTS. Hans W. Sollinger,[1] Laura L. Christensen,[2] Fu L. Luan,[3] Dennis M. Heisey,[1] Alan B. Leichtman,[2,3] Akinlolu O. Ojo,[2,3] Robert A. Wolfe,[2,3] Friedrich K. Port.[2] *[1]University of Wisconsin, Madison, WI; [2]SRTR/URREA, Ann Arbor, MI; [3]University of Michigan, Ann Arbor, MI.*

Diabetic kidney and pancreas transplant candidates can receive either a simultaneous kidney and pancreas transplant (SPK) or a kidney alone transplant (KA) from a living (LD) or cadaveric (CAD) donor, possibly to be followed by a pancreas after kidney (PAK) transplant. Improved understanding of patient survival for varying sequences of kidney and pancreas transplantation is necessary to optimize clinical decisions. Methods: We analyzed 10,109 transplant recipients waitlisted for an SPK between 1/1/90 and 2/28/01. Additional death ascertainment was obtained from the SS Death Master Files. Time-dependent Cox regression models were used to compare death rates of those who received a PAK following a LD or CAD KA transplant with those who had not (yet) received a PAK. Death rates were also compared for those receiving a PAK within 1 year vs. greater than 1 year after KA transplant. Additional Cox models compared death rates for patients receiving a CAD-KA, a LD-KA, and a SPK transplant. Multivariate analyses were adjusted for donor and recipient demographics, ischemia time, HLA matching, PRA, and year of first transplant. Results: Among SPK candidates who initially received only a KA, multivariate models showed no significant difference in death rates for patients after receiving a PAK (RR=1.20, p=0.28) compared with those not yet receiving a PAK for LD and CAD combined, with similar RR results for both LD-KA (N=804) and CAD-KA (N=876). For PAK within 1 year of KA (N=250), mortality after PAK was not statistically significantly higher than without PAK (RR=1.47, p=0.06), with similar RR results for both LD and CAD. For PAK occurring more than 1 year post-KA (N=126), mortality after PAK was not statistically different than without PAK (RR=0.87, p=0.62), with similar RR results for LD and CAD. Models adjusting only for donor type yielded similar results. Median length of follow-up post-PAK was 697 days for LD-KA and 745 days for CAD-KA. Among all SPK candidates, LD-KA recipients (N=804) had a RR of death of 0.53 (p<0.0001) and SPK recipients (N=8429) had a RR of death of 0.70 (p<0.0001), compared with CAD-KA (N=876) (LD vs. SPK: RR=0.75, p=0.02). Similar results were found when controlling for time on dialysis. Conclusions: Among SPK candidates, patient survival was better for LD-KA vs. SPK and for SPK vs.CAD-KA. No significant patient survival advantages were found for PAK compared with KA during 2 years median follow-up.

Abstract# 668

PREGNANCY OUTCOMES IN FEMALE PANCREAS-KIDNEY TRANSPLANT RECIPIENTS. Carolyn H. McGrory,[1] Lisa A. Coscia,[1] Michael J. Moritz,[2] Vincent T. Armenti.[1] *[1]Surgery, Thomas Jefferson University, Philadelphia, PA; [2]Surgery, Drexel University College of Medicine, Philadelphia, PA.*

The purpose of this study was to examine the outcomes of 47 pregnancies (49 outcomes, 2 sets of twins) in 34 female pancreas-kidney (P/K) recipients reported to the National Transplantation Pregnancy Registry (NTPR). Data were collected via questionnaires, phone interviews and hospital records. There were 39 (80 %) livebirths, 6 (12%) spontaneous abortions, 3 (6%) therapeutic abortions (one twin reduction), and 1 (2%, one twin) ectopic. Exocrine drainage was to bladder in 24 and enteric in 10 (6 conversions). Mean transplant to conception interval was 3.8 ± 2.3 yrs. Maintenance immunosuppression during pregnancy was cyclosporine-based in 40 (Sandimmune® 25, Neoral® 15) and tacrolimus-based in 7. Maternal comorbid conditions during pregnancy included: hypertension 36/46 (78%), infections 23/46 (50%), and pre-eclampsia 14/39 (36%). Rejection occurred during 3/44 (7%) pregnancies; all three recipients went on to have graft losses. Two recipients required intrapartum nephrostomies, both removed postpartum. There was no gestational diabetes reported. Of the 39 liveborn, 20 (54%) were delivered by cesarean section, one complicated by a tear to the duodenum of the graft. Mean gestational age was 35 ± 2.9 wks; 29 (74%) were premature (<37 wks). Mean birthweight was 2159 ± 680 gms, compared to cyclosporine-treated kidney only recipients with a mean of 2490 gms; 23 (59%) were low birthweight (<2500 gms). Twenty-two (56%) infants had neonatal complications with one neonatal death in a severely premature infant. At last follow-up of the children, all 38 were reported to be healthy and developing well. One child was recently diagnosed with attention defecit hyperactive disorder and has asthma. There were 6 graft losses within 2 yrs postpartum: 3 K (2 retransplanted, 1 dialysis), 1 P (insulin), 2 P-K (1 re-transplanted, both subsequently died). Current maternal graft function was reported as adequate in 24 (71%) of recipients. The remaining 8 recipients reported the following current graft function: 3 (P adequate, dialysis), 3 (P not functioning, K adequate), 1 (P reduced, K adequate) and 1 (P adequate, K reduced). CONCLUSIONS: Pregnancy after pancreas-kidney transplantation appears to be well tolerated in the majority of recipients as evidenced by the absence of gestational diabetes and a low incidence of rejection. Birthweights are lower compared to kidney only recipients. As yet there is no evidence for an increase in the incidence or pattern of birth defects in the newborn.

Abstract# 669

POLYOMA VIRUS INFECTION IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANT RECIPIENTS: LEADING CAUSE OF RENAL GRAFT LOSS IN FIRST TWO YEARS POST TRANSPLANT. Gerald S. Lipshutz,[1] Harish Mahanty,[2] Sandy Feng,[1] Ryutaro Hirose,[1] Peter G. Stock,[1] Sang-Mo Kang,[1] Chris E. Freise.[1] *[1]Department of Surgery, University of California, San Francisco, San Francisco, CA; [2]Department of Surgery, University of California, San Francisco-East Bay, Oakland, CA.*

Background: With the introduction of more potent immunosuppressive agents, rejection rates have decreased markedly in simultaneous pancreas kidney transplant (SPK) recipients. However, with more intense immunosuppression, opportunistic infections such as polyoma virus have been more frequent. The purpose of this report is to outline the clinical course of SPK patients who developed documented polyoma infection in the transplanted kidney. Methods: A retrospective review of 154 consecutive SPK recipients from 1996 to 2002 was performed. Induction and maintenance immunosuppression, surgical complications, rejection episodes, and opportunistic infections were reviewed. Patients who developed biopsy-proven polyoma virus infection in the renal allograft were identified. Results: Seven patients (4.5%) were identified who developed polyoma. All had received induction therapy with either OKT3 (5 mg/day for 10.5 days [avg]) or thymoglobulin (5.8 mg/kg [avg]). Patients without polyoma had received similar induction. Maintenance immunosuppression included Prograf/MMF in four patients, CsA/MMF in two, and CsA/Azathioprine in one. Time to diagnosis was an average of 364 days (range 252-706) after transplantation Two patients had undergone treatment for kidney rejection prior to the diagnosis of polyoma. Immunosuppression was decreased in all patients when polyoma was identified and more recently, Cidofovir has been administered. Despite these interventions, 5 of the 7 lost kidney function (creatinine >5.0 or resumption of dialysis). However, none of the 7 developed pancreatic abnormalities as demonstrated by normal blood glucose and amylase and no requirement for exogenous insulin. Two patients underwent LRRT >1 year after polyoma diagnosis; both have normal kidney function (Cr <1.5) at four years follow-up. Polyoma virus was the leading cause of renal loss in this cohort of patients. Conclusions: Polyoma is a serious concern for SPK transplant recipients. The pancreas, however, is spared from clinical evidence of infection, and no rejection was noted when immunosuppression was decreased.. These graft losses appear to be a penalty of more potent immunosuppression, and a better treatment strategy is needed to prevent renal graft loss when polyoma is diagnosed. Retransplantation can be considered based on our limited experience.

Abstract# 670

SUCCESSFUL REVERSAL OF REFRACTORY ACUTE REJECTION WITH CAMPATH 1H IN PANCREAS TRANSPLANT RECIPIENTS. Santosh Potdar,[1] Ron Shapiro,[1] Ngoc Thai,[1] Ashok Jain,[1] Paramjeet Randhawa,[2] Anthony Jacob Demetris,[2] Amit Basu,[1] Amedo Marcos,[1] John J. Fung,[1] Thomas E. Starzl.[1] *[1]Thomas E Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA.*

Patients and Methods: Between September 2002 and November 2002, 9 pancreas transplant recipients (SPK5, PAK 1,PTA 3) were treated with Campath 1H for refractory rejection.All had been transplanted between July 2001 and July 2002. There were 5 males and 4 females,whose median age was 47 years (range 19-58).All patients had enteric exocrine drainage. All patients had recived Thymoglobulin, 5 mg/kg, as preconditioning pre-operatively and were treated with tacrolimus monotherapy (target level 10ng/ml) post-operatively.Weaning of tacrolimus was started 100 days after transplant. Patient follow up included lipase,glucose,c-peptide,and serum creatinine.Elevated lipase levels were evaluated by biopsy(Pancreas,Kidney and Pancreas or Kidney alone). Rejection was treated with steroids,reversal of weaning,addition od rapamycin, or antibody tratment, depending on grade of rejection.Treatment with campath 1H utilized premedication with iv methylprednisolone (500-1000mg). RESULTS: Six patients had mild pancreas rejection(SPK3,PAK1,PA2) which was initially treated with 1.5 gm to 2.0 gms of iv methylprednisolone.Persistent elevation of lipase prompted repeat biopsy, which showed no improvement (5 patients) or worse rejection (1 patient). All six were then trated with 2 or 3 doses of Campath 1H (30 mg) depending on the lipase levels.repeat biopsy on post campath days 10-14 showed significant improvement of rejection in all 6 patients (no ACR=3,minimal ACR=3). Lipase levels also fell significantly.Three patients(SPK 2, PA 1) had moderate to severe rejecton on pancreas biopsy and were treated with 1gm iv methylprednisolone and 2-3 doses of Campath 1H (30mg), depending on lipase levels in response to the first campath dose. The post-campath treatment biopsy showed no rejection in 2 patients. The third paient refused follow-up biopsy, but his lipase had normalized. No infectious complications were seen in all 9 patients at 1 month after treatment.One patient required hospitalization for neutropenic precautions(wbc 0.5), which responded to Neupogen. CONCLUSION: Campath 1H is an effective agent for the reversal of steroid-resistant rejection in pancreas transplantation.In addition, it appears to be effective for the treatment of moderate to severe rejection in pancreas transplant patients.

IMMUNOSUPPRESSION AND REJECTION IN LIVER TRANSPLANTATION

Abstract# 671

EFFECTIVE AND SAFE STEROID-FREE IMMUNOSUPPRESSION WITH A TACROLIMUS / DACLIZUMAB REGIMEN AFTER LIVER TRANSPLANTATION. Olivier Boillot,[1] David A. Mayer,[2] Karim Boudjema,[3] the MASTER Study Group. *[1]Service de Transplantation, Hôpital Edouard Herriot, Lyon, France; [2]The Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Centre de Chirugie Digestive et Hepato-Biliare, Hôpital Pontchaillou, Rennes, France.*

Objectives: This open, randomised, multicenter, 3-month study compared a dual tacrolimus plus steroids (Tac/steroids) regimen with a steroid-free immunosuppressive regimen of tacrolimus plus daclizumab induction therapy (Tac/Dac) in adult liver transplant recipients. **Methods:** In both groups, the initial Tac dose was 0.15 mg/kg/day given orally in 2 doses. Subsequent doses were adjusted to trough levels of 10-20 ng/ml in the first 6 weeks and 5-15 ng thereafter. Both groups received an intraoperative i.v. bolus of 500 mg methylprednisolone. The Tac/steroid group received orally 15-20 mg/day prednisone during month 1, 10-15 mg/day during month 2, and 5-10 mg/day during month 3. The Tac/Dac group received the first dose of 2 mg/kg Dac prior to reperfusion; a second dose of 1 mg/kg Dac was given between days 7 and 10. **Results**: A total of 708 patients were randomised (1:1). Ten patients were not eligible for analysis. The full analysis set comprised 347 patients in the Tac/steroids group and 351 in the Tac/Dac group. Baseline characteristics were balanced between the groups. Mean tacrolimus dose during month 3 was 0.11 mg/kg/day in both groups; mean whole blood trough levels during month 3 were 10.9 ng/mL (Tac/steroids) and 10.6 ng/mL (Tac/Dac). Fifty-four (15.6%) patients in the Tac/steroids group and 78 (22.2%) patients in the Tac/Dac group were withdrawn, mostly because of adverse events or protocol violations; 81.0% (Tac/steroids) and 73.8% (Tac/Dac) completed the study. The incidence of biopsy-confirmed acute rejection was 28.5% in the Tac/steroids group and 27.6% in the Tac/Dac group (p=ns.); the incidence of corticosteroid-resistant acute rejection was 6.3% and 2.8% (p=0.027). Twenty-eight grafts (8.1%) were lost in the Tac/steroids group vs. 36 grafts (10.3%) in the Tac/Dac group. Twenty patients (5.8%) in the Tac/steroids group and 25 patients (7.1%) in the Tac/Dac group died. The reported early recurrence of HCV infection was similar (Tac/steroids 27%, Tac/Dac 22%). Differences between Tac/steroids and Tac/Dac were reported in the incidences of the following adverse events, regardless of the relationship to study medication: diabetes mellitus (15.3% vs. 5.7%, p<0.001), and CMV infection (11.5% vs. 5.1%, p=0.002). **Conclusion:** Overall, both regimens were safe and effective. The Tac/Dac regimen showed some advantage in terms of lower incidences of diabetes and viral infection, and a lower incidence of steroid-resistant acute rejection.

Abstract# 672

PREGNANCY OUTCOMES IN FEMALE LIVER TRANSPLANT RECIPIENTS ON CYCLOSPORINE VS TACROLIMUS. Scott W. Cowan,[1] Lisa A. Coscia,[1] John S. Radomski,[2] Michael J. Moritz,[3] Vincent T. Armenti.[1] *[1]Surgery, Thomas Jefferson University, Philadelphia, PA; [2]Surgery, Our Lady of Lourdes, Camden, NJ; [3]Surgery, Drexel University College of Medicine, Philadelphia, PA.*

The purpose of this study was to analyze pregnancy outcomes in female liver recipients maintained on calcineurin inhibitor based regimens and reported to the National Transplantation Pregnancy Registry (NTPR) over the last 12 years. Data were collected from 28 tacrolimus-based (41 pregnancy outcomes) and 74 cyclosporine-based recipients (Sandimmune®/Neoral®/Gengraf®, 125 pregnancy outcomes) via questionnaires, telephone interviews and hospital records. Analysis was by generalized linear (GEE) regression models. Variables included comorbid medical conditions before and during pregnancy, maternal graft function and newborn outcomes.

	cyclosporine	tacrolimus	p value
Transplant to conception interval (yrs)	4.1±3.1	2.8±2.1	0.007
Hypertension before pregnancy	51/121 (42%)	8/38 (21%)	0.048
Hypertension during pregnancy	49/122 (40%)	10/40 (25%)	0.104
Preeclampsia	25/94 (27%)	5/34 (15%)	0.185
Diabetes before pregnancy	3/122 (3%)	6/38 (16%)	0.024
Diabetes during pregnancy	2/122 (2%)	6/40 (15%)	0.016
Rejection before pregnancy	60/116 (52%)	27/37 (73%)	0.054
Rejection during pregnancy	11/124 (9%)	3/38 (8%)	0.878
Livebirths	92/125 (74%)	29/41 (71%)	0.691
Mean gestational age (wks)	37±3.6	37±3.4	0.946
Mean birthweight (gms)	2655±795	2828±824	0.369

Factors that were significant included: transplant to conception interval, hypertension before pregnancy, and diabetes before and during pregnancy. Tacrolimus dose was not changed in 68% of pregnancies versus 55% for cyclosporine (p=0.010). Mean serum creatinines were similar before and during pregnancy. Graft loss within 2 yrs of pregnancy occurred in 4% tacrolimus-treated and 9.5% cyclosporine-treated recipients. One recipient in the tacrolimus group reported a pregnancy with exposure to mycophenolate mofetil. A healthy infant was delivered at 37 wks weighing 2608 gms, with no structural malformations. CONCLUSIONS: Based on this analysis of pregnancies in liver transplant recipients reported to the NTPR, while there are some differences in maternal conditions, there do not appear to be significant differences in recipient graft and newborn outcomes when calcineurin inhibitor regimens are compared. Thus, data to date suggest that both regimens may be of similar efficacy with regard to pregnancy safety.

Abstract# 673

THREE MONTH INTERIM RESULTS OF LIS2T A MULTICENTER, RANDOMIZED STUDY COMPARING CYCLOSPORINE MICROEMULSION WITH C2 MONITORING AND TACROLIMUS IN *DE NOVO* LIVER TRANSPLANTATION. G. A. Levy,[1] F. Sanjuan,[2] G. Grazi,[2] Y. Wu,[2] P. Marrotta,[2] O. Boillot,[2] F. Muelbacher,[2] D. Samuel.[2] *[1]Medicine, Toronto General Hospital, Toronto, ON, Canada; [2]on Behalf of LIS2T Study Group.*

Introduction: Previous studies comparing cyclosporine and tacrolimus were based on trough monitoring. The benefit of C2 monitoring for cyclosporine has since been recognised. The present study was designed to compare the efficacy and safety of a cyclosporine regimen (Neoral®) based on C2 monitoring to a tacrolimus regimen based on trough monitoring in the prevention of rejection after *de novo* liver transplantation. **Methods:** 500 patients were enrolled in 50 sites from 17 countries. We report the results of a planned interim analysis of the first 300 patients who attained 3 months post-transplant. Within 24 hours post-transplant patients were randomised to either cyclosporine (NEO) or tacrolimus (TAC). NEO was administered at an initial dose of 10.0-15.0 mg/kg/day, TAC at 0.1-0.15 mg/kg/day. Both arms received in addition steroids or steroids plus azathioprine. During the first 3 months daily doses of NEO were adjusted to target C-2h levels of 800 to 1200 ng/mL whilst TAC to target C-0h levels of 5 to 15 ng/mL. **Results:** 152 patients were randomized to NEO and 148 to TAC representing the intent to treat population on which the analyses have been performed. The rates of acute rejection were 27.6% in the NEO arm vs. 27.0% in the TAC arm (p=ns) despite the fact that the majority of patients receiving NEO achieved target levels later

than patients receiving TAC (Day 7 vs Day 2). The severity of rejections was comparable (BANFF criteria). The incidence of graft loss or death was NEO=11.8% vs. TAC=12.2% (p=ns). Among the causes of graft loss, more hepatic artery thrombosis was observed with TAC (TAC=8 vs NEO=4). In hepatitis C positive patients (52 in each arm), the rejection rate was NEO=19.2% vs. TAC=23.1% (p=ns) whereas in HCV negative patients it was 32% (NEO) versus 29.2% (TAC) (p=ns). Significantly more diabetes: TAC= 13.4% vs NEO= 5.84% (p<0.05) and diarrhea: TAC= 24.8% vs NEO= 8.44% (p<0.0002) were reported in patients receiving TAC. The incidence of hypertension was TAC=26.8% vs NEO=35.7% (p=ns). Mean serum creatinine at 3 months was NEO=106 umol/L (range 51-256) vs TAC=106.9 umol/L (range 53-442). **Conclusions:** *De novo* liver transplant recipients treated with Neoral C2 or tacrolimus both have a low acute rejection rate whether HCV positive or negative. The use of Neoral was associated with a significantly lower incidence of diabetes and diarrhea.

Abstract# 674

THE EFFICACY OF SIROLIMUS CONVERSION IN LIVER TRANSPLANT PATIENTS WHO DEVELOP RENAL DYSFUNCTION ON CALCINEURIN INHIBITORS. Patrick Lam, Atsushi Yoshida, Kimberly Brown, Marwan Abouljoud, Fadi Dagher, Iman Bajjoka, Dilip Moonka. [1]*Department of Gastroenterology and Transplantation Surgery, Henry Ford Hospital, Detroit, MI.*

Background Renal dysfunction represents a significant source of morbidity following orthotopic liver transplant (OLT) and is associated with the use of calcineurin inhibitors (CI). This study evaluates the efficacy of sirolimus (SLR) in preserving renal function in patients who have developed renal insufficiency after OLT while being maintained on a CI. Methods This is a nonrandomized, retrospective study of 28 OLT patients who were converted from a CI to SLR. Patients were eligible for conversion once their serum creatinine (Cr) was persistently greater than 1.8 mg/mL after excluding other causes of renal failure. The primary end-point was Cr over time. Other parameters included the Hgb, WBC, platelet count, and lipid levels. Results 28 OLT patients with renal dysfunction were converted to SLR at a mean of 789 days after OLT and have been followed for a mean of 270 days. Of these 28 patients, 7 (25%) were unable to tolerate SLR and 1 died of malignancy. Of the remaining patients, 6 (21%) progressed to ESRD despite the use of SLR and the other 14 patients (50%) have been maintained on SLR with stable renal function. On an intention to treat basis, the 28 patients had a drop in Cr of 0.38 mg/dL (p=0.029) at week 4 with a small increase at week 24 of 0.08 (p=0.794). However, the subset of 14 patients who did not develop ESRD had a decline in Cr of 0.64 mg/dL at week 4 (p=0.018), 0.56 at week 12 (p=0.039), 0.57 at week 24 (p=0.049) and 0.43 at week 48 (p=0.306). The patients who developed ESRD were compared to the patients with stable renal function on SLR. While there were no statistically significant differences between the two groups, the patients who developed ESRD were converted later after OLT (1223 vs 498 days), had a higher Cr at conversion (2.8 vs 2.3 mg/dL), and a lower creatinine clearance (36 vs 53 mL/min). In the 14 patients with stable renal function maintained on SLR, there were no significant changes in the Hgb, WBC, and platelet count after week 24; however there was a significant rise in cholesterol (p<0.05) which persisted at all time points. Conclusion The use of sirolimus to preserve renal function after OLT was limited by the number of patients unable to tolerate drug (25%) and the number of patients who developed ESRD (21%). However, a subgroup of patients (50%) had an improvement in Cr that persisted at 24 weeks. These patients may have benefited from earlier conversion and more preserved renal function at the time of conversion.

Abstract# 675

RAPAMUNE CONVERSION IN LONG-TERM LIVER TRANSPLANT PATIENTS IS EFFECTIVE IN RECOVERING RENAL FUNCTION. Edmund Q. Sanchez,[1] Nicholas Onaca,[1] Mark Thomas,[1] Takehisa Ueno,[1] Vandad Raofi,[1] Robert M. Goldstein,[1] Marlon F. Levy,[1] Srinath Chinnakotla,[1] Sherfield Dawson,[1] Henry Randall,[1] Giovanna Saracino,[1] Goran B. Klintmalm.[1] [1]*Baylor Regional Transplant Institute, Baylor University Medical Center, Dallas, TX.*

Background: The benefit of rapamycin is evaluated in chronic nephrotoxicity in liver transplant patients. **Mehtods:**Our prospectively maintained database of 2005 liver transplants (Ltx) was reviewed. Patients (Pts) were converted from CNI to rapamycin (rapa) as the main immunosuppressive agent for nethrotoxicity or rejection. Glomerular filtration rates (GFR) by glofil method and serum creatinine (sCr) were obtained pretransplant, 3 mo., and yearly on both conversion and control pts. GFR and sCr were obtained before and after conversion. Target rapa levels were kept between 10-15 ng/mL in pts <3 mos from transplant and 5-10 ng/mL in the remaining pts. Pts were maintained on mycophenolate mofetil. **Results**:32 pts (16 male, 16 female) with mean age at Ltx of 50.3 ± 12.8 yrs were converted (27 for nephrotoxicity and 5 for acute rejection). The median time to initiating rapa from time of transplant was 177.5 days (range 4-4534). The median exposure time was 380 days (range 4-1969 days). Case control analysis was based on age, sCr, and liver disease. There were 30 pts at 1 yr, 18 pts at 2 yrs, and 5 pts at 3 yrs. There were 17 pts (56.7%) and 8 pts (44.4%) at 1 yr and 2 yrs respectively with continued improvement in GFR. Also, 8 pts (26.7%) at 1 yr and 5 pts (27.8%) at 2 yrs displayed progressive decline in GFR. The GFR and sCr data is demonstrated in the table:

Time point	GFR (control)	GFR (rapa)	sCr (control)	sCr (rapa)	P (vs.case control)
Pre-transplant	77 ± 24.7	84.7 ± 49.2	1.5 ± 1.0	1.9 ± 1.5	NS
Pre conversion	N/A	33.7 ± 17.2	N/A	1.8 ± 1.6	NS
3 mo	59.6 ± 26.6	41.9 ± 26.1*	1.5 ± 0.6	1.7 ± 1.2	*P=0.05
1 yr	55.4 ± 27.8	53.9 ± 26.2	1.7 ± 0.6	1.8 ± 1.2	NS
2 yr	51.2 ± 23.7	49.8 ± 22.9	1.4 ± 0.4	1.8 ± 0.6*	*P=0.02

No pts progressed to hemodialysis. There was 1 episode of acute cellular rejection after conversion treated with steroids. Rapa was discontinued in 12 (34.3%) cases due to side effect. **Conclusion:**The renal benefit of rapa in Ltx pts tolerating conversion has been demonstrated. 65.7% of pts tolerated rapa conversion. Of these, 56.7% and 44.4% demonstrated continued increase in GFR and 1 and 2 yrs respectively. Conversion is an effective strategy in improving renal function in pts with CNI nephrotoxicity and can be done without increased rejection rates. Prospective randomized controlled studies should further validate these results.

Abstract# 676

IMPACT OF TACROLIMUS (TAC) VERSUS CYCLOSPORINE (CSA) ON LONG-TERM RENAL FUNCTION AND CARDIOVASCULAR (CV) RISK FOLLOWING LIVER TRANSPLANTATION. Michael R. Lucey.[1] [1]*For the Transplant Therapy Outcomes Study Group, University of Wisconsin-Madison Medical School, Madison, WI.*

Purpose: This study aims to examine the impact of immunosuppressive regimens in routine clinical practice on long-term renal function and CV risk following liver transplantation. **Methods:** This is a retrospective study projected to include 1000 patients (pts) who received a primary liver transplant between 1/97 and 9/98 at 11 US transplant centers. We report renal impairment, hypertension, hyperlipidemia, and post-transplant diabetes mellitus (PTDM—use of insulin or oral agents for ≥30 days in pts with no diabetic history) in 506 pts. Data are reported on an intent-to-treat (first use) basis. **Results:** Immunosuppressive therapy: antilymphocyte antibody induction—19%; TAC—42%; CSA—58%; corticosteroids–80% at 1 month, 40% at 3 years. At 3 years, 20% of pts were receiving mycophenolate mofetil.

	Month	TAC N=212	CSA N=294	P-value
SCr (mg/dL)	6	1.29±0.56	1.50±0.55	‹.05
Mean ± SD	12	1.27±0.49	1.64±1.15	‹.05
	24	1.28±0.52	1.60±0.56	‹.05
	36	1.35±0.56	1.71±1.11	‹.05
Systolic BP (mmHg)	6	130±21	131±21	
Mean ± SD	12	131±19	139±21	‹.05
	24	133±20	143±18	‹.05
	36	133±21	140±19	‹.05
Diastolic BP (mmHg)	6	81±15	82±16	
Mean ± SD	12	81±12	84±11	
	24	83±12	83±13	
	36	83±12	84±12	
Antihypertensives/pt		0.80±1.15	1.16±.94	‹.05
Antihyperlipidemics/pt		0.07±0.26	0.22±0.41	‹.05

Renal function was significantly better in pts receiving TAC. Systolic BP was significantly lower in TAC-treated pts. There were no significant differences in the mean serum levels of atherogenic lipids between groups. Cholesterol levels were normal while triglyceride values were above normal, with no difference between groups. Significantly fewer agents were required to control blood pressure and serum lipid levels in TAC-treated pts. The incidence of PTDM was equivalent. **Conclusions:** Analysis of 50% of the projected data from a large multicenter cohort of liver transplant pts shows renal function and systolic BP were significantly better in pts maintained on TAC. In addition, TAC-treated pts required fewer medications to control blood pressure and serum lipid levels. Diastolic BP, lipid levels, and PTDM were equivalent in both groups.

Abstract# 677

TACROLIMUS (TAC) VERSUS CYCLOSPORINE (CSA) FOLLOWING LIVER TRANSPLANTATION: IMPACT ON LONG-TERM OUTCOMES IN ROUTINE CLINICAL PRACTICE.

Michael R. Lucey.[1] [1]*For the Transplant Therapy Outcomes Study Group, University of Wisconsin-Madison Medical School, Madison, WI.*

Purpose: Calcineurin inhibition with TAC or CSA is a mainstay of immunosuppressive therapy following liver transplantation. However, the relative advantages of TAC or CSA remain in doubt. This retrospective study examines the impact of immunosuppressive regimens on long-term outcomes in liver transplant patients (pts). **Methods:** We report on 506 of a projected 1000 consecutive liver transplant pts from 11 US centers. Pts included in the study received a primary liver transplant between 1/97 and 9/98 and were followed up to 3 years after transplantation or to graft loss (requirement for retransplantation or death due to graft failure). We used electronic Synapse AXON technology to enter data and generate results and statistics. Data are reported on an intent-to-treat (first use) basis. **Interim cohort:** 58% male, 86% Caucasian, 91% cadaveric organ recipients. Mean age at transplant—45.7±15.8 years. Mean MELD score—17.0±9.0. Pre-transplant diagnoses—61% non-cholestatic cirrhosis (32% HCV-associated cirrhosis; 26% alcoholic liver disease), 16% cholestatic cirrhosis, 16% multiple diagnoses. Immunosuppression: antilymphocyte antibody induction 19%; TAC 42%; CSA 58%; corticosteroids 80% at 1 month, 40% at 3 years. At 3 years, 20% of pts were receiving mycophenolate mofetil. **Results:** Pt survival was equivalent in both treatment groups. Of 75 deaths in 3 years, 20 pts were on TAC, 40 on CSA, and 13 pts on no calcineurin inhibitor. Causes of death—infection 29%, cardiovascular events 14%. Graft loss was significantly more common in pts receiving CSA (11% vs 5%; *P*=0.027). Freedom from acute rejection over the first year was 41.5% and 48.9% for TAC- and CSA-treated pts, respectively (*P*=0.03). The incidence of corticosteroid-resistant acute rejection in the first year was 12.0% for TAC pts, and 9.6% for CSA pts (*P*=0.67).

	TAC N=212	CSA N=294	P-value
Mean dose (mg/day)			
- Month 6	9.4±22.7	341±105	
- Month 36	7.0±19.8	229±90	
Mean trough (ng/mL)			
- Month 6	9.6±4.3	258±98	
- Month 36	11.9±44.6	169±128	
Pt survival*	86%	83%	0.337
Graft survival*	95%	89%	0.027

*Kaplan-Meier estimates based on N=473

Conclusions: Analysis of 50% of projected data shows equivalent pt survival in liver transplant recipients initiated on either TAC- or CSA-based immunosuppression. There was a significant benefit in graft survival among pts on TAC.

Abstract# 678

CAMPATH 1H WITH TACROLIMUS IMMUNOSUPPRESSION IN ADULT LIVER ALLOTRANSPLANTATION: OUR EXPERIENCE WITH 19 CASES.

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INTRODUCTION: We report our experience with Campath 1H in combination with Tacrolimus, in 19 cases of adult cadaveric liver transplantation. **MATERIALS AND METHODS:** Campath 1H was administered at 4 doses, pre transplant, immediately post transplant and on post operative days 3 and 7. Maintenance immunosuppression, consisted of half the usual dose of Tacrolimus (levels 5-10 ngr/ml). Rejection episodes were treated with steroids or OKT3 according to their severity. Tacrolimus was substituted with cyclosporine (neoral) or sirolimus, in case of serious side effects. Patients with hepatitis C were excluded from this protocol in fear of recurrence of the disease under Campath 1H. The follow up was 1-11 months, (average: 4 months). **RESULTS:** 19 patients received 19 grafts. Their underlying liver disease was Laennec (n=7), Cryptogenic (n=7), or Primary Biliary Cirrhosis (n=4) and Primary Sclerosing Cholangitis (n=1). **Mortalities:** One patient suffered a cerebral infarct intraoperatively and died 12 days later. **Tacrolimus side effects:** Two patients were converted to neoral and steroids and one to sirolimus and steroids for tacrolimus related neurotoxicity (n=2) and nephrotoxicity (n=1). **Campath side effects**: A patient developed urticarial rash during the administration of Campath 1H. **Rejection:** 4 patients had clinical evidence of mild rejection confirmed by biopsy in 1 case. All were successfully treated with steroids. Two of them had the steroids withdrawn within 2 weeks after treatment and the other two are in the process of withdrawal (8 mgr and 12 mgr medrol, QD). **Opportunistic infections:** There was a case of herpes zoster which resolved with treatment. Another patient presented staphylococcal pneumonia and ARDS. The latter has not resolved as yet. **CONCLUSION:** Campath 1H in combination with half the usual dose of tacrolimus, seems to be an effective steroid free maintenance immunosuppression regimen in adult liver transplantation. Opportunistic infections were not frequent and rejections encountered till now were mild. The majority of the patients are maintained on low dose Prograf monotherapy.

Abstract# 679

ANTITHYMOCYTE GLOBULIN-BASED INDUCTION PROTOCOL IN ADULT LIVER TRANSPLANT PATIENTS.

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Introduction Induction with T-cell depleting immunosuppressive drugs in liver transplantation is controversial. This study examined the safety of, and incidence and timing of rejection in liver transplantation with an immunosuppressive protocol that utilized antithymocyte globulin (ATG, Sangstat), tacrolimus, and steroids. Additional subgroup analysis compared hepatitis C (HCV)-infected and non-infected patients. **Methods** 106 Adult patients received 109 liver transplants over 15 months. 6 Patients who died in the perioperative period, prior to receiving ATG, were excluded. All patients had at least 2 months posttransplant follow-up. Postoperatively, patients received 3 doses of ATG (2 mg/kg) given every other day, steroids tapered to 20 mg/day by postoperative day 7, and tacrolimus started postoperative day 3-4 (trough levels 10-12). Elevations in liver numbers in the first 4 weeks were investigated with Doppler ultrasound followed by ERCP. If these tests were non-diagnostic, biopsy was performed to evaluate for rejection. **Results** 96% of patients are alive. No graft was lost to rejection. 10% of patients had episodes of rejection. A cumulative total of 6%, 8% and 10% of patients had biopsy proven rejection at 4, 8 and greater than 8 weeks, respectively. All rejection episodes were reversed with a steroid pulse, and no patient had steroid resistant rejection. No patient has had more than 1 episode of rejection. 3 of 10 patients with rejection had autoimmune hepatitis and have been placed on mycophenolic acid in addition to prograf and steroids. Two patients experienced vascular complications (hepatic artery thrombosis). ATG related drug events were limited to fever, chills, tachycardia, and worsening PO2 and O2 saturation. No patient required reintubation as a result of ATG. No patients had posttransplant lymphoproliferative disorder. 42% of patients had HCV. 12% of HCV patients and 9% of non-HCV patients had rejection. 31% of HCV-patients had biopsy proven hepatic recurrence of their hepatitis occurring at 4 weeks to 10 months post-transplant. **Conclusion** ATG induction therapy is associated with good patient and graft survival, low incidence of rejection, and minimal detrimental side effects. Patients with HCV had a slightly higher incidence of rejection than non-HCV patients and had a high recurrence of hepatitis C.

Abstract# 680

LIVER TRANSPLANT RECIPIENTS HOMOZYGOUS FOR CTLA-4 GENE +49 HAVE A REDUCED RISK OF ACUTE REJECTION.

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Naïve T-cells require for activation two signals: the first is generated by binding of the T cell receptor to MHC-peptide complex and the second by interaction of CD28 with the co-stimulatory molecules CD80 and CD86. Activated T-cells can express a second co-stimulatory molecule ligand, CTLA-4 (CD152), which generates an inhibitory signal in the T-cell. The outcome of T-cell activation is dependent on a balance between interactions of CD28 and CTLA-4 with co-stimulatory molecules. The aim of this study was to investigate whether single-nucleotide polymorphisms (SNP) in the CD86- and CTLA-4 genes of liver-transplant donors and recipients are associated with acute rejection. CD86 gene +1057 G/A SNP was determined in donors and recipients. CTLA-4 gene -318 C/T SNP, and +49 A/G SNP were determined in recipients. DNA was isolated from PBMC of 135 recipients and from spleen cells of 73 donors. All patients had a minimal follow-up of 1 month after transplantation, and none of them was treated with a IL-2 receptor-a blocking antibody. SNP were determined by PCR with allele-specific primers. Differences in allele distributions between rejectors and non-rejectors were statistically tested by the Fisher's Exact test. CD86 +1057 G/A genotype distributions in donors and in recipients were not associated with acute rejection. CTLA-4 +49 G/G genotype was significantly more prevalent among non-rejecting recipients than among rejectors (14% versus 2%; p=0.03). Only 1 out of 13 CTLA-4 +49 G/G recipients (8%) experienced acute rejection(s) compared with 40% of A/A or A/G recipients. Thirteen % of non-rejectors were either CTLA-4 -318 T/T or G/T versus 26% of the rejectors, but this difference did not reach statistical significance (p=0.07). Conclusion: Liver transplant recipients homozygous for CTLA-4 +49 G have a reduced incidence of acute rejection. Since this polymorphism results in an amino acid substitution in the signal peptide of CTLA-4, the association with rejection may be related to differences in intracellar trafficking of the CTLA-4 protein in T-cells.

Abstract# 681

RAPAMYCIN INHIBITS DENDRITIC CELL DEVELOPMENT AND FUNCTION IN VIVO. Holger Hackstein,[1,2,3] Timucin Taner,[1,3] Adrian E. Morelli,[1] Angus W. Thomson.[1] *[1]Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Immunology and Transfusion Medicine, University of Giessen, Giessen, Germany; [3]H.H. and T.T. contributed equally.*

Rapamycin (RAPA) is a potent immunosuppressive macrolide hitherto believed to mediate its action primarily via suppression of lymphocyte responses to IL-2 and other growth factors. Recently, in vitro data from our group and others provided new insight into the immunosuppressive effect of the drug, by indicating that RAPA impairs dendritic cell (DC) activation. By using two independent in vivo models we have further analyzed systematically the effects of RAPA on DC function and DC development. Mice (C57BL/10) were injected for 7-10 days with RAPA (0.5 mg/kg/d) or drug vehicle to study drug effects under steady-state conditions. Additionally, to explore RAPA's effects under dynamic conditions, we expanded DC in vivo by injecting the endogenous growth factor Flt3 Ligand (Flt3L; 10 µg/d; 10d) in combination with either RAPA or vehicle. To investigate RAPA's effect on a per cell basis, we analyzed DC costimulatory molecule upregulation and cytokine production after LPS stimulation (± IL-4). T cell stimulatory capacity of in vivo generated DC (± RAPA) was investigated by performing adoptive transfer experiments into naive, fully allogeneic recipients (C3H). The data show that RAPA significantly decreased (40-50% reduction; $p<0.002$) DC numbers in bone marrow and spleen, both under steady-state and dynamic conditions. These effects are not due to apoptotic cell death as shown by Annexin-V/ 7-AAD staining. In Flt3L-treated animals the inhibitory effect of RAPA on DC expansion was apparent from the appearance and significant reduction in weights and cellularity of the spleen ($p=0.0004$). Independent from its effects on the total DC pool, RAPA significantly suppressed DC costimulatory molecule upregulation (CD54, CD80, CD86), TNF-alpha and IL-4 induced IL-12p70 production and DC endocytic activity (each $p<0.05$). T cells of mice that had been injected with bead-sorted, in vivo-expanded DC from RAPA-treated donors, showed significantly reduced T cell proliferation, IL-2 and IFN-gamma production after restimulation with donor alloantigen (each $p<0.05$). In conclusion, we propose that RAPA effectively suppresses DC development and function in vivo. These novel findings indicate that RAPA interferes with immune responses at the earliest stages and have clinical implications for RAPA-based therapy of transplant rejection, DC-triggered autoimmune diseases and unexpectedly, hematological malignancies with activating Flt3 mutations.

Abstract# 682

INHIBITION OF STAT 4 PHOSPHORYLATION AND IL-18 RECEPTOR EXPRESSION: PATHWAYS OF DC IMPAIRMENT BY RAPAMYCIN. Lianfu Wang, Po-Hui Chiang, Xiaoyan Liang, Ran Tao, John J. Fung, Shiguang Qian, Lina Lu. *[1]Thomas E. Starzl Transplantation, University of Pittsburgh, Pittsburgh, PA.*

Rapamycin (rapa) has a unique mechanism of action distinct from that of the calcineurin inhibitors. The immunosuppressive effect of rapamycin on T cells has been extensively studied, but its influence on antigen presenting cells is less understood. In this study, DC were exposed to rapa during propagation from B10 ($H2^b$) bone marrow (BM) in the presence of GM-CSF and IL-4, and extensively washed at the end of culture. DC surface molecule expression was determined by flow cytometry. DC allostimulatory function were assessed by MLR and CTL *in vitro*, and influence of allograft rejection in vivo. Cytokine profiles were analyzed by ELISA and RNase protection assay. NF-kB activity and Stat4 activation were detected by gel shifting assay and western blotting, respectively. The proliferative responses of C3H ($H2^k$) spleen T cells stimulated by B10 rapa-DC were significantly inhibited, and the antigen-specific CTL activity was suppressed by 90% compared with control. This was associated with reduced production of both Th1 and Th2 cytokines (IFN-g, IL-4, IL-5, IL-10 and IL-13). In contrast to administration of normal DC that accelerated rejection of B10 cardiac allografts (MST 6 days vs 10 days in control, $p<0.05$), rapa-DC significantly prolonged allograft survival (MST 18 day, $p<0.05$ compared with control). Exposure to rapa did not alter DC yield or the expression of MHC, costimulatory molecules and IL-12. DC NF-kB activity was also not suppressed. However, rapa markedly inhibited IL-12 induced Stat4 activation and expression of inducible IL-18 receptor (IL-18Rb) in DC. It has also been shown that Stat4 mediated DC expression of IFN-g and other mediators. IFN-g production in rapa DC was decreased, but addition of exogenous IFN-g did not reverse the low MLR of rapa DC, and DC deficient in IFN-g elicited normal MLR. These data suggest that other factors may mediate rapa inhibition to DC. IL-12 and IL-18 receptors on DC regulates activation by an autocrine activity of IL-12. This may be an additional explanation for the inhibitory activity of rapa. Taken together, these data indicate that rapa does not affect DC maturation, but significantly impairs DC allostimulatory function, probably mediated by inhibition of DC Stat4 phosphorylation and IL-18 receptor expression.

Abstract# 683

RAPAMYCIN-TREATED, ALLOANTIGEN-PULSED RECIPIENT DENDRITIC CELLS INDUCE ANTIGEN-SPECIFIC T CELL HYPORESPONSIVENESS. Timucin Taner,[1] Holger Hackstein,[2] Angus W. Thomson.[1] *[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Institute of Clinical and Transfusion Medicine, Justus-Liebig University, Giessen, Germany.*

Background: Dendritic cells (DC) are uniquely well-equipped professional antigen-presenting cells (APC) regarded as both initiators and regulators of immune responses. Donor-derived and recipient DC both contribute to graft rejection by inducing T cell activation and proliferation via the direct and indirect allorecognition pathways, respectively. Evidence has also accumulated however that DC, particularly in an immature state, can promote tolerance and prolong allograft survival. Rapamycin is well recognized for its potent inhibitory effects on T cell proliferation, but recent evidence indicates that it can also impair DC function. Here we have examined the impact of exposure of DC to rapamycin on their subsequent capacity for alloAg presentation via the indirect pathway.**Methods:** Myeloid DC were propagated from C3H/J ($H\text{-}2K^k$) mouse bone marrow, in the presence or absence of a clinically relevant concentration of rapamycin (5 ng/ml). The capacity of the DC to activate syngeneic T cells following pulsing with alloAg [freeze-thawed donor (C57BL/10; $H\text{-}2K^b$) splenocytes] was assessed by thymidine uptake. *In vivo* priming of T cells was investigated by *ex vivo* anti-donor proliferative responses, following i.v. infusion of highly-purified rapamycin-treated or control alloAg-pulsed syngeneic DC. **Results:** Rapamycin significantly inhibited spontaneous and LPS-induced maturation of DC, as assessed by costimulatory and MHC-II molecule expression. Exposure to rapamycin had no effect on the uptake of fluorescein-labeled lysates. Whereas the alloAg-pulsed control DC effectively stimulated naive syngeneic T cells, those exposed to rapamycin were much inferior stimulators. Compared with mice given purified syngeneic alloAg-pulsed control DC, those given rapamycin-treated DC exhibited marked T cell hyporesponsiveness to *ex vivo* challenge with allogeneic APC or alloAg-pulsed syngeneic APC. The hyporesponsiveness was donor-specific as evidenced by unimpaired reactivity of T cells that have been primed *in vivo* with rapamycin-treated, third party Ag-pulsed DC. **Conclusions:** These results show that when alloAg is presented to T cells by rapamycin-treated DC via the indirect pathway, the outcome is induction of Ag-specific hyporesponsiveness to subsequent challenge through either the direct (i.e. by donor DC) or indirect (i.e. by recipient DC) pathways. They also have novel implications for the use of rapamycin at the level of Ag presentation by DC for targeting of both acute and chronic rejection in a single strategy.

Abstract# 684

SIROLIMUS INHIBITS CELL GROWTH AND THE EXPRESSION OF CYCLIN PROTEINS IN EBV-ASSOCIATED B CELL LYMPHOMAS DERIVED FROM PATIENTS WITH PTLD. Cynthia E. Balatoni,[1] Ronald R. Nepomuceno,[1] Sheri M. Krams,[1] Olivia M. Martinez.[1] *[1]Department of Surgery, Stanford University School of Medicine, Stanford, CA.*

Immunosuppressive drugs are thought to influence the development of EBV-associated post-transplant lymphoproliferative disorder (PTLD) primarily through inhibition of EBV-specific T cells. However, it is also possible that these drugs have direct effects on EBV-infected B cells that could effect tumor development and progression. Along these lines our lab has shown that cyclosporine and tacrolimus promote the survival of EBV-infected B cell lines from patients with PTLD. In contrast, we observed that sirolimus (10 ng/mL) inhibits the proliferation of EBV-infected B cell lines (n=3) by approximately 70%. This reduction in proliferation could be attributed to an arrest in the G1 phase of the cell cycle, because sirolimus treatment caused an increase in the proportion of cells in the G1 phase compared to the proportion of cells in S and G2/M. We hypothesized that this arrest was due to alterations in the expression of cell cycle proteins. In this study, the expression of cell cycle proteins unique to G1 were examined by Western Blot in sirolimus-treated and untreated EBV-infected B cell lines from patients with PTLD. Treatment with sirolimus resulted in a decrease of cyclin D3 protein expression by 58-72% in three EBV+ B cell lines. Sirolimus also reduced cyclin D2 expression in the cell lines by 17-39%, while cyclin E expression was not affected. Since previous studies have shown that IL-10-induced proliferation in normal B cells is associated with increased cyclin D3 and since our lab has determined that EBV-infected B cells depend on IL-10 as an autocrine growth factor, we examined the effect of sirolimus on IL-10 production. Treatment with sirolimus (10 ng/mL) resulted in a dose-dependent inhibition of IL-10 (>75%) in three EBV+ B cell lines. Thus, our data indicates that sirolimus inhibits proliferation of EBV+ B cells from patients with PTLD by attenuating IL-10 production and the associated expression of the cyclin D3 protein. These results provide insight into the growth pathways of EBV+ B cell lymphomas and suggest that sirolimus may be beneficial in inhibiting the development of PTLD.

Abstract# 685

THE SPHINGOSINE-1-PHOSPHATE RECEPTOR AGONIST FTY720 ENHANCES MIGRATORY RESPONSES OF DENDRITIC CELLS TO CCL21 AND PROMOTES THEIR TH2-POLARIZING ACTIVITY. Yuk Y. Lan,[1,2] Patrick T. Coates,[1,3] F. J. Duncan,[1,3] Bridget L. Colvin,[1,3] Volker Brinkmann,[4] Angus W. Thomson.[1,2,3] *[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Immunology, University of Pittsburgh, Pittsburgh, PA; [3]Surgery, University of Pittsburgh, Pittsburgh, PA; [4]Novartis Pharmaceuticals, Basel, Switzerland.*

Introduction: FTY720 is a novel immunosuppressive agent that causes reversible lymphopenia, with sequestration of lymphocytes from the peripheral circulation to lymph nodes and Peyers' patches. As FTY720 is a structural homologue of sphingosine-1-phosphate (S1P), endothelial differentiation gene receptors [EDGR] (the molecular targets of S1P) have been suggested as its putative site of action on lymphocytes. The influence of FTY720 on dendritic cell (DC) function is unknown.We hypothesized that FTY720 might regulate DC trafficking and function and favor their Th2-inducing capacity via EDGR cell signalling. **Methods**: Murine immature bone marrow-derived DC (BMDC) were generated in GM-CSF for 7 days. $CD11c^+$ cells were purified by immunomagnetic bead separation then treated for 90 min with clinically relevant concentrations of the active metabolite FTY720-P (10^{-5}M, 10^{-6}M, and 10^{-7}M) prior to in vitro functional assays. DC were assessed for EDGR expression by RT-PCR and for surface Ag expression by flow cytometry and MLC, respectively. Cytokine secretion was quantified by ELISA and DC chemotactic responses were assessed using transwell plates. **Results**: Using RT-PCR, expression of EDGR1, EDGR3 and EDGR4 was confirmed in BMDC and also identified in renal and splenic DC mobilized in vivo by the hematopoietic growth factor Flt3Ligand. FTY720-P did not alter BMDC functional status, as assessed by flow cytometric analysis of surface costimulatory molecule (CD40, CD80, and CD86) expression and allostimulatory capacity of the DC for naive allogeneic T cells in proliferation assays. However, analysis of the supernatants of MLC for signal cytokines revealed that pre-exposure of BMDC with FTY720-P induced a Th2 shift in a dose-dependent manner. In migration experiments, FTY720-P treated BMDC exhibited enhanced chemotactic response to S1P and the constitutively expressed CC chemokine CCL21 (secondary lymphoid chemokine), but not to the "inflammatory" chemokines CCL20 (MIP-3α) and CCL5 (RANTES). **Conclusions**: These data demonstrate for the first time that murine BMDC express the putative FTY720 target EDGR and that exposure of BMDC to FTY720 promotes migration to S1P and CCL21 without affecting their allostimulatory function. The capacity of FTY720 to affect DC migration may play an important role in its mechanism of action.

Abstract# 686

IDENTIFICATION OF PUTATIVE MEDIATORS OF THE FTY720-INDUCED SIGNALTRANSDUCTION PATHWAY IN HUMAN LYMPHOCYTES. Torsten Boehler,[1] Johannes Waiser,[1] Sebastian Bork,[1] Hans H. Neumayer,[1] Klemens Budde.[1] *[1]Nephrology, Charité Campus Mitte, Humboldt-University, Berlin, Germany.*

BACKGROUND: FTY720(FTY) is the first member of a new class of therapeutics to prevent allograft rejection. In contrast to calcineurin-, TOR- and IMPDH-inhibitors, FTY does not inhibit lymphocyte proliferation. FTY exerts its immune-modulating activity by altered lymphocyte trafficking leading to peripheral lymphopenia. Recently G-protein coupled EDG-receptors were identified as a molecular target of FTY, however very little is known about the signal transduction pathway of FTY in lymphocytes. METHODS: In order to identify mediators of the FTY signaling cascade a proteomic approach (Becton Dickinson Powerblot) was used. Peripheral human lymphocytes, treated for 12 hours with 0.1µM FTY, were simultaneously analyzed for the differential expression of 622 cellular signal transduction proteins. All experiments were performed in triplicates. RESULTS: Out of 504 proteins detected in lymphocytes we identified 7 differential expressed proteins (FTY versus control) linked to cytoskeletal organisation: 1. Cofilin, a G- and F-actin binding protein was 5.2±1.9 decreased. 2. The expression of Moesin, a regulator of the interaction between cytoskeletal and membrane proteins was 1.4±0.1-fold increased. 3. We detected a 2.0±0.5-fold increase of KAP3A, an accessory protein for the microtubule translocator kinesin. 4. The expression level of MAP2B, a protein involved in assembly of microtubules was 2.3±0.7-fold elevated. 5. EPLIN-107, a protein associated with cytoskeletal structures, such as stress fibers and focal adhesions was 1.5±0.4-fold reduced. 6. PTEN, a phosphatase interacting with PI3K/AKT signal transduction and cytoskeletal dynamics was 2.3±0.3-fold increased. 7. Rho, an important regulator which orchestrates the reorganization and formation of the actin cytoskeleton was 3.7±1.4-fold increased. In addition we detected changed expression levels of proteins associated with cell adhesion: The expression of ILK-53, an integrin linked kinase, localized to focal adhesion plaques and interacting with the cytoplasmatic domains of integrin b1 and b3 was +1,9±0,7-fold increased. Interestingly FTY increased 2.3±0.6-fold integrin beta 1but decreased 3.1±0.4-fold integrin beta 3. CONCLUSION: Using a proteomic approach, we identified putative mediators of the FTY signal transduction pathway in human lymphocytes. The data suggest that FTY interacts with integrins and the organisation of the cytoskeleton. These features of FTY720 might play an important role in FTY-altered lymphocyte motility.

Abstract# 687

THE BLOOD OF "OPERATIONALLY TOLERANT" RECIPIENTS OF KIDNEY ALLOGRAFTS IS CHARACTERIZED BY AN INCREASE IN $CD4^+CD25^+$ T CELLS. Stephanie Louis,[1] Sophie Brouard,[1] Magali Giral,[1] Alexandre Dupont,[1] Jean-Paul Soulillou.[1] *[1]ITERT INSERM U437, CHU Hotel-Dieu, Nantes, France.*

Graft survival depends on a strong and long-term immunosuppression. Nevertheless, some patients preserve their graft despite immunosuppression withdrawal. Recently, we showed that the blood of these patients had an abnormal TCR Vβ transcriptome, combining strongly altered CDR3-LD, high Vβ/HPRT transcript ratios and low cytokine Th1/Th2 accumulation, suggesting an active process of peripheral T cell modulation. In this study, we analyzed the phenotype of blood lymphocytes in a small cohort of these subjects in comparison with kidney recipients in other clinical contexts. **Methods**: Recipients with a functional graft without an immunosuppression (drug-free DF, n=5) or under low doses of steroid monotherapy (< 10mg)(LD, n=11) for more than 3 years were referred to as "operationally tolerant". Normal individuals (NL, n=6), patients with chronic rejection (CR, n=8) or a stable graft function under immunosuppression (Sta, n=9) were also studied. The phenotype of mononuclear blood cells was analyzed in detail via four-color flow cytometry. **Results**: Whereas the majority of the cell populations studied (NK, HLA DR^+, $CD28^-CD8^+$ and Va $24^+CD8^-CD4^-$ T cells) showed no difference between the groups, DF patients presented a significant increase in $CD4^+CD25^+$ T cells (195 cells/µl of blood ± 76 vs 86 ± 39 for CR, $p<0.05$) as well as the B cell population (261 cells/µl of blood ± 121 vs 50 ± 61 for Sta, $p<0.05$). LD patients, also considered as "operationally tolerant" on the basis of their clinical history and Vb transcriptional pattern, showed, like DF recipients, an increase in their $CD4^+CD25^+$ T cell/T cell ratio (0.108 ± 0.051 for LD and 0.146 ± 0.066 for DF vs 0.063 ± 0.031 for CR, $p<0.05$). However LD patients had less TCR$\alpha\beta^+$ cells (memory $CD45RA^-CD62L^-CD4^+$ as well as naive $CD45RA^+CD62L^+CD4^+$ T cells) than DF recipients ($p<0.05$). **Conclusions**: "Operationally tolerant" patients (ie DF and LD patients) exhibited an increase in $CD4^+CD25^+$ T cells, described in the literature as having regulatory properties, and suggesting peripheral T cell regulation in sorted CDR3-LD altered families in these patients. This hypothesis is supported by the absence of Th1/Th2 cytokine accumulation, the increase in IL2Rα chain receptor transcript levels and the selected TCR bias in these patients. The differences between DF and the LD recipients suggest an effect of long-term steroid monotherapy (even at low doses) on blood phenotype which may interfere with the mechanisms of tolerance maintenance.

Abstract# 688

COMPARISON OF THE EFFECTS OF IMMUNOSUPPRESSIVE AGENTS CYCLOSPORIN A AND FK506 ON ENDOTHELIAL STRUCTURAL DYSFUNCTION. Chumpon Wilasrusmee,[1] Gaurang Shah,[1] David Bruch,[1] Dilip S. Kittur.[1] *[1]Surgery, SUNY Upstate Medical University, Syracuse, NY.*

INTRODUCTION: Previously in a novel model for transplant endothelialitis we have demonstrated that Cyclosporin A (CyA) profoundly affects the morphological function of the endothelial cells while FK506, another calcineurin inhibitor, has no such effect. To discern the mechanisms by which CyA and FK506 exerts these effects on microvascular capillaries, we studied the release of prostacyclin (PGI_2) and endothelin-1 and the expression kinetics of genes that are involved in the growth and differentiation of vascular ECs. **METHODS:** TGF-b 1 and pre-pro Endothelin-1 (ppET-1) was determined by RT-PCR assays in RNA isolated from three sets of human aortic endothelial cells(HAECs): 1) HAECs that formed capillary-tube like structures on Matrigel at 24 hours after culture, 2) HAECs that were incubated with CyA or FK506 at the onset of culture on Matrigel for 24 hour, and 3) HAECs capillary tubes that were incubated with CyA or FK506 for 24 hours. **RESULTS:** TGF-b 1mRNA was expressed in HAECs before, during and after capillary tube formation. Complete inhibition of TGF-b 1 gene expression was noted in HAECs when CyA induced inhibition of capillary tube formation and induced disruption of mature capillary tubes. ppET-1 was expressed in resting HAECs in culture and during capillary tube formation but disappeared from HAECs once capillary tubes matured (24 hrs after the onset of experiment). In CyA treated HAECs, however, ppET-1 expression was significantly induced suggesting that this induction was a factor in the disruption of the capillary tubes by CyA. Incubation of HAECs with FK506 did not change the pattern of ppET-1 expression. PGI_2 release was significantly increased in both CyA and FK506 treated group while ET-1 released was significantly increased only in CyA treated group ($p < 0.05$ with ANOVA and Bonferroni test). **CONCLUSION:** Although FK506 has similar immunosuppressive properties as CyA, it does not cause endothelial dysfunction which was demonstrated by both *in vitro* endothelial capillary tube assay and in a *in vivo* subcutaneous Matrigel plug model for angiogenesis (unpublished results). The gene expression of ET-1 provides a clue to this difference since CyA influence the gene expression of ET-1 to a greater extent than FK506. Similar difference was also noted when ET-1 release was measured. This difference in the effect of the two immunosuppressive agents has implication for long term graft survival.

Abstract# 689

DONOR PRETREATMENT WITH CARBON MONOXIDE-INHALATION INHIBITS iNOS OVEREXPRESSION AND ATTENUATES ISCHEMIA/REPERFUSION INJURY AFTER RAT LIVER TRANSPLANTATION. Takashi Kaizu,[1] Nakao Atsunori,[1] Brian T. Bucher,[1] Leo E. Otterbein,[2] Fang Liu,[2] David A. Geller,[1] Noriko Murase.[1] *[1]Surgery, Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA.*

Stress response genes, such as heme oxygenase-1 (HO-1) or inducible nitric oxide synthase (iNOS), are known to be involved in ischemia/reperfusion (I/R) injury and have benefical effects. Carbon monoxide (CO), the byproduct of heme degradation by HO-1, has also been shown to provide protection against oxidative stress, similar to that seen with HO-1. This study was designed to examine whether donor pretreatment with CO impacts subsequent hepatic cold I/R injury and induction of iNOS. **Methods:** Orthotopic syngeneic LEW rat liver transplantation (OLT) was performed after 18 hrs preservation in cold UW. Donor rats were exposed to a non-toxic dose of CO (250 ppm) in a CO chamber for 24 hrs before harvesting. Air gas was used as negative control. Rats were sacrificed 3-24 hrs post-transplant and severity of I/R injury was evaluated by serum AST levels and histopathology. Expression of HO-1, iNOS, and heat shock protein 70 (HSP70) were analyzed by Western blots in the donor liver after CO treatment and prior to OLT. After OLT, iNOS and HO-1 protein levels, and caspase-3 activity were measured. **Results:** CO-inhalation for 24 hrs markedly increased HSP70, but not HO-1 or iNOS expression in the donor liver. After OLT, CO pretreatment markedly inhibited hepatic iNOS expression, and significantly improved serum AST levels and tissue necrosis, compared with air-treated control. Caspase-3 activity was significantly increased above baseline in control liver grafts 3 hrs after OLT, and this was significantly abrogated by donor CO-inhalation. (Table, mean±SE, n=2-6 animals per group, $*P < 0.05$) There was no difference in HO-1 expression after OLT between CO- and air-treated liver grafts. **Conclusion:** These results demonstrate that exogenous CO pretreatment leads to inhibition of iNOS overexpression and attenuates hepatic cold I/R injury. The protective effect of donor CO pretreatment is possibly mediated by HSP70 induction and down-regulation of caspase-3 activity and appears independent from endogenous HO-1 expression. Thus, low-dose CO inhalation would be a novel therapeutic strategy to combat hepatic cold I/R injury.

	Caspase-3 activity (ΔF/min/μg protein)	iNOS expression (band density)		AST (IU/L)			
Group	3h	3h	6h	24h	6h	24h	48h
Air control	3.6±0.3	3449±2293	7127±83	1070±596	3317±578	5547±979	1992±467
CO (250 ppm)	2.5±0.1*	982±456*	3490±2248*	140±9*	1213±100	1981±381*	820±313

Lung Transplantation: Toward Improved Outcomes

Abstract# 690

P-GP ACTIVITY IS DOWN-MODULATED IN CD4+ BUT NOT CD8+ LUNG ALLOGRAFT INFILTRATING T CELLS DURING ACUTE CELLULAR REJECTION. Vera S. Donnenberg,[1] Gilbert J. Burckart,[1] Adriana Zeevi,[1] Bartley P. Griffith,[2] Aldo Iacono,[3] Kenneth R. McCurry,[1] John W. Wilson,[4] Albert D. Donnenberg.[3] *[1]Surgery, University of Pittsburgh, Pittsburgh, PA; [2]Surgery, University of Maryland, Baltimore, MD; [3]Medicine, University of Pittsburgh, Pittsburgh, PA; [4]Biostatistics, University of Pittsburgh, Pittsburgh, PA.*

Many immunosuppressive drugs are P-gp substrates. In lung transplantation the upregulation of P-gp activity in graft infiltrating T cells may directly decrease intracellular concentrations of these agents. Our objective was to assess P-gp activity in T cells from the lung and peripheral blood. We measured P-gp activity in CD4 and CD8 T-cells from bronchoalveolar lavage (BAL) of 5 healthy volunteers and 27 lung allograft recipients with and without a history of acute cellular rejection (ACR, as indicated by a lung biopsy of ≥2+) on 88 occasions over 2.5 years. Activity was assessed in the absence or presence of the P-gp inhibitor verapamil (50uM). Cells were stained for expression of CD3, CD4, CD8 and CD14. For basal (in vivo) activity, cells were loaded (15 min) with the fluorescent substrate Rhodamine 123 (R123). For in vitro activity, cells were returned to culture and R123 efflux measured at 3 hrs. The effect of inhibitor was calculated as the difference in R123 fluorescence intensity between paired cultures with and without inhibitor. Transplant patients had significant CD8 skewing, whereas control subjects had CD4/CD8 ratios similar to peripheral blood. In healthy subjects, basal P-gp activity was greatly upregulated in both CD4 (35% P-gp active) and CD8 (63%) lung T cells, as compared to peripheral T cells (<10% for CD4 and CD8). Basal P-gp activity was also elevated in BAL T cells from transplant recipients, compared to their peripheral blood T cells, but was lower than that observed in control BAL. During ACR, we found no evidence that P-gp activity is increased in BAL T cells. CD4+ BAL T cells had a reduction in basal and in vitro induced P-gp activity in ACR patients. P-gp activity in CD8+ BAL T cells was similar in patients with and without ACR. Our observations indicate that normal and allograft lung T cells have increased in vivo P-gp activity, and therefore should eliminate P-gp substrate drugs rapidly. Although this may result in resistance to T-cell directed therapy, P-gp is activity is actually reduced by the time the resulting pathology of ACR becomes apparent. The data argue against the hypothesis that exposure to drug substrates selects for drug resistant BAL T cells with high P-gp activity, and support the interpretation that ACR or its treatment, actually depresses P-gp activity in CD4+, but not CD8+, T-cells locally and systemically.

Abstract# 691

A PROSPECTIVE STUDY OF THE CLINICAL IMPACT OF COMMUNITY ACQUIRED RESPIRATORY VIRUS INFECTIONS IN LUNG TRANSPLANT RECIPIENTS. Deepali Kumar,[1] Shaf Keshavjee,[1] Genevieve Miyata,[1] Lianne Singer,[1] Atul Humar.[1] *[1]Multi-Organ Transplant Program, University of Toronto, Toronto, ON, Canada.*

Background Community acquired viral respiratory tract infections (RTI) have been proposed to have significant clinical impact in lung transplant recipients including sustained detrimental effects on lung function. However, this has not been assessed in a prospective study. Methods: This was a prospective case-control study in lung transplant patients. Cases were identified based on symptoms compatible with a community acquired RTI. Asymptomatic controls were selected from the lung transplant population and were matched for time from transplant. Controls were selected during summer months and cases were accrued year-round. All patients had nasopharyngeal and throat swabs for respiratory virus antigen detection and culture. All patients had pulmonary function tests at regular intervals for 3 months from enrolment. Rates of rejection, drop in FEV-1, and bacterial and fungal superinfection over a 3 month follow-up period were compared in the two groups. Results: 31 lung transplant patients with RTI infection were identified and compared to 31 controls. A microbial etiology was identified in 8/31 (26%) patients and included RSV (n=3), influenza (n=2), and parainfluenza (n=3). All patients presented with upper RTI symptoms and 2/31 (6.5%) progressed to lower tract infection. An adverse clinical event (rejection, bacterial or fungal superinfection) during the 3 month follow-up period occurred in 9/31 cases vs. 0/31 controls; p=0.002. Clinically treated or biopsy proven acute rejection (grade 2 or higher) occurred in 7/31 cases vs. 0/31 controls; p=0.01. The number of patients experiencing a 20% or more decline in FEV-1 by 3 months was 9/31 (29%) cases vs. 0/31 controls (0%); p=0.002. The mean percent change in FEV-1 over 3 months was negative 8% for cases vs. positive 3% for controls (p=0.01). Conclusions: Community acquired respiratory viruses have significant clinical impact on lung transplant recipients. They are associated with the development of rejection and can result in sustained drops in lung function.

Abstract# 692

PALIVIZUMAB, AN RSV-SPECIFIC MONOCLONAL ANTIBODY, IN COMBINATION WITH INTRAVENOUS IMMUNOGLOBULIN AND INHALED RIBAVIRIN DECREASES THE ACUTE AND CHRONIC MORBIDITY AND MORTALITY ASSOCIATED WITH RESPIRATORY SYNCYTIAL VIRUS PNEUMONIA IN LUNG TRANSPLANT RECIPIENTS. Martin R. Zamora,[1] David Cassatt,[2] Todd Grazia,[1] Tony Hodges,[1] David Weill,[1] Mark Nicolls.[1] *[1]Pulmonary Sciences and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, CO; [2]MedImmune, Inc., Gaithersburg, MD.*

Background: RSV causes significant morbidity in lung transplant (LTx) recipients. In addition to its acute effects, RSV has been associated with the development of bronchiolitis obliterans syndrome (BOS) or chronic rejection. Palivizumab is approved for the prevention of RSV in high-risk infants and has been used for therapy of RSV pneumonia in bone marrow transplant recipients. Our purpose was to describe the pharmacokinetics and activity of palivizumab as part of combination therapy for RSV pneumonia in LTx recipients. **Methods:** Patients with RSV pneumonia confirmed by bronchoalveolar lavage (GpA, n=18) received a single dose of palivizumab 15mg/kg IV, inhaled ribavirin 2g tid x 5 days, Solumedrol 10mg/kg/d x 3, and IVIG 500mg/kg x 1. Pharmacokinetic activity was determined in 10 patients. Clinical outcomes for all 18 were compared (Fisher's Exact) to a historical LTx cohort treated with steroids±inhaled ribavirin and those receiving GpA therapy more than 2 weeks after symptom onset (GpB n=9). **Results:** Mean peak serum concentration of palivizumab was 356mg/ml, with a mean half-life of 19.3 days and an AUC of 3929mg*day/ml. At 30 days, 60% of the patients had serum concentrations > 40mg/ml; this serum level was found to provide > 99% reduction in RSV titers in pulmonary tissue in infected cotton rats. Follow-up nasal washes demonstrated viral clearance in 17/18 patients by day 5 of therapy. All 18 GpA patients survived their RSV pneumonia versus 6/9 in GpB ($p<0.03$). No GpA patient required mechanical ventilation vs 3/9 in GpB. Mean FEV1 fell by 19.6±12.4% acutely and returned to 99.2±10.5% of baseline 30 days post-therapy in the GpA patients. 5 episodes of acute rejection occurred within 90 days in GpA patients (0.28 episodes/pt) versus 18 in GpB (2 episodes/pt) ($p<0.05$). All surviving GpB patients (n=6/6) developed BOS by 142±105 days versus three (16.5%) GpA patients by 183±42 days ($p<0.07$) with 281 days of follow-up. In addition, all GpA patients tolerated palivizumab infusion with no untoward effects observed. **Conclusion:** These results suggest that combination therapy with palivizumab, IVIG, inhaled ribavirin, and corticosteroids is safe, is well tolerated, and decreases the acute and chronic morbidity associated with RSV pneumonia in lung transplant recipients.

Abstract# 693
WITHDRAWN

Abstract# 694
LONG-TERM FUNCTIONAL OUTCOME AFTER LIVING-DONOR LUNG TRANSPLANTATION IN ADULTS. Michael E. Bowdish,[1] Felicia A. Schenkel,[1] Richard G. Barbers,[1] Craig J. Baker,[1] Vaughn A. Starnes,[1] Mark L. Barr.[1] *[1]University of Southern California, Los Angeles, CA.*

Objective: Living-donor lobar lung transplantation was developed as a procedure for patients considered too ill to await cadaveric transplantation. We compared the functional outcomes in long-term survivors (>90 days) of adult-to-adult living-donor lobar transplantation to those undergoing bilateral cadaveric lung transplantation. **Methods:** A single-center cohort analysis of the functional outcome of 62 live-donor lobar and 46 cadaveric lung transplant recipients who survived >90 days after surgery was performed. Mean follow-up was 3.3 ± 3.2 years. **Results:** There was no difference in conditional 1, 3, and 5-year actuarial survival in this cohort (83, 79, and 73% in cadaveric recipients; 82, 65, and 62% in live-donor recipients; p=0.56). Survival among all live-donor and cadaveric recipients (n=131) was lower in the live-donor group (p=0.03), likely due to the increased severity of illness at the time of transplantation. Among long-term survivors, there was no difference in the incidence of acute rejection, bronchial stenosis, bronchiolitis obliterans syndrome, or rates or types of infection. After one month, mean percent predicted FVC_1, FEV_1, and FEF_{25-75} were comparable and remained so throughout the follow-up period. Maximal exercise, heart rate, peak VO_2, anaerobic VO_2 threshold, and ability to maintain oxygen saturation were also comparable. **Conclusion:** Concerns about the ability of adult-to-adult living-lobar transplantation to provide adequate pulmonary function are unwarranted. In those patients surviving >90 days after transplantation, living-donor lobar transplantation provides comparable pulmonary function and exercise capacity to bilateral cadaveric lung transplant recipients. Given differences in philosophical and ethical acceptance of live organ donors, these results are important if this procedure is to be considered an option at more pulmonary transplant centers.

Abstract# 695
IMPACT OF LUNG TRANSPLANT OPERATION ON BRONCHIOLITIS OBLITERANS SYNDROME (BOS) IN PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS (IPF). Denis Hadjiliadis,[1] Cecilia Chaparro,[1] Robert H. Messier,[2] Carlos Gutierrez,[1] Tom K. Waddell,[1] Mark P. Steele,[2] Lianne G. Singer,[1] Robert D. Davis,[2] Michael A. Hutcheon,[1] Scott M. Palmer,[2] Shaf Keshavjee.[1] *[1]Toronto Lung Program, University of Toronto, Toronto, ON, Canada; [2]Duke Lung Transplant Program, Duke University, Durham, NC.*

Purpose: The optimal transplant operation for patients with IPF remains unknown. Previous studies of patients with emphysema suggest that bilateral lung transplantation results in lower rates of BOS and improved long-term survival. Methods: All lung transplant recipients with a pre-transplant diagnosis of IPF (not related to connective tissue disease) at the University of Toronto (n=69) and at Duke University (n=36) were retrospectively reviewed. BOS analysis was performed in recipients surviving longer than six months after their transplant operation (Toronto: n=52; Duke: n=26). Data collected were age, gender, date and type of transplant, survival, presence and time of BOS. BOS was defined as a 20% or more drop in FEV1 from the post-transplant baseline. The freedom from BOS and survival curves were evaluated by the Kaplan-Meier method and they were compared by the log-rank test. Results: 78 (bilateral n=35, single n=43) patients met our criteria. Freedom from BOS was similar at 1, 2 and 3 years post-transplant (bilateral vs. single: 1-year, 91.2% vs. 87.8%; 2-years, 65.5% vs. 73.1%; 3-years 60.8% vs. 69.7%; p>0.2). Survival of bilateral (n=49) and single (n=56) lung transplant recipients was similar: 79.6% vs. 87.5% at 1-month , 67.4% vs. 62.2% at 1-year, 51.6% vs. 54.0% at 3-years and 38.5% vs. 51.1% at 5-years (p>0.2). Age, gender and transplant center had no effect on rates of BOS. Conclusion: Bilateral and single lung transplantation result in similar rates of BOS in patients with IPF. In contrast to patients with emphysema, patients with IPF receiving bilateral lung transplants have no survival advantage over single lung transplant recipients with IPF. Further studies are needed to address the reason for the differences between patients with IPF and emphysema.

Abstract# 696
IMPACT OF PROSTANOID THERAPY AND TRANSPLANTATION ON THE OUTCOME OF PATIENTS WITH PRIMARY PULMONARY HYPERTENSION. Michael Dandel,[1] Dagmar Kemper,[1] Yuguo Weng,[1] Sead Mulahasanovic,[1] Hans B. Lehmkuhl,[1] Manfred Hummel,[1] Roland Hetzer.[1] *[1]Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, Berlin, Germany.*

Background: Without treatment, patients with primary pulmonary hypertension (PPH) survive on average less than 3 years after diagnosis. We assessed the outcome of PPH patients referred by our institution for tranplantation in order to evaluate the survival benefits of both transplantation and pretransplant prostanoid therapy. **Methods:** The analysis included all PPH patients (60 women, 28 men) referred by our institution for transplantation between January 1991 and June 2002. Transplantation has now been performed in 39 of these patients (23 heart-lung and 16 double-lung transplantations). Since 1996 we have used a stepwise therapeutic regimen which includes beraprost tablets or iloprost inhalation in the earlier stages of the disease and intravenous iloprost infusion in NYHA class IV patients. **Results:** Only 14 patients underwent neither prostanoid therapy (for various reasons) nor transplantation (8 died before transplantation, 6 are still on the waiting list). The median survival after diagnosis in this group was only 22 ± 15 months. The other 74 patients were treated with prostanoids or with transplantation or with both. The median survival after PPH diagnosis in this group was 73.5 ± 25.3 months, with a survival rate of 76.2% at 3 years. The 54 patients treated with prostanoids (19 were transplanted, 35 not or not yet transplanted) had significant higher survival rates ($p < 0.01$) since diagnosis of PPH than the 34 patients not treated with prostanoids (20 of these were transplanted, 14 not or not yet transplanted). Thus, the 3-year actuarial survival after diagnosis was 81.0 % in patients treated with prostanoids and 59.2% in those without prostanoids. The mortality rate on the waiting list for transplantation was 14.8% in the prostanoid group and 26.6% in the group without prostanoid treatment ($p<0.01$). The actuarial post-transplant survival was 50.0 ± 9% at 5 years. At the present time, 27 patients are being treated with prostanoids. Listing for transplantation could be postponed during this treatment (mean duration 25.2 ±12.6 months) in 26 patients, although the mean time since diagnosis reached alereadу 58.0 ± 54.4 months. **Conclusions:** Since use of prostanoids increases the 3-year actuarial survival after PPH diagnosis and reduces the mortality rate on the waiting list, it is an effective bridging-to-transplant therapy. Transplantation in combination with prior prostanoid administration provides important survival benefits for PPH patients.

Abstract# 697
SINGLE CELL DETECTION OF IFN-g PRODUCTION IDENTIFIES RENAL AND LUNG TRANSPLANT RECIPIENTS AT HIGH RISK FOR EARLY IMMUNE COMPLICATIONS. Karen Mohler,[1] Angela Burnette,[1] Kay Savik,[3] Marshall Hertz,[3] Arthur Matas,[3] Mark Steele,[2] Scott Palmer,[2] Robert D. Davis,[2] Nancy L. Reinsmoen.[1] *[1]Pathology, Duke University Medical Center, Durham, NC; [2]Medicine, Duke University Medical Center, Durham, NC; [3]Medical Center, University of Minnesota, Minneapolis, MN.*

Alloreactive T cells are primary mediators of acute rejection (AR) and are also thought to play a role in brochiolitis obliterans syndrome (BOS) in lung transplant recipients (recips), and biopsy proven chronic rejection (CR) in kidney recips. The Enzyme-Linked Immunosorbent Spot Assay (ELISpot) has been used to measure cytokine-producing memory T cells that are presumably generated by exposure to environmental antigens, which cross-react with donor histocompatibility antigens. The aim of our study was to determine if the pretransplant frequency of donor specific IFN-g producing T cells identified recipients at high risk for acute rejection and subsequent CR/BOS. We tested peripheral blood mononuclear cells (PMBC) from 15 lung and 24 kidney recipients, transplanted at our two centers, for IFN-g producing cells by incubating the recipient cells for 18 hours with CD3 depleted donor or third party PBMC expressing donor HLA-DR antigens. The IFN-g produced by single cells was detected by a secondary enzyme-linked antibody and spots were counted using an automated computer-assisted image analyzer. The overall responses of the lung recips were much higher than that observed for kidney recips. We attribute this difference to increased exposure to environmental antigens in lung recips due to their underlying disease. The results were analyzed by dividing the frequencies per 3 x 10^5 cells into groups of low (Lung: <300 spots/, Kidney: <40 spots), intermediate (Lung: 300 – 600 spots, Kidney: 40 – 60 spots) and high responders (Lung: >600 spots, Kidney: >60 spots). Of the 21 recips with early AR or BOS (< 6 mo. posttransplant, and one lung recip, who had BOS 3 at 855 days), 16 were intermediate or high responders as determined by the ELISpot IFN-g response. Twelve of the 18 recips with no early AR or BOS, were low responders (chi-square=8.4, p=0.015). Thus, identification of recipients with preexisting intermediate or high frequencies of donor specific memory T cells appears to identify those at high risk for early immune complications and may allow clinicians to guide the use of appropriate immunosuppressive therapy and targeted interventions aimed at improving long-term graft survival.

Abstract# 698

VALGANCICLOVIR FOR CMV PROPHYLAXIS AFTER LUNG TRANSPLANTATION: INTERIM RESULTS. Lisa M. Bolin,[1] Jordan Dunitz,[1] S. Park,[1] S. K. Savik,[1] Marshall I. Hertz.[1] *[1]University of Minnesota and Fairview-University Medical Center, Minneapolis, MN.*

Previous studies have shown that oral and intravenous (IV) ganciclovir are effective in reducing the incidence and severity of CMV disease after lung transplantation (LT). In 10/01, our hospital instituted a protocol using oral valganciclovir in place of ganciclovir for CMV prophylaxis due to its superior oral abosrption. We present here the interim results comparing oral valganciclovir with historical comparison groups of daily oral and three-times-a-week (TIW) IV ganciclovir. **Methods:** Recipients with D+ and/or R+ CMV status transplanted between 10/01 and 07/02 were included. Patients who did not survive to 90d post-LT and those who did not leave the ICU were excluded. Patients received valganciclovir from days 8-90 (900mg/d, adjusted for WBC). Primary endpoints were CMV antigenemia, CMV shedding (+CMV culture on surveillance BAL), and CMV disease (signs/symptoms of systemic illness or pneumonitis, +CMV culture, and no alternative diagnosis). **Results:** 22 patients have been treated with oral valganciclovir (10F, 12M; average age 49.2 ± 11.6 yr). All are >90d from transplant; mean time from transplant is 9.2 ± 2.4 mos.

			Comparative Data				
	Valgan-ciclovir	**Valgan-ciclovir**	**PO Ganciclovir**	**PO Ganciclovir**	**TIW IV Gan**	**TIW IV Gan**	
	90 days	180 days	90 days	180 days	90 days	180 days	p-value
CMV shedding	0	19%	6%	40%	4%	43%	.04
CMV Ag	0	33%	2%	26%	ND	ND	.92
CMV disease	0	5%	0	18%	3%	25%	.03
Survival	100%	95%	100%	98%	92%	81%	.06
BOS grade 2	NA	0	NA	3%	NA	3%	.51

percentages based on time to event using Kaplan-Meier method

There was significantly less CMV disease and CMV shedding in the valganciclovir group compared with the ganciclovir groups. After stopping the valganciclovir, only one patient was diagnosed with CMV disease; symptoms improved with valganciclovir therapy. **Conclusions:** In this small study population, oral valganciclovir suppressed CMV shedding and disease during treatment significantly more than either oral or IV (TIW) ganciclovir. After stopping valganciclovir, there was a lower incidence of CMV disease than was seen after ganciclovir treatment. This project was funded in part by NIH Training Grant #T32 HL07741.

Abstract# 699

TOLEROGENIC PROTOCOL IN HUMAN LUNG TRANSPLANTATION. Kenneth R. McCurry,[1] Adrianna Zeevi,[1] Diana B. Zaldonis,[1] Alin Girnita,[1] Aldo T. Iacono,[2] Thomas E. Starzl.[1] *[1]Department of Surgery, University of Pittsburgh, Pittsburgh, PA; [2]Division of Pulmonary Medicine, University of Pittsburgh, Pittsburgh, PA.*

Between 6/02 and 10/02, we performed 16 cadaveric lung transplants under an immunosuppressive protocol that is postulated to facilitate clonal exhaustion-deletion and acquired tolerance. The therapeutic principles are: 1) pretransplant recipient T-cell depletion (3-5 mg/kg Thymoglobulin^{R} IV prior to allograft revascularization), and 2) minimal postoperative immunosuppression (tacrolimus (tac) with a trough target of 10-15 ng/ml) and 5mg/day prednisone (pred). One patient died of multisystem organ failure (MOF) with associated aspergillus infection (58 days). The remaining 15 are alive after 29-155 days, 7 without additional immunosuppression. In the other 8, symptomatic Grade 2 (1 patient) or Grade 3 (7 patients) rejection developed. These were reversed in 5 patients with steroid boluses or a steroid bolus and pred taper, before resuming baseline therapy. In the other 3, rejection control was accomplished with 1-3 doses of 30 mg campath-1H and (in 2) an increase in baseline pred to 20mg/day. Two of the 3 campath-treated patients had donor-specific HLA antibodies (1 de novo class I and the other preexisting class II). Nine patients are more than 2 months posttransplant. In 5, CD3 counts have returned to normal. Eight of 9 are on tac (trough 11.1±1.3 ng/ml) and 5 mg/day pred while one is on tac (10.9 ng/ml) and 20 mg pred. There have been no CMV, EBV, or other virus-associated infections. One patient developed a bacterial pneumonia and 2 have had positive BAL culture without symptoms. The 15 survivors have good allograft function. Retrospective review of the 15 recipients transplanted prior to initiating this protocol revealed: 13 received daclizumab induction and 2 received posttransplant Thymoglobulin^{R} while all received tac, perioperative solumedrol taper followed by pred 20 mg/day and azathioprine or MMF (unless infectious issues dictated otherwise). One patient died of MOF (day 18). In the first 3 months posttransplant, there were 7 pulmonary infections and 1 staph empyema and 8/15 required augmented immunosuppression for rejection. At 2 months, 12 of 15 were on triple immunotherapy. **CONCLUSION**: Adherence to the principles of tolerogenic immunosuppression can lead to good early outcomes following lung transplantation. The results are consistent with the hypothesis that prior lymphoid depletion and minimal post-transplant immunosuppression permits the evolution of variable donor specific nonreactivity. This is currently being evaluated with an eye to weaning of immunosuppression in selected patients.

Abstract# 700

IMPACT OF THE MDR-1 C3435T POLYMORPHISM ON TACROLIMUS DOSES, TROUGH CONCENTRATIONS AND DELAYED GRAFT FUNCTION IN RENAL TRANSPLANT RECIPIENTS. Dany Anglicheau,[1,2] Celine Verstruyft,[3] Marie H. Schlageter,[4] Laurent Becquemont,[3] Philippe Beaune,[1] Christophe Legendre,[2] Eric S. Thervet.[1,2] *[1]Unite INSERM U490, Universite des Saints-Peres, Paris, France; [2]Service de Nephrologie et Transplantation, Hopital Saint-Louis, Paris, France; [3]Service de Pharmacologie, Hopital Saint Antoine, Paris, France; [4]Service de Medecine Nucleaire, Hopital Saint-Louis, Paris, France.*

Introduction Tacrolimus, characterized by large inter-individual pharmacokinetic variations, is a substrate for P-glycoprotein (P-gp). P-gp is the product of the multidrug-resistance (MDR-1) gene. A genetic polymorphism (C3435T) of MDR-1, correlated with intestinal expression and in vivo activity of P-gp, has been recently described. We postulated that this polymorphism is associated with pharmacokinetic characteristics of tacrolimus in renal transplant recipients. Methods Using the TaqMan technology, we genotyped for the MDR-1 C3435T polymorphism, 75 renal transplant recipients treated by tacrolimus. One month (M1) after tacrolimus initiation, we correlated, MDR-1 genotype with tacrolimus daily dose and the ratio of tacrolimus trough concentration (mg/l) to the corresponding 24-h dose (mg/kg) (C0aj). COaj reflects the dose of tacrolimus needed to achieve target blood levels. We also analyzed the intensity of delayed graft function (DGF) defined as the delay between transplantation and the first spontaneous creatinine decrease (days). Results The MDR-1 wild-type genotype (CC) was observed in 26 patients, whereas 33 patients were heterozygous (CT) and 16 were homozygous for the mutation (TT). At M1, the median daily dose of tacrolimus was significantly higher in CC patients compared with CT and TT patients (0.192 mg/kg/d, 0.154 mg/kg/d, and 0.132 mg/kg/d respectively ; CC vs CT, p=0.03; CC vs TT, p=0.03; CT vs TT, p=ns). The values of median C0aj were 61.2, 77.4 and 92.6 for CC, CT, and TT genotypes respectively (CC vs CT, p=0.058; CC vs TT, p=0.02; CT vs TT, p=ns). Tacrolimus was started on the first day after transplantation in 42 patients. In this population, mean delay for spontaneous creatinine decrease was 3.5 days. This delay was 1.6, 3.4, and 7.8 days for CC, CT and TT genotypes respectively. Conclusion Genotype monitoring of MDR-1 gene reliably predicts the optimal dose of tacrolimus in transplant recipients and the intensity of DGF. Patients with no mutation (CC) are more likely to extrude tacrolimus from intestinal cell and a higher daily dose is needed to achieve adequate blood levels. This genotype characterization can be easily performed before transplantation and will help to individualize the initial daily dose needed by a specific patient in order to obtain an adequate immunosuppression without increased risk of nephrotoxicity.

Abstract# 701

CONVERSION FROM CYCLOSPORINE TO SIROLIMUS IN KIDNEY TRANSPLANT RECIPIENTS ALTERS THE PHARMACOKINETIC PROFILE OF MYCOPHENOLATE. Jean Louis Bosmans,[1] Jacques Sennesael,[2] Pierre Wallemacq,[3] Gert A. Verpooten.[1] *[1]Department of Nephrology, University Hospital Antwerp; [2]Department of Nephrology, AZ VUB Jette, Brussels; [3]Department of Toxicology, University Hospital St-Luc, Brussels, Belgium.*

Elective conversion from cyclosporine (CsA) to sirolimus (SRL) based immunosuppression likely prevents the development of chronic calcineurin-inhibitor nephrotoxicity in kidney transplant recipients. Since CsA decreases the exposure (AUC) to mycophenolic acid (MPA) and increases the exposure to mycophenolic glucuronic acid (MPAG), it could be anticipated that conversion from CsA to SRL induces significant alterations of the pharmacokinetic profile of concommittant therapy with mycophenolate mofetil (MMF). This hypothesis was tested in a prospective study in 31 stable kidney transplant recipients, with a serum creatinine < 2.5 mg /dl. Recipient characteristics were: age: 49±12 y, gender: 20 male/11 female, 30 cadaveric transplants, 30 first kidney transplants. Conversion was performed at 7±1 month after transplantation. One month prior to the conversion the dose of MMF was reduced from 1g b.i.d. to 0.75g b.i.d. During conversion, the dose of CsA was weekly reduced by 25%, while SRL was started at an initial dose of 2 mg/d, and increased to reach trough levels of 5 to 15 ng/ml. During the study prednisolone was kept constant at 5 to 10 mg/d. A pharmacokinetic profile of MPA and MPAG, with blood sampling at baseline, 30, 60, 90 min, 2,4,6,8 and 12 hr was performed at conversion (n=29), at 2 m (n=27), and at 9 m (n=19). MPA and MPAG concentrations were simultaneously determined by means of HPLC-UV. At 2 m the AUC for MPA increased to 138% (95% CI: 113-168%; p=0.003), the trough level of MPA increased to 267% (95% CI: 188-378%; p<0.001), and the half-life of MPA increased to 172 % (p<0.001). The Tmax and Cmax remained unchanged. In addition, the AUC for MPAG decreased to 66 % (95% CI: 56-78 %; p <0.001) at 2 m. These alterations remained constant at 9 m (p=ns vs 2 m). Infectious complications were associated with a higher AUC for MPA at 2 m (55±18 vs 41±10; p=0.014) and at 9 m (55±19 vs 38±9; p=0.020), while they were unaffected by trough levels of SRL at 2 m (13±5 vs 10±5 ng/ml; p=ns) and 9 m (13±6 vs 10±4 ng/ml; p=ns). Conversion from CsA to SRL consistently increases the exposure to mycophenolic acid in kidney transplant recipients. Our study indicates that this increased exposure to MPA may have implications on the incidence of infectious complications.

Abstract# 702

INTESTINAL CYP 3A4 ACTIVITY DECLINES PARALLEL WITH DECREASING CORTICOSTEROID DOSES IN RENAL TRANSPLANT PATIENTS. Wim Lemahieu,[1] Bart Maes,[1] Kristin Verbeke,[2] Yves Vanrenterghem.[1] *[1]Internal Medicine, Division of Nephrology, UZ Gasthuisberg KULeuven, Belgium; [2]Laboratory of Radiopharmaceutical Chemistry, UZ Gasthuisberg KULeuven, Belgium.*

Background: Catabolism by intestinal and hepatic cytochrome P450 3A4 (cyp 3A4) and excretion by P-glycoprotein (Pgp) have major influences on oral bio availability of calcineurin inhibitors. High doses of corticosteroids (CS) induce both enzymes. In this study, the effect of decreasing CS doses on the in vivo activity of intestinal and hepatic cyp 3A4 and Pgp in renal transplant recipients was studied. **Methods:** ^{14}C Erythromycin was used as a probe for cyp 3A4 and Pgp after intravenous and oral administration on 2 consecutive days in order to estimate hepatic and intestinal enzyme activity respectively. Catabolism by cyp 3A4 generates $^{14}CO_2$ that is exhaled. The fraction of tracer that escapes demethylation by cyp 3A4 and re-excretion in the gut lumen by Pgp is excreted in the urine. Recovery of ^{14}C at time infinite (*m*) in breath reflects activity of cyp 3A4 directly and *m* in urine corresponds inversely with Pgp activity. 11 renal transplant (tx) patients performed the test at one week and 3 months after tx. All patients received induction immunosuppressive therapy with corticosteroids and FK 506. The dose of corticoids amounted to 500 mg at day 0, 40 mg at day 1, 20 mg at day 2 and was further tapered to 4 mg/d at day 90. The test results at one week and 3 months after tx were compared using the paired student's t test. **Results:** As shown in the table, hepatic and intestinal cyp 3A4 activity decreased with time after tx, though the difference was only significant for intestinal cyp 3A4 activity. Intestinal Pgp activity decreased also, in contrast to an increased hepatic Pgp activity as a function of time. These differences however were not significant. **Conclusions:** After renal tx, changes in hepatic cyp 3A4 and Pgp activity did not alter significantly as a function of time. In contrast, a significant decrease of intestinal cyp 3A4 activity was observed, paralleling tapering of CS in time. This suggests a stronger inductive effect of CS on intestinal cyp 3A4 than on it's hepatic counterpart.

cyp 3A4 and Pgp activity at day 7 and 90 post tx

	IV : hepatic		PO : intestinal	
	breath test: m	*urine test: m*	*breath test: m*	*urine test: m*
	cyp 3A4	**1-Pgp**	**cyp 3A4**	**1-Pgp**
day 7	8.77	8.75	1.79	1.96
day 90	8.45	7.85	1.17	2.35
%change	-4 (p=0.10)	-10 (p=0.20)	***-34 (p=0.04)***	+18 (p=0.16)

Abstract# 703

TACROLIMUS PHARMACOGENETICS: A SINGLE NUCLEOTIDE POLYMORPHISM ASSOCIATED WITH CYTOCHROME P4503A5 EXPRESSION IDENTIFIES PATIENTS WHO FAIL TO ACHIEVE TARGET BLOOD CONCENTRATIONS DURING THE FIRST WEEK AFTER RENAL TRANSPLANTATION. Iain A. M. MacPhee,[1] Salim Fredericks,[2] Tracy Tai,[2] Petros Syrris,[3] Nicholas D. Carter,[3] Atholl Johnston,[4] Lawrence Goldberg,[5] David W. Holt.[2] *[1]Renal Medicine, St. George's Hospital Medical School, London, United Kingdom; [2]Analytical Unit, St. George's Hospital Medical School, London, United Kingdom; [3]Medical Genetics, St. George's Hospital Medical School, London, United Kingdom; [4]Clinical Pharmacology, Barts and the London School of Medicine, London, United Kingdom; [5]Renal Medicine, Royal Sussex County Hospital, Brighton, United Kingdom.*

Previously, we have shown that the dose-normalised blood concentrations of tacrolimus at 3 months after renal transplantation were related to a single nucleotide polymorphism (SNP) in the CYP3AP1 pseudogene (A/G_{-44}), that is commoner in Black subjects and strongly associated with hepatic CYP3A5 activity. Patients with a G-allele require two-fold higher doses of tacrolimus to achieve target concentrations. This study addresses the question as to whether concentration-controlled dosing with tacrolimus during the early period after transplantation can overcome this problem. Results for 178 renal transplant recipients transplanted between 1995 and 2001 were examined (CYP3AP1 –44 genotype: AA, n=125, AG/GG n=53). Target blood tacrolimus trough concentrations, measured 3 times weekly for 2 weeks, were 15-20 µg/L during the first week, then 10-15 µg/L for the next 3 months. The mean blood tacrolimus concentration during the first week was significantly lower for patients with the G-allele (Median 13.5 vs 18.5 µg/L, p<0.0001). More importantly, there was a significant delay in achieving target blood concentrations for patients with a G-allele (Table). A significantly higher proportion of AA patients had at least one blood tacrolimus concentration above target during the first week (73.6% vs 35.8%, p=0.003). There was no significant difference in the rate of biopsy-confirmed acute rejection over the first three months post-transplant but the episodes of rejection occurred earlier in the AG/GG group (median 7 days vs 12 days, p=0.006). In conclusion, initial dosing with tacrolimus, based on a knowledge of the CYP3AP1 genotype and, subsequently, guided by concentration measurements, has the potential to increase the proportion of patients achieving target blood concentrations early after transplantation.

CYP3AP1 genotype and failure to achieve target blood tacrolimus concentrations

Patients with all blood tacrolimus concentrations below target (%)

	Week 1			Week 2		
Ethnic group	AA	AG/GG	Chi-squre p	AA	AG/GG	Chi-square p
All	11 (8.8%)	21 (39.6%)	<0.0001	5 (4%)	9 (17%)	0.005
White	8 (7.8%)	12 (75%)	<0.0001	5 (4.9%)	3 (18.8%)	0.05

Abstract# 704

FTY720 TARGETS THE G-PROTEIN-COUPLED RECEPTORS S1P1, S1P3, S1P4 AND S1P5: ATTEMPTS TO DISSECT RECEPTORS MODULATING LYMPHOCYTE TRAFFIC AND HEART RATE. Volker Brinkmann,[1] Martin Lipp,[2] Christian Bruns,[1] Peter Heining,[3] Rainer Albert,[1] Hiestand Peter,[4] William Rust.[1] *[1]Transplantation Research, Novartis Pharma AG, Basel, Switzerland; [2]Molecular Tumor- and Immunogenetics, Max Delbrueck Center for Molecular Medicine, Berlin, Germany; [3]Preclinical Safety, Novartis Pharma AG, Basel, Switzerland; [4]Arthritis and Bone Metabolizm, Novartis Pharma AG, Basel, Switzerland.*

FTY720, combined with Neoral or Certican (Everolimus/RAD), has proven to be effective in renal transplantation in man. The profound anti-inflammatory properties of FTY720 relate to a sequestration of lymphocytes to secondary lymphatic tissue, thus away from inflammatory sites and grafted organs. FTY720 does not impair T cell activation, expansion, and memory to systemic infection, suggesting a novel mechanism not observed with classical immunosuppressants. We found that FTY720 is rapidly phosphorylated in vivo, and that phosphorylated FTY720-P signals as potent agonist at four of five G-protein-coupled receptors (GPCRs) for sphingosine-1-phosphate (S1P), namely S1P1 (Edg1), S1P3 (Edg3), S1P4 (Edg6) and S1P5 (Edg8), but not S1P2 (Edg5). Stimulation of lymphocytes by FTY720-P increased their intrinsic mobility, and *in vivo* accelerated homing to lymph nodes in a CCR7/CD62L-independent manner. Studies in S1P receptor-deficient mice, together with S1P receptor expression analysis in T cells, suggested S1P1 (coupled to $G\alpha_{i/o}$ protein) to be a key player in FTY720-P-induced lymphocyte homing. In analogy to the natural serum lipid S1P, FTY720-P induced a mild negative chronotropic effect (NC) in humans, suggesting additive effects of S1P and FTY720-P on S1P receptors expressed on atrial myocytes. Indeed, the NC was a stereoselective, PTX-sensitive process that could be mimicked *in vivo* in rats and *ex vivo* in an isolated rabbit heart model. *In vitro* experiments further revealed that FTY720-P could reduce adenylyl-cyclase activity and cAMP accumulation, functional consequences that have also been associated with S1P-mediated reduction of pacemaker activity and beta2-adrenergic receptor blockade. These findings, together with the expression profile of S1P receptors on sino-atrial node cells, suggest an involvement of $Ga_{i/o}$-coupled S1P1 (and perhaps S1P5) in the NC. Recent animal studies provided evidence that FTY720 monotherapy at low dosing is highly effective in models of multiple sclerosis and lupus erythematosus, and prevents the onset of autoimmune diabetes in NOD mice as well as the rejection of transplanted allo-isets. All together these data suggest that the novel sphingolipid drug FTY720 may have broad application in various settings of transplantation and autoimmunity.

Abstract# 705

FTY720 DISPOSITION IS NOT AFFECTED BY DEMOGRAPHIC OR CLINICAL FACTORS IN DE NOVO KIDNEY TRANSPLANT PATIENTS. J. M. Kovarik,[1] C. H. Hsu,[1] A. Skerjanec,[1] G. J. Riviere,[1] R. Schmouder.[1] *[1]Novartis Pharmaceuticals, Basel, Switzerland and East Hanover, NJ.*

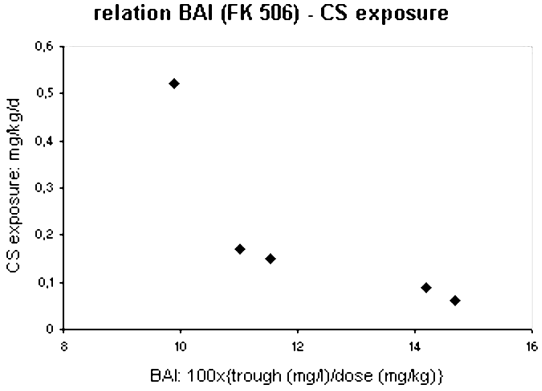
The population pharmacokinetics of the novel lymphocyte homing agent FTY720 were assessed in 163 de novo kidney transplant patients to explore whether demographic or clinical covariates identify patient subgroups in which drug exposure may vary from the population average. **Study design and methods:** In this open-label study, patients were randomized to receive FTY 0.25 mg/day (n = 42), 0.5 mg/day (n = 43), 1 mg/day (n = 40), or 2.5 mg/day (n = 38) with Neoral and corticosteroids for 3 months. Predose blood samples were collected once weekly during treatment and over a 4-week washout period after the last dose. A total of 2,439 FTY blood concentrations (15.0 ± 5.6 per patient) were analyzed at a central lab. Nonlinear mixed-effects modeling with a one-compartment pharmacokinetic model was used to estimate the pharmacokinetic parameters. **Population pharmacokinetics:** Population average clearance (CL/F) was 10.8 L/h with 55% interpatient variability, apparent distribution volume (V/F) was 3280 L with 40% variability, and the absorption half-life was 1.5 h. The derived elimination half-life was 8.8 days. Residual variability in blood concentrations was 28%. **Demographic covariates:** None of the common demographic covariates had a significant influence on CL/F: age (19 - 69 years), weight (40 - 115 kg), sex (56% men), or ethnicity (74% white, 11% black, 3% Asian, 12% other). Of these, only weight modestly influenced V/F albeit to a clinically insignificant extent. **Clinical covariates:** A doubling in baseline alkaline phosphatase resulted in a modest 23% increase in CL/F. Renal function as assessed by serial serum creatinine from baseline and weeks 7, 8,

and 11 (range, 114 - 1432 umol/L) did not influence exposure. Indicators of hepatic function including bilirubin (1 - 39 umol/L), AST (7 - 283 U/L), ALT (3 - 373 U/L), and albumin (25 - 46 g/L) did not impact on exposure. CL/F was not different in diabetics (n = 31) compared with nondiabetics. CL/F was also not influenced by comedication with the beta-blockers atenolol (n = 29), labetolol (n = 16), metoprolol (n = 41), propranolol (n = 14). **Conclusions:** (1) Dose adjustment of FTY on the basis of weight (mg/kg) does not appear necessary; (2) FTY blood concentrations remained stable despite changes in renal function posttransplant; (3) Concomitant use of beta-blockers did not alter the pharmacokinetics of FTY; (4) No special patient populations were identified in this analysis for which FTY dose regimens need to be modified.

Abstract# 706

ORAL BIOAVAILABILITY OF FK 506 RAISES PARALLEL WITH DECREASING CORTICOSTEROID DOSES IN RENAL TRANSPLANT PATIENTS. Wim Lemahieu,[1] Kathleen Claes,[1] Pieter Evenepoel,[1] Dirk Kuypers,[1] Bart Maes,[1] Yves Vanrenterghem.[1] *[1]Internal Medicine, Division of Nephrology, UZ Gasthuisberg KULeuven, Belgium.*

Background: Catabolism by intestinal and hepatic cytochrome P450 3A4 (cyp 3A4) and excretion by P-glycoprotein (Pgp) is considered to have a major influence on oral bio availability of FK 506. Since it is known that high doses of corticosteroids (CS) induce both enzymes, the effect of changing CS exposure on the oral bio availability of FK506 was studied. **Methods:** A cohort of 203 renal transpant patients was analysed. At transplantation (tx), all received induction with steroids (500 mg methylprednisone) in addition to FK506 and MMF. Afterwards, CS doses, starting from 20 mg/d, were progressively tapered. CS exposure was calculated as mean daily dose in mg/kg body weight during the time intervals: day 0-30, 31-60, 61-90, 91-180 and 181-365 post tx. Bio availability of FK506 was calculated as an index (BAI): {through level (mg/l) / dose (mg/kg)}multiplied by 100 at 30, 60, 90, 180 and 365 days post tx. CS exposure and BAI were compared at the given time intervals with one way ANOVA. **Results:** CS exposure dropped significantly ($p<0.0001$) from 0.58 at 1 month to 0.17, 0.15, 0.09 and 0.06 at 2, 3, 6 and 12 months post tx respectively. Parallel, BAI raised by 11% from 9.9 at 1 month to 11 at 2 months ($p=0.0003$), by 16% to 11.54 at 3 months, by 43% to 14.2 at 6 months and by 48% to 14.69 at 12 months post tx ($p<0.0001$). As shown in the figure, BAI increases in correlation with decreasing corticoid exposure in function of time after tx. **Conclusions:** In renal transplant patients higher doses of CS were associated with lower oral bio availability of FK506, suggestive for the inducing effects of CS on cyp 3A4 and Pgp.



Abstract# 707

PERIPHERAL BLOOD FTY720 PHARMACOKINETIC/ PHARMACODYNAMIC (PK/PD) MODELING IN RENAL TRANSPLANTED RECIPIENTS. Sung I. Park,[1] Cláudia R. Felipe,[1] Paula G. Machado,[1] Riberto Garcia,[1] Andrej Skerjanec,[2] Robert Schmouder,[2] 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; [2]Novartis Pharmaceuticals, East Hanover, NJ.*

INTRODUCTION: FTY720 is a lymphocyte homing drug that induces peripheral blood lymphopenia. The relationship between FTY720 dose or blood concentration and peripheral lymphopenia is not clear. This study investigates models of FTY720 PK/PD relationships in the blood compartment. **METHODS:** 23 kidney transplant recipients were randomized to receive FTY720 (0.25, 0.5, 1.0 or 2.5 mg QD) or MMF (2gm/day) in combination with Neoral and steroids. FTY720 was administered for 12 weeks post-transplant. FTY720 dose, blood concentrations and peripheral blood lymphocyte counts were obtained weekly in all 5 groups, before and at weeks 4 to 12 after transplantation. Peripheral blood lymphocyte counts from MMF group were used to calculate the effect when FTY720 dose or concentrations were equal to zero. The PD effect was calculated as a % reduction compared to the lymphocyte count before the administration of the first dose of FTY720 or MMF. FTY720 blood concentrations were measured by HPLC/MS/MS method. PK/PD modeling was utilized to find the best-fit model of the correlation between % reduction in peripheral lymphocyte count and increasing doses or blood concentrations of the FTY720. **RESULTS:** Mean age was 40 years, 61% white, 61% males and mean BMI was 22.8±2.6 kg/m². FTY720 dose associated with best efficacy in preventing acute rejection was 2.5 mg/day. Mean FTY720 concentrations were 0.36±0.05 (0.25 mg), 0.73±0.12 (0.5 mg), 3.26±0.51 (1 mg), and 7.15±1.41 ng/mL (2.5 mg). Between weeks 4 to 12, best-fit PK/PD modeling for dose-effect or concentration-effect relationship was the simple E_{max} model [$E = (E_{max} * C) / (C + EC_{50})$, where E is the effect at a given concentration C, Emax is the maximum effect attributed to the drug, and EC_{50} is the drug concentration which produces 50% of maximum effect]. For dose-effect relationship, $E_{máx}$=87,8±5,3% and ED_{50}=0,48±0,08 mg (r^2=0,94). For concentration-effect relationship $E_{máx}$=78,3±2,9% and EC_{50}=0,592±0,091 ng/mL (r^2=0,89). **CONCLUSION:** According to the PK/PD model, EC_{50} was achieved at FTY720 doses of 0.5 mg and blood concentrations of 0.6 ng/mL. Since FTY720 PK are dose-linear and effective doses of FTY720 are 2.5 and 5 mg/day, the immunosuppressive effect of FTY720 may depend upon induction of high degree of lymphopenia (~80%) and/or be associated with other FTY720 effects out of the blood compartment, perhaps in secondary lymphoid tissues where lymphocyte home.

Abstract# 708

PHARMACOKINETICS OF MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANT IMMUNOSUPPRESSION: RISKS OF USING A FIXED DOSE REGIMEN. Kazuharu Uchida,[1] Yoshihiro Tominaga,[1] Toshito Haba,[1] Akio Katayama,[1] Susumu Matsuoka,[1] Norihiko Goto,[1] Tsuneo Ueki,[1] Tetsuhiko Sato,[1] Asami Takeda,[1] Kunio Morozumi,[1] Takaaki Kobayashi,[2] Hirosi Takagi,[3] Akimasa Nakao.[2] *[1]Dept. of Transplant Surgery, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan; [2]Dept. of Surgery II, Nagoya University, Nagoya, Aichi, Japan; [3]Surgery, JR Tokai General Hospital, Nagoya, Aichi, Japan.*

Pivotal pharmacokinetic studies that evaluated Mycophenolate Mofetil (MMF) in renal transplantation have demonstrated low intrapatient and interpatient variability resulting in the adoption of fixed dose recommendations for MMF therapy. We investigated comparative pharmacokinetics, including intrapatient and interpatient variability over time up to the 6th postoperative week; to evaluate the MMF fixed dose regimen in renal transplantation . Study population and Methods: The study included 45 de novo renal transplant recipients treated with prednisolone, MMF and CNI's, (CsA = 24, FK= 21). Drug exposure in the first four hours post-dose (AUC) of CNI's and mycophenolic acid (MPA) were measured once or twice a week from the 4th postoperative day to the 6th postoperative week. MMF dosing was initiated with a fixed dose at 3g/day (BID) from the 2nd postoperative day, with a dose change to 2 g/day from the 15th or 29th postoperative day. Results&Conclusion: The study data demonstrate that interpatient variability in MPA pharmacokinetics is high (CV; 50-80%), although intrapatient variation is lower (CV; 25-45%). The MPA C0 level increased gradually, reaching a steady state at the 2nd-3rd postoperative week (2-3 fold from baseline), and lowered after changing the dose to 2 g. The MPA AUC in the same patients remained steady without declining even after decreasing the dose from 3 g/day to 2 g/day. Our evaluation of MPA pharmacokinetics demonstrates that MPA interpatient variability is high, and that the trough level elevates gradually over time without MMF dose changes. The MPA AUC changes are not dose-dependant. These results indicate a potential risk for variable MPA exposure with a MMF fixed dose regimen, and suggest TDM of MPA for individualization of MMF doses.

1. MPA mean values plus inter-patient variation for C0 (mcg/mL) and AUC0-4h (mcg·h/mL)

Day(MMF dose)	#4d (3g)	#14d (3g)	#35 (2g)	#42d (2g)
Mean C0 (%CV)	1.3±0.7(75.8)	0.8±2.1(76.7)	3.7±3.0(82.5)	3.7±2.3(61.0)
Mwan AUC(%CV)	39.1±21.7(55.5)	37.7±18.6(49.3)	44.5±22.5(50.6)	45.5±21.1(46.4)

2. Intrapatient variability during the administration period for 3 g/day and 2 g/day fixed doses per patient

	3g-period	2g-period
Mean C0 (%CV)	2.6±1.6(45.8)	3.1±1.5(39.8)
Mean AUC(%CV)	41.9±12.0(26.9)	40.2±10.1(25.1)

Abstract# 709

DIFFERENCES IN HEALTH INSURANCE ARE ASSOCIATED WITH ACCESS TO THE KIDNEY TRANSPLANT WAITLIST.

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[1]SRTR/URREA, Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI; [3]Massachusetts General Hospital, Boston, MA; [4]Virginia Commonwealth University, Richmond, VA; [5]Lifelink Transplant Institute, Tampa, FL.

Background: Previous studies have assessed cadaveric renal transplantation rates among different patient groups, and multivariate analyses have shown that minorities, females, the elderly, and diabetics were relatively less likely to receive a renal transplant. This analysis examines the relationship between the type of insurance (primary and secondary) at initiation of dialysis (ESRD) and access to the transplant waitlist. **Methods:** We used national (CMS) data for insurance status and characteristics of all dialysis patients at time of first dialysis and SRTR data for time of first waitlisting. The study population consists of 258,391 dialysis patients (age < 65) beginning dialysis between 1995 and 2001. Relative rates of waitlisting (RR-WL) from ESRD onset were calculated for kidney dialysis patients by type of insurance using a Cox regression model of time to waitlisting (censored at death, living donor transplant, or end of study on 6/30/2002). Pre-emptive waitlists were excluded. The model was adjusted for age, gender, diagnosis, race, incidence year, ethnicity, 20 comorbidities, type of dialysis facility, and geography (state). **Results:** The table below shows the relative waitlisting rate by type of insurance coverage. Patients with Medicare only, Medicaid only, or Medicare and Medicaid only have significantly lower waitlisting rates than do other patients.

Insurance Coverage	N	%	RR-WL	p-value
Medicare Only	19,784	7.7	1.00	ref
Medicaid Only	51,989	20.1	0.93	<.01
Medicare and Medicaid Only	19,871	7.7	0.88	<.01
Employer Group Health Insurance Only	18,041	7.0	1.97	<.01
Medicare and Any Other Insurance	75,026	27.2	1.27	<.01
Other Medical Insurance	41,603	16.1	1.55	<.01
No Medical Insurance Listed	36,909	14.3	1.03	0.20

Patients with only Medicaid insurance had an overall waitlisting rate 34% lower (RR-WL=0.66, p<0.01) than patients with all other types of insurance. Although this RR-WL varied by state (22% to 68% lower), it remained statistically significant in 43 states. **Conclusions:** These newly reported results by state reveal dramatic geographic differences in access to the kidney transplant waitlist by patient insurance status at initiation of dialysis.

Abstract# 710

KIDNEY TRANSPLANTATION RATES FROM THE WAITLIST REVEAL DISPARITIES IN ACCESS BY INSURANCE STATUS.

Alan B. Leichtman,[1,2] Valarie B. Ashby,[1,2] Robert A. Wolfe,[1,2] Friedrich K. Port,[2] Akinlolu O. Ojo,[1,2] Francis L. Delmonico,[3] Winfred W. Williams, Jr.,[3] Robert D. Higgins,[4] Denise Y. Alveranga,[5] Philip J. Held.[2]
[1]University of Michigan, Ann Arbor, MI; [2]URREA, Ann Arbor, MI; [3]Massachusetts General Hospital, Boston, MA; [4]Virginia Commonwealth University, Richmond, VA; [5]Lifelink Transplant Institute, Tampa, FL.

Background: Minorities, females, and the elderly have been shown to be less likely to receive cadaveric donor kidney transplants. This analysis looks at the relationship between patient insurance at the time of entry onto the transplant waitlist and cadaveric transplant access. **Methods:** Transplant rates (RR-Tx) for waitlisted patients were calculated using a Cox regression model (censored at removal from waitlist or end of study [6/30/02]) among 112,319 registrants entering the kidney waiting list for the first time from 1995-2001. Waitlist dates before first dialysis were moved to onset of ESRD. Pre-emptive transplants were excluded. The model was adjusted for age, gender, diagnosis group, blood type, race, ethnicity, waitlist year, previous transfusions, state of residence, initial PRA, time from first dialysis to waitlisting, dialysis modality at waitlist, and HLA antigens. **Results:** The table below shows adjusted transplantation rates by type of insurance coverage. Patients with Medicare only, Medicaid only, and HMO/PPO only have significantly lower waitlisting rates than do patients with private or multiple types of insurance.

Insurance Coverage	N	%	RR-Tx	p-value
Medicare Only	13,009	11.0	1.00	ref
Medicaid Only	7,171	6.1	0.99	0.68
Medicare + Other	40,627	34.4	1.09	<.01
Private Only	24,147	20.4	1.05	0.01
HMO/PPO Only	6,602	5.6	0.86	<.01
Private/HMO/PPO + Other	19,717	16.7	1.11	<.01
Other source of payment	5,860	5.0	1.05	0.09
Missing source of payment	1,046	0.9	0.85	0.20

Waitlisted patients with only Medicaid insurance had an overall transplantation rate in the U.S. 10% lower (RR=0.90, p<0.01) than patients with all other types of insurance. The disparity was greater than 10% for 20 states (3 of them with p<0.05), while in 6 states Medicaid patients had significantly higher rates (p<0.05). **Conclusions:** These results indicate substantial disparities in access to cadaveric transplantation for waitlisted patients by insurance type and by geography. These disparities are less extreme than those observed in access to the waitlist itself. Patients with insurance types (HMO/PPO only, Medicare only, and Medicaid only) at waitlist are most disadvantaged.

Abstract# 711

COST EFFECTIVENESS OF EXTENDED MEDICARE COVERAGE OF IMMUNOSUPPRESSIVE MEDICATIONS TO LIFE IN RENAL TRANSPLANTATION.

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A substantial number of renal transplant recipients lose Medicare coverage of immunosuppressive medications after 36 months post-transplant. One's ability to afford these medications is correlated with non-adherence to treatment and graft loss, especially among patients of lower socioeconomic status. Woodward et al.(AJT 1:69-73, 2001) determined that extending Medicare immunosuppression coverage from one to three years correlated with a 27% greater improvement in graft survival in the majority of patients stratified by income. We sought to compare the economic costs and quality of life benefits of extension of immunosuppressive coverage to life. Methods: The United States Renal Data System (USRDS) was analyzed for recipients of renal transplants from 1995-1999. A Markov model was designed to assess the outcomes of patients who receive a renal transplant. This model compared current immunosuppressive coverage of 3 years to a model representing lifetime immunosuppressive medication coverage, both measured over a 20-year period. Probabilities of all outcomes were calculated including 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. Results: The previously reported graft loss reduction from extending immunosuppression coverage translates into an increase in overall survival from 55.4% with current coverage to 61.7% after 20 years with lifetime coverage. In addition, lifetime immunosuppressive medication coverage produced an average of 0.30 additional QALYs per individual transplant over existing coverage. Since Medicare spends approximately $79,400 per QALY to care for wait-listed patients on dialysis, we felt it reasonable to follow that precedent for renal transplantation. We found that the QALY benefit of lifetime immunosuppression coverage would be cost-effective relative to dialysis if the average annual cost of immunosuppression to Medicare were $5,570. Conclusions: The average annual cost of immunosuppression can be considerably higher than the cost-effective threshold calculated here. However, providing lifetime coverage through Medicare as secondary insurance, available to patients without alternatives, those truly at risk, may yield the previously observed benefits of extended coverage while bringing the average cost of lifetime coverage down to cost-effective levels.

Abstract# 712

DID INSURANCE REDUCE RACIAL DISPARITIES IN KIDNEY GRAFT SURVIVAL?

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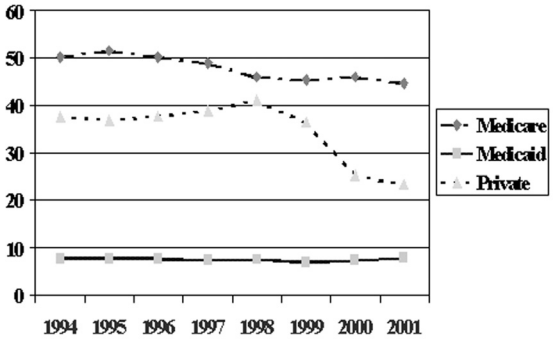
Purpose: It had been previously reported that the additional two years of immunosuppression insurance benefits Medicare added between 1993 and 1995 effectively eliminated graft survival differences associated with income disparities. (*Am J Transplant*, 2001) The current study determined whether that same additional immunosuppression coverage had an equally beneficial effect on the graft survival differences associated with ethnicity. **Methods:** We first merged patient-level clinical data from the USRDS-distributed UNOS registry with median family income for each patient's ZIP code from the 1990 Census. We then compared only the first cadaveric single-organ renal transplants performed in 1992-3 in the highest and lowest income quartiles with the similar transplants performed in 1995-7. We used Cox Proportional Hazards models to compare graft survival in the second and third years post-transplant among i) the 4,441 patients transplanted in 1992 and 1993 that survived at least one year and ii) the 6,496 set of similar patients transplanted between 1995 and 1997. (Medicare maintenance immunosuppression insurance benefits were available only to the second of these 2 cohorts.) **Results:** In a model controlling for other significant donor, recipient, and transplant characteristics, the extra two years of Medicare immunosuppression insurance more than eliminated the 23% (Hazard Ratio Confidence Interval, HRCI, 1.05 to 1.44; P=0.009) greater graft loss associated with the lowest incomes. But the extra Medicare insurance produced no such beneficial impact on the greater graft loss associated with black ethnicity. Black ethnicity was associated with a 42% additional graft failure (HRCI 1.09 to 1.85, P=0.009). On top of that, the lowest income black recipients were associated with an additional 32% graft loss (HRCI 1.02 to 1.72; P=0.037). All variables testing for the value of the extra Medicare insurance benefits to blacks generally and to low-income blacks in particular were highly insignificant. **Conclusions:** Gaston (*Am J Transplant*, 2002) and others have expressed

concern about racial disparities in kidney graft survival. Our results imply that while removing the financial barrier to immunosuppression medications was sufficient to eliminate the disparities associated with low incomes, it did nothing to eliminate the concurrent racial disparities.

Abstract# 713

ECOMOMIC & RACIAL DISPARITIES FOR KIDNEY TRANSPLANTATION IN THE US. Ross B. Isaacs,[1] Rasheed Balogun,[1] Robert B. Davis,[1] Peter I. Lobo,[1] Kenneth Brayman,[2] Wida Cherikh.[3] *[1]Department of Medicine, University of Virginia, Charlottesville, VA; [2]Department of Surgery, University of Virginia, Charlottesville, VA; [3]Department of Biostatistics, United Network Organ Sharing, Richmond, VA.*

Intro: Despite the known success of renal transplantation as a life saving modality, important disparities for access to transplantation persist for minorities and working and non-working poor patients. The purpose of our study was to assess trends in wait list and transplant rates in the US underserved ESRD population over the last 8 years. Methods: We performed a prospective analysis comparing all ESRD patients (n = 281,590) using the USRDS database, and all wait listed (WL;n = 153,477) and CRTx pts(n = 60,427) using the UNOS database during two separate eras from 4/1/94 (when insurance data first became available) and 12/31/02. 1 and 5 year transplant rates were analyzed by race, ethnicity, insurance and employment status and level of education. Racial categories included African American (AA), Hispanic (H), Caucasian(C) and other. Results: (See Table and figure below)) Conclusion: Insurance status adversely influenced placement on the wait list and transplant rates more in AA's and H than C for both eras, but has worsened over the last few years. In addition, these disparities have increased waiting times for AA to 3 times that of C. Our data suggest more efforts are needed to remove financial barriers to transplantation especially in the minority working poor to prevent worsening racial disparities.



Transplant rates by era (%)

Insurance Status	WL - C	1 yr Tx Rate - C	WL-AA	1 yr Tx Rate - AA	WL-H	1 yr Tx Rate - H
Before 1995						
Medicare	46.4	34.5	60.4	20.9	55.3	26.8
Medicaid	4.7	34.5	9.8	19.5	14.8	20.8
Private	44.9	34.1	26.3	22	25.5	23.6
After 1995						
Medicare	42	29.3	55	16.5	52.9	18.8
Medicaid	4.2	27.2	9.6	15.8	14.3	14.8
Provate	41.7	27.9	25	15.8	19.9	17.6

Abstract# 714

THE ECONOMICS OF PEDIATRIC KIDNEY TRANSPLANTION. Paul Hmiel,[1] Mark A. Schnitzler.[2] *[1]Pediatrics, Washington University School of Medicine, Saint Louis, MO; [2]Health Administration Program, Washington University School of Medicine, Saint Louis, MO.*

The survival of children with chronic kidney disease has improved dramatically over the last two decades, but little is known of the economic costs of their disease relative to adults with chronic kidney disease. Data were drawn from the United States Renal Data System (USRDS) on 6,405 cadaveric renal transplant recipients between 1995 and 1999 under the age of 40 at transplant, with Medicare as their primary payer. Pediatric age groups were defined as 0-24 months (n= 8), 2- 5 years (n= 68), 6-11 years (n= 116), 12-15 years (n= 174), and 16-19 years (n= 269), and compared to non-diabetic adults age 20-40 (n= 5,770). Reported costs are average Medicare payments for all covered medical services and supplies adjusted for race, gender, preemptive transplant, type of dialysis, HLA mismatches, cause of end-stage renal disease, and year of transplant. Costs for transplant were significantly higher in all pediatric age groups compared to non-diabetic adults, with the incremental cost for children aged 2- 5 years were increased 11%, those 6-11 years were increased 22%, adolescents were increased by +9%, and older teens minimally increased at +4%. Children under the age of 5 years sustained higher costs for the first post-transplant year as well, +34% for ages 2-5. Children between 6 and 11 demonstrated minimal increases in costs, and while the adolescents and teens had a 12% increase, these trends did not reach statistical significance.

Incremental Cost of Pediatric Kidney Transplants Relative to Adults

Age Group	n	Transplant	p	First Year	p
2- 5 years	68	$3,815	0.031	$11,778	0.009
6-11 years	116	$7,402	0.0001	$1,754	NS
12-15 years	174	$3,185	0.005	$4,313	NS
16-19 years	269	$1,530	NS	$4,187	NS

Mean cost difference relative to non-diabetic adults, aged 20-40 years; NS: $p > 0.05$

Costs associated with pediatric kidney transplantation in children were higher for all age groups relative to the adults aged 20-40, with age inversely related to cost. This effect continued into the first year following transplant, although the differences were less pronounced. The basis for these differences requires more detailed analysis.

Abstract# 715

PAYMENT FOR LIVING DONORS – WHAT PRICE IS COST-EFFECTIVE? Arthur J. Matas,[1] Mark Schnitzler.[2] *[1]Surgery, University of Minnesota, Minneapolis, MN; [2]Washington University, St. Louis, MO.*

Currently, the supply of kidneys (from cadaver and living donors) does not meet the demand; each year more patients go on the waiting list than are transplanted. As a consequence, the waiting time for a cadaver kidney continues to get longer, and there is renewed debate about payment for living donors. To facilitate this debate, we studied what amount of payment would be cost-effective for society—i.e., what costs would be saved (if any) by removing a patient from the waiting list using a paid (living unrelated—LURD) donor-vendor. A Markov model was developed to calculate the expected average cost and outcome benefits of increasing the organ supply and reducing waiting times by adding paid LURD organs to the available pool. Factors included in the model were patient survival, cost on dialysis, graft survival, death with function, death after graft loss, cost of donor acquisition, cost of tx, maintenance cost with graft function, and cost of return to dialysis for LURD transplantation. Tx patient and graft survivals were estimated from the US Renal Data System and calculated using Cox regression analysis for 2,757 LURD recipients, and 24,333 dialysis patients wait-listed for transplantation; costs were calculated from the prospective of Medicare (the primary insurer for ESRD in the US) using linear regression. Results were adjusted to mean patient characteristics for recipient age, race, gender, immunologic sensitization, diabetes, donor age and gender, and cause of ESRD. Wait list patient survival while on dialysis was calculated by the methods of Wolfe et al. (NEJM 341:1728, 1999); results were adjusted for age, race, gender, and cause of ESRD. (The model was solved for the first vendor recipients, who would already have waited on dialysis. If there were large numbers of vendors, waiting time would be shortened resulting in potentially increased savings to society.) **Results**: Transplantation from an LURD saved $102,000 (US – 2002 dollars) and provided 2.8 quality-adjusted life years (QALYs) gained. Adding the value of QALYs, a LURD tx saves $336,000, assuming society values additional QALYs from transplantation at the rate paid per QALY while on dialysis. **Conclusion:** At the minimum, a vender program saves society >$100,000 per tx and provides QALYs for the ESRD population. Thus, society could break even while paying $100,000/kidney vendor. Currently, living donors receive no payment. It is possible that if a vendor program were started, many donors now who would donate altruistically would then also want some payment. This would reduce the amount for the break-even point.

Abstract# 716

IMPACT OF UNOS-DEFINED EXPANDED DONOR POOL ON TRANSPLANT CENTER COSTS. David B. Leeser,[1] Christopher Handley,[1] Colleen Reilly,[1] Clarence Foster,[1] Benjamin Philosophe,[1] Alan Farney,[1] Stephen T. Bartlett,[1] Eugene J. Schweitzer.[1] *[1]Department of Surgery, Division of Transplantation, University of Maryland Medical System, Baltimore, MD.*

Introduction. In October 2002, UNOS created a separate waiting list for recipients willing to accept kidneys from a defined set of expanded criteria donors. Prior studies have demonstrated that transplantation of kidneys from certain groups of expanded criteria donors is more expensive to Medicare than those from ideal donors. This study examined transplant hospitalization charges as a surrogate for the increased costs an individual transplant center might expect from transplanting kidneys from the newly defined donor pool. **Methods**. Data pertaining to 1,001 kidney transplants performed at a single center between 3/98 and 2/01 were collected. Cadaver transplants from donors fitting the new UNOS definition were identified ("Expanded CAD" n = 150), and compared to an ideal group of 0 mismatch cadaver kidneys ("Ideal CAD" n = 72), and to the live donor transplants ("LD" n = 385) with respect to transplant hospitalization length of stay (LOS) and charges, as well as graft and patient survival. **Results**. The table shows that patients receiving Expanded CAD kidneys had a 50% longer LOS than those receiving Ideal CADs, and 140% longer than LDs. Corresponding hospital charges were 19% greater for Expanded CAD versus Ideal CADs, and 37% greater than for LDs. As expected, three-year graft survival was significantly lower for the Expanded CAD group than for Ideal CAD or LD transplants. **Conclusion**. Recipients will benefit from the expanded donor pool arising from the new UNOS allocation system. However, transplant centers and reimbursement systems will incur additional costs related to both the transplant admission and higher graft failure rates.

	LOS	Charge	3-yr Graft	3-yr Patient
Expanded CAD	12 days	$79,600	66%	82%
Ideal CAD	8 days ($p < 0.0001$)	$66,900 ($p < 0.0001$)	85% ($p = 0.003$)	90% (p = NS)
LD	5 days ($p < 0.0001$)	$58,100 ($p < 0.0001$)	88% ($p < 0.0001$)	93% ($p < 0.0005$)

Abstract# 717

MANAGING ENLARGING KIDNEY WAIT LISTS: A MODEL FOR DETERMINING THE PROBABILITY FOR A 0-ANTIGEN MISMATCH OFFER. Lee Ann Baxter-Lowe,[1] Harish Mahanty,[1] Calvin Lou,[1] Peter Bacchetti,[1] John Roberts.[1] *[1]Univeristy of California, San Franciso, San Francisco, CA.*

Management of the enlarging cadaver wait list is logistically challenging and expensive. Large wait lists might be more efficiently managed by prioritizing medical evaluations based upon likelihood of receiving an offer for a kidney (e.g., consider waiting time, sensitization, and HLA type). Toward this end, we previously reported a method for using HLA haplotype frequencies to determine the probability that a patient will be 0-antigen mismatched (0-MM) with organ donors in the US. The goal of this investigation was to develop and validate a model that uses these probabilities along with ABO blood groups to predict the likelihood that a patient will receive an offer for a 0-MM kidney from the next 5000 donors (approximately 1 year). **Methods:** A model for predicting the probability of an offer for a 0-MM kidney was developed using HLA haplotype frequencies for the major US racial groups (Mori et al., 1997) along with HLA type, race, and ABO blood group for the UNOS cadaveric kidney donors from 1991 to 2000. The model was used to determine the probability that each patient on the UCSF wait list between 07/12/00 and 01/18/02 (n=3,382) would receive an offer for a 0-MM kidney from the next 5000 donors. The predictions were compared to actual offers after adjusting for time on the waitlist. **Results:** Approximately 70% of the patients had <20% probability of receiving an offer from the next 5000 donors (~1 year). The racial/ethnic composition of this low probability population was Caucasian (28%), Asian (23%), African American (21%), Hispanic (17%), and other (11%). The remaining patients with probabilities ≥20% were predominantly Caucasian (63%), Hispanic (18%), and African American (9%). Only 11% of patients had >90% probability of receiving an offer; this population was predominantly Caucasian (77%) with relatively low representation of Hispanics (11%), African Americans (8%), Asians (1%), and others (3%). Nearly all patients with a probability of 100% actually received an offer; 76% of these received multiple offers (two with more than 20 offers). When the probabilities for each patient are ordered into deciles, there is excellent agreement with the observed offers. **Conclusions:** It is currently feasible to reliably determine the likelihood that each patient will receive an offer for a 0-MM kidney. This information is useful for counseling patients, prioritizing medical evaluations for growing wait lists, and making decisions regarding acceptance of marginal 0-MM organs.

Chronic Rejection and Chronic Allograft Nephropathy

Abstract# 718

HLA ANTIBODIES APPEAR BEFORE CHRONIC REJECTION OF KIDNEY TRANSPLANTS. Paul I. Terasaki. *[1]Terasaki Foundation Laboratory, Los Angeles, CA.*

According to at least 33 publications, HLA antibodies which appear AFTER transplantation are associated with chronic rejection. As evidence that antibodies cause rejection, four published studies show that HLA antibodies appear in the serum of patients BEFORE organ transplant failure. The present study among multiple transplant centers was undertaken to determine if chronic rejection could be predicted by the prior appearance of HLA antibodies PROSPECTIVELY. As part of a cooperative study in 24 international centers, 3,161 patients who had functioning kidney grafts for more than one year were examined for antibodies during a short four month period from January 2002 to April 2002. Then in July, 2002, after passage of 3-6 months, the centers were asked which patients had failed over this relatively short follow-up period. Among a clean subset of 1629 patients who did not have antibodies pre-transplantation, 4.6% of patients had failed in this period. In the 212 patients who developed antibodies, 3.3% failed as compared to 1.3% failure among 1,417 patients who did not develop antibodies. This difference was statistically significant (p=0.05). If deaths were counted as failures, 3.8% of those who developed antibodies failed as compared to 1.8% of those who did not develop antibodies (p=0.05). Although based currently on small numbers, those patients who developed *de novo* antibodies had a significantly greater chance of failure. Of the chronic failures, 28% had antibodies before failure and could be assigned as immunologic and the remainder may have been the result of non-immunological factors such as senescence, drug toxicity, hyperfiltration, recurrence of disease, etc . Also among the deaths 12% could be attributed to HLA antibodies and the remainder to non-immunologic factors. A one year follow-up will be performed in February, 2003, and will be available for the AST meeting in May. If this trend continues, monitoring patients for antibodies should prove useful in identifying those at risk and in altering immunosuppression to forestall chronic rejection.

Abstract# 719

HUMORAL ALLOREACTIVITY AFTER KIDNEY TRANSPLANTATION: AN IMPORTANT RISK FACTOR FOR CHRONIC ALLOGRAFT DYSFUNCTION. Antonina Piazza,[1] Elvira Poggi,[1] Oreste Buonomo,[2] Giuseppina Ozzella,[1] Alessandra E. Scornajenghi,[1] Daniela Settesoldi,[1] Carlo U. Casciani,[2] Domenico Adorno.[1] *[1]Istituto Trapianti d'Organo, CNR, Rome, Italy; [2]Department of Surgery, Tor Vergata University, Rome, Italy.*

The relationship between humoral alloreactivity towards mismatched HLA antigens of graft and chronic allograft dysfunction has not been yet clarified. This study aimed to investigate the relevance of HLA donor-specific-antibodies (DSA) on chronic graft dysfunction and graft loss in kidney transplantation. Pre- and post-transplant sera from 290 recipients (mean follow up: 4.6±2.9 years) were screened for HLA-DSA by flow cytometric technique (flow cytometry crossmatch and beads coated with purified HLA class I or II antigens). Two patients showed preformed HLA class II DSA and both lost the graft for acute rejection (ARj) while 51 (17.6%) developed HLA-DSA after transplantation. A higher incidence of HLA-A DSA was revealed in comparison to HLA-B DSA (53.7% vs. 18.6%, p=0.001). Most HLA-class I DSA were CREG-specific antibodies (66.7%), mainly due to A1, A3 and A9 mismatches (MMs). On the contrary, incidence of HLA-DR and HLA-DQ DSA were similar (63.3% vs. 59.1%). It is noteworthy that 23.1% of HLA-DQ DSA occurred in patients who received zero HLA-DR mismatched grafts. Analysis of HLA-MMs revealed a higher incidence of HLA-A and B MMs in DSA-Pos patients than in DSA-Neg ones (HLA-A MMs: 1.03±0.67 vs. 0.86±0.65, P=0.004; HLA-B MMs: 1.05±0.69 vs. 0.89±0.62, P=0.004). More acute rejections (ARj) and more chronic-graft-dysfunctions/graft losses (CGD/GL) occurred in DSA-Pos than in DSA-Neg patients (ARj: 41.2% vs. 11.8%, P<0.00001; CGD/GL: 49% vs. 17.7%, P<0.00001). Incidence of CGD/GL was higher not only in the HLA-class II DSA-Pos (41.7%) but also in the HLA-class I DSA-Pos patients (54.5%). Analysis of the cohort of DSA-Pos/ARj-Neg patients demonstrated the relevance of DSA production on graft outcome. CGD/GL was in fact more frequent in DSA-Pos/ARj-Neg than in DSA-Neg/ARj-Neg ones (44.8% vs. 15.8%, P=0.0007). Focusing on immunosuppressive treatment of DSA-Pos patients, we observed that 20% of CGD/GL occurred in patients receiving Mycophenolate-Mofetil, while the remaining 80% occurred in patients treated with Azathioprine. Our findings define DSA development as a negative prognostic factor for renal graft outcome, independent from ARj but strongly associated to HLA-MMs. DSA adverse effect might be modulated with immunosuppresants able to control both T- and B-cell responses. In conclusion humoral alloreactivity is a cause rather than a consequence of chronic-graft-dysfunction or graft loss.

Abstract# 720

CAN WE CHANGE THE PROGRESSIVE DECAY IN GRAFT FUNCTION AFTER THE ONSET OF CHRONIC ALLOGRAFT NEPHROPAHTY (CAN) IN THE RECIPIENTS OF KIDNEY ALLOGRAFTS? (PRELEMINARY RESULTS OF THE ONGOING STUDY). Ravinder K. Wali,[1] Amber Richards,[4] Rochelle Cunningham,[1] Cinthia Drachenberg,[2] Emilio Ramos,[1] Meredith Brisco,[4] Ann M. Wiland,[3] Ben Philosophe,[3] Stephen Bartlett,[3] Matthew Weir.[1] *[1]Department of Medicine, University of Maryland School of Medicine, Baltimore, MD; [2]Department of Pathology, University of Maryland School of Medicine, Baltimore, MD; [3]Department of Surgery and Division of Transplantation, University of Maryland School of Medicine, Baltimore, MD; [4]School of Medicine, University of Maryland School of Medicine, Baltimore, MD.*

One of the alloantigen-independent mechanisms for chronic allograft nephropathy could be CNI-related nephrotoxicity. Methods: Between May 2000 and June 2002, 289 patients with biopsy proven diagnosis of CAN and increased creatinine were included in the study. In this preliminary report of 107 patients. 88 patients were on tacrolimus and others on cyclosporine therapy. After the histological diagnosis of CAN, CNI was stopped and replaced with sirolimus at a dose to a goal 12-hour trough level of 12 to 15 ng/ml. Sirolimus was stopped in 7 patients due to adverse events and censored from this analysis. All CrCl measurements are mean of three values and calculated by Nankivell Formula. Baseline CrCl was ≤ 29 ml/min (n= 30), 30 to 65 ml/min (n=52) and ≥ 66ml/min (n=18). Based on the difference in the CrCl (Δ CrCl) before and after conversion, patients were grouped in three categories; Δ group 1(≤ zero); n= 30, Δ group 2 (increase by 1 to 15 ml/min); n= 38 and Δ group 3 (increase in CrCl ≥ 16 ml/min); n=32.

Distribution of CrCl in Whole Group and Delta CrCl groups
(before and after the converison of CNI to sirolimus)

		Post-Sirolimus Delta CrCl Groups			
	Whole Group (n=100)	≤zero (n=30)	1-15 (n= 38)	≥ 16 (n=32	p
Pre-sirolimus CrCl (mean±SD)	41.5±24.6	47.4±24.3	47.8±23.6	28.4±19.4	0.004
Post-sirolimus CrCl (mean±SD)	53.7±25.6	40.9±23.5	54.4±23.0	64.8±28.8	0.003

CrCl: calculated by Nankivell Formula, each CrCl is the mean of three values.

During the mean (±SD) follow-up of 17.7 ± 7.6 months, repeat biopsies were performed in 29 patients, 5 had AR , graft loss (n=13) and (n=6) died. Improvement in CrCl was noted in 70% of the cohort and most significant improvement was in the group with lowest mean baseline CrCl (28.4±19.4) with almost 50% increase in follow-up CrCl to 64.8 ± 25.8 (p<0.000). Rate of death and graft loss were highly significant in Δ group 1 as compared to other groups p=0.004 (long rank test). Conclusions: These results indicate that conversion of CNI to sirolimus in patients with CAN is associated with a significant improvement in graft function and this benefit is more pronounced in the group with less than 30 ml CrCl at baseline. We are in the process of analyzing the data in the rest of 182 patients.

Abstract# 721

EARLY ISCHAEMIC AND IMMUNOLOGICAL INJURY, AND LATE CALCINEURIN INHIBITOR TOXICITY, LEAD TO CHRONIC ALLOGRAFT NEPHROPATHY. Richard J. Borrows,[1] Jeremy R. Chapman,[1] Caroline Fung,[1] Philip J. O'Connell,[1] Richard D. Allen,[1] Brian J. Nankivell.[1] *[1]Renal and Transplant Units, Westmead Hospital, Sydney, Australia.*

Objective: With improvements in immunosuppression, and a reduction in early graft loss, chronic allograft nephropathy (CAN) has become a major cause for graft loss. The events leading to CAN remain unclear, possibly as such events may not be clinically apparent. We aimed to evaluate the role of both clinical and sub-clinical events leading to CAN. **Method:** 868 prospective sequential protocol kidney transplant biopsies (from 120 patients followed up for 10 years) were examined for evidence of both immunological and non-immunological damage. The Banff scoring system was used. **Results:** Grade I CAN was almost universal as early as 12 months (94%). Early CAN was characterised by interstitial fibrosis and tubular atrophy and resulted from ischaemia-reperfusion injury (histological ATN), particularly if severe, leading to dialysis-dependency (p<0.05). Early, severe rejection (resistant to steroid therapy) and sub-clinical rejection (SCR) in the first year also contributed to CAN at 12 months (p<0.01 and <0.001 respectively). SCR was seen in 42% of 3 month biopsies, was predicted by a prior episode of clinical rejection, was seen less commonly with tacrolimus or mycophenolate based therapies (both p<0.05), and decreased with time. Beyond 12 months the development of interstitial fibrosis and tubular atrophy slowed, but there was progressive development of arteriolar hyalinosis, which correlated with interstitial fibrosis and led to increasing glomerulosclerosis on subsequent biopsies. This calcineurin-inhibitor nephrotoxicity (CIT) was the major factor implicated in the development/worsening of CAN on later biopsies and was almost universal by 10 years (92.4%), even in grafts with normal 12-month histology. The severity of CAN worsened with time such that the prevalence of grade II CAN at 5 and 10 years was 64% and 85% respectively. Worsening CAN was associated with a progressive fall in GFR. Chronic cellular infiltrates (duration >2 years) were uncommon (5.8% of biopsies), and later biopsies revealed little in the way of chronic vascular damage (mean cv score=0.25), probably as this compartment had suffered little preceding acute histological damage. **Conclusion:** This series of protocol biopsies has allowed identification of both clinically obvious and sub-clinical events preceding, and leading to, the development of CAN, without evidence of ongoing immune-mediated damage. CIT was the major cause for late histological damage.

Abstract# 722

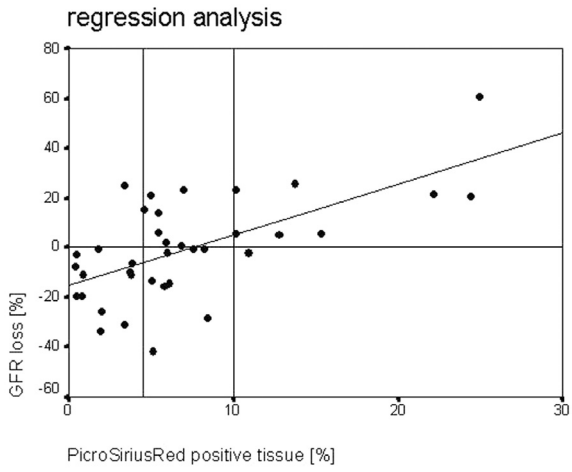
DEVELOPMENT OF DONOR-SPECIFIC ANTI-HLA ANTIBODIES POST RENAL TRANSPLANTATION IS ASSOCIATED WITH ACUTE REJECTION AND CHRONIC ALLOGRAFT NEPHROPATHY. Alin L. Girnita,[1] Jake A. Demetris,[1] Parmjeet Randhawa,[1] Edward Gray,[2] Rene Duquesnoy,[1] Ron Shapiro,[2] Velma Scantlebury,[2] Mark Jordan,[2] Jennifer Woodward,[2] Noriko Murase,[2] Thomas E. Starzl,[2] Adriana Zeevi.[1] *[1]Pathology, University of Pittsburgh and TE. Starzl Transplantation Institute, Pittsburgh, PA; [2]Surgery.*

The purpose of this study was to determine if humoral response pre- and post-transplantation (Tx) correlates with development of acute rejection (RA) and chronic allograft nephropathy (CAN). The follow-up period for 90 kidney recipients was from 3 to 18 mo. All patients received pre-Tx Thymoglobulin infusion and Tacrolimus-monotherapy, with steroid use only for episodes of biopsy-proven rejection. We used commercial ELISA kits for assessing the presence and specificity of IgG anti-HLA antibodies (HLAab). RESULTS: Only 2/90 patients had PRA>25%, when screened by CDC pre-Tx, while by ELISA 19 patients exhibited PRA>25% (p< 0.00001). One of the 19 pre-Tx sensitized patients had donor-specific HLAab. De novo sensitization occurred within 3 mo in 5 patients, all with donor-specific class I HLAab, between 3-6 mo in 2 patients that had class I+II HLAab (1/2 donor-specific) and 5 patients developed de novo HLAab after 9 mo, all class II and in 3/5 donor-specific. Three patients who had class I HLAab pre-Tx developed after 6 mo donor-specific class II HLAab. We observed that de novo producers had a higher prevalence of class I HLAab in the first 6 mo, followed class II thereafter (p<0.01). A significant (p<0.02) proportion of ELISA+ patients (24/26) developed acute cellular rejection grade RA≥2 in comparison with ELISA-group (45/64). Furthermore, there was a strong correlation (p<0.005) between ELISA+ group and presence of Banff Grade 1B or higher RA (22/26 ELISA+ versus 33/64 ELISA-). All 11 patients who exhibited donor-specific HLAab and 11/12 de novo HLAab producers had Banff Grade 1B or higher RA (p<0.01). CAN was seen in 14/26 ELISA+ patients while only 19/64 ELISA- showed the same histological changes (p<0.05). 10/11 donor-specific HLAab producers and 9/13 class I+II HLAab producers had CAN (p<0.0002 and p<0.01, resp). CONCLUSION: Our results indicate that ELISA-detected HLAab in kidney Tx may serve as markers for patients at risk for more severe and chronic allograft rejection. Class I HLAab correlated better with high-grade acute rejection, while class I+II HLAab correlated with both high-grade acute and chronic rejection. The frequency of de novo sensitization may also increase with time post-Tx. Sequential monitoring of humoral response post-Tx may be useful in the clinical management of kidney recipients.

Abstract# 723

COMPUTER-ASSISTED QUANTIFICATION OF FIBROSIS IN CHRONIC ALLOGRAFT NEPHROPATHY (CAN) BY PICROSIRIUSRED(PSR)-STAINING: A NEW TOOL FOR PREDICTING LONG TERM GRAFT FUNCTION. Lars Pape,[1] Gisela Offner,[1] Juergen Strehlau,[1] Jochen H. H. Ehrich,[1] Michael Mengel,[2] Paul C. Grimm.[3] *[1]Pediatric Nephrology, Medical School of Hannover, Hannover, Germany; [2]Pathology, Medical School of Hannover, Hannover, Germany; [3]Pediatric Nephrology, University of California in San Diego, San Diego, CA.*

CAN has become the most important limiting factor for long term transplant survival. There has been no reliable method for the quantification of interstitial fibrosis, the histomorphologic correlate of CAN, in renal grafts. Therefore we have used staining with PSR followed by computerized image analysis for this matter. Renal biopsies were performed in 56 children (mean age 13.7 ± 3.6 years) after a mean period of 4.6 ± 3.1 years after transplantation because of significant increases in s-creatinine. All biopsies were stained with PSR. Video polarisation microscopy was performed of the whole renal cortex; glomeruli were excluded. The percentage of fibrotic tissue was calculated by computerized image analysis. The glomerular filtration rate (GFR) of the patients was calculated at the time point of renal biopsy and two years later. Linear regression analysis was performed between the intensity of graft fibrosis and the changes in the GFR. There was a significant correlation (r = 0.62, p<0.001) between the percentage of PSR positive fibrous tissue and the decrease of the GFR in the first two years after renal biopsy . When the amount of PSR positive tissue was below 5%, 82% of the patients had an increase in GFR after two years. 93% of the patients with an amount of >10% of fibrosis experienced a worsening of renal function. When comparing patients with stable GFR with patients having a decrease in GFR a significantly different amount of PSR positive tissue was found (p=0.008). The quantification of fibrosis by PSR staining was found to be an adequate tool for estimating long term graft function. As the early detection of allograft fibrosis may lead to therapeutic changes, this staining procedure, in combination with other methods, may be an easy, fast and inexpensive step in the improvement of graft survival.



Abstract# 724

ANTIBODIES TO GBM ANTIGENS AND GLOMERULAR C4D DEPOSITS IN CHRONIC TRANSPLANT GLOMERULOPATHY.

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Chronic transplant glomerulopathy (CTG) is an uncommon feature of which the pathogenesis is unknown. In a Fisher to Lewis rat model of CTG we found IgG antibodies against the glomerular basement membrane (GBM) and identified perlecan as one of the antigens recognized (Am J Path 2002;160:1301). We studied risk factors, glomerular deposition of C4d and the presence of anti-HLA and 'anti-GBM' antibodies to establish the role of the humoral immune response in clinical CTG. From a cohort of 1111 kidney transplants (1983-2001) with at least 6 months of graft function we identified 18 cases with CTG showing double contours of the GBM on light microscopy. To assess the risk factors, this group was compared with 739 patients with a stable function using multivariate Cox regression analysis. Paraffin sections of 11/18 biopsies were stained with polyclonal C4d antibodies and sera of 13/18 patients were tested for anti-HLA antibodies and antibodies reactive with total GBM isolates. Patients with chronic rejection and absence of glomerular lesions were used as controls. CTG was diagnosed at 7.5 ± 3.2 years. Panel reactive antibodies at time of transplantation, RR 1.23 (1.05-1.45) per 10% increase, and late acute rejection episodes, RR 7.58 (1.81-31.73) were independently associated with CTG. We found glomerular C4d deposits in a capillary pattern in 10/11 biopsies showing CTG and in 1/14 controls. Donor-specific anti-HLA antibodies were present in 6/13 patients and in 2/9 controls. Antibodies to GBM but not recognizing the Goodpasture antigen, were detected in 9/13 patients and in 2/10 controls. Immunoabsorption using a column with purified IgG obtained from a patient with 'anti-GBM' antibodies yielded an antigen (complex) of >250 kDa. All patients with GBM reactivity had antibodies to this purified antigen coated in ELISA. Pre-sensitization and late acute rejection episodes were the identified risk factors that support an immunological pathogenesis in CTG. Glomerular C4d deposits established in situ humoral rejection. Besides donor specific anti-HLA antibodies we found tissue specific antibodies directed to a single GBM antigen (complex). Therefore, chronic transplant glomerulopathy should be considered a manifestation of humoral rejection.

Abstract# 725

CANDESARTAN REDUCES EXPRESSION OF ANGIOTENSIN II AND TGF-BETA AND INCREASES DNA REPAIR ENZYME IN HUMAN CHRONIC ALLOGRAFT NEPHROPATHY.

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Chronic allograft nephropathy (CAN) is a chronic scarring process associated with increased tissue expression of angiotensin II (AII) and transforming growth factor beta-1 (TGF-β1), DNA damage (8-oxoG generation) and apoptosis (TUNEL, caspase-3). MYH is a DNA mismatch repair enzyme during oxidative damage in cells. We have started a randomized controlled trial comparing candesartan (16-32 mg qd) or amlodipine (5-10 mg qd) plus additional non-ACE inhibitor antihypertensive therapy in renal transplant patients with biopsy-proven CAN and dwindling renal function to see if chronic AII blockade will improve outcome. All patients had significant reduction (>50%) of cyclosporine and and received mycophenolate mofetil and low dose prednisone. Mean creatinine (CR) at the start of the study was 3.5 mg/dl. (2.8 years post-transplant). The results of the first and second biopsies before and after (13.6 months) randomized therapy is shown below. ***p<0.05 vs 1BX**.

		AII	AT-1R	AT-2R	TGFβ1	TGFβ1R
Candesartan	1BX	2.9±0.2	2.6±0.2	0.3±0.1	3.6±0.1	3.7±0.1
(n=16)	2BX	0.9±0.2*	2.0±0.1*	0.9±0.1*	2.3±0.2*	1.7±0.2*
Amlodipine	1BX	2.5±0.1	2.8±0.2	0.3±0.1	2.7±0.1	2.8±0.1
(n=14)	2 BX	3.2±0.2*	2.7±0.2	0.2±0.1	3.1±0.2*	3.2±0.1*
		8-oxoG	MYH	TUNEL	Caspase-3	
Creatinine (mg/dl)						
Candesartan	1 BX	2.9±0.2	2.3±0.2	1.9±0.1	3.1±0.2	3.4±0.2
	2 BX	0.9±0.2*	2.9±0.1*	0.8±0.1*	2.2±0.2*	2.9±0.2*
Amlodipine	1 BX	2.5±0.1	2.2±0.2	1.8±0.1	2.9±0.1	3.5±0.1
	2 BX	2.7±0.2	2.1±0.2	1.7±0.1	2.8±0.2	3.3±0.1

AII, TGFβ1 and their receptors (AT1R, AT2R an TGFβ1R) were primarily localized in glomeruli and renal tubular cells and were evident in renal tissue in patients without CAN (score: 0.2-0.4). Within one year of treatment, candesartan attenuated renal tissue expression of AII, TGFβ1 and their receptors. Candesartan reduced DNA damage and apoptosis and increased renal tissue expression of DNA repair enzyme. AII receptor blockade may help protect against scarring and loss of renal function in patients with CAN.

Abstract# 726

CHRONIC REJECTION AFTER KIDNEY TRANSPLANT - HAVE WE REACHED THE LIMIT OF IMMUNOLOGIC INTERVENTION?

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Background: Previously we have shown a decrease in the incidence of chronic rejection (CR) in kidney transplant recipient, which paralleled a decrease in the acute rejection (AR) rates. This suggested the primary importance of immunologic factors in long-term graft function. The last 5 years have seen AR rates drop to <10%, but has CR followed a similar trend? **Results:** Between Jan92 to Dec01, 1442 primary kidney transplants were performed (495 CADs, 947 LDs). Recipients were analyzed and compared in 2 groups: those transplanted between 1992-1996 (Era1, n=701), and those transplanted between 1997-2001 (Era2, n=741). Demographics for the 2 groups were similar, but the proportion of LD transplants was higher in the more recent era (68.1% vs. 63.0%, p=0.04). Mean donor age,peak PRA, and incidence of DGF did not differ significantly. The incidence of AR has significantly decreased in the recent era. At 6 months posttransplant, 31% for Era1 recipients vs. 8% for Era2 recipients (p=0.001). By 1 year posttransplant, 32% vs.12% (p=0.001). This, however, has not translated to a significant decrease in the incidence of chronic rejection. By 5 years posttransplant, 15% of Era1 recipients had biopsy-proven CR vs. 15% for Era 2 recipients (p=ns). A multivariate analysis was performmed to determine significant risk factors for CR in each era. The following variables were included: AR status, initial graft function (DGF vs not), donor source, donor age, recipient age, HLA mismatch, and immunosuppressive protocol. In Era 1 significant variables were acute rejection (RR=8.6, p<0.001), DGF (RR=3.9, p=0.001), cadaver donor source (RR=1.6, p=0.04), and recipient age (<50 vs. >50, RR=1.8, p=0.02). In Era 2 , AR was again the most significant variable (RR=8.1, p<0.001). Donor age also remained a significant variable (RR=3.1, p=0.0004). However, donor source, DGF, and recipient age were no longer significant. Also not significant were the individual immunosuppressive protocols (CsA vs FK, steroid avoidance vs. maintainence, MMF vs sirolimus). **Conclusions:** Non-immunologic factors such as DGF, donor source, and recipient age seem to have less impact on risk for CR in the current era; AR remains the most significant risk factor. While AR rates have decreased significantly in the last decade, the incidence of CR has not followed suit. One possible explanation is that AR episodes in the current era have a more significant long-term impact.

Abstract# 727

SUPPRESSION OF CD8 T CELL MEMORY RECALL BY CD4+CD25+ REGULATORY T CELLS.

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Introduction: Regulatory T cells (Treg) play an important role in the maintenance of immune tolerance by suppressing naive T cells, but it is not known whether they also influence memory cell function. Since memory T cells hinder transplantation tolerance, we investigated whether CD4+CD25+ Treg cells suppress allograft rejection mediated by CD8 memory T cells. Methods: CD4+CD25+ Treg cells were isolated by magnetic cell separation and cell sorting from either naive or allostimulated B6 (H2b) mice (mice immunized 10 weeks earlier with BALB/c splenocytes). In both cases, Treg cells had a CD44low phenotype. CD8+CD44high memory T cells were purified from B6 mice 10 weeks after allogeneic stimulation with BALB/c splenocytes. The cell populations were then adoptively transferred either singly or in combination into splenectomized alymphoplastic (aly/aly-spleen, H2b) recipients of BALB/c skin transplants. aly/aly-spleen mice fail to mount a primary alloimmune response but reject allografts promptly if they are adoptively transferred with memory T cells, thus serving as an in vivo model of memory T cell function. Results: The transfer of naive or antigen-induced Treg cells alone did not lead to allograft rejection while the transfer of CD8+CD44high memory T cells alone caused prompt skin rejection (MST = 22 days, n=6). The co-transfer of antigen-induced Treg cells and CD8 memory T cells delayed allograft rejection significantly (MST = 60 days, n = 6). In contrast, naive Treg cells failed to delay graft rejection mediated by CD8 memory T cells (MST = 24 days, n=6). The suppressive function of Treg cells was dependent on CD30 as the transfer of CD30-/- Treg cells failed to significantly inhibit rejection mediated by CD8 memory T cells (MST = 31 days, n = 7). This finding was confirmed by treating skin recipients with anti-CD30L following co-transfer of Treg and memory cells (MST = 35 days, n =6). Finally, we demonstrated that both CD4+CD25+Treg and CD8 memory T cells infiltrate the allografts but that CD8 memory T cell apoptosis in the graft is significantly increased in the presence of Treg (19% vs. 7% if memory T cells were transferred alone). Increased CD8 memory T cell apoptosis was CD30-dependent. Treg cells did not influence the proliferation of CD8 memory T cells. Conclusion: Antigen-induced but not naive Treg cells suppress CD8 memory T cell recall. This suppressive function is largely dependent on CD30 expression on Treg cells, suggesting that CD30 serves as a costimulatory pathway that leads to immune regulation.

Abstract# 728

HIGH-RESOLUTION REAL-TIME IMAGING OF T CELL INTERACTIONS WITH DENDRITIC CELLS IN INTACT LYMPHOID TISSUE. Ronald N. Germain,[1] Sabine Stoll,[1] Jérôme Delon,[1] Tilmann Brotz,[2] Owen Schwarz.[3] *[1]Laboratory of Immunology, NIAID, NIH, Bethesda, MD; [2]Experimental Immunology Branch, NCI, NIH, Bethesda, MD; [3]Confocal Imaging Facility, NIAID, NIH, Bethesda, MD.*

Purpose: To provide a real-time, high-resolution view of the cell interaction events involved in T cell adaptive immunity. Methods: A new method has been developed for imaging the interaction of dendritic cells and T cells within lymphoid tissues. Bone marrow-derived dendritic cells are dye labeled and then pulsed with specific antigenic peptides before adoptive transfer s.c. into mice to permit migration via lymphatics into draining lymph nodes. Naïve T cells from TCR transgenic mice are labeled with a different color dye and transferred i.v. into the same animals. After various periods of time, the draining lymph node is either removed for 4D (volume x time) visualization over as many as 15 hours, or viewed directly in the intact animals using multiphoton methods. Results: Some data suggest that T cells require a stable physical association with one DC lasting several hours to commit to cell division, whereas other data suggest that T cells add up short (10-15 minute) bursts of signaling occurring during transient contact with a series of presenting cells, until the cell responds. These two views have come from experiments performed in vitro in either dispersed liquid culture or collagen gel matrices. Using our new microscopic methods, we observed that the vast majority of T cells show monogamous adherence to a single antigen-bearing DC throughout an observation period of up to 15 hrs, eventually resulting in T cell activation and cell division. This prolonged contact was paralleled by the formation of a visible synapse, visualized as exclusion of CD43-GFP from the region of T cell-DC contact. These data indicate that T cell activation follows long-term association of lymphocytes with individual antigen-bearing DC rather than many brief encounters with several presenting cells. They also reveal the power of this imaging method for understanding T cell behavior in a physiological setting. This technique has also been used to visualize interaction of CD8 T cells with DC, and, using bone marrow retrovirus transduction or knock-in mice, will allow observation of the movement / interaction of multiple membrane proteins or signaling molecules, the tracking of early effector cell development, the migration of helper T cells to B cell zones for initiation of germinal center formation, and possibly the visualization of effector cell activity in tissue sites including grafts.

Abstract# 729

PROLONGATION OF HEART ALLOGRAFT SURVIVAL BY EXOSOMES DERIVED FROM DONOR DENDRITIC CELLS IS CHARACTERIZED BY A DECREASE IN ANTI-DONOR RESPONSES. Hélène Pêche,[1] Michèle Heslan,[1] Claire Usal,[1] Sébastian Amigorena,[2] Maria Cristina Cuturi.[1] *[1]ITERT-INSERM U437, Nantes, France; [2]INSERM U520-Institut Curie, Paris, France.*

Introduction : Exosomes are antigen presenting vesicles, which express functional Major Histocompatibility Complex (MHC) class I and class II, and T-cell costimulatory molecules (L. Zitvogel 1998, Nat Med). We have shown that allograft rejection can be delayed by priming allograft recipients with exosomes derived from donor Bone Marrow Dendritic Cells (BMDC). **Materials and methods :** Exosomes were purified from the culture supernatants of BMDC as described previously (Zitvogel 1998, Nat Med). Rat recipients of class I and II histoincompatible heart allografts were treated before transplantation (days –14 and –7) with 10 µg of exosomes derived from donor or host BMDC and allograft survival was monitored. **Results :** We show here that 10 µg of donor type exosomes modulated allograft rejection and induced allograft tolerance in some recipients (91±105 days n=7, ie: >200 days n=2 and 33±34 days n=5, compared to untreated recipients : 6.7±2 days, n=8). Furthermore, this effect was donor specific since syngeneic exosomes did not significantly prolong allograft survival. In order to define the mechanisms involved in allograft prolongation, the anti-donor responses were examined 5 days after transplantation. Compared to untreated recipients, a lower proliferation against donor antigens of CD4+ T cells from exosome treated rats was observed. We also observed significant decrease in the total amount of graft infiltrating cells (GIC) (44% decrease compared to untreated recipients GIC). This was accompanied by a general decrease in inflammatory cytokines and a significant reduction in the IFNg mRNA expression in the grafted hearts of exosome treated rats. **Conclusion :** These results suggested that the presentation of donor MHC antigens by donor-type exosomes before transplantation is a good way of manipulating allograft responses. It has been shown that exosomes can, in vivo, induce an antigen specific immune response when they are pulsed with antigen and that this effect is indirect and mediated by dendritic cells (Thery et al Nat immunol 2002). However, we described here a direct effect in vivo of non antigen pulsed exosomes on the modulation of immune responses, suggesting that like dendritic cells, exosomes can either stimulate or regulate antigen specific immune responses. We also showed that this effect is mediated by a decrease in the anti-donor response that could be due to the induction of alloreactive CD4+ T cell anergy or deletion.

Abstract# 730

INDUCTION OF ACTIVATED T CELL APOPTOSIS BY LIVER DERIVED B220$^+$ DC DEPENDS ON EXPRESSION OF LYMPHOTOXIN (LT) β . Xiaoyan Liang, Shiguang Qian, Lianfu Wang, John J. Fung, Lina Lu. *[1]Thomas E. Starzl Transplantation, University of Pittsburgh, Pittsburgh, PA.*

Mouse liver allografts are spontaneously accepted, which is associated with overwhelming activated T cell apoptotic death. The underlying mechanisms are unclear. We have recently identified novel liver-derived B220$^+$ lymphoid DC propagated from mouse liver in the presence of IL-3 and CD40 ligand that were phenotypically mature, but poor stimulators of ^{3}[H]TdR incorporation by allogeneic spleen T cells. Interestingly, addition of caspase inhibitor peptide zVAD-fmk into the MLR culture restored T cell proliferation, indicating that the low thymidine uptake results from extensive activated T cell apoptosis. TUNEL staining revealed that ~30% of activated T cells underwent apoptosis following culture with allogeneic liver B220$^+$ DC, while only <10% were TUNEL positive after culture with bone marrow (BM)-derived myeloid DC. Apoptotic cells were evenly distributed in CD4$^+$ and CD8$^+$ populations. Administration of B10 (H2^b) liver B220$^+$ DC (2 x 10^6) dramatically prolonged survival of B10, but not third party (BALB/c; H2^d) heart allografts in C3H (H2^k) recipients (MST 37 days vs. 10 days in PBS controls, and 5 days in the group treated with BM myeloid DC). A higher incidence of apoptotic cells was detected in draining LN and spleen. These data indicate that the *in vivo* and *in vitro* tolerogenic activity of liver B220$^+$ DC is associated with induction of activated T cell apoptosis. Interestingly, supernatants of B220$^+$ DC/T cell cultures induced more apoptosis in activated T cells, than those from myeloid DC/T cell cultures (27% vs 13%). T cell apoptosis induced by *gld* (FasL deficient) B220$^+$ DC was slightly reduced (30%), indicating a partial role of Fas ligation in apoptosis. However, liver B220$^+$ DC induced similar apoptosis in TNFR (either p55 or p75) deficient T cells, suggesting that TNF and lymphotoxin (LT) α may not be important ligands. Encouraged by results of RNase protection assay that expression of LTβ mRNA was extraordinary high compared with BM-derived myeloid DC, we proposed a role of LTβ in induction of T cell apoptosis, and found that liver B220$^+$ DC from LTβ$^{-/-}$ mice were poor inducers of allogeneic T cell apoptosis (only 4.38%) and stimulated profound T cell proliferative responses (cpm 61.1 x 10^3 vs 3.60 x 10^3 in wild controls). These data suggest that apoptosis inducing capacity of liver B220$^+$ DC is largely dependent on expression of LTβ.

Abstract# 731

BLOCKADE OF BOTH STAT4 AND NF-kB PATHWAYS POTENTIATES TOLEROGENICITY OF DENDRITIC CELLS. Shi-Ho Wang, Lina Lu, Lianfu Wang, John J. Fung, Shiguang Qian. *[1]Thomas E. Starzl Transplantation, University of Pittsburgh, Pittsburgh, PA.*

Administration of immature dendritic cells (DC) inhibits T cell responses and prolongs allograft survival. The tolerogenic effect is however, limited due to late activation of DC by interaction with recipient allogeneic T cells. Decoy oligodeoxyribomucleotides (ODN) specific for NF-kB prevent DC maturation in response to cytokines, but do not block maturation in response to allo-T cell stimulation. We investigated immune responses of DC engineered with NF-κB ODN and sirolimus both *in vivo* and *in vitro*, since sirolimus has been shown to block Stat4 phosphorylation, a pivotal molecule mediating signaling DC activation. B10 (H2^b) mouse bone marrow-derived DC propagated in GM-CSH and IL-4 were transduced with NF-κB ODN (10 µM). Sirolimus (20 ng/ml) was added on day 3 and extensively washed out at the end of culture. DC phenotype was evaluated by FACS. The allostimulatory function was determined by MLR and CTL assays *in vitro*. Cytokine profiles were analyzed by ELISA and RNase protection assay. For *in vivo* evaluation, DC were injected into C3H (H2^k) recipients 7 days prior to a B10 cardiac transplantation. Grafts were inspected for cytokine expression by RNase protection assay, and apoptosis of graft infiltrating lymphocytes (GIC) by TUNEL staining. Both DNA binding capacity of NF-κB (assayed by gel shift) and IL-12-stimulated Stat4 phosphorylation (determine by Western blotting) were completely blocked in sirolimus/ODN DC. Expression of CD40, CD80 and CD86 (not MHC class I, II and CD11c) was markedly inhibited by ODN alone. This inhibition appeared resistant to LPS activation, but not resistant to allo-T cell stimulation. Importantly, the inhibition of costimulatory molecule expression on sirolimus/ODN DC maintained despite allo-T cell stimulation, indicating the stable immature status. The proliferative responses of C3H spleen T cells were significantly inhibited by stimulation with B10 ODN DC (cpm 37316±2149), and more profound inhibition was achieved by stimulation with sirolimus/ODN DC (14588±1561, both $p<0.05$, compared with 73761±4265 of control DC). Donor-specific CTL activity (4h ^{51}Cr release) was also suppressed in ODN group, and further suppressed by sirolimus/ODN DC. The supernatant levels of IL-2 and IFN-γ were correlated with this suppressive pattern in MLR and CTL assays. Injection of C3H mice with sirolimus/ODN DC significantly prolonged B10 cardiac allograft survival, associated with increased apoptosis of GIC. In conclusion, DC engineered to inhibit both Stat4 and NF-κB pathways enhance their tolerogenicity.

Abstract# 732

MDR1 P-GLYCOPROTEIN IS A DENDRITIC CELL/ MACROPHAGE DIFFERENTIATION SWITCH IN ANTIGEN PRESENTING CELL MATURATION. Shona S. Pendse,[1,2] Sam Behjati,[1,2] Mohamed H. Sayegh,[1,2] Markus H. Frank.[1,2] *[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Nephrology, Children's Hospital, Harvard Medical School, Boston, MA.*

We have shown that in vitro blockade of human MDR1 P-glycoprotein (P-gp) results in inhibition of alloantigen-dependent T cell activation via both T cell and antigen presenting cell (APC)-dependent pathways, through blockade of alloimmune IFN-γ and IL-12 production. Here we demonstrate that P-gp, via an IL-12-dependent pathway, functions as a critical switch determining monocyte differentiation into the dendritic cell vs. macrophage lineage during APC maturation and resultant Th1 vs. Th2 responses. Purified CD14+ monocytes cultured in the presence of IL-4 and GM-CSF, in the absence of P-gp blockade, differentiated into APCs of the immature dendritic cell phenotype, as evidenced by loss of CD14 expression (4% vs. 99% positive cells compared to unstimulated controls), de novo CD1a expression (60% vs. 0%), and augmented CD80 (75% vs. 37%) and CD86 (27% vs. 5%) expression at 7 days (flow cytometry). In contrast, addition of the specific pharmacologic P-gp antagonist PSC833 (5 μM) to IL-4/GM-CSF- stimulated cultures, resulted in complete blockade of CD1a induction (0% positivity) and down-regulation of CD80 expression (8% vs. 75%), but specific enhancement of CD86 expression (71% vs. 27%) and de novo CD68 expression on 95% of cells, consistent with a macrophage phenotype. These strongly adherent macrophage-type APCs, when harvested, washed and irradiated (1750 rad) on day 7, stimulated 3-day allogeneic PBMC proliferation 75% less efficiently than untreated controls ($p<0.05$). This finding correlated with 97% inhibition of the relative abundance of IFN-γ-producing Th1 cells ($p<0.05$) (Elispot), but preservation of the frequency of IL-5-secreting Th2 cells, demonstrating a marked Th1 to Th2 switch of the alloimmune response. Addition of exogenous IL-12, a putative P-gp transport substrate, during the 7-day APC maturation culture significantly restored the functional capacity of PSC833-treated APCs to effect alloimmune PBMC proliferation ($p<0.05$), demonstrating that P-gp functions as a differentiation switch in APC maturation at least in part via its role in IL-12 regulation. Our findings define a novel role for P-gp as a switch in dendritic cell vs. macrophage differentiation and resultant Th1 vs. Th2 alloimmune responses. Furthermore, these findings suggest that P-gp may be involved in determining the balance of cellular and humoral immunity, and that P-gp modulation may thus have potential future therapeutic applicabilities in allotransplantation, autoimmunity and infectious disease.

Abstract# 733

BLOCKADE OF ALLOREACTIVE RESPONSES USING SPLENOCYES DECORATED WITH A NOVEL FORM OF FASL PROTEIN FOR IMMUNOMODULATION. Narendra P. Singh,[1] Esma S. Yolcu,[1] Nadir Askenasy,[2] Haval Shirwan.[1] *[1]Institute for Cellular Therapeutics and Microbiology and Immunology, University of Louisville, Louisville, KY; [2]Laboratory of Experimental Hematology, Schneider Children's Medical Center of Israel, Petach, Tikva, Israel.*

FasL-induced apoptosis is important in tolerance to self-antigens. This feature of FasL has been exploited for immunomodulation to prevent allograft rejection with conflicting results. This apparent discrepancy may arise from the complex post-translational regulation of FasL and the diverse functions performed by different forms of FasL. Although FasL is synthesized as a type II membrane protein, it is cleaved from the cell surface by metalloproteinases. Apoptosis is mediated by the membraneous form of FasL whereas the soluble form is ineffective in apoptosis and may serve as an anti-apoptotic factor by competing with the membraneous form for Fas binding. Also, soluble FasL serves as a chemotactic factor for neutrophils and may perpetuate graft rejection by recruiting neutrophils into the graft. Therefore, generation of a FasL molecule with potent apoptotic activity that lacks the anti-apoptotic and chemotactic functions may prove an effective strategy for immunomodulation. Toward this end, we generated two forms of FasL molecule using an insect expression system; a chimeric form consisting of the extracellular portion of rat FasL fused with core streptavidin (SA-FasL) and a soluble form containing the extracellular domains of FasL (sFasL). SA-FasL formed tetramers and higher structures with potent apoptotic activity on Fas expressing cell lines and alloantigen-activated lymphocytes. SA-FasL was displayed on the surface of biotinylated splenocytes for an extended period of time in vitro and in vivo. Immunization of PVG.1U rats with allogeneic ACI splenocytes decorated with SA-FasL resulted in complete systemic blockade of alloreactive responses without any detectable effect on reactivity to third party antigens. Also, splenocytes decorated with SA-FasL blocked secondary alloreactive responses in rats pre-sensitized with donor antigens. Alloinhibition was dependent on a functional Fas/FasL pathway as demonstrated by using Fas-deficient mice. This novel method of rapid and durable cell-surface display of chimeric proteins offers an entirely new means of intervention in the areas of autoimmunity and transplantation. This approach possesses the simplicity, safety, and efficacy required to make it a clinically relevant alternative to DNA-based gene therapy for the treatment of a broad spectrum of immune disorders. Supported in parts by grants to from JDRF (1-2001-328), NIH (R21 DK61333 and R01 AI47864), and an AHA Postdoctoral Fellowship (0120396B).

Abstract# 734

RESTRICTION OF ALLORESPONSIVE T CELL RECEPTOR REPERTOIRE IS INDUCED BY INDIRECT PRESENTATION OF ALLOCHIMERIC CLASS I MHC SEQUENCES. Yuan Zhai, Natalya V. Semiletova, Xiu-Da Shen, Kaushik Mukherjee, Ronald W. Busuttil, Jerzy W. Weglinski, Rafik M. Ghobrial. *[1]Surgery, UCLA Medical School, Los Angeles, CA.*

Background: Allochimeric class I MHC molecules, that contain donor-type ($RT1A^u$) immunogenic epitopes displayed on recipient-type ($RT1A^a$) sequences, were shown to induce transplantation tolerance of WF ($RT1^u$) heart allografts in ACI ($RT1^a$) recipients following peri-or post-trsansplant administration, in the presence of subtherapeutic CsA. Our previous studies demonstrated intract allograft architecture, absence of chronic rejection and minimal T cell infiltration. The current study addressed the mechanisms by which allochimeric sequences may affect responding T cells. **Methods:** Splenocytes and graft-infiltrating-lymphocytes (GILs) were isolated from cardiac allografts of either allochimeric tolerant (>100 days) or rejecting (day 9) recipients. T cell receptor (TCR) Vβ chain spectrotyping was performed by first 22 Vβ-specific RT-PCR followed by run-off reactions with a fluorescence-labeled interanl constant region primer. The products were separated in ABI capillary sequencer and visulized with Genotyping software. **Results:** Immunohistological examination of allochimeric-induced long-term (>100 days) tolerant WF cardiac allografts in ACI recipients revealed absence of ICAM-1, monocytes, CD8α+ cells, marked reduction of B cells and presence of only clusters of CD4+ T cells. This was in sharp contrast to rejecting allografts that demonstrated abundance of monocytes, B cells, CD8α+ but absence of CD4+ cells. Unlike naive T cell repertoires with Gaussian distributions in all 22 Vβ spectrotyping profiles, GILs from tolerant recipients had alterred Vβ spectrotyping profiles, with dominant size peaks representing preferential clonal expansions, in limited numbers of Vβ gene families (Vβ 7, 15). This repertoire change was allograft-specific, since it was not detected in splenocytes or LN cells from the same tolerant animal. In contrast, GILs isolated at day 9 from rejecting recipients (untreated, or treated with unmuted donor-type class I molecule) demonstrated unrestricted clonal expansions in all Vb gene families. Clonally expanded Vβ genes in tolerant GILs are currently under Jβ-specific immunoscope analysis to identify TCR sequences. **Conclusions:** Allochimeric MHC class I sequences may induce tolerance, in part, by selecting the alloresponsive T cell repertoire. Indirect presentation of allochimeric class I molecules induces a unique tolerant state characterized by restricted T cell clonal expansion.

Abstract# 735

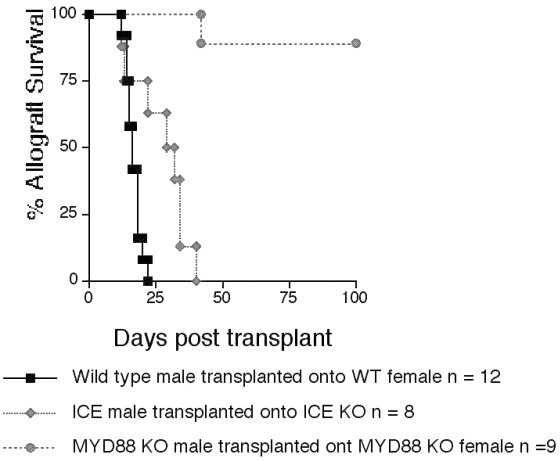
IMPACT OF CMV INFECTION ON IMMUNE REGULATION IN ACCEPTED MURINE CARDIAC ALLOGRAFTS. Alice A. Bickerstaff,[1] Charles H. Cook,[1] Charles G. Orosz.[1] *[1]Surgery/Transplant, The Ohio State University, Columbus, OH.*

C57BL/6 mice accept DBA/2 cardiac allografts when treaed with gallium nitrate (GN). This is associated with the development of donor-reactive regulatory T cells, which produce the anti-inflammatory cytokines TGFβ and IL10 (*Transplantation* 69: 1517, 2000). Thus, allograft acceptor mice fail to make DTH responses to donor alloantigens unless TGFβ or IL10 are serologically neutralized at the challenge site. In tissues, these cytokines promote linked antigen non-responsiveness. For example, tetanus-toxoid (TT) pre-sensitized allograft acceptor mice respond in DTH assays to challenge with TT, but not if the TT is mixed with donor alloantigens. In general, immune regulation of allogeneic responses can extend to third party antigens when they are co-localized at the same tissue site, leading to the process of "infectious tolerance" (*Science* 259, 974,1993). This regulatory T cell activity is also operative within allografts, leading to the possibility that infectious agents which find their way into accepted allografts may actually be protected from immune destruction by the local anti-inflammatory responses to donor alloantigens. To test this hypothesis, 60 day allograft acceptor mice were infected by IP injection with CMV (2x10E4 pfu Smith Strain murine CMV). After 30 days, they were tested to determine if infectious virus was present in the allograft, whether the virus had induced anti-viral immunity, and whether the virus had influenced the immune responses to donor alloantigens. All cardiac allografts were infected, and displayed DNA and mRNA for the late viral antigen glycoprotein B of CMV, as detected by RT-PCR. The CMV infected mice generated anti-CMV T cell immunity, which was manifest as strong, positive CMV-reactive DTH responses. Further, the infected allograft acceptors retained donor-reactive regulatory T cell reactivity, i.e., they displayed no donor-reactive DTH responses, but would do so if TGFβ was serologically neutralized at the challenge site. Further, they lost DTH responses to CMV when it was mixed with donor alloantigens (linked antigen non-responsiveness). Notably, these mice did <u>not</u> display a CMV-induced compromise of allograft survival or of donor-reactive regulatory T cell function, nor did they develop "infectious tolerance" toward CMV. In general, they appeared to concurrently manage two different types of immune responses to CMV and graft alloantigens in an independent manner, despite the fact that both antigenic systems were co-localized within the accepted allograft.

Abstract# 736

TOLL-LIKE RECEPTOR SIGNALING IS REQUIRED FOR ACUTE ALLOGRAFT REJECTION. Daniel R. Goldstein,[1] Fadi G. Lakkis,[1] Shizuo Akira,[2] Bethany M. Tesar.[1] *[1]Internal Medicine, Yale University, New Haven, CT; [2]Host Defense, Osaka Universitty, Osaka, Japan.*

Introduction Toll like receptors (TLR) are germ line encoded receptors on dendritic cells (DC) that are critical in innate immune recognition of microbial pathogens. However, their role in transplantation is unknown. As TLRs signal via the adaptor protein, MyD88, we investigated the role of TLRs in allograft rejection using MyD88 KO mice (B6 background). We also utilized caspase-1 deficient recipients (ICE KO, B6) to control for MyD88 signaling mediated by IL-1 and IL-18 (IL-1 and 18 are converted to active forms via caspase 1). Specifically, we utilized an HY mismatched skin allograft model with either donor, recipient or both deficient in MyD88. **Results** 8/9 MyD88 KO female recipients were unable to reject MyD88 KO male skin (see figure), MST>100days vs. wild type recipients (WT male→WT female) 16 days, p=0.0001. ICE KO had a modest prolongation in allograft survival (MST =32 days, p=0.01 vs. WT, although significantly inferior to MyD88 KO, p =0.0001).



The presence of intact MyD88 signaling in either the allograft (WT male→MyD88 K0 female) or in the recipient (MyD88 KO male → WT female) reconstituted rejection (both MST = 44 days, p =0.001 vs. MyD88 KO male→MyD88 KO female). Analysis of allograft draining lymph nodes revealed reduced number of mature DC in MyD88 KO recipients vs. WT recipients (6.4 x10^5 MyD88 KO vs. 4.33 x 10^6 WT p=0.0001). This lead to reduced number of anti graft CD8+ T cells quantified by HY tetramers (2.0% MyD88 KO vs. 15.0% WT, p =0.01). Finally, TH1 immunity was reduced in draining lymph nodes of MyD88 KO vs.WT (interferon γ gene expression, by real time PCR: x5 fold increase in MyD88 KO vs. 20 fold increase WT, p=0.0001). **Conclusion** By controlling DC maturation and TH1 immunity, MyD88 signaling is critically important for rejection of HY incompatible skin allografts. This shows that TLRs can be activated by transplantation and not solely by infections and are critical for initiation of an alloimmune response.

Abstract# 737

EXPRESSION PROFILING IDENTIFIES PROGNOSTIC AND THERAPEUTIC GENE MARKERS FOR ACUTE RENAL ALLOGRAFT REJECTION. Minnie M. Sarwal,[1] Mei-Sze Chua,[1] Xin Chen,[2] Szu-Chuan Hsieh,[1] Oscar Salvatierra,[3] Brown Patrick.[2] *[1]Pediatrics; [2]Biochemistry; [3]Surgery, Stanford University, Stanford, CA.*

AIM: Heterogeneity in acute renal allograft rejection is difficult to predict by conventional clinical and pathological information. We recently identified at least 3 molecularly distinct sub-groups (AR-I, AR-II and AR-III) of acute rejection by DNA microarray analysis of 67 stable and dysfunctional renal allograft biopsies. We hypothesized that differentially regulated genes in AR groups would distinguish molecular categories of acute rejection and serve as correlates of graft function recovery, graft loss and glucocorticoid resistance. METHODS: Gene expression profiles of tissue biopsies from 67 grafts (n=21 with acute rejection) were obtained by hybridization to DNA microarrays (12, 440 human genes) and unsupervised hierarchical clustering. We used *Significance Analysis of Microarrays* and *R* to identify genes significantly differentially regulated across rejection groups, and *Kaplan-Meier survival* analysis to correlate gene expression to clinical parameters such as graft function recovery, graft loss and glucocorticoid resistance. We validated these results by immunohistochemical staining for significant genes such as CD20, PCNA, CD4 and CD8. RESULTS: AR-1 was a T and B cell rich rejection, mediated largely by INFg. AR-II utilized cytokines/ complement and AR-III, rapid cellular turnover. High expression of INFg, B cell genes and CD20 correlated with graft loss (Kaplan-Meier survival, p=0.0002). Additionally, CD20+ biopsy density (>250 cells/hpf) strongly correlated with graft loss in the 21 acute rejection samples studied (p=0.0009). Intra-graft CD20+ density correlated with glucocorticoid resistance in AR-I. Using a retrospective independant test series of 31 acute rejection biopsies confirmed that intra-graft CD20+ aggregates of > 250 cells/hpf correlated again with glucocorticoid-resistance (p=0.0001). Additionally, Kaplan-Meier analysis showed graft loss correlated significantly with genes involved in cell turnover, apoptosis, leukocyte trafficking and migration. CONCLUSIONS: Using DNA microarrays and survival analyses, we have identified several genes which may have valuable diagnostic (for glucocorticoid resistance) and prognostic (for graft loss) indications. Of particular interest is a robust B cell signature, which correlated strongly with glucocorticoid resistance and graft loss in acute rejection patients. Leukocyte trafficking, apoptpsis and cell cycling have been identified as important pathways assisting recovery of the graft in acute rejection.

Abstract# 738

IMMUNE PROFILING: MOLECULAR MONITORING OF INTRAGRAFT AND PERIPHERAL IMMUNE RESPONSES IN RENAL ALLOTRANSPLANTATION USING A NOVEL 384-WELL MICRO FLUIDIC CARD FOR GENE EXPRESSION ANALYSIS BASED ON REAL TIME QUANTITATIVE PCR (TAQMAN® REAGENTS). Steven Hoffmann,[1] C. Ted Rigl,[2] Robert Kampen,[1] Shashi Amur,[2] Jenny Park,[1] Patrick Blair,[1] Allan Kirk.[1] *[1]Transplantation and Autoimmunity Branch, NIDDK, Bethesda, MD; [2]Applied Biosystems, Foster City, CA.*

Comprehensive RT-PCR gene expression studies must incorporate aspects of gene mining, transcript profiling and quantification. To better define the transcriptional events following allograft transplantation, we examined gene transcripts derived from human renal allograft protocol biopsies and peripheral blood mononuclear cells (PBMC). We employed the use of novel 384-well Micro Fluidic Cards (Applied Biosystems) and RT-PCR which allowed for rapid and reproducible quantification of 24, and ultimately 96, gene targets, in quadruplicate, from <100ng of template. Individual biopsies and cDNA pools from 52 patient biopsies were grouped by Banff criteria as: post-reperfusion (IRI), stable allograft (SF), subclinical (SCR) or clinical acute rejection (ACR) and normalized to normal kidneys. PBMC expression levels were compared to intragraft transcripts. Sample isolation, integrity and reproducibility were assessed via serial dilutions [neat(100ng), 1:4, 1:16, 1:64, 1:256, 1:1024] of cDNA samples and established by standard PCR for select target genes using Micro Fluidic Cards and individual single well PCR runs. Inter- and intra-assay variation was minimized all targets demonstrated amplification efficiencies >90% for inclusion in this study. Significant elevation of cell stress and inflammatory transcripts (IL6, IL8, TNFα, ECE) predominate IRI biopsies. Although biopsies were qualified as SF, continual elevation of TNFα, TGFβ and C3 may be involved in early chronic changes. SCR biopsies demonstrate significant elevation of APC (CD80/86, CD154), cytotoxic (perforin, Granulysin) and T cell activation (CD3, CD25, PD1, Tbet) transcripts compared to SF. Enhanced elevation in T cell activation (CD3, CD28, CD152, Rantes, PD1, Gr B, FasL) was exhibited during ACR. PBMC, obtained during ACR, did exhibit elevated levels of cytotoxic (perforin, Gr B, FasL) transcripts. However, APC activation and inflammatory transcripts in SCR or SF did not mirror levels seen in intragraft biopsies. Transcriptional profiles obtained with this novel methodology provide ultra-sensitive molecular diagnostics beyond routine histology. Indeed, these studies underscore the necessity for a sensitive and precise quantitative measurement of the allograft microenvironment to correctly diagnose renal dysfunction to ultimately individualize patient immunotherapy.

Abstract# 739

DOCK2, A HAEMATOPOIETIC CELL-SPECIFIC CDM FAMILY PROTEIN IS REQUIRED FOR TRANSPLANTATION REJECTION. Yoshinori Fukui,[1] Fan Pan,[2] Carmen Wynn,[2] Ogert Fisniku,[2] Mei-shiang Jang,[2] Masakazu Kobayashi,[2] Hongsi Jiang.[2] *[1]Department of Immunobiology and Neuroscience, Kyushu University, Kyushu, Japan; [2]Basic Science, Fujisawa Research Institute of America, Evanston, IL.*

DOCK2, a CDM family protein, is specially expressed in haematopoietic cells. Our previous study demonstrated that migration of lymphocytes, but not other cell types, is severely disrupted in DOCK2 –deficient mice. To further characterize the requirment and effect of DOCK2 during transplant rejection, we examined transplant rejection response of mice completely deficient of DOCK2. **M.M:** Heterotopic cardiac allografts were transplanted using both C57BL/6J (B6) DOCK2 -/- and DOCK2 +/- deficient mice as recipients, and wide-type (WT) Balb/c mice as donors, and vice versa. B6 rag-1 -/- recipients with WT Balb/c heart grafts were used as an experimental control. Graft survival was determined by abdominal palpation and confirmed by histology. **Results:** BALB/c cardiac graft survival was significantly prolonged in B6 DOCK2 -/- recipients at the median survival time (MST) of 57 days, when compared to WT (MST: 11 days) and B6 DOCK2 +/- (MST: 7 days) controls. 4 of 7 B6 DOCK2 -/- recipients with Balb/c cardiac allograft survived more than 50 days. There was no significant prolongation of allograft survival when B6 DOCK2 -/-cardiac grafts were used as donor. As an experimental control, B6 Rag1-/- recipients accepted Balb/c cardiac grafts indefinitely. **Conclusion:** DOCK2 plays an important role in organ transplantation rejection. Strategies to regulate CDM family protein and its related pathway molecules will be an important and exciting challenge in the area of transplantation immunosuppression.

Abstract# 740

THE T CELL CAN LINK EARLY ISCHEMIC INJURY AND LONG TERM GRAFT DYSFUNCTION. Melissa J. Burne-Taney,[1] Naoko Yokota,[1] Hamid Rabb.[1] *[1]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.*

Renal ischemia reperfusion injury (IRI) is associated with delayed graft function, increased acute rejection and decreased long term allograft function. The mechanisms linking these are unknown. The T cell has recently been demonstrated to be an important modulator of renal IRI *(J Clin Invest 108:1283, 2001).* We hypothesized that once the T cell is activated during IRI it can harbor long term "memory", and that this "memory" can lead to long term changes that predispose to allograft fibrosis. C57BL/6 mice underwent 60 min warm unilateral IRI or sham surgery and were maintained for 6 weeks. Serum creatinine (SCr) was measured and kidneys were analyzed for tubular injury, myeloperoxidase (MPO) levels for neutrophil and macrophage recruitment, and CD4 infiltration. Spleens from these mice were analyzed for CD4 and CD8 levels and intracellular cytokine expression. SCr rose immediately following IRI compared to sham mice but were similar at 6 weeks postischemia. Histological analysis revealed extensive tubular injury consisting of a pronounced loss of tubular architecture and infiltration of inflammatory cells at 6 w postischemia. Sham and contralateral kidneys revealed no tubular injury. MPO levels where significantly increased at 6 weeks postischemia compared to sham and contralateral kidneys. CD4 infiltration was also increased in IRI kidneys. Splenic CD4 and CD8 numbers were similar between groups. However, intracellular cytokine staining revealed a significant increase in T cell IFN-γ in IRI spleens compared to sham spleens. To determine if this ischemic memory had functional and structural consequences, we adoptively transferred lymphocytes from long term IRI animals and sham mice to normal mice. Recipient mice were then maintained for 6 weeks. SCr rose significantly in normal mice by 2 weeks after transfer of IRI lymphocytes compared to mice receiving sham lymphocytes. Changes in tubular histology, evident by fibrosis, were observed in mice receiving IRI lymphocytes. Mice receiving sham lymphocytes showed no change in histology. These data demonstrate that renal IRI leads to long term phenotypic changes in extra-renal T cells. A "memory" for injury in T cells results that can produce kidney fibrosis. Linking the T cell between early ischemic events and long term scarring is a novel mechanism by which "non-immune" and "immune" systems interplay in determining graft outcome.

Abstract# 741

ACTIVATION OF FOCAL ADHESION KINASE (FAK) IN CHRONIC ALLOGRAFT NEPHROPATHY (CAN). Xaio-Ling Jiang,[1] Frank Thomas,[1] Judy Thomas.[1] *[1]Surgery, Univ. of Ala., Birmingham, AL.*

CAN is the largest cause of allograft failure. A canonical feature of CAN is a progressive increase in extracellular matrix (ECM) The signaling mechanisms and molecular pathology of CAN are poorly defined. FAK is a major signaling kinase in assembly and proliferation of ECM and is central to the transmembrane integrin signaling between the cell cytoskeleton and the ECM. We studied a well-characterized model of CAN in rhesus kidneys 2 — 4 years post transplant. Five animals had no clinical or histopathology features of CAN while 3 animals had CAN. A total of 31 histopathology sections. Were analyzed in these kidneys at similar times post transplant (1300 plus days, (p value < 0.05) Using immunohistochemistry , we compared FAK activation and prominence of ECM proteins in the two groups. Activation of FAK was assessed in CAN using a MC antibody that recognized phosphorylation of the tyrosine residue at position 861 (phospho-FAK861) indicative of FAK activity Immunohistochemical analysis of positive staining scores with anti-FAK antibody showed a significant increase of 3.1-fold in phospho-FAK861 levels in CAN compared with LST ($p<0.01$). High expression of FAK correlated with expression of TGF-b1, aVb3, ECM proteins, Vitronectin, but not aVb5, aVb1, a3b1. Thus, activation of FAK during overexpression of aVb3 is an integral feature of CAN. Consistent with this hypothesis, adenoviral transfection of TGF-b1 in COS-7 kidney cells resulted in a 3.5 fold increase in phospho-FAK861 and AVb3 double staining positive cells. These results indicate that FAK is prominent in CAN. Thus, activated FAK is a signature molecule for CAN. Finally, agents blocking the signaling by FAK may be useful in drug therapy of CAN These results show that activation of FAK occurs predominantly via aAVb3 integrin engagement and can be mediated by elevation of intracellular TGFb1 concentration. AVb3 integrin ligation rapidly increased FAK activity which has significant implications for pathology process in CAN. Thus, FAK activation is required for increased aVb3 ligation after TGFb1- induced CAN.

Abstract# 742

GRAFT ARTERIOSCLEROSIS DEVELOPS IN THE ABSENCE OF HOST HUMORAL IMMUNITY BUT NEOINTIMAL SMOOTH MUSCLE CELL PROLIFERATION REQUIRES PRESENCE OF ALLOANTIBODIES. Behzad Soleimani,[1] Andreas Katopodis,[2] Grazyna Wieczorek,[2] Philip I. Hornick,[1] Kenneth M. Taylor,[1] Christoph Heusser.[2] *[1]Department of Cardiac Surgery, Imperial College School of Medicine, London, United Kingdom; [2]Transplantation Research, Novartis Pharma, Basel, Switzerland.*

Graft Arteriosclerosis (GA) is the leading cause of late graft failure in solid organ transplantation. The pathogenesis of GA is poorly understood and is likely to be multifactorial. The role of anti-donor antibodies in particular remains unclear. In this study we investigated the contribution of donor specific alloantibodies in development of GA in an experimental model of the disease. **Method:** Orthotopic carotid artery transplantation was performed between B10A(2R)(H-2^{h2}) donor mice and B-cell deficient $\mu MT^{-/-}$ knockout or wild type ($\mu MT^{+/+}$) recipients. Grafts were harvested at 35 days and subjected to histomorphometry and immunohistochemistry. Serum samples collected during the study period were assayed for alloantibody titre by flow cytometry. For serum transfer experiments, anti-donor serum was raised by multiple immunizations of wild-type animals with donor splenocytes. **Results:** Alloantibodies were detectable in wild-type recipients within 7 days of transplantation and reached a plateau at three weeks. The antibody isotypes were predominantly IgG1 and IgG2b. As anticipated, B cell deficient ($\mu MT^{-/-}$) mice did not mount an IgG response. Wild-type recipients developed marked intimal thickening at 35 days. Surprisingly, allografts harvested from B-cell deficient mice did also develop GA which was comparable in severity with wild-type recipients (mean neointimal areas of 39644±10874 and 33059±4650 μm^2respectively, n=6 per group, P=0.6). Both groups had intimal infiltration with CD45+, CD3+ and F480+ (macrophages) cells. However, immunostaining for α-actin revealed that, whereas allografts from wild type recipients showed marked intimal smooth muscle cell (SMC) proliferation, neointima in B-cell deficient recipients lacked SMC's. Post-transplantation administration of anti-donor serum to $\mu MT^{-/-}$ recipients by weekly intraperitoneal injections did not influence the severity of GA (mean intimal area of 28878±10245 *vs* 39644±10874 μm^2, n=6 per group, P= 0.49) but, significantly, it did restore neointimal SMC population. **Conclusion**: Alloantibodies are detectable in experimental GA though their absence in knockout recipients does not influence the size of the neointima. However, alloantibodies do appear to be necessary for SMC proliferation in the neointima. Therapeutic modulation of host humoral immunity may therefore be of value in prevention of GA in clinical transplantation.

Abstract# 743

VISUALIZATION OF THE ACTIVATION, RETENTION, AND ENRICHMENT OF DIRECT ALLOANTIGEN-REACTIVE $CD8^+$ T CELLS WITHIN TRANSPLANTED AIRWAY TISSUES UNDERGOING CHRONIC REJECTION. David M. Richards,[1] Stacy L. Dalheimer,[1] Marshall I. Hertz,[1] Daniel L. Mueller.[1] *[1]Center for Immunology and Department of Medicine, University of Minnesota Medical School, Minneapolis, MN.*

Transplantation is a viable option for many diseases that result from end-stage organ failure. Advances in systemic immunosuppression have increased the early survival of transplanted organs so that chronic graft dysfunction now emerges as the greatest threat to long-term patient survival. T lymphocytes appear to play an important role in long-term graft rejection; however, the investigation of these cells has been complicated by the fact that lymphoid populations are very heterogeneous in antigen (Ag) specificity, and the important graft target Ag are unknown. Many of the $CD8^+$ T cells recovered from airway allografts can be shown to express high levels of CD44 and CD69, suggesting a recent Ag recognition event. Nevertheless, it has not been possible to prove that these cells are reactive to unique Ag expressed by the graft tissues. To address this, we have developed a model system that allows for the investigation of graft-infiltrating T cells with known alloantigen specificity. Using an established model of chronic graft rejection (the heterotopic transplantation and eventual fibrosis of allogeneic tracheas) together with an adoptive transfer of TCR-transgenic T cells (from 2C mice whose T cells are uniformly $CD8^+$ and L^d-specific) into the recipient mice, we have tracked a representative population of directly alloreactive T cells. These $CD8^+$ T cells are shown here to be retained and enriched within allograft tissue that bears their antigen (class I - L^d). Grafts from C57BL/6 animals transplanted with both L^d-positive (BALB/c – H-2^d) and L^d-negative (CBA – H-2^k) allografts were both filled with activated endogenous

$CD8^+$ T cells. Nevertheless, only the L^d-bearing BALB/c grafts were infiltrated with 2C cells. These 2C cells appeared to have undergone at least 7 rounds of cell division at the time of their recovery from the grafts, and they were uniformly high for CD44 and CD69. They were enriched from ~0.2% of $CD8^+$ T cells in the draining lymph nodes to ~12% of $CD8^+$ T cells within the L^{d+} grafts. An immunohistochemical analysis confirmed that they infiltrate the sub-epithelial space within the transplanted airways. These results suggest that direct class I alloantigen-reactive $CD8^+$ T cells may participate in the targeting and destruction of airway epithelial cells that eventually leads to the fibroproliferative process responsible for airway obliteration. This work was supported by NIH grants T32 HL07741 and PO1 AI50162.

Abstract# 744

ROLE OF MATRIX METALLOPROTEINASES 2 AND 9 IN SKIN AND CARDIAC ALLOGRAFT REJECTION. Hiroyuki Amano,[1,2] Shoji Koga,[2] Satoshi Hirohata,[3] Masayoshi Miura,[1] Suneel Apte,[3] Hiroshi Toma,[2] Andrew C. Novick,[1] Robert Fairchild.[1] *[1]The Glickman Urological Inst., Cleveland Clinic Fdn., Cleveland, OH; [2]Dept. Urology, Tokyo Women's Medical Univ., Tokyo, Japan; [3]Dept. Biomedical Engineering, Cleveland Clinic Fdn., Cleveland, OH.*

INTRODUCTION: The factors directing T cell infiltration into allografts to mediate rejection remain poorly defined. The infiltration of leukocytes into the allograft parenchyma must include a process that digests the extracellular matrix supporting the cellular structure of the tissue. Matrix metalloproteinases (MMPs) are enzymes that mediate the digestion of extracellular matrix proteins such as collagen and laminin. During activation T cells express MMP 2 and MMP9. The goal of the current study was to investigate the potential role of these MMPs in the rejection of skin and cardiac allografts. METHODS: C57BL/6 (H-2b) mice and MMP-9/- mice on a 129 background received heterotopic heart allografts from MHC mismatched A/J (H-2a) donors or skin allografts from BALB/c (H-2d) donors. The allografts were retrieved from each group at the time of rejection and immunohistochemistry was performed to identify graft infiltrating cell populations. Recipients were depleted of CD4+ or CD8+ T cells by treatment with 200 ug specific mAb on three consecutive days before transplantation and every four days after transplantation. Whole cell RNA was isolated from allografts at various times post-transplant and was tested for expression of MMP2 and MMP9 by Northern blot and ribonuclease protection analyses. Graft homogenates were prepared at the time of rejection and tested by gel zymography to detect the presence of proenzyme and active forms of MMP2 and MMP9. RESULTS: Heart allografts were rejected in wild-type recipients on day 7-8 post-transplant and in MMP9-deficient recipients from day 11-22. Rejecting allografts in MMP-9 deficient mice had decreased cellular infiltration, particularly with CD4+ T cells. MMP-9 accepted syngeneic heart and skin grafts indefinitely. At the time of rejection in wild-type recipients, heart allografts had high levels of MMP9 and low levels of MMP2. MMP9 activity was absent in recipients depleted of CD4+ T cells. Using antibody-mediated depletion of allograft recipients as well as CD4- and CD8-knockout recipients, expression of MMP9 was only observed during CD4 T cell mediated rejection whereas MMP2 was observed in all rejecting allografts. CONCLUSIONS: MMP-9 activity is required for optimal rejection of complete MHC-mismatched heart allografts and is dependent on CD4+ T cells. The results suggest that T cell graft infiltration may be attenuated by interfering with the function of these enzymes.

Abstract# 745 Poster Board #-Session: P1-II

POLYMORPHISMS IN THE INTERLEUKIN-10 PROMOTER GENE AND ABILITY FOR INTERLEUKIN-10 PRODUCTION ARE ASSOCIATED WITH POST-TRANSPLANT SQUAMOUS CELL CARCINOMA. Eric Alamartine,[1] Patricia Berthoux,[1] Christophe Mariat,[1] Frederic Cambazard,[1] Francois Berthoux.[1] *[1]Nephrology and Dermatology, University Hospital, Saint Etienne, France.*

Risk factors for cancer after transplantation may include genetic susceptibilities, such as the control of cytokine production. Interleuklin-10 (IL-10) is implicated in tumorigenesis, and polymorphisms in its gene promoter correlate with differential amount of production. We investigated a possible association between IL-10 gene promoter polymorphisms and the occurrence of post-transplant skin carcinoma. 70 kidney transplant recipients (KTR) who developed a squamous cell carcinoma or a basal cell carcinoma were examined for polymorphisms in the IL-10 gene promoter using PCR-based methods. Single base pairs mutations were studied at positions –1082, -819 and –592. We compared genotypes; GCC, ACC and ATA haplotypes and production predicted phenotypes (low is GCC negative, high is GCC homozygous). The IL-10 secretion capability was tested by in vitro stimulation of peripheral mononuclear cells. These patients were compared to 70 healthy controls and to 70 matched KTR without cancer. All the following results were statistically significant. IL-10 genotypes were differently distributed in kidney transplant recipients who developed a skin carcinoma, but especially a squamous cell carcinoma. In these patients, the frequency of the GCC haplotype was higher (56% Vs 32% in unaffected-KTR) and the frequency of the ATA haplotype was lower (14% Vs 34%). Subsequently, we found a shift in the predicted phenotypes from the low production phenotype (12% Vs 47%) to the high production phenotype (25% Vs 11%). Secretion of IL-10 was strongly correlated to the production predicted phenotype, and was higher in patients who developed a squamous cell carcinoma (917 pg/ml) than in the others (649 pg/ml in unaffected-KTR, 699 pg/ml in KTR with a basal cell carcinoma). These results indicate that IL-10 gene polymorphisms and IL-10 production capability may contribute to the development of post-transplant skin squamous cell carcinoma.

Abstract# 746 Poster Board #-Session: P2-II

LIVER TRANSPLANTATION FOR PRIMARY OR METASTATIC LIVER MALIGNANCIES IN CHILDREN. J. F. Buell,[1] M. Gupta,[1] T. M. Beebe,[1] J. Trofe,[1] M. J. Hanaway,[1] T. G. Gross,[1] R. R. Alloway,[1] E. S. Woodle.[1] *[1]Division of Transplantation, The University of Cincinnati, Cincinnati, OH.*

Transplantation for the treatment of primary and metastatic liver malignancies has been met with varying degrees of success in the adult population. The rate of recurrence in a large degree has been related to tumor histology and stage at transplantation. The purpose of this study was to examine the results of liver transplantation for hepatic malignancies in children and evaluate risk factors for recurrence and mortality. **Methods:** All pts that underwent liver transplantation for either primary or metastatic malignancies to the liver were identified. Demographics, tumor histology, treatment, recurrence, and survival data were collected. **Results:** Forty-seven (46) pediatric pts were identified with 22 hepatoblastomas (HB), 22 hepatocellular carcinomas (HCC), 2 sarcomas. Gender was evenly divided (23 male and 23 female). Thirty-one (31) patients were Caucasian, 5 African-American, and 10 were not identified. Mean age of the entire group at transplant was 9.5 ± 4.9 years of age. Pre transplant therapy included chemotherapy alone in 9 HB pts, and combined therapy in 5, (chemo and surgery, radiation and surgery, or chemo, radiation, and surgery). Three HCC pts received chemo alone, while 3 received a combination of chemo and surgery. Surgical resection of the primary sarcoma was performed prior to transplantation. Interestingly, all HB recurrences occurred in pts receiving pre transplant chemo as monotherapy. Mean follow-up post transplant for the total cohort was 27.6 ± 27.8 mos (range 0.3 – 129.1 mos). **Conclusions:** Treatment of hepatic malignancies whether primary or metastatic in pediatric patients, by liver transplantation appears to mimic that seen in the adult population. Five-year survival rates are 55% or greater in both HCC and HB.

	HB (n=22)	HCC (n=22)	Sarcoma (n=2)
Mean Age at Tx (yrs)	3.2 +/- 2.4	10.0 +/- 4.7	16.4 +/- 1.3
Gender (M:F)	10:12	13:9	1:1
Median Time DX to TX (mos)	10 (0 to 64 mos)	17 (0 to 93 mos)	0
Recurrence Rate (%)	5 (23%)	1 (5%)	2 (100%)
Median Time to Recurrence (mos)	12.4 (2.1 to 16.6 mos)	1 (1mo)	12.2 (4 to 17mos)
Dead to Disease	4 (20%)	1 (5%)	2 (100%)
Median Time to Death after Tx (mos)	6 (0.3 To 20.0 mos)	16 (3.4 to 59.0 mos)	33.4 (6 to 42.7 mos)
1,3,5-yr Pt Survival	73%, 55%, 55%	77%, 64%, 59%	0%

Abstract# 747 Poster Board #-Session: P3-II

ARE DIABETICS LESS LIKELY TO DEVELOP MALIGNANCIES FOLLOWING KIDNEY AND KIDNEY-PANCREAS TRANSPLANTATION? Maureen A. McBride,[1] Wida S. Cherikh,[1] H. M. Kauffman,[1] Jude Maghirang,[1] Sandy Feng,[2] Douglas W. Hanto.[3] *[1]Research Department, United Network for Organ Sharing, Richmond, VA; [2]Division of Transplantation, University of California, San Francisco, CA; [3]Division of Transplantation, Beth Israel Deaconess Medical Center, Boston, MA.*

Purpose: To determine whether diabetic patients receiving either cadaveric kidney (KI) or simultaneous kidney-pancreas (KP) transplants are less likely to develop post-tx malignancies (any cancer, PTLD, skin cancer, or solid tumor) compared to non-diabetic patients. **Methods:** All adult primary cadaveric KI and KP transplant recipients reported to the UNOS/OPTN database (1/1/97-12/31/00) who had at least 7 days of follow-up were included. Three transplant groups were considered: KI without diabetes, KI with diabetes, and KP patients. Multivariate Cox regression models were used to determine the effect of transplant group on the risk of developing any post-tx malignancy, PTLD, skin cancer, and solid tumor. These models controlled for various recipient (age, gender, ethnicity) and transplant characteristics (HLA mismatch, induction and discharge maintenance immunosuppression, delayed graft function, and early acute rejection). Patients who developed cancer after 814 days post-tx were censored to ensure comparable follow-up among the transplant groups. **Results:**There were 17,112 KI non-diabetics, 5,441 KI diabetics, and 3,015 KP patients. The overall incidence of cancer in each group was 2.95%, 1.97%, and 1.56%, respectively (chi square $p<0.001$). The table shows the relative risk (RR) and 95% confidence interval (CI) of developing each type of post-tx malignancy for KI diabetics and KP recipients compared to non-diabetic KI recipients.

Relative Risk (95% CI) of Developing Post Transplant Malignancy

	Any Cancer	PTLD	Skin Cancer	Solid Tumor
Non-Diabetic KI	1.00	1.00	1.00	1.00
Diabetic KI	0.62 (0.50, 0.76)	0.79 (0.46, 1.34)	0.51 (0.35, 0.74)	0.60 (0.47, 0.77)
Diabetic KP	0.70 (0.50, 0.98)	1.19 (0.68, 2.09)	0.66 (0.36, 1.21)	0.61 (0.39, 0.95)

Of note, IL-2 induction was associated with a significantly reduced risk of development of any non-PTLD cancer. **Conclusions:** Diabetic KI patients are significantly less likely to develop any post-tx malignancy, skin cancer, and solid cancers than non-diabetic KI patients. KP patients are less likely to develop any cancer and solid tumors. There was no difference in the likelihood of developing PTLD for any transplant group. There were also no differences in the risk of developing any cancer including PTLD, skin and solid tumor between diabetic KI and KP patients.

Abstract# 748 Poster Board #-Session: P4-II

DE NOVO MALIGNANCIES FOLLOWING LUNG TRANSPLANTATION. M. Gupta, T. D. Merchen, M. R. First, J. Trofe, T. G. Gross, T. M. Beebe, R. R. Alloway, M. Hanaway, E. S. Woodle, J. F. Buell. *[1]IPITTR, Division of Transplant Surgery, University of Cincinnati, Cincinnati, OH.*

Immunosuppression in solid organ transplant recipients is associated with an increased risk of de novo malignancy following transplantation. Moreover, intensive immunosuppression may be associated with higher mortality with de novo malignancies. We therefore examined our experience with de novo malignancy in lung transplant recipients (LTR). **Methods**: Our database was searched for LTR who developed de novo malignancies following transplantation. **Results**: 43 patients were identified who developed malignancies following lung transplantation: 27 males and 16 females. Mean age at the time of lung transplant was 50.9±16.1 years, and mean time between transplantation and diagnosis of malignancy was 25.0±24.6 months. Mean follow-up after transplant and diagnosis was 35.2 and 11.2 months respectively. Etiologies of end stage lung disease included: COPD (20), idiopathic pulmonary fibrosis (9), cystic fibrosis (6), sarcoidosis (1), and other (7). Maintenance immunosuppressive therapy consisted of azathioprine, cyclosporine, and prednisone in 38 patients, tacrolimus 8 patients and mycophenolate mofetil 6 patients. Antibody therapy was utilized in 24 patients. Skin cancer (19) was the most common malignancy encountered; squamous cell (8), basal cell (6), combined squamous and basal cell (2), Kaposi's sarcoma (2) and malignant melanoma (1). Lung cancer was seen in 13 patients; adenocarcinoma (4), small cell (3), unspecified (4), neuroendocrine (1), and Kaposi's sarcoma (1). Breast cancer occurred in three females. Other cancers included (prostate 1, GI tract 3, head & neck 2, kidney 1, misc. 1). Overall survival was 67% (14/43 deaths). Survival for those with skin cancer (74%) compared to those with non-skin cancer (63%) was not statistically different (p=0.44). The survival and recurrence rates for patients who developed lung cancer was 54% and 46% respectively. Of the fourteen deaths, 8 were due to recurrent malignancy (57%); skin cancer (2, BCC and SCC) and non-skin cancer (6; lung 5, GI tract 1). Death due to recurrence, therefore, was 11% for those with skin cancers compared to 25% for those with non-skin cancers (p=0.22). **Conclusions**: De Novo malignancies in LTR show the following characteristics: 1) the most common malignancy is skin cancer which carries a 11% risk of death due to recurrent cancer, 2) post transplant de novo lung cancer has a high mortality rate, and 3) death due to recurrent cancer is higher for non-skin cancers compared to skin cancers.

Abstract# 749 Poster Board #-Session: P5-II

LUNG TRANSPLANTATION IN PATIENTS WITH PREEXISTING MALIGNANCIES. Manish Gupta, Todd D. Merchen, M. Roy First, Jennifer Trofe, Thomas G. Gross, Thomas M. Beebe, Rita R. Alloway, Michael J. Hanaway, E. Steve Woodle, Joseph F. Buell. *[1]IPITTR, Divison of Transplant Surgery, University of Cincinnati, Cincinnati, OH.*

In general, thoracic organ transplant recipients are considered to require more intense immunosuppression than abdominal organ transplant recipients. It is therefore possible that the risk of recurrence of pre-existing malignancies may be higher in thoracic organ transplant recipients. To evaluate this possibility, we reviewed the clinical course of lung transplant recipients with a history of pre-existing malignancy. **Methods**: Our database was searched for lung transplant recipients with a history of malignancies prior to transplantation. **Results**: 21 recipients were identified: 8 males and 13 females. Mean age at transplantation was 42.9±19.1 years, and time from malignancy diagnosis to transplantation was 55.5 months. Five patients were diagnosed at the time of transplant. Etiologies of end-stage lung disease included: COPD (8), idiopathic pulmonary fibrosis (5), lung cancer (2), cystic fibrosis (2), and miscellaneous (4). Pre-existing cancer histologies included: lung adenocarcinoma (n=7), colorectal adenocarcinoma (n=1), Hodgkin's lymphoma (n=2), leukemia (n=2), skin (n=3), renal cell (n=1), neuroendocrine (n=1), and unspecified (n=4). Most patients (19) received azathioprine, cyclosporine, and prednisone as maintenance immunosuppressive therapy. 7 of 21 patients received antibody therapy for either rejection or induction therapy. Tacrolimus was used in 1 patient. Median follow-up after transplant was 9.3 months, range (0.8-46.4 months). An overall mortality of 42.9% was observed with death due to recurrent malignancy (lung cancers, n=4 and basal/squamous cell skin cancer, n=1), lung allograft rejection (n=2), infection (n=1), and CVA (n=1). The overall risk of death due to recurrent malignancy was 24%. Mean time to death after transplant was 15.4±14.5 months. Of 5 patients with diagnosis of cancer at the time of transplant, 3 died, all of recurrent lung cancer. **Conclusions**: Lung transplantation in patients with pre-existing cancer is associated with high risk of death due to recurrent cancer (24%). Synchronous lung cancer in the explanted lung appears has a particularly high risk of recurrence (60%)

Abstract# 750 Poster Board #-Session: P6-II

PREEXISTING THYROID CANCER IS ASSOCIATED WITH A LOW RISK OF RECURRENCE FOLLOWING SOLID ORGAN TRANSPLANTATION. Manish Gupta, Todd D. Merchen, Jennifer Trofe, Thomas G. Gross, Thomas M. Beebe, Rita R. Alloway, Michael J. Hanaway, E. Steve Woodle, Joseph F. Buell. *[1]IPITTR, Division of Transplant Surgery, University of Cincinnati, Cincinnati, OH.*

Thyroid cancers are, in general, thought to be biologically indolent. The biologic behavior of thyroid cancer in solid organ recipients, however, has not been defined. The purpose of this study was to examine the clinical courses of patients with thyroid cancers who underwent solid organ transplants (txp). **Mehtods**: A retrospective analysis of all recipients with history of pre-txp thyroid cancers in our database was performed. **Results**: 27 patients were identified who were diagnosed with and treated for thyroid cancer prior to txp. Two patients had recurrence prior to txp and were treated prior to transplantation. Recipients included 23 kidneys, 1 liver, 1 heart, 1 lung, and 1 pancreas. Mean age at transplantation was 44.0±12.5 years with a median wait period of 40.1 months (range 0.8-415.3 months) between diagnosis and transplantation. Median follow-up was 26.5 months (0.8-206.9 months) after transplantation. Gender distribution included 10 males and 17 females with 20 Caucasians, 1 African-American, and 6 others. Tumor histology included papillary (55.6%), follicular (11.1%) and unspecified (33.3%). Node positive disease was encountered in 2 (7.4%) patients with papillary and follicular histology in each instance. All patients had surgical therapy with partial or complete thyroidectomy. Maintenance immunosuppression included prednisone (n=27), cyclosporine (n=21), mycophenolate mofitil (n=1), and azathioprine (n=17). Antibody therapy was used in 14 patients. 21 patients (77.8%) were free of disease and alive at the end of follow-up. The overall recurrence rate after txp was 7.4% (2/27) with a single mortality (3.2%) due to recurrent disease. The overall mortality in the study group was 18.5% (5/27) with a mean survival of 53.8±75.4 months. The causes of death were stroke (n=1), CMV infection (n=1), post transplant lymphoproliferative disease (n=1), unknown (n=1) and recurrent disease (n=1). Antibody therapy did not seem to play a role in recurrence. **Conclusions**: Biological behavior of thyroid cancer in solid organ transplantation appears to be similar to that in general population, in that the overall risk of recurrence and death due to recurrent cancer is low. This observation suggests that solid organ transplantation can be performed in patients with history of thyroid cancer with a low risk of recurrence.

Abstract# 751 Poster Board #-Session: P7-II

NEOPLASIA AND POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER FOLLOWING PEDIATRIC HEART TRANSPLANTATION-INCIDENCE AND OUTCOME. Jonah N. K. Odim,[1] Hiroko Kunitake,[1] Fernando Mendoza,[1] Hyde Russell,[1] Mark Plunkett,[1] Azie Alikhani,[1] Hillel Laks,[1] Heart Transplant Team.[1] *[1]Dept. of Surgery, Div. of Cardiothoracic Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Introduction Prolonged nonspecific immunosuppression following heart transplantation is a risk factor for the development of neoplasia. We sought to determine the incidence of malignancy and post-transplant lymphoproliferative disorder (PTLD) following pediatric cardiac transplantation at our institution and nationally using the United Network of Organ Sharing (UNOS) database. **Methods and Results** We retrospectively surveyed the medical records of all 154 pediatric patients (mean age 9.31+/-6.10 yrs, range 16 days to 20.53 yrs) undergoing 170 heart transplantations from 1984 to 2002 for the development of malignancy and PTLD. Six cases (3.9%) of PTLD were identified over a mean follow-up period of 38.6+/-45.2 months. We subsequently reviewed the data on all pediatric patients who underwent cardiac transplantation in the UNOS database. In this group of 4110 patients, there were 181 (4.4%) cases of post-transplant malignancies and PTLD.

Table 1. Cancer and PTLD subgroups

	Institution CA+PTLD	Institution Non-CA+PTLD	UNOS CA+PTLD	UNOS Non-CA+PTLD
Patient Number	6	148	181	3929
Mean age at Tx (yr)	11.2+/-6.9	9.3+/-6.1	6.9+/-6.3	7.0+/-6.6
Female gender	50%	54.5%	48%	42%
CHD	100%*	37%	40%	46%
CMV mismatch (+dnr/-rcpt)	50%	20.1%	31%	30%
Mean follow-up (mo)	64.5+/-45.4	37.6+/-45.0		

*p=0.004

Table 2. Characteristics of Patients developing PTLD at our Institution

Gender	Age at Tx (yr)	Dx	CMV mismatch (+dnr/-rcpt)	Immuno	Time Tx to Dx (mo)	Neoplasia (B Cell)	Site	Time Dx to death (mo)	Cause of death
1. F	7.36	CHD	N	CYA	3.9	Y	GI	38.5	Lymphoma
2. F	13.25	CHD	Y	CYA	112.4	Y	Cervical LN	16.1	Lymphoma
3. M	9.68	CHD	N	CYA	64.2	N	Eye	52.3	TCAD
4. F	17.66	CHD	Y	CYA	29.6	Y	GI	0.6	Lymphoma
5. M	18.64	CHD	Y	FK	30	Y	Meninges, Chest, GI	11	Lymphoma
6. M	0.31	CHD	N	FK	28.4	N	Cervical LN, Chest, Abd	alive	alive

Conclusion Lymphoproliferative neoplasia continues to represent the most common proliferative disorder after pediatric transplantation and is associated with important morbidity and mortality. The incidence of PTLD and maligancy at our institution (3.9%) was similar to that found nationally (4.4%). While all patients at our institution who developed lymphoproliferative disorders carried the diagnosis of congenital heart disease (CHD), this was not observed in the larger UNOS database.

Abstract# 752 **Poster Board #-Session: P8-II**

PREOPERATIVE SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA IN LIVER TRANSPLANT CANDIDATES: IMAGE ISN'T EVERYTHING. Jeffrey Rogers,[1] Kenneth D. Chavin,[1] Angello Lin,[1] David Lewin,[3] Ira Willner,[2] Adrian Reuben,[2] Prabhakar K. Baliga.[1] *[1]Departments of Surgery; [2]Medicine; [3]Pathology, Medical University of South Carolina, Charleston, SC.*

Background: The optimal screening protocol for hepatocellular carcinoma (HCC) before liver transplantation (OLT) is controversial. **Aim:** To evaluate the ability of our Program's screening protocol to preoperatively detect HCC, and to examine the impact of this on patient survival. **Methods:** 424 consecutive adult OLT at our institution were retrospectively reviewed. Preoperative HCC screening consisted of abdominal ultrasound (US) every 6 months and serum alpha-fetoprotein (AFP) every 3 months until OLT. Patients with suspicious lesions on US or AFP>20 ng/ml were further evaluated with biphasic, contrast-enhanced helical CT or with MRI. **Results:** Seventeen of 424 (4%) OLT recipients had HCC identified on explant pathology. There were 15 men and 2 women, mean age 46 ± 15 years. Mean follow-up was 17 ± 20 months. The most common indications for OLT in patients with HCC were hepatitis C (53%) and Laennec's cirrhosis (17.6%). There was a trend toward more hepatitis C infection in patients with HCC (53%) compared to those without HCC (32%), p=0.07. Screening US and AFP detected (DET) only 8/17 HCC preoperatively (47.1%). Nine of 17 HCC were discovered incidentally (INC) in the explant (52.9%). Seven of 8 patients with DET HCC had a liver mass identified on US; 6 of these underwent confirmatory CT or MRI. One of 8 patients with DET HCC had a normal US and an elevated AFP, with subsequent confirmation of a liver mass on MRI. AFP correlated poorly with presence of HCC, with only 3/17 patients demonstrating elevated preoperative AFP, all in the DET group. Fifty six percent of INC and 75% of DET HCC were either stage I or II (p=0.14). One- and 3-year actuarial patient survival for INC and DET was comparable: 63%, 63% and 72%,72%, respectively (p=0.9). **Conclusions:** Despite the poor ability of US and AFP to detect HCC preoperatively, the similar long-term survival between patients with INC and DET HCC suggests that routine surveillance with CT or MRI would be unlikely to significantly alter management strategy for the vast majority of OLT candidates. In this series, only 2/424 patients (0.5%) would have been excluded as OLT candidates had surveillance imaging detected their advanced HCC preoperatively. Better screening and risk stratification tools are needed to more accurately predict which patients require more extensive imaging in order to allow for most efficient utilization of diagnostic resources.

Abstract# 753 **Poster Board #-Session: P9-II**

RECENT INCIDENCE OF PTLD AMONG RENAL TRANSPLANT PATIENTS AT 20 TRANSPLANT CENTERS IN THE U.S. Jacqueline Travasso,[1] Donnie P. Funch,[1] Alexander M. Walker.[1] *[1]Epidemiology, Ingenix, Auburndale, MA.*

Recent reports of PTLD incidence among renal transplant patients have been in conflict, with some suggesting that the rates are increasing while others suggest declines in rates. *Methods.* With the assistance of the United Network for Organ Sharing (UNOS) and 20 large U.S. transplant centers, we identified and verified PTLD cases through 2001 who had received a renal only transplant between July 1, 1995 and December 31, 1998. UNOS supplied the total number of renal only transplants performed at these centers by year as well as breakdowns by age and donor type. R*esults.* Of 149 reported cases, 108 were confirmed as having PTLD. The three-year cumulative incidence was 0.6% for s and 3.5% for children. Yearly rates suggested a decline in PTLD for the population (1.2% in 1995 to 0.4% in 1998). Numbers were too small in the pediatric population to provide stable estimates. Recipients of cadaver kidneys had higher rates of PTLD than recipients of kidneys from living donors (cumulative rates: 0.7% vs. 0.5%). Because the percentage of living donors increased over time, we limited a secondary analysis to recipients of cadaver kidneys. This subgroup also showed a reduction in rate from 1.3% for 1995 down to 0.7% for 1998. *Conclusion.* There was a consistent patterns of reduction in PTLD incidence from 1995 through 1998, not explained by variations in follow-up time or donor type. There was no evidence to suggest that the introduction of new and more effective transplant therapies during this period resulted in an increase in PTLD risk.

Abstract# 754 **Poster Board #-Session: P10-II**

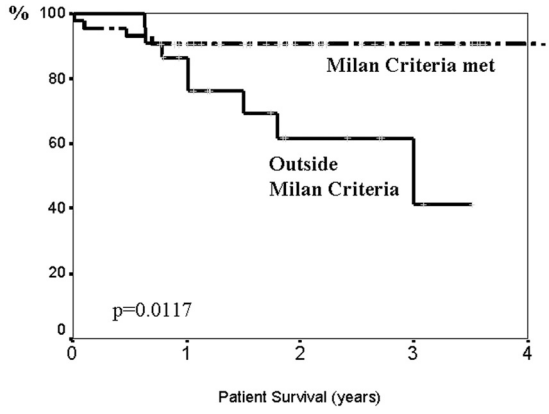
OUTCOME OF HEPATOCELLULAR CARCINOMA POST ORTHOTOPIC LIVER TRANSPLANTATION (OLT): IMPACT OF TUMOR SIZE AND SURGICAL TECHNIQUE. Bashar Aqel,[1] H. Bonatti,[1] R. Paz,[3] V. Machicao,[1] R. C. Dickson,[1] J. Steers,[2] C. Hughes,[2] J. Nguyen,[2] Denise Harnois.[1] *[1]Gastroenterology and Hepatology; [2]Department of Surgery; [3]Department of Radiology, Mayo Clinic-Jacksonville, FL.*

Background: Patients (pts) with cirrhosis are at risk of HCC (3%/year). Patients transplanted within Milan criteria (MC)(single lesion<5cm, <3lesions none >3cm) undergoing OLT have 4-year survival of 84%. Piggyback (Pb),recipient vena cava sparing technique, is used forOLT with good outcome. The impact of this technique in pts with HCC has not been described. Aim: Report the outcome of OLT for HCC (based on MC) using the Pb. Methods: Longitudinal cohort analysis of all pts transplanted for HCC between 03/98-12/01. Results: During study period, 75 pts were transplanted for HCC (11pts with incidental tumor). Nine pts required 11 re-OLT. All pts were transplanted using Pb technique. Pre OLT CT scan in 64pts was reviewed by a single radiologist and pts stratified according to MC. Fourty-two pts met MC (MC+) and 22pts were outside at time of OLT (MC-). Pre-OLT treatments were:chemoembolization (61pts), alcohol ablation (1), radiofrequency ablation (1), and 1 surgical resection. Pre-OLT data in the two groups is shown

table1

	MC+	MC-	p-value
Pt age(yr)	59	55	0.74
Donor age(yr)	61	49	0.19
Hepatitis C(HCV)	50%	54.8%	0.466
Hepatitis B	9.1%	19%	0.254
waiting time(days)	32	42	0.8
Pt death(%)	9.5%	36.4%	0.013
Death due to tumor(%)	4.8%	27.3%	0.016

Recurrence was seen in 14 pts, eight of them died with tumor progression. Median time of recurrence was 247days(range112-917). None of the pts with incidental tumor had recurrence. Multivariate analysis revealed that vascular invasion on explants (RR 4.7, p=0.016) and pre-OLT tumor data (MC-) (RR 4.4, p=0.031) were predictors of tumor recurrence. Pt survival (Kaplan-meier)among the two groups was significantly different (see figure). Conclusions: 1.Pb technique has no negative impact on outcome of pts transplanted with HCC 2.MC is a good predictor of pt survival 3.Tumor recurrence is dependent on tumor size and vascular invasion.



Abstract# 755 **Poster Board #-Session: P11-II**

EXTENDED INDICATION OF ADULT LIVING DONOR TRANSPLANTATION FOR ADVANCED HEPATOCELLULAR CARCINOMA. Hiroyuki Furukawa,[1] Tsuyoshi Shimamura,[2] Maeng Bong Jin,[1] Tomomi Suzuki,[2] Masahiko Taniguchi,[2] Masahiro Hattori,[2] Toshiya Kamiyama,[2] Michiaki Matsushita,[2] Satoru Todo.[2] *[1]Department of Organ Transplantation and Regenerative Medicine, Hokkaido University School of Medicine, Sapporo, Japan; [2]Department of General Surgery, Hokkaido University School of Medicine, Sapporo, Japan.*

The indication of cadaveric liver transplantation for hepatocellular carcinoma (HCC) is extremely limited because of organ shortage. Since adult living donor liver transplantation (LDLT) was established as a rather safe procedure, the indication of LDLT for HCC have been expanded in our center, using methods of micrometastasis (MM: AFP-mRNA PCR) or FDG-PET. Materials and methods: From Sep 97 to Nov 02, twenty-six patients were evaluated for liver transplantation. LDLT was considered when the patients had either an advanced liver disease or hepatoma not treatable with surgical resection or medical treatment. LDLT was indicated if the candidate was negative for metastasis with FDG-PET and for MM (alpha-fetoprotein mRNA PCR in peripheral blood and bone marrow) in addition to no extrahepatic metastasis and vascular invasion. Number or size of the tumor was not accounted. Chemotherapy and transarterial chemoembolization were applied perioperatively. Lamivudine / HbsAb immunoglobulin for hepatitis B, and Interferon / ribavirin for hepatitis C were used to prevent the disease recurrence. Results: Seven of 26 cases were excluded from candidacy because of tumor thrombus in the major portal vein, and MM was checked in the rest of the patients. Four patients had positive MM, of whom three had lymphnode metastasis and excluded from candidacy. Two had positive FDG-PET as well. One with positive MM had LDLT (case 9) with consenting high chance of tumor recurrence. Fifteen patients underwent LDLT (table). Four have been awaiting the liver transplantation. Ten cases are surviving from 2 to 32 months (median 9 months), and five died; one (case 9) with and four without tumor recurrence. Conclusion: The indication of LDLT could be extended to even advanced hepatoma (stage 4a) if the stringent evaluation using micrometastasis / FDG-PET and proper perioperative management are applied.

indication and outcome of LDLT for HCC

case	age/sex	hepatitis	C-P stage*	tumor stage	AFP-mRNA	outcome
1	55F	HCV	C	1	(-)	dead (4m)
2	54M	HBV	B	4a	(-)	alive (32m)
3	47F	HBV	B	1	(-)	alive (29m)
4	60M	HCV	C	1	(-)	dead (3m)
5	59M	HCV	B	4a	(-)	dead (15m)
6	46M	HBV	A	4a	(-)	alive (17m)
7	37M	HBV	C	1	(-)	alive (16m)
8	50M	HCV	C	4a	(-)	alive (13m)
9	55M	HBV	B	4a	(+)	dead (9m) (rec)†
10	54M	HBV	B	4a	(-)	alive (11m)
11	57M	HBV	B	4a	(-)	dead (4m)
12	58F	HCV	C	1	(-)	alive (3m)
13	50M	HBV	A	4a	(-)	alive (2m)
14	52F	HBV	A	2	(-)	alive (1m)
15	45M	HCV	C	4a	(-)	alive (1m)

*Child-Pugh stage, †only one tumor recurrence

Abstract# 756 **Poster Board #-Session: P12-II**

PREEXISTING TESTICULAR CANCER IN SOLID ORGAN TRANSPLANT RECIPIENTS. T. D. Merchen,[1] M. Gupta,[1] M. J. Hanaway,[1] J. Trofe,[1] T. M. Beebe,[1] T. G. Gross,[1] R. R. Alloway,[1] J. F. Buell,[1] E. S. Woodle.[1] *[1]Israel Penn International Tumor Registry, Univ. of Cincinnati, Cincinnati, OH.*

Testicular cancer is primarily a disease of young men with a 5 year survival of 95%. As a highly treatable disease that occurs in a young patient population, testicular cancer is a malignancy that is occasionally encountered in potential transplant (txp) recipients and may be associated with low recurrence rates. **METHODS**: Demographics, tumor histology, recurrence rates and survival data were analyzed in patients who had undergone solid organ transplantation with a history of preexisting testicular cancer. **RESULTS**: Twenty-six men with preexisting testicular cancer were identified. Mean age at diagnosis was 29.8 years and mean age at txp was 43.5 years. The majority of patients were Caucasian (n= 23, 88.5%) with the remaining patients' race unknown. Most patients underwent kidney transplantation (n=17, 65.4%), with the remaining undergoing heart (n=6, 23.1%) and liver transplantation (n=3, 11.5%). Twelve renal txps were cadaveric and five living related. Testicular cancers encountered included: seminomas (n=12, 4 6%), nonseminomas (n=9, 35%), and unknown histology lesions (n=5, 19%). The median time interval from diagnosis of testicular cancer to txp was 151.3 months

	Seminomas	Nonseminomas
Mean Age at Diagnosis	28.1 years (+/- 11.5)	30.0 years (+/- 8.8)
Orchiectomy	100% (n=12)	100% (n=9)
Radiation Therapy	66% (n=8)	22% (n=2)
Median Time to Txp	147.4 months	151.3 months
Median Follow-up After Txp	16.5 months	19.9 months
Recurrence Rate	17% (n=2)	11% (n=1)
Mortality Due to Recurrence	8% (n=1)	11% (n=1)
Overall Mortality	42% (n=5)	11% (n=1)

CONCLUSIONS: Testicular cancer in the general population is a successfully treated disease of the young. In our review we have found patients with preexisting testicular cancer who undergo solid organ txp to be at a low to moderate risk of recurrence and mortality despite a long waiting time prior to transplant. This data suggests that the impact of immunosuppression on recurrence of cancer in this population should not prohibit solid organ transplantation.

Abstract# 757 **Poster Board #-Session: P13-II**

PRE-EXISTING BLADDER CANCER IN SOLID ORGAN TRANSPLANT RECIPIENTS. T. D. Merchen,[1] M. Gupta,[1] M. J. Hanaway,[1] J. Trofe,[1] T. M. Beebe,[1] T. G. Gross,[1] R. R. Alloway,[1] J. F. Buell,[1] E. S. Woodle.[1] *[1]Israel Penn International Tumor Registry, Univ. of Cincinnati, Cincinnati, OH.*

Bladder cancer in the general population is the second most common genitourinary malignancy. As such, it is occasionally seen in the history of potential transplant (txp) recipients. Methods: Demographic, tumor histology, recurrence rate, and mortality data from our data bank was analyzed on patients who had undergone solid organ transplantation with a history of preexisting bladder cancer. **Results**: Eighty-one patients with preexisting bladder cancer were identified. The population was 84% (n=68) men and 16% (n=13) women. The mean age at diagnosis was 47.3 (+/- 14.1) and mean age at txp 54.3 (+/- 12). The overall median wait from diagnosis to txp was 33.8 months. The organs transplanted included: kidney 82.7% (n=67), heart 11.1% (n=9), liver 2.5% (n=2), lung1/2% (n=1), kidney/pancreas 1.2% (n=1), and pancreas 1.2% (n=1). The types of cancer encountered were: transitional cell carcinoma 70.4% (n=57), unspecified histology 13.6% (n=11), adenocarcinoma 6.2% (n=5), and papillary carcinoma 9.9% (n=8). Treatments included: transurethral resection of the bladder (TURB)/fulguration 63% (n=51), cystectomy 30.9% (n=25) and TURB/intravesicular chemotherapy (IVCTX) 6.2% (n=5).**Conclusion**: In our review, we found no significant difference in recurrence rates based on either a two, five or over five-year wait period. There was only a small increase in mortality due to bladder cancer seen in those pts transplanted with a wait time less than 2 years. However, due to the small number of pts for comparision no definitive conclusions could be drawn. Despite this drawback, it would appear prudent to obtain a 2 year-wait period prior to transplanatation. Even when faced with recurrence, overall survival is favorable (77 to 80%).

Wait Time (yrs)	< 2 yrs	2 to 5 yrs	> 5 yrs	Total
N	35	22	20	77
Recurrence	6/35 (17%)	4/22 (18%)	4/20 (20%)	14/77 (18%)
Death to Disease	5/35 (14%)	2/22 (9%)	2/20 (10%)	9/77 (12%)
Overall Survival	77%	77%	80%	78%

Abstract# 758 **Poster Board #-Session: P14-II**

DE NOVO TESTICULAR CANCER IN SOLID ORGAN TRANSPLANT RECIPIENTS. T. D. Merchen,[1] M. Gupta,[1] M. J. Hanaway,[1] J. Trofe,[1] T. M. Beebe,[1] T. G. Gross,[1] R. R. Alloway,[1] J. F. Buell,[1] E. S. Woodle.[1] *[1]Israel Penn International Tumor Registry, University of Cincinnati, Cincinnati, OH.*

Testicular cancer in the general population has excellent five-year survival rates often exceeding 95%. Survival after de novo testicular cancer in the transplant population, however, remains to be defined. The purpose of the present study was to analyze survival and risk factors that influence survival in de novo testicular cancer solid organ transplant recipients. **METHODS:** Patients with de novo testicular cancer were identified in our database and demographic and outcome data was examined. **RESULTS:** Nineteen patients with de novo testicular cancer after solid organ transplant were identified. The majority of patients were Caucasian 53% (n=10), with the remaining patients being of unknown races 42% (n=8), or African American 8% (n=1). The mean age at transplant was 31.6 years +/- 7.9 and the mean age at diagnosis was 36.9 years +/- 7.8. All 19 patients underwent kidney transplant with 58% (n=11) receiving cadaveric kidneys and 42% (n=8) receiving living related kidneys. The testicular tumors encountered were: seminoma 74% (n=14), nonseminoma 21% (n=4), and Leydig cell tumor 5% (n=1). All 19 patients underwent orchiectomy with the majority of patients with seminomas as well undergoing radiation therapy 57% (n=8). The patients with seminomas had a recurrence rate of 7% (n=1) with this single patient alive with disease at 52.8 months follow-up after diagnosis. An overall mortality in the seminoma population of 21% (n=3) was identified with deaths due to progressive disease in 14 % (n=2) and sepsis in 7% (n=1) of patients. There were no episodes of recurrence or deaths due to progressive disease in the five nonseminoma patients. Median time for follow-up after diagnosis was 19.4 months. **CONCLUSION:** Similar to the general population, post-transplant testicular cancer has a low recurrence rate and high survival.

Abstract# 759 **Poster Board #-Session: P15-II**

COLON CANCER IN SOLID ORGAN TRANSPLANT RECIPIENTS. Naga G. Yadlapalli,[1] Wida S. Cherikh,[2] Myron Kauffman,[2] Martha Pavlakis,[1] Douglas W. Hanto.[1] *[1]Divisions of Nephrology and Transplantation, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [2]Research Department, United Network for Organ Sharing(UNOS), Richmond, VA.*

Introduction: The aim of this study is to examine the clinical and pathological characteristics of colon cancer in liver and kidney transplant recipients and its effect on patient and graft survival.**Methods**: This is a retrospective study based on the information obtained from UNOS. **Results:** There were a total of 231,554 patients who received solid organ transplants (kidney, kidney pancreas, liver, heart, lung and heart-lung) between 1988-2000 of whom 118 developed colon cancer (0.5%). The time between transplantation and the diagnosis was less than one year in 6%, 1-3 yrs in 36%, 4-6ys in 25%, 7-9yrs in 20%, and beyond 10yrs in 8.5% of cases. The correlation between Duke's staging of colon cancer (D.S) and changes in immunosuppressive regimen (I.R.) in kidney, and liver transplant recipients are shown in figure1,2, respectively. **Conclusions: 1)** the majority of colon cancers (67%) manifested within 6 years after transplantation. **2)** Patients with Duke stage C colon cancer had a shorter patient and graft survival as compared to patients with stage A and B. **3)** Kidney transplant recipients who had no changes made in immunosuppressive regimen (either reduced/ stopped) had a better patient and graft survival as compared to those who had changes made in their immunosuppressive regimen (either reduced/stopped). The reverse was observed in liver transplant recipients.

Table.1

Kidney Transplant Recipients with Colon Cancer (61)

		Patient Survival ♦			Graft Survival ♦		
		1Yr	3Yr	5Yr	1Yr	3Yr	5Yr
D.S	A	62	19	9	59	16	9
	B	50	14	7	50	14	7
	C	47	13	0	47	13	0
I.R	No change	65	23	6	66	19	6
	Reduced/ stopped	47	10	6	43	10	6

Table.2

Liver Transplant Recipients with Colon Cancer (31)

		Patient Survival ♦			Graft Survival ♦	
		1Yr	3Yr	5Yr	1Yr	3Yr
D.S	A	53	35	24	53	35
	B	27	9	0	27	7
	C	0	0	0	0	0
I.R	No change	27	13	6	27	13
	Reduced/ stopped	63	31	19	63	31

Abstract# 760 **Poster Board #-Session: P16-II**

DETECTION OF MULTIFOCAL HEPATOCELLULAR CARCINOMA IN PATIENTS UNDERGOING LIVER TRANSPLANTATION: ARE CURRENT SCREENING STUDIES RELIABLE? Fadi Y. Dagher, Richard N. Berri, Atsi Yoshida, Kimberly Brown, Dilip Moonka, Iman Bajjoka, Marwan S. Abouljoud. *[1]Division of Transplant Surgery, Henry Ford Hospital, Detroit, MI.*

OBJECTIVE: Recognition of Multifocal Hepatocellular Carcinoma (MFHCC) is important in the management of patients awaiting liver transplant. The final outcome of MFHCC patients is not well defined. The goal of this study was to determine the sensitivity of the current diagnostic tests and to define the outcome of this subgroup of patients. **METHODS:** 251 adult Liver transplant patients were reviewed from January 1992 to July 2002 at a single tertiary urban Liver Transplant Center. Pathological explants were reviewed and two groups of patients were identified; Focal HCC (FHCC) and MFHCC (defined as diffuse disease or the presence of more than 3 lesions). The prevalence of clinically suspected FHCC and MFHCC were evaluated by pre-transplant radiological studies including CT, MRI, US, and level of alpha fetoprotein (AFP). These results were compared to the explant pathology. The outcome of both groups was evaluated by the rate of recurrence. **RESULTS:** Twenty five patients (10%) had hepatocellular carcinoma at explantation. Seven patients of this group had MFHCC (28% of HCC). Twenty one patients (84%) were male (21/25=84% vs. 137/226=60%, p=0.05), 18 patients had viral etiology (18/25 = 72% vs. 71/226 = 35%, p=0.03) and 20 patients were caucasians (20/25 = 80% vs. 176/226 = 77% p= 0.2). The median follow-up was 25 and 17 months in the FHCC and MFHCC respectively. Highly elevated AFP(> 100) was present in 2 patients (28%) in the MFHCC group but only in 1 patient (5%) in the FHCC group. Multimodality pre-transplant screening (CT, MRI, US) was able to detect all (18/18, 100%) of the FHCC patients but only 1/7 (14%) in the MFHCC group (p=0.05). Recurrence of carcinoma was identified in 2/7(35%) patients in the MFHCC group (3 and 6 months post-transplant) but in none of the patients (0/18) of the FHCC group. **CONCLUSIONS:** 1.HCC is more prevalent in males with viral etiology. 2.The recurrence of HCC in multifocal disease patients is more common after transplantation. 3.Multimodality preoperative radiological screening is not sensitive in detecting MFHCC. 4. Highly elevated AFP (>100) is more prevalent in the multifocal disease. 5. We conclude that elevated AFP in the absence of radiological signs of HCC and in high risk populations (male and viral etiology) should raise the suspicion for MFHCC and possibly warrant a diagnostic laparoscopy and multiple biopsies.

Abstract# 761 **Poster Board #-Session: P17-II**

DE-NOVO MALIGNANCIES AFTER LIVER TRANSPLANTATION: INCIDENCE AND PROGNOSIS. Ivo W. Graziadei,[1] Karin Nachbaur,[1] Alfred Koenigsrainer,[2] Raimund Margreiter,[2] Wolfgang Vogel.[1] *[1]Dept. of Gastroenterology & Hepatology, University of Innsbruck, Innsbruck, Austria; [2]Dept. of Transplant Surgery, University of Innsbruck, Innsbruck, Austria.*

De-novo malignancies are of essential importance in the long term survival after liver transplantation (LT), as they are the second leading cause of late death in LT recipients following cardiovascular complications. Estimates of developing de-novo malignancies range from 4 – 16%. Its pathogenesis is multifactorial, however, long term use of immunosuppression delineates the major risk factor. Therapeutic options of de-novo tumors are extremely limited and thus, the prognosis dismal. The aim of this study was to characterize the incidence, types, time to diagnosis and prognosis of de-novo malignancies in our LT-institution. A total of 502 patients underwent LT in our center between 1882 and 2001; pediatric cases and reLT were excluded. The mean follow-up period was 4.8 (range: 0.1 – 18) years. Immunosuppression consisted of Cyclosporine /FK 506 in combination with prednisolone (taper within 3 months) and azathioprine /mycophenolate mofetil (within the first year). Thirty-six patients developed 38 de-novo malignancies, corresponding to an incidence of 7.6%. Twenty-five patients were men, 11 female with an mean age of 54 ys. The mean time to diagnosis was 45 (range 2-154) months following LT. Interestingly, nine tumors (24%) occurred within the first year post LT. De-novo tumors were as follow: lung 6, oropharynx 6, skin 4, lymphomas (PTLD) 4, pancreas 3, prostate 3, breast 2, renal cell 2, de-novo hepatocellular 1, karposi's sarcoma 1, uterine 1, vesical 1, stomach 1, esophageal 1 and metastatic of unknown primary carcinoma 1. Fourteen patients (39%) died within a mean follow-up period of 15 (0.7-94) months. The total 1 and 3-year survival rates were 62% and 38%. Patients with lymphoma, lung and pancreatic cancer had the shortest survival time (median survival: 4.0, 6.2 and 14.3 months, respectively). A specific immunosuppression did not show significance in tumor development. Alcohol-related cirrhosis was the main indication for LT and the only calculated risk factor for oropharyngeal and lung (in combination with nicotine) cancer. Our study indicates that de-novo malignancies are an important complication after LT with a poor prognosis due to limited therapeutic options. Therefore, careful long-term screening protocols are mandatory in order to detect these malignancies earlier and consequently improve long-term survival of LT recipients.

Abstract# 762 **Poster Board #-Session: P18-II**

DE NOVO NODE POSITIVE SQUAMOUS CELL CANCER IN SOLID ORGAN TRANSPLANT RECIPIENTS. T. D. Merchen,[1] M. Gupta,[1] M. J. Hanaway,[1] J. Trofe,[1] T. M. Beebe,[1] T. G. Gross,[1] R. R. Alloway,[1] E. S. Woodle,[1] J. F. Buell.[1] *[1]Israel Penn International Tumor Registry, Univ. of Cincinnati, Cincinnati, OH.*

Squamous cell carcinoma (SCC) of the skin is the most common malignancy observed in solid organ transplant recipients. Although most skin cancers in transplant recipients can be effectively treated by surgical excision alone, a small but significant minority of patients present with metastatic lymph node involvement. The purpose of this study was to analyze the biologic behavior of de novo, lymph node positive SCC of the skin in organ transplant recipients. **Methods**: Patients with aggressive node positive SCC were identified and patient and tumor demographics were examined and related to survival characteristics. **Results**: 35 patients were identified with node positive SCC at a mean age of 52.4 ± 11.4 yrs with a median time to occurrence post-transplantation of 50.8 months. These pts received: 13 heart, 19 kidney and 3 liver transplants. Sites involved: head and neck (n=22; 63%), extremities (n=10; 29%), torso (n=4; 11%). Seventeen (49%) patients presented with multiple sites or diffuse disease. Late metastases presented in patients in the lung (n=8), CNS (n=1), and liver (n=1). Immunosuppressive therapy included: antibody induction (n=13; 37%), calcineurin inhibitors (n=25; 71%), and azathioprine (n=34; 97%). Thirty (89%) patients underwent surgical excision as primary therapy. Five patients who did not undergo resection were treated with chemotherapy/radiation therapy (n=1), chemotherapy alone (n=1), radiation therapy alone (n=1), and no therapy (n=2). Most patients undergoing surgical resection received radiation therapy (n=20; 66%). The remainder received surgery alone or resection plus chemotherapy. Median follow-up time after diagnosis was 19.6 months. Twelve recurrences (34%) were noted after resection at a median time to recurrence of 11.5 months (4.3 – 63.9 months). Actuarial 1, 3, and 5-yr survival for the entire group was 77, 36, and 20%. Addition of radiation to surgery did not improve survival with 1, 3, and 5-yr survival of 63%, 29%, and 16%. Overall, deaths were due to malignancy in 88% of the 18 patients who died (n=16). **Conclusions**: Node positive SCC is an aggressive lesion with high mortality. Addition of radiation therapy to standard resection did not improve outcome. Further evaluation is necessary to identify risk factors for the development of these malignancies and the practice of immunosuppression reduction and combined therapy.

Abstract# 763 **Poster Board #-Session: P19-II**

ASSOCIATION BETWEEN MYCOPHENOLATE MOFETIL (MMF) AND DEVELOPMENT OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER IN RENAL TRANSPLANT PATIENTS IN THE U.S. Donnie P. Funch,[1] Hnin Hnin Ko,[1] Jacqueline Travasso,[1] Julie Mechlowicz,[1] Alexander M. Walker.[1] *[1]Epidemiology, Ingenix, Auburndale, MA.*

Little is known about the association between the widely used immunosuppressant MMF and the development of post-transplant lymphoproliferative disorder (PTLD). *Methods.* A multi-center case-control study was conducted at 20 large U.S. transplant centers. All participants received a renal only transplant on or after July 1, 1995. PTLD cases were reported by centers and confirmed by external review. The United Network for Organ Sharing (UNOS) identified controls matched on center, date of transplant, and age. Data were abstracted by center personnel for cases and up to 4 controls per case. Abstracted information included detailed data on all initial and maintenance immunosuppressants, antivirals, and rejection therapies as well as demographics, pre-transplant viral status, risk factors for rejection (race, donor type, HLA mismatches, prior transplant, months on dialysis) and rejection experience. *Results.* Data were collected for a total of 108 PTLD cases and 404 controls. Cases were similar to controls on demographic factors. There was a weak association between some of the risk factors for rejection and use of MMF such that patients with some risk factors were more likely to receive MMF. When the total amount of MMF received over the follow-up period was examined, a univariate analysis found nonsignificant increases in risk with each tertile of MMF use. Conditional logistic regression was used to assess the impact of MMF while adjusting for other therapies and known risk factors. Overall, there was no association between basic immunosuppression and PTLD risk. Individuals on double therapy or triple therapy with MMF were similar to individuals on triple therapy with no MMF. There was also no dose response relationship when MMF risk was adjusted for other factors. For PTLD cases, the cumulative amount of MMF received was not associated with any characteristics of PTLD or with survival. *Conclusion.* There is no evidence suggesting an increase in PTLD risk with use of MMF. The nonsignificant dose-response effect noted with the univariate analysis points to the danger of examining simple associations between MMF use and PTLD outcome without adjusting for other factors. The association between MMF use and some of the risk factors for rejection may create some confounding since rejection is a risk factor for PTLD.

Abstract# 764 **Poster Board #-Session: P20-II**

EDUCATION REGARDING SKIN CANCER RISK IN KIDNEY TRANSPLANT RECIPIENTS: WE NEED TO DO BETTER. Ryutaro Hirose,[1] Michael Mendelson,[1] Deborah B. Adey,[1] Stephen J. Tomlanovich,[1] John P. Roberts.[1] *[1]Departmemts of Surgery and Medicine, University of California, San Francisco, San Francisco, CA.*

Purpose: The goal of this study is to determine level of understanding of renal transplant recipients regarding risk factors for skin caners, and their behavior concerning sun exposure. **Methods**: A survey of 951 adult renal transplant recipients being followed in our renal transplant clinic was performed. The survey was performed by a voluntary questionnaire regarding history of skin cancer and potential risk factors completed by renal transplant patients. The patients that were given the questionnaire had all received their kidney transplants more than one year prior to the survey. All (100%) of self-reported cancers were confirmed by pathologic examination. **Results:** The incidence of skin cancer in the surveyed population was 108/951 (11.3%). Despite the ethnic diversity in the surveyed group of renal transplant recipients as a whole (45% Caucasian, 23% Hispanic, 20% Asian, 10% African), 101 of the 108 (93%) patients with skin cancer were Caucasian. Before kidney transplant, 63% seldom or never used sunblock when outside, and 23% sunbathed regularly. After transplant, 47% of the respondents still seldom or never use sunblock. Disappointingly, approximately one-third (34%) of all respondents reported that no health care provider had ever advised them to avoid the sun, 36% currently make no effort to avoid sun exposure. Even in the subset of those patients that have had skin cancer, 19% reported that no health care provider had ever advised avoidance of sun exposure! Only 53% of respondents has ever seen a dermatologist for any reason, and only 23% have regular follow-up visits with a dermatologist. **Conclusions:** The incidence of skin cancer in our renal transplant population is high. The great majority of renal transplant recipients with skin cancer are Caucasian. Low rates of skin cancers are seen in Hispanics, Asians, and African Americans living in the same geographical area. Improved education of transplant patients regarding skin cancer etiology, prevention and risk modification is needed.

Abstract# 765 **Poster Board #-Session: P21-II**

TRANSARTERIAL CHEMOEMBOLIZATION OF HEPATOMAS PRIOR TO TRANSPLANTATION: A PILOT STUDY. George N. Tzimas,[1] Marc Deschenes,[2] Marvin Wexler,[1] Caroline Rochon,[1] David Valenti,[3] Elliot Alpert,[2] Jeffrey S. Barkun,[1] Jean I. Tchervenkov,[1] Peter P. Metrakos.[1] *[1]General Surgery/ Section of Transplantation, Royal Victoria Hospital, Montreal, QC, Canada; [2]Hepatology, Royal Victoria Hospital, Montreal, QC, Canada; [3]Radiology, Royal Victoria Hospital, Montreal, QC, Canada.*

Background: Liver Transplantation (LT) for the treatment of hepatomas (HCCs) has been shown to have excellent results as long as the "UNOS" criteria are adhered to. Several groups have recently reported good results with pts having disease beyond the "UNOS" criteria by applying neoadjuvant chemotherapy. Furthermore transarterial chemoembolization (TACE) of HCCs has been shown to prolong survival in patients with unresectable HCC. Currently, data for carboplatin-based TACE is missing. **Aim:** To assess the impact of neoadjuvant, carboplatin-based TACE on patient survival with HCCs beyond the UNOS criteria. **Materials and Methods:** Between 1997 and 2002, 16 patients were enrolled. HCV was the etiology of cirrhosis in the majority of the cases (Table 1).Criteria included: **a)** single HCC more than 5 cm **b)** up to 3 HCCs more than 3 cm **c)** More than 3 HCCs less than 3 cm each **d)** no main portal vein invasion **e)** no evidence of extrahepatic disease. TACE was performed with carboplatin-lipiodol emulsion and was followed by gelfoam pellet embolization.While treated, pts were followed with monthly AFP, US and CT. Radiographic response and/or AFP decrease was followed by LT. **Results:** 14 patients were transplanted. 2 pts were dropped-off because of disease progression. The average size in the explants was 4.2±1.08 cm for the single lesion and 6.48±0.5 cm for the multiple lesion group. 42% of pts had microvascular invasion, 92% of HCCs had extensive tumor necrosis (>50%), while no pts had positive nodes.There were a total of 2 recurrences.Out of 3 deaths only one was attributed to recurrent HCC. The one, three and five year survival was **75%** (Kaplan-Meier). 4 pts (25%) developed TACE-related complications, while the procedural complication rate was 8%. **Conclusions:** TACE with carboplatin is a safe procedure. It effectively down-stages tumors, while it gives patients the needed "test of time" while waiting for LT. This strategy allowed for transplantation of a group of patients previously thought to be inoperable.

Recipient Characteristics

Etiology	HCV (5 pts, 35%)	HBV (3 pts, 21%)	Other (6 pts, 44%)
Child-Pugh Score	A (5 pts, 35%)	B (4 pts, 30%)	C (5 pts, 35%)
TNM	II (8 pts, 57%)	IIIA (4 pts, 30%)	IIIB (2 pts, 14%)
CLIP Score	1 (5 pts, 35%)	2 (4 pts, 30%)	≥3 (5 pts, 35%)

CLIP=The Cancer of The Liver Italian Program Investigators Score

KIDNEY: DRUG COMPARISON, COMBINATIONS AND NEW STRATEGIES

Abstract# 766 **Poster Board #-Session: P22-II**

BENEFITS OF BASILIXIMAB INDUCTION THERAPY IN LIVING DONOR TRANSPLANTATION. Anne M. Wiland,[1] Jeffrey C. Fink,[2] Benjamin Philosophe,[1] Stephen T. Bartlett.[1] *[1]Transplant Services, University of Maryland Medical Center, Baltimore, MD; [2]Nephrology, University of Maryland School of Medicine, Baltimore, MD.*

Induction therapy in living donor transplantation is controversial due to increased medication costs and favorable outcomes observed without induction. Outcomes that may improve using induction include rejection rates, infectious complications and hospital readmissions. Use of induction therapy may enable the decrease of overall maintenance immunosuppression such as lowering drug level goals or eliminating prednisone altogether. In this study we examine living donor transplant recipients (LRTs) who received basiliximab induction, tacrolimus (FK506) with a target trough level of 10-12 ng/mL, mycophenolate mofetil (MMF) ±prednisone (study group) and compared them retrospectively to LRTs who did not receive induction therapy but received maintenance therapy with FK506 (goal trough=12-15 ng/mL), MMF and prednisone (no induction group). Rejection rates, hospital readmissions 3 months after transplant, length of stay (LOS) and infection rates were compared. 57 LRTs (35 related and 22 unrelated) received basiliximab and 72 LRTs (49 related and 23 unrelated) received no induction. There was no difference in age, sex, CMV status, peak PRA or AB and DR mismatch between the groups. Median length of follow-up was significantly shorter in the basiliximab therapy group (8.3±0.54 vs. 17.8±0.75 months, p=0.001). The initial mean LOS was shorter in the basiliximab group (6.8±3.6 vs. 10.1±21.7 days, p=0.26), however, the median LOS was the same (5 days). The overall rejection rate in the basiliximab group was 15.8% compared to 20.8% in the LRTs who were not induced (p=0.81), however the 6-month rejection rate was comparable (14.0% vs.13.9%, respectively, p=0.9). Reasons for hospital readmissions in the non-induced LRTs included opportunistic infections (1 Nocardia, 4 CMV, 1 Aspergillus) whereas no opportunistic infection readmissions occurred in the basiliximab-induced LRTs. No difference was observed in overall mean LOS for hospital readmission days 3 months after transplant (2.96±6.7 no induction vs. 2.7±5.8 days basiliximab, p=0.83). 4 LRTs in the no induction group had renal biopsies revealing polyoma virus (BK). Prednisone was eliminated in 30/57 (52.6%) LRTs in the basiliximab group. The most recent mean serum creatinine is 1.36±0.39 mg/dL in the basiliximab group and 1.57±0.88 in no induction group (p=0.11). This study demonstrates that basiliximab induction therapy in LRTs allows for reduction in overall maintenance immunosuppression potentially decreasing infectious complications without increasing rejection rates.

Abstract# 767 **Poster Board #-Session: P23-II**

CAN MONITORING WITH NEORAL® TROUGH LEVEL PREVENT SUBCLINICAL REJECTION IN KIDNEY TRASNPLANTATION? Atsushi Aikawa,[1] Kenji Arai,[1] Takeshi Kawamura,[1] Masanori Okamoto,[1] Masaki Muramatsu,[1] Toshihiro Itabashi,[1] Takehiro Ohara,[1] Ken Sakai,[1] Sonoo Mizuiri,[1] Akira Hasegawa,[1] Takayoshi Kosugi,[2] Kazuhiro Matsuo,[2] Ayumu Kusano,[2] Masahiko Obayashi,[2] Minoru Kurokawa.[2] *[1]Department of Nephrology, Toho University, School of Medicine, Tokyo, Japan; [2]Department of Pharmacy, Toho University Omori Hospital, Tokyo, Japan.*

[Purpose] We studied whether subclinical rejection (SR) can be avoided only by monitoring of Neoral® trough level. [Methods] Twenty living related kidney transplant recipients were enrolled in this study. Protocol biopsy was carried out 1 month and 1 year post-transplantation and AUC0-4 was measured at the same time. Immunosuppression was consisted of Neoral®, steroid and azathioprine or mizoribine or MMF. The dose of Neoral® was adjusted to trough level 200-300ng/ml within 2months and 100-150ng/ml 1 year post-transplantation. The doses of immunosuppressants had not been changed for the 2-4 weeks before the procedure of 1 year protocol biopsy. SR signified borderline or the severer rejection classified pathologically by Banff criteria without any clinical signs and symptoms of rejection. [Results] Six patients (30%) had SR and 14 had no SR 1 month post-transplantation. C0, C2 and AUC0-4 did not differ significantly in between patients with and without SR (Table 1).

Table 1. Subclinical rejection and Neoral® monitoring 1month post-transplantation

	C0(ng/ml)	C2(ng/ml)	AUC0-4(ng·hr/ml)
Subclinical Rejection(+)	221±93	1208±207	3510±744
Subclinical Rejection(-)	291±73	1166±453	3788±1171

Eleven patients (55%) had SR and 9 had no SR 1 year post-transplantation. C2 and AUC0-4 were relatively low, although not significant, in patients with SR compared with those without SR (Table 2). In contrast C0 did not differ between patients with and without SR (Table 2). AUC0-4 was less than 2000 ng·hr/ml in all patients with SR.

Table 2. Subclinical rejection and Neoral® monitoring 1year post-transplantation

	C0(ng/ml)	C2(ng/ml)	AUC0-4(ng·hr/ml)
Subclinical Rejection(+)	118±28	454±148	1424±284
Subclinical Rejection(-)	114±26	622±98	2064±532

Cyclosporine toxicity did not present in any biopsy specimen. [Conclusion] SR appeared more frequently 1 year than 1 month post-transplantation. Only Neoral® trough level monitoring failed to prevent SR particularly 1year post-transplantation. SR should be avoided not by monitoring with NEO trough level but with AUC0-4 (preferably more than 2000ng·hr/ml 1year post-transplantation).

Abstract# 768 **Poster Board #-Session: P24-II**

IN VIVO OXIDATIVE STRESS AND LIPID PEROXIDATION IN RENAL TRANSPLANT RECIPIENTS AFTER CONVERSION FROM CYCLOSPORINE TO TACROLIMUS. Waichi Wong,[1] Nina Tolkoff-Rubin,[1] R. Preston Mason,[2] Francis L. Delmonico,[1] Seema Baid,[1] Mary F. Walter,[3] Winfred Williams,[1] A. Benedict Cosimi,[1] Manuel Pascual.[1] *[1]Renal and Transplantation Units, Massachusetts General Hospital, Boston, MA; [2]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA; [3]Elucida Research, Beverly, MA.*

Introduction: Oxidative modification of low-density lipoproteins (LDL) contributes to endothelial dysfunction and atherosclerosis. The most common cause of death in renal transplant recipients (RTR) is cardiovascular disease (CVD). Recent data suggest that oxidative stress biomarkers such as plasma lipid hydroperoxides (LOOH) and malondialdehyde (MDA) may be independently associated with cardiovascular events. Since tacrolimus (Tac) is associated with a lower incidence of hypertension and hyperlipidemia compared to cyclosporine (CsA), we determined if conversion from CsA to Tac may result in a better lipid profile and lower *in vivo* evidence of LDL lipid peroxidation. **Methods**: This was an open-label, single-arm prospective study. Twenty-two adult RTR who were > one year after renal transplantation, receiving CsA-based immunosuppression and had a total serum cholesterol > 200 mg/dl were enrolled. Tac was substituted for CsA to target trough levels of 5-8 ng/ml. Fasting lipid profile, LOOH and MDA were measured at baseline and 6 months post conversion to Tac. LOOH levels were measured using ferrous oxidation with xylenol orange and MDA levels were measured as thiobarbituric acid reactive substances by high power liquid chromatography coupled with ultraviolet detection. **Results**: Six months after conversion, there was significant improvement in total cholesterol (250 ± 50 to 207 ± 28 mg/dl, P<0.001), LDL cholesterol (155 ± 43 to 119 ± 25 mg/dl, P<0.001), LOOH levels (19.47 ± 15.13 to 5.94 ± 5.85 mM, p<0.01) and MDA levels (5.79 ± 3.72 to 2.98 ± 1.66 mM, p=0.01). The percentage of LOOH and MDA decrease was 4.0-fold and 2.8-fold superior to that of total cholesterol and 3.0-fold and 2.1-fold greater than that of LDL, respectively. There were no significant differences in serum creatinine, HDL cholesterol, and triglycerides before and after conversion. **Conclusions**: Conversion from CsA to Tac was safe and associated with a significant decrease in total and LDL cholesterol, as well as LOOH and MDA levels. The decrease in LDL lipid peroxidation products was between 2-4 fold greater than that of total or LDL cholesterol. These novel findings indicate that the potential benefits of Tac use compared to CsA include not only lower LDL and total cholesterol, but also lower levels of LDL oxidation products; all of these factors should decrease the risk of CVD.

Abstract# 769 **Poster Board #-Session: P25-II**

COMPARISON OF TWO DOSAGES OF THYMOGLOBULIN USED AS INDUCTION FOR INITIAL T-CELL CLEARANCE IN KIDNEY TRANSPLANTATION. Waichi Wong,[1] Manuel Pascual,[1] Francis L. Delmonico,[1] Winfred Williams,[1] A. Benedict Cosimi,[1] Nina Tolkoff-Rubin.[1] *[1]Renal and Transplantation Units, Massachusetts General Hospital, Boston, MA.*

Introduction: THYMOGLOBULIN (THYMO), a rabbit derived polyclonal antibody, is currently used as an effective induction agent in renal transplantation as well as for rescue therapy for resistant rejection. Initial T-cell clearance has proved to be one of the most accurate measures of the efficacy of such antibodies. Recent data suggest that a short (3-day) course of THYMO induction is as effective as a 7-day induction course. However the optimal dose of THYMO for induction remains debated. In this study, we compared the effects on T-cell clearance profiles within the first 3 months of transplantation of two different dosages of THYMO. **Methods:** This was an open-label prospective study. A total of fourteen renal transplant recipients (RTR) received a THYMO-based induction protocol, with tacrolimus, prednisone and mycophenolate mofetil (MMF). The first seven consecutive RTR received 1.0 mg/kg/day of THYMO, while the following seven RTR received 1.5 mg/kg/day. THYMO induction was administered daily for a total of three days. T-cell (CD3, CD4, CD8) and B-cell (CD19) subsets were measured by flow cytometry using FACSCalibur on days 3, 7, 14, 30 and 90 post transplant. **Results**: THYMO induction was well tolerated in all patients. Measurements of T-cell subsets revealed equivalent initial T-cell clearance by the two regimens, but more complete and prolonged T-cell depletion after 14 days by the1.5 mg/kg/d dose. In particular, CD3 T-cell subsets were comparable on day 3 (6 ± 2 vs 14 ± 5 cells/mm³, NS), and day 7 (106 ± 46 vs 175 ± 107 cells/mm³, NS), but lower on day 14 (152 ± 56 vs 597 ± 368 cells/mm³, p=0.07), day 30 (331 ± 102 vs 988 ± 248 cells/mm³, p<0.05), and day 90 (371 ± 137 vs 1024 ± 201 cells/mm³, p<0.05) in the 1.5 mg/kg/d versus 1 mg/kg/d groups, respectively. CD4 and CD8 T-cell subsets had similar trends. No acute rejection, CMV infection, or other opportunistic infection was noted in either group. There was 100% patient and graft survival. **Conclusions:** A three-day course of THYMO induction at 1.5 mg/kg/d was found to be safe and provided significantly more complete and more prolonged T cell clearance than 1.0 mg/kg/d. No rejection occurred in any of the patients studied. A more profound and sustained T-cell clearance in the first three months post transplant may translate into a decreased risk of immunological injury and improved long-term outcomes after renal transplantation.

Abstract# 770 **Poster Board #-Session: P26-II**

THE USE OF DACLIZUMAB, TACROLIMUS AND MYCOPHENOLATE MOFETIL IN AFRICAN-AMERICAN AND HISPANIC FIRST RENAL TRANSPLANT RECIPIENTS. Gaetano Ciancio,[1] George Burke,[1] Adela Mattiazzi,[1] Zvi Leibovici,[1] Anil Vaidya,[1] David Roth,[2] Warren Kupin,[2] Anne Rosen,[1] Joshua Miller.[1] *[1]Surgery, University of Miami, Miami, FL; [2]Medicine, University of Miami, Miami, FL.*

Introduction and Objective: Limited data are available on the use of tacrolimus and mycophenolate mofetil in conjunction with anti IL-2 receptor antibody, particularly in higher risk groups of patients. This study compared differences in incidence of acute rejection between African-American, Hispanic and Caucasian first renal transplant recipients. **Methods:** We studied 233 sequential recipients of first renal transplant. Of the 233 patients, 37 (16%) were African-Americans, 85 (36.5%) were Hispanics and 111 (47.5%) were Caucasians. All received Daclizumab (1 mg/kg) on the day of surgery, and every other week for a total of 5 doses, Mycophenolate mofetil was started on day 1 (1 gm/bid) and tacrolimus was withheld until serum creatinine was < 4 mg/dl, and steroids. There was at least 1 HLA DR antigen compatibility match present between all donors and recipients. **Results:** At 1 year, patient and graft survival for African-Americans was 97% and 95%, 98% and 98% for Hispanics and 96% and 95% for Caucasians, respectively, and were not statistically different. Biopsy-proven acute rejection episodes were 8.1% in African-Americans, 4.7% in Hispanics and 4.5% in Caucasians. Recurrent rejection episodes have not been seen. The incidence of infection requiring hospitalization appeared to be low among the 3 groups. **Conclusions:** The addition of daclizumab to our immunosuppression protocol is safe and effective in decreasing biopsy-proven acute rejection and improving renal allograft survival in African-American and Hispanic renal transplant recipients in the first year posttransplantation which may have an eventual impact in long-term graft survival.

Abstract# 771 **Poster Board #-Session: P27-II**

COMPARISON OF TACROLIMUS (FK506) AND SIROLIMUS(SRL) COMBINATION WITH FK506 AND MYCOPHENOLATE MOFETIL (MMF) IN KIDNEY TRANSPLANT RECIPIENTS WITH STEROID AVOIDANCE. Aparna Kumar,[1] Daniel Lee,[1] Sheng G. Xiao,[1] Michael J. Moritz,[1] Billie Fyfe,[2] Michael Heifets,[3] Debra Sierka,[4] Mysore S. Anil Kumar.[1] *[1]Surgery/Transplant, Drexel University College of Medicine, Philadelphia, PA; [2]Pathology, Drexel University College of Medicine, Philadelphia, PA; [3]Tranplant, Hahnemann University Hospital, Philadelphia, PA; [4]Medicine/nephrology, Drexel University College of Medicine, Philadelphia, PA.*

Chronic steroid therapy is toxic and may accentuate the side effects of FK506. A prospective steroid free therapy study was carried out with FK506/MMF(group 1) and FK506/SRL (group 2) to determine the efficacy, safety and advantages of these 2 combinations and to avoid steroid related side effects. 49 primary kidney recipients with low PRA were randomized into 2 groups with comparable demography. All recipients were induced with basiliximab and were given 2 doses of methylprednisolone; 250mg on day 0 and 125 mg on day 1 and then discontinued. Group 1 was given 2g of MMF/day and in Group 2, SRL dose was adjusted to maintain blood levels of around 10ng/ml. Blood FK506 levels in both the groups were maintained at 10-15ng/ml. Acute rejections (AR) were diagnosed by biopsy and treated with pulse doses of steroids. Protocol biopsies were done to diagnose chronic allograft nephropathy (CAN) and subclinical acute rejections (SCAR). Kidney function was assessed by serum creatinine and creatinine clearance. Twenty-nine recipients were in Group 1 and 20 in Group 2. AR were seen in 14% of MMF and 5% of SRL(p=ns). SCAR was seen in 14% of MMF and 15% of SRL groups. In MMF group, CAN was absent in 47%, mild in 28% and moderate in 25% and in SRL group, CAN was absent in 50%, mild in 30% and moderate in 20%. Serum creatinine was 1.7 and 1.8 mg% and creatinine clearance was 74 and 59 mls/minute in MMF and SRL groups respectively. One year patient survival was 100% in Group 1 and 95 % in Group 2, and graft survival was 95% and 95% in groups 1 and 2. The incidence of bone marrow depression, gastrointestinal side effects and hyperlipidemia were similar in the 2 groups.There was no incidence of delayed wound healing in SRL group. In FK506/MMF group one African American recipient(3%) developed post transplant diabetes mellitus(PTDM) and in FK/SRL group none had PTDM.Our data indicates that steroid free combination of FK506/MMF and FK506/SRL provide comparable patient and graft survival with a similar incidence of graft function,CAN, and acute rejection. Incidence of FK506 associated PTDM was significantly reduced in both the groups compared to previous reports in the literature.

Abstract# 772 **Poster Board #-Session: P28-II**

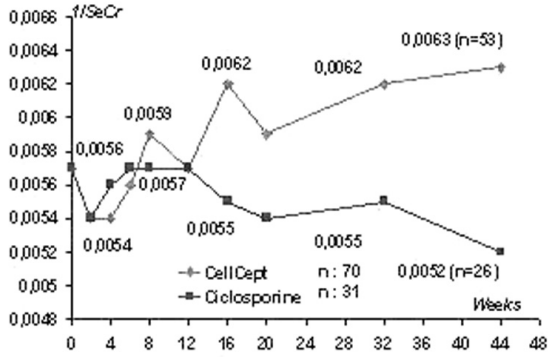
STEROID AVOIDANCE WITH TACROLIMUS AND MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANTATION. Richard Borrows,[1] Jen van Tromp,[1] Thomas Cairns,[1] Megan Griffith,[1] Nadey Hakim,[1] Adam McLean,[1] Andrew Palmer,[1] Vassilios Papalois,[1] David Taube.[1] *[1]Renal and Transplant Units, St.Mary's Hospital, London, United Kingdom.*

Early data from the US suggest that steroid avoidance regimes [steroids for 7 days post transplant] in combination with Tacrolimus [Tac] and Mycophenolate [MMF] are associated with good patient and allograft survival, with a low incidence of rejection and post transplant diabetes mellitus [PTDM]. In this study, we report our results from West London, which has a high proportion of non-Caucasoid recipients [>50%], using a steroid avoidance regime [Prednisolone 1mg/kg/day at day 0 and stopped at day 7] with Tac and MMF. 101 patients [65m, 36f, mean age 43 years, 73 cadaver and 28 live donor, mean HLA mismatch 2.2 antigens] were enrolled into the study. Mean follow up was 14 months [range 1-29 months]. Baseline immunosuppression consisted of Tac [levels adjusted to 10–15 ng/ml] and MMF [750 mg twice daily, and increased, if tolerated to a maximum of 2g daily]. Twenty-five patients who were either sensitised or receiving a poorly matched allograft [3 or more mismatches] also received CD25 monoclonal antibody induction. Allograft rejection was diagnosed by biopsy and treated with IV methyl prednisolone [MP] and oral prednisolone [P] 30 mgs daily, tapering to 10 mgs by 3 months. Overall patient survival is 99% [1 death due myocardial infarction at 13ms] and graft survival 98% when censored for this death. Two other grafts were lost [1 renal vein thrombosis; 1 from chronic rejection as a result of non compliance]. Nineteen patients [19%] have experienced rejection, with 1 episode occurring before steroid withdrawal. With the exception of the single non-compliant patient, all episodes were reversed with methyl prednisolone and prednisolone. Mean estimated GFR [by Cockroft-Gault] was 55.5 (±17), 56.0 (+/-17) and 57.0 (+/-18) mls/min at 3, 6 and 12 months respectively. There were few significant infections [tissue-invasive CMV disease in 2 patients, BK virus nephropathy in 2 patients, multi-resistant urinary tract infections in 3 patients] and 1 patient has developed malignancy [carcinoma of the breast]. 1 patient developed recurrence of original disease [dense deposit disease]. Only 3/101 patients [3%] have developed PTDM, compared with an incidence of 20% in our historic control group. There was an insignificant mean weight gain at 12 months of 5.2[±1.7] kg . This study shows that this steroid avoidance regime with Tac and MMF produces excellent short-term results in a non-US, predominantly non-caucasoid patient group, with few side effects.

Abstract# 773 **Poster Board #-Session: P29-II**

RENAL FUNCTION EVALUATION AFTER HALF DOSE REDUCTION OF NEORAL® IN COMBINATION WITH CELLCEPT® IN RENAL TRANSPLANT PATIENTS WITH ALTERED RENAL FUNCTION: PRELIMINARY 12 MONTHS RESULTS: RANDOMIZED, OPEN, MULTICENTRIC, PROSPECTIVE, CONTROLLED STUDY. M. Kessler. *[1]Transplantation-Nephrology, Brabois Hospital, Nancy, France.*

Introduction: Although pathophysiology of CAN is multifactorial, progressive but significant reduction of CsA, or even complete discontinuation, in pts with failing renal function (RF) can improve or slow this deterioration. CellCept® (CC) is a potent IS without vascular/metabolic toxicities, and should allow a new therapeutic approach in renal transplant (RT) pts with altered RF. **Objectives**: Evaluate long term benefits of a strategy consisting in CC introd. (2 g/d) over 4 weeks followed by CsA half dose reduction over 4 weeks, on RF evolution in pts with altered RF. **Methods**: 103 RT pts, with CsA based IS therapy, 1st or 2nd CAD or LRD > 1 yr and < 10 yrs with a serum creatinine (SCr) :150-300 µmol/L, were randomised (2:1 ratio). 101 pts were analysed in ITT at 12 m. **Baseline Parameters**: 84 reached 12 m. and 31 pts have completed 2 yrs. There was no significant difference in baseline parameters between 2 groups. Median follow up is 526 days in CC group and 524 days in CsA group. At end of Phase I (8 weeks; CC introd. and CsA half dose) T0 of CsA was 79±54 ng/ml in CC group and 133±49 in CsA group. **Results**: **Safety**: At 32 m. after randomisation, 17 pts have been withdrawn. 1st causes were: in CC group: GI disorders (5); in CsA group: RF deterioration (2). At 12 m. proteinuria was 0.56±0.74 g/24h in CC group (n=36) and 0.71±0.94 in CsA group (n=21). **Efficacy**: **at 12 m,** 1/SCr increased in CC group from 0.0057 to 0.0063 (SCr: 177 to 159 µmol/L), while it decreased in CsA group from 0.0057 to 0.0052 (SCr: 177 to 193 µmol/L). A complete statistical analysis will be shown (101 pts).



Conclusion: In RT pts with altered RF, CellCept® introd. followed by a significant CsA reduction is a safe approach with a benefice on RF at 12 m. The design of this study for long term evaluation will give us possibility to confirm these results.

Abstract# 774 **Poster Board #-Session: P30-II**

INDUCTION VERSUS NO INDUCTION THERAPY IN KIDNEY TRANSPLANTATION – CONSIDERING DIFFERENT PRA LEVELS AND DIFFERENT INDUCTION THERAPIES. Maria Cristina R. Castro,[1] Liliam M. P. Araujo,[1] Sami Arap,[1] Elias David-Neto,[1] Luiz Estevam Ianhez.[1] *[1]Renal Transplantation Unit, Sao Paulo University, Sao Paulo, SP, Brazil.*

Graft survival and acute cellular rejection (ACR) rates are extremely influenced by the presence of anti-HLA antibodies. To evaluate the rate of ACR and long-term results in different levels of anti-HLA sensitization, using no induction or different induction therapies, we analyzed 763 patients transplanted from Jan/95 until Dec/2001 at our unit. 213 patients received induction therapy: 71 patients received THYMOGLOBULIN, 66 SIMULECT and 44 OKT3. Follow-up time was at least one year for all groups. SIMULECT had older recipients and OKT3, more female patients. SIMULECT and OKT3 had more black patients, THYMO and OKT3 more retransplants. Patients who received induction had also longer dialysis time. PRA was very low in the no-induction group (Med: 7%) and about the same in the SIMULECT and the THYMO group (Med: 30%). OKT3 was the most sensitized group (Med=59%). Dialysis at the first week post transplantation was frequent, mainly in the induction groups (43 x 65%, p< 0,005). Fewer patients had rejections episodes in the THYMO group (20% x 50%, p=0,02). A 2nd episode of rejection was also less frequent in the THYMO group (7%x20%, p<0,05), and occurred significantly later (59dx23d, p<0,01). We classified all patients by their level of sensitization (<10%, 10-50% and >50%), and we could see that THYMO showed the lower rejection rates in all levels (Med= 20%,p=0,001). When analyzing specifically those patients with PRA > 50%, the THYMO group showed significantly lower rejection rates (12%x50%, p=0,02). The relative risk of

rejection was reduced 2.3 times with THYMO versus no-induction in these hyper-immunized patients. At this level of sensitization, there was no significant difference on graft loss and death with a functioning graft. Death in the THYMO group was caused equally by infection and cardiovascular disease. There was only a tendency to more CMV disease in the THYMO group (33%x23%, p=0,08), but no death was related to CMV. Two PTLD were diagnosed, both in the no-induction group. Renal function at the end of the 1st. year was better in the THYMO group (1,3 mg/dl). In conclusion, THYMOGLOBULIN showed lower one-year ACR rates in all PRA level groups when compared to no-induction, SIMULECT and OKT3. No significant differences in CMV infection, tumors and patient survival were detected in all groups at the end of the 1st year.

Abstract# 775 **Poster Board #-Session: P31-II**

SIROLIMUS-BASED THERAPY WITH OR WITHOUT CYCLOSPORINE: LONG-TERM FOLLOW-UP IN RENAL TRANSPLANT PATIENTS. Jose M. Morales,[1] Josep M. Campistol, Henri Kreis, Georges Mourad, Josette Eris, Francesco P. Schena, Josep M. Grinyo, Marco Castagneto, Nathalie Castaing, James T. Burke. *[1]Hospital 12 de Octubre, Madrid, Spain.*

Purpose: This ongoing, open-label study in renal transplantation assesses the long-term safety of sirolimus (Rapamune®, SRL), administered with cyclosporine (CsA; SRL+CsA group) or as base therapy without CsA (SRL group). **Methods:** The recruitment for this extension protocol started in April 1998 for patients who either had completed a Wyeth Research-sponsored study in solid organ transplantation or had achieved a protocol-designated endpoint after at least 3 months of study participation. Data are available for 158 patients remaining on originally assigned therapy (SRL+CsA or SRL). **Results:** In the SRL+CsA group (98 patients), 1 was subsequently switched to tacrolimus. In the SRL group (60 patients), 17% were receiving SRL combined with azathioprine and 2% SRL combined with MMF. All patients were initially receiving steroids. Mean total exposure was 1093 days (including prior study participation). The SRL group had significantly higher mean calculated glomerular filtration rates (GFR, Nankivell method) from months 4 to 36 after transplantation. The slope of GFR, calculated beginning at 6 months after transplantation, was negative for SRL+CsA (-3.56 mL/min per year, p<0.001), indicating declining renal function, but was positive for SRL (0.93 mL/min per year, p=0.257), indicating improving renal function; the difference between treatments was highly significant (-4.45 mL/min per year, p<0.001). During this extension study, the incidences of the following treatment-emergent adverse events were significantly higher in the SRL+CsA group: increased creatinine, anemia, hypertension, gingival hyperplasia, nausea, headache, and upper respiratory infection. No adverse events were significantly more frequent in the SRL group. No malignancies were reported in patients receiving CsA-free therapy, whereas 9.2% of those receiving SRL+CsA (p<0.01) had malignancies. Discontinuations from the SRL+CsA group were 27.6% compared with 15% from the SRL group (p=NS). The frequencies of acute rejection (6.1% vs 0%), graft loss (2% vs 0%, deaths censored), and death (2% vs 1.7%) were not significantly different (SRL+CsA vs SRL, respectively). **Conclusions**: SRL-based therapy without CsA is a safe alternative to SRL+CsA immunotherapy and provides long-term improvement in renal function without increased risk of late acute rejection.

Abstract# 776 **Poster Board #-Session: P32-II**

CLINICAL MONITORING OF MYCOPHENOLIC ACID (MPA) CONCENTRATIONS IN KIDNEY TRANSPLANT RECIPIENTS USING AREA UNDER THE CURVE AND TROUGH CONCENTRATION CORRELATION. Joan S. Kramer,[1] Charles F. Shield III,[1] Glenn A. Wiens.[1] *[1]Kidney Transplant Program, Via Christi Regional Medical Center, Wichita, KS.*

Introduction: Parameters for therapeutic drug monitoring of mycophenolic acid (MPA) have yet to be determined. This study in kidney transplant recipients measured MPA levels to determine area under the curve (AUC) for mycophenolate mofetil (MMF) dosage requirements. AUC values and corresponding trough concentrations were studied for correlation purposes. Methods: Forty-one consecutive patients (63% male), average age 41.68 (14-65), during the previous 9 months received MMF (n = 35 < 24 months post transplant). Seven patients (17%) were insulin dependent diabetics. Thirty-six patients (88%) received tacrolimus, MMF and prednisone for immunosuppression; initial MMF doses were 1000mg bid (n = 35), < 1000mg bid (n = 4), and >1000mg bid (n = 2). MPA AUC values were calculated using the trapezoidal AUC method; MMF dose administration was observed immediately after time 0. Blood MPA concentrations were measured at time 0, 30, 60, 90, 120, and 240 minutes. All MMF dosage adjustments were based on a target AUC of 20 ug . h/mL. Results: All 4 patients receiving MMF < 1000mg had AUC results < 20 ug . h/mL and MPA trough concentrations of 1 - 2 ug/mL (n = 3) and 2.1 - 3 ug/mL (n = 1). One patient (MMF > 1000mg bid) achieved an AUC value > 20 ug . h/mL. Ten patients (MMF 1000mg bid) reached the AUC target of > 20 ug . h/mL with MPA trough concentrations of 1 - 2 ug/mL (3 patients), 2.1 - 3 ug/mL (3 patients), and > 3 ug/mL (4 patients). Twenty-five patients (71%) receiving MMF 1000mg BID had a target AUC < 20 ug . h/mL; MPA trough concentrations measured < 1 ug/mL (2 patients), 1 - 2 ug/mL (19 patients), and 2.1 - 3 ug/mL (4 patients). MMF doses were increased in twenty patients; MPA AUCs have been completed in 10 patients. Seven patients (70%) had an AUC > 20 ug . h/mL; MPA trough concentrations were 1 - 2 ug/mL (5 patients), 2.1 - 3 ug/mL (1 patient), and > 3 ug/mL (1 patient). Subanalysis of the 7 patients with insulin dependent diabetes showed that 6 patients had AUC values < 20 ug . h/mL, indicating a need for dosage adjustment. Conclusion: The majority of patients receiving MMF 1000mg bid are not receiving enough drug based on AUC results. Although it would appear that the patients studied requiring MMF dosage increases had measured MPA trough concentrations < 2 ug/mL, not enough data is available to suggest a relationship between AUC and MPA trough results for dosage adjustment. Clinical correlation of AUC results with rejection is being studied.

Abstract# 777 **Poster Board #-Session: P33-II**

CONCENTRATION-CONTROLLED USE OF SIROLIMUS (SRL) ASSOCIATED WITH REDUCED EXPOSURE OF CYCLOSPORINE (CSA) IN BLACK PATIENTS. Paula G. Machado,[1] Cláudia R. Felipe,[1] Sung I. Park,[1] Riberto Garcia,[1] Marcelo Franco,[2] Fernando Alfieri,[3] Dulce E. Casarini,[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; [2]Pathology, Universidade Federal de São Paulo, São Paulo, SP, Brazil; [3]Laboratórios Wyeth, São Paulo, SP, Brazil.*

In a phase III trial black patients receiving CSA and prednisone required a higher dose (5 mg) of SRL to show reduced incidence of acute rejection (AR) compared to azathioprine. Due to the large inter-individual variability in SRL concentrations, the purpose of this prospective trial was to define target SRL whole blood trough concentrations in this high-risk population. **Methods**: Kidney transplant recipients of black ethnicity received CSA 8-10 mg/kg/day, prednisone (30 mg), and a 15 mg loading dose followed by 5 mg fixed daily doses of SRL till day 7 when they were randomized to target SRL trough blood concentration of 8-12(GI) or 15-20(GII) ng/mL (HPLC). **Results**: 70 patients (64 living/6 cadaveric, mean age 34.4±11.6 years, 67% male, mean HLA mm 3.3±1.1) were randomized to GI (N=34) and GII (N=36). Mean follow up time was 420 days (30-705) and 58 patients reached 6 months. There were no deaths and 3 graft losses (GI=1; GII=2). The overall incidence of biopsy-proven acute rejection at month 6 was 10% [GI: 11.7 % (1IA, 2 IIA and 1 IB) vs. GII: 8.3% (1 IA, 1 IB and 1 IIB), p=0.706]. At 1, 3, and 6 months, mean CSA concentrations were 178±85 vs. 163±89, 113±96 vs. 98±55, 77±57 vs. 66±36 ng/mL (ns), respectively. Accordingly, mean SRL trough concentrations were 17.7±9.2 vs. 19.2±9.8 (ns), 12.1±5.4 vs. 20.7±8.2 (p< .001), 10.6±6.1 vs. 17.9±5.5 ng/mL (p< .001). At 6 months, mean SRL doses were higher in GII (3.2±1.7 vs. 5.0±3.2 mg/day, p=.02) but mean prednisone doses were comparable (10.5±8.9 and 9.5±7.8 ng/mL, ns). There were no differences in mean creatinine (1.6±.04 and 1.9±1.1 mg/dL, p=0.187), cholesterol (263±152 vs. 253±59 mg/dL) or triglyceride concentrations (263±183 vs. 249±142 mg/dL), but patients in GII had lower hemoglobin values (14.5±1.9 vs. 13±2.4 g/dL, p=.02). There were no differences comparing the incidence of infections, thrombocytopenia, leukopenia or in the number of SAEs in each group (GI=21 vs. GII=27). **Conclusion**: High risk transplant patients of black ethnicity showed very low incidences of acute rejection and good graft function using a SRL/CSA concentration-controlled strategy and early reduction in cyclosporine exposure. SRL target therapeutic concentration early after transplantation appears to be at least 15 ng/mL.

Abstract# 778 **Poster Board #-Session: P34-II**

IMPACT OF ANTIBODY INDUCTION ON OUTCOME IN HIGH-RISK LIVING DONOR RENAL TRANSPLANT RECIPIENTS. Shamkant Mulgaonkar,[1] Ashok Kumar,[1] Paresh Kaneria,[1] Luigi Bonomini,[1] Nita Shah.[1] *[1]Dep't of Transplantation, Saint Barnabas Medical Center, Livingston, NJ.*

Cadaveric donor organ shortage combined with increasing demand, has resulted in dramatic growth in living donor (LD) kidney transplantation. Due to advances in surgical techniques of donor nephrectomy with shortened donor recovery time and excellent graft and patient survival, use of unrelated LDs has also increased. LD organ transplant recipients generally are considered at low risk of rejection and often do not receive induction therapy, but certain factors may increase recipient risk, warranting antibody use. **Methods**: This study was a single center retrospective analysis of all LD kidney transplants performed from January 1998 to August 2001. Patients were stratified into high and low risk groups. High risk criteria were prior transplant, PRA>20%, 6 antigen mismatch, and 2 DR mismatch. African American race, ABO incompatibility, and positive crossmatch, although considered high risk characteristics, were not included. Outcomes at 1 year were examined based on whether or not induction therapy was administered, and if so, what type.Outcome parameters were serum creatinine (SCr), graft survival (GS), acute rejection (AR) and serious adverse events (SAEs). **Results**: Of 230 LD transplants, 152 were low risk and 78 were high risk. Patients in both groups received either antibody induction (basiliximab, equine antithymocyte globulin [ATG], or rabbit ATG) or no antibody induction, and triple maintenance immunosuppression. There was no significant benefit of antibody induction in the low risk group, and no increase in SAEs. High risk patients who did not receive antibody therapy had an increased AR rate. There was no difference in SCr, GS,or AR of patients treated with basiliximab compared to patients treated with rabbit ATG; SAEs in these patients were similar.

Low Risk	**n**	**SCr (mg/dL)**	**GS (%)**	**AR (%)**	**SAEs (%)**
No antibody	113	1.5	97.3	16	24
Basiliximab	27	1.34	92.5	11	6
Equine ATG	8	1.5	87.5	12.5	2
Rabbit ATG	4	1.5	100	25	2
High Risk	**n**	**SCr (mg/dL)**	**GS (%)**	**AR (%)**	**SAEs (%)**
No antibody	36	1.7	97	39	8
Basiliximab	26	1.65	92.3	19.2	4
Equine ATG	6	2.3	100	33	4
Rabbit ATG	10	1.87	90	20	4

Conclusion: It is important to separate low risk from high risk LD transplant recipients. Low risk patients probably do not require antibody induction. For high risk LD transplant patients, antibody induction is beneficial in reducing the incidence of AR without measurably increasing adverse events. When high risk patients were compared according to whether they received basiliximab or rabbit ATG, no difference in GS, incidence of AR, or SAEs was observed.

Abstract# 779 **Poster Board #-Session: P35-II**

SUBSTITUTION OF SIROLIMUS (SRL) FOR STEROIDS IN LONG-TERM RENAL TRANSPLANT RECIPIENTS TREATED WITH CYCLOSPORINE (CSA). Barry D. Kahan, Jeanette Podbielski,[1] Lai Dejian,[2] Charles Van Buren.[1] *[1]Div Immunology and Organ Transplantaion, Univ of Texas Medical School, Houston, TX; [2]Univ of Texas School of Public Health, Houston, TX.*

Objective: Can SRL be substituted for steroids among long-term CsA-treated renal transplant recipients afflicted with a variety of steroid-induced complications? **Methods**: 30 recipients (19 men/11 women) aged 25-68 years bearing successful renal allografts for 9-204 months (mean±SD=58±27.7; median=47) requested steroid withdrawal due to a variety of complaints. The study was approved by the Human Subjects Committee; all patients signed informed consent documents. A 6-week withdrawal program began 1 week after initiation of SRL as a 6mg loading dose and 2mg daily doses, which were subsequently adjusted to achieve trough concentrations of 10± 3ng/mL. CsA exposure was concomitantly reduced by >50% to preserve renal function at pre-enrollment levels. The mean values (±SD) of clinical and laboratory data at 1, 3, 6, 12, and 24 months were subjected to ANOVA. A Quality Of Life (QOL) questionnaire specifically addressing medical and social complications associated with steroids was administered at baseline, 12, and 24 months. **Results**: 26/30 (87%) of patients were successfully withdrawn and are currently stable. The discontinuations include 1 patient who withdrew consent; 2 graft losses at 7 and 11 months after steroid withdrawal (44 and 113 months posttransplant) due to chronic rejection; and 1 patient with elevated serum creatinine (Scr) due to recurrent primary disease, hemolytic uremic syndrome, whose graft remains functional (Scr=2.2 mg/dL). There were no episodes of acute rejection. Comparison of group mean baseline vs. 1 year post-withdrawal values revealed no significant change in Scr (+0.12mg/dL), cholesterol (CHO; -14.7mg/dL), triglycerides (TG; +18.2), or systolic (-0.6mm Hg) and diastolic (+0.04) blood pressures. Pairwise comparisons of pre vs post values in individual patients using the nonparametric Wilcoxon test showed no significant difference: Scr (p=0.07), CHO (p=0.21), TG (p=0.37), and systolic (p=0.15)/diastolic (p=0.13) blood pressures. Benefits of steroid withdrawal were evidenced by responses on the QOL instrument, including improved activity (moderate to full) in all patients, increased capacity to climb 3 flights of stairs (41 to 88%), ability to walk more than a mile (41 to 62%), decreased gingival hypertrophy (29 to 50%), return to desired body weight (12 to 35%), and satisfaction with appearance (6 to 38%). **Conclusions**: SRL substitution for steroids was successful in most patients with subjective improvements in their QOL.

Abstract# 780 **Poster Board #-Session: P36-II**

SUCCESSFUL RAPID DISCONTINUATION OF PREDNISONE IN HIGH-RISK KIDNEY TRANSPLANT RECIPIENTS. Khalid Khwaja,[1] Massimo Asolati,[1] James Harmon,[1] Kristen Gillingham,[1] Abhinav Humar,[1] Raja Kandaswamy,[1] Arthur Matas.[1] *[1]Surgery, U of Mn, Mpls, MN.*

Immunosuppressive protocols incorporating prednisone avoidance or rapid discontinuation of prednisone have been shown to be successful in low risk transplant recipients (e.g., first transplant, immediate graft function). We report on rapid discontinuation of prednisone in a higher risk cohort. Between 10/1/2000 and 10/1/2002, we transplanted 78 high risk kidney transplant recipients (22 retransplants; 6 African American; 9 Native American; 21 delayed graft function [DGF]; 46 peak PRA >10% [24 peak PRA >50%]), using a rapid discontinuation of prednisone protocol. Many recipients had >1 high risk factor: African Am & DGF (2), African Am & 2nd tx (1); DGF & PRA >10 (1), DGF & PRA >50 (1), DGF, 2nd tx, & PRA >50 (1); 2nd tx & PRA >10 (3), 2nd tx & PRA >50 (9), 3rd tx & PRA >50 (1); Native Am & PRA >10 (1), Native Am, 2nd tx, & PRA >50 (1). Immunosuppression consisted of polyclonal antibody (Thymoglobulin) (1.25-1.5 mg/kg for 5 days, the first dose given in the operating room); Prednisone (P) (Solu-medrol, 500mg intraop, P 1mg/kg on postop day 1, 0.5mg/kg on postop days 2 & 3 and 0.25mg/kg on postop days 4 &5); calcineurin inhibitor; and either mycophenolate mofetil or sirolimus. All received ganciclovir prophylaxis. For those with DGF, antibody and 5 mg/day P was continued until urine output increased and serum Cr level decreased (maximum 10 doses); at that time calcineurin inhibitors were started and both antibody and P were stopped. For the entire group (including PRA >10) (n=78), actuarial 3-year graft survival was 96%; patient survival, 96%. Ninety-eight percent of recipients were free of acute rejection at 1- and 3- years posttx; 99% were free of biopsy-proven chronic rejection at 3-years postx. When the data was restudied using a definition for high PRA of >50% (n=63), results were similar: 3-year actuarial graft survival, 95%; patient survival, 94%; acute rejection-free graft survival, 93%; and chronic rejection-free survival, 98%). In the entire group, there have been only 3 graft losses (1 from acute tubular necrosis/primary nonfunction, 1 from death with function, and 1 from a technical/vascular complication). 2 recipients died (1 after graft failure): 1 from fungal sepsis, and 1 myocardial infarction. **Conclusions.** We report >90% rejection-free graft survival using a rapid discontinuation of P in a higher risk kidney transplant cohort. We conclude steroids can be rapidly discontinued in higher risk kidney transplant recipients without compromising graft outcome.

Abstract# 781 **Poster Board #-Session: P37-II**

A PROSPECTIVE, RANDOMIZED, PHASE IV COMPARATIVE TRIAL OF THYMOGLOBULIN® VERSUS SIMULECT® FOR THE PREVENTION OF DELAYED GRAFT FUNCTION AND ACUTE ALLOGRAFT REJECTION IN RENAL TRANSPLANT RECIPIENTS. Hamid Shidban,[1] Mazen Sabawi,[1] Mercedes Puhawan,[1] Sali Aswad,[1] Rafael G. Mendez,[1] Robert Mendez.[1] *[1]National Institute of Transplantation, Los Angeles, CA.*

Introduction The initial immunosuppressive induction regimen may have a significant impact on early allograft function and can delay or prevent rejection. The theoretical advantages of anti-lymphocyte induction include avoiding the use of calcineurine inhibitors in the immediate post-transplant period and thus reducing potential nephrotoxicity. **Study objectives** Measure the impact of Thymoglobulin® vs. Simulect® on delayed graft function (DGF) and acute rejection rates post-transplant in first cadaveric renal transplant patients. **Material and Method** Single center, prospective, randomized, open label Phase IV study. First cadaveric renal transplant patients between December 2001 and September 2002, who met enrollment eligibility criteria were randomized to this trial on a 2:1 ratio (75 patients: 50 in Thymoglobulin® arm & 25 in Simulect® arm). All patients were given Neoral®, Cellcept® and Prednisone as maintenance immunosuppressive regimen. Thymoglobulin was started on day of transplant (1.5 mg/Kg/day IV infusion for 5 days not to exceed 100 mg daily); Simulect also started on transplant date (two doses: 20 mg IV at transplant and day 4). **Results** (See Table below) **Conclusions** The interim analysis of the data shows patients who received Thymoglobulin at time of transplant had a significantly lower rate of delayed graft function. Acute rejection was less in the Thymo group at three months post transplant, although it is not statistically significant. The increased DGF percentage in the Simulect group may be attributed to early use of Cyclosporine post transplant at day 1. More follow up and data collection up to one year post transplant will be done for long-term impact of these two medications on graft and patient survival.

	Thymoglobulin®	**Simulect®**	**P-Value**
Patients Number	50	25	
R-Gender Male	36 (72%)	18 (72%)	NS
R-Gender Female	14 (28%)	7 (28%)	NS
R-Age Mean	52 (±11)	54 (±14)	NS
R- Age > 50 Y	30 (60%)	19 (76%)	NS
D- Gender Male	27 (54%)	19 (76%)	NS
D- Gender Female	23 (46%)	6 (24%)	NS
D- Age Mean	46.5 (±15)	48.1 (±13)	NS
D-Age > 50 Y	23 (46%)	15 (60%)	NS
CIT Mean	26.8 (±8)	26.2 (±7)	NS
DGF	22 (44%)	19 (76%)	0.010
Acute rejection at 3 Months	4 (8%)	3 (12%)	NS

Abstract# 782 Poster Board #-Session: P38-II

SIROLIMUS(SRL) AS 'RESCUE' TREATMENT IN KIDNEY AND KIDNEY-PANCREAS TRANSPLANTATION IN PATIENTS RECEIVING TACROLIMUS(TAC)-BASED IMMUNOSUPPRESSION. Amit Basu,[1] Mohan Ramkumar,[1] Edward Gray,[1] Henkie Tan,[1] Mark L. Jordan,[1] Amadeo Marcos,[1] John J. Fung,[1] Thomas E. Starzl,[1] Ron Shapiro.[1] *[1]Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA.*

We studied the efficacy and safety of using SRL to salvage renal and pancreatic allografts. **Patients and Methods**:58 patients(41 kidney[*group A*] and 17 kidney-pancreas[*group B*]) underwent transplantation between 10/12/90 and 6/10/01.6(10%) were from living donors;12(21%) were re-transplants.Indications for initiating SRL were chronic allograft nephropathy(43%), refractory rejec-tion(26%), calcineurin inhibitor toxicity(14%), hemolytic-uremic syndrome(7%), mycophenolate(MMF) toxicity(3%) and glucose intolerance(7%).SRL was started 465+/-600 days post-transplant.Patients were followed for 577+/-289 days.35(60%) pts were converted to SRL from MMF,10(17%) pts were converted from Azathioprine,6(10%) pts were converted from TAC, & 7(12%) had SRL added to their regimen. **Results**: In GroupA(GpA), there were 1.3+/-1.1 rejection episodes/patient before SRL and 0.5 +/-0.6 episodes after SRL was started.In GroupB(GpB), there were 1.3 +/-0.9 rejection episodes before SRL and,0.4+/ 0.6 after SRL.In **GpA**, 9(22%) grafts failed; 8 of these occurred when >300 days elapsed between transplant and starting SRL.There were 7(17%) deaths: cardiac causes(4),subarachnoid hemorrhage(1),viral infection(1) and non-compliance(1).In **GpB**, 7(41 %) pancr-eata failed; 6 occurred when >450 days elapsed between transplant and starting SRL.2 patients died;1 from cardiac ,1 for unkno-wn reasons.Renal function remained stable or improved, and glucose control improved in both the groups regardless of the reason for conversion(Table).Hypertension was controlled with fewer medications, and lipid levels were managed by diet and statins. **Con-clusions**:Reduced rates of rejection were noted after starting SRL treatment in both groups. To be effective as a rescue agent, SRL should be started <300 days post-transplant.SRL had few side effects which precluded its use, and was well tolerated.

	kidney transplants(n=31)					kidney-pancreas transplants(n=12)				
	0 mo	1 mo	3 mo	6 mo	12 mo	0 mo	1 mo	3 mo	6 mo	12 mo
creat(mg/dl)	3.66(1.65)	4.06(2.7)	3.99(3.23)	3.48(3.13)	3.11(2.5)	1.81(0.92)	1.76(0.8)	2.05(1.0)	1.79(0.92)	1.73(0.48)
gluc(mg/dl)	112(53)	123(84)	119(57)	104(28)	97(16)	142(94)	142(91)	129(87)	106(54)	135(85)
WBC	7(3.4)	6.9(3.4)	6.8(3.2)	6.9(3.6)	6.6(2.8)	6.4(2.1)	6.2(3.4)	6.3(2.9)	6.2(2.8)	6.9(3.0)
platelets	205(99)	192(86)	203(91)	210(88)	230(106)	238(61)	273(98)	255(77)	230(83)	277(105)
SRL level(ng/ml)		6.9(4.65)	7.8(4.57)	6.2(3.45)	9.5(4.26)		5.9(4.43)	8.9(6.08)	8.6(9.07)	6.9(2.72)
SRL dose(mg/d)		4.4(1.82)	4.8(2.01)	4.5(2.33)	4(1.91)		4.2(1.91)	4.2(1.67)	3.8(2.22)	4.3(1.7)
Cholesterol	204(54)	249(88)	223(54)	246(54)	193(47)	188(47)	191(40)	188(49)	202(24)	203(19)
TG	206(94)	310(209)	254(91)	423(379)	196(30)	145(65)	159(87)	179(152)	159(98)	170(107)
	All values are mean(SD).SRL=Sirolimus.WBC=White blood cells.TG=Triglycerides									

Abstract# 783 Poster Board #-Session: P39-II

A RANDOMIZED PROSPECTIVE TRIAL OF CALCITRIOL VERSUS ALENDRONATE FOR THE TREATMENT OF OSTEOPENIA IN RENAL TRANSPLANT RECIPIENTS. John R. Jeffery,[1] William D. Leslie,[2] Martin E. Karpinski,[1] Peter W. Nickerson,[1] David N. Rush.[1] *[1]Medicine, Health Sciences Centre, Winnipeg, MB, Canada; [2]Medicine, St. Boniface Hospital, Winnipeg, MB, Canada.*

Osteopenia is a well known complication of renal transplantation, which results in an increased risk of fractures. The purpose of this study was to compare the effect of treatment with vitamin D or a bisphosphonate on bone mineral density (BMD). *Methods:* We randomized stable, osteopenic patients (T score ≤-1.0) to treatment with calcitriol 0.25 mgm (Group D) or alendronate (Group A) 10 mg daily. All patients were given 500 mg of elemental calcium daily. Criteria for entry were stable renal function, estimated GFR >35 ml/min, no previous therapy for bone disease, and no recent esophagitis or gastritis. Randomization was stratified for gender and diabetes. BMD was re-measured after approximately 1 year of treatment. A group of non-osteopenic patients (n=35) were used for comparison (Group C). BMD was measured in the lumbar region and the total proximal femur using two cross-calibrated dual energy X-ray absorptiometers. *Results*: Sixty-one were randomized into Group D and 55 into Group A. Fifty-two and 46 completed therapy respectively. Analysis was by completion of treatment. Gender, donor source, diabetes, age, GFR, creatinine, baseline BMD were not different between the 2 groups. Group D patients were 9.0 years post transplant vs. 6.76 years for group A (P=0.067). In Group D, BMD increased from 1.016 to 1.033 g/cm² (P=0.02) in the lumbar region, and from 0.83 to 0.857 (P=0.023) at the femur. In Group A, the respective increases were 0.984 to 1.025 (P=0.0001), and 0.809 to 0.836 (P=0.0003). Lumbar BMD increased by 0.015 g/cm² per year in Group D and 0.034 in Group A (P=0.058). At the femur the respective numbers were 0.024 and 0.025 g/cm² per year (P=0.93). In Group C, lumbar BMD decreased by –0.005 g/cm² per year and increased at the femur by 0.004 g/cm² per year. Group A patients had a significantly greater increase in BMD than Group C at both regions. Group D had a significantly greater increase than Group C at the lumbar area (P=0.005), but not at the femur (P=0.14). *Discussion*: Both calcitriol and alendronate along with supplemental calcium increased BMD in renal transplant patients with osteopenia after 1 year of treatment. There was a strong trend for alendronate being more effective in the lumbar, but not the femoral region.

Abstract# 784 Poster Board #-Session: P40-II

TACROLIMUS(FK506) AND CYCLOSPORINE(CSA) BASED STEROID FREE IMMUNOSUPPRESSION IN KIDNEY TRANSPLANT RECIPIENTS-A COMPARATIVE STUDY OF GRAFT SURVIVAL(GS), GRAFT FUNCTION(GF) AND CHRONIC ALLOGRAFT NEPHROPATHY(CAN). Daniel Lee,[1] Sheng G. Xiao,[1] Aparna Kumar,[1] Michael J. Moritz,[1] Billie Fyfe,[2] Susan Stabler,[3] Michael Heifets,[4] Robert Chvala,[4] Mysore S. Anil Kumar.[1] *[1]Surgery/Transplant, Drexel University College of Medicine, Philadelphia, PA; [2]Medicine/Nephrology, Drexel University College of Medicine, Philadelphia, PA; [3]Transplant, Hahnemann University Hospital, Philadelphia, PA.*

Steroid therapy aggravates the side effects of FK506 and CSA such as diabetes(PTDM) and hypertension. To avoid these side effects and to compare the efficacy and safety of steroid free FK506 and CSA therapy a clinical trial was carried out in 91 primary non-sensitized kidney recipients to study GS, GF and CAN at 1 year. Basiliximab induction was used in all recipients.Two doses of methylprednisolone, 250mg on day 0 and 125 mg on day 1 were given and then discontinued. In both groups, Mycophenolate Mofetil(MMF) or Sirolimus(SRL) were used as adjuvants. Acute rejections(AR) were diagnosed by biopsy and treated with 2G of intravenous methylprednisolone and resistant rejections by Thymoglobulin infusion. Protocol biopsies were carried out at 1, 6 and 12 months to study subclinical rejections(SCR) and chronic allograft nephropathy(CAN). Graft function was assesed by serum creatinine and calculated creatinine clearance. 49 were in FK506 and 42 in CSA groups with comparable demography. Follow up is for 60 to 850 days. At 1 year, patient survival was 98% and 100% and graft survival was 92% and 90% in FK and CSA groups respectively. Serum creatinine levels at 1 year was 2.1 and 2.0mg% and creatinine clearances were 63 and 60mls/minute in FK and CSA groups respectively. Incidence of AR was14% and 14%, SCR 14% and 16% in FK506 and CSA groups respectively(p=ns). In FK506 group CAN was absent in 57%, mild in 23% and moderate to severe in 20%. In CSA group CAN was absent in 60%, mild to moderate 20% and severe in 20%(p=ns). PTDM was 0% in CSA and 3% in FK506 groups(p=ns). Requirement of antihypertensive medicines was not different in the 2 groups. We conclude that both FK506 and CSA based steroid free therapy are equally effective in preventing AR and prolonging patient and graft survival at 1 year with a reduced incidence of PTDM. Graft function, CAN, AR and SCR in the 2 groups are similar at one year. Further evaluation of these patients is required.

Abstract# 785 Poster Board #-Session: P41-II

THE EFFECT OF DOUBLE FILTRATION PLASMAPHERESIS (DFPP) ON BASILIXIMAB PHARMACOKINETICS IN ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION. Kazuhide Saito,[1] Hidemitsu Wakatsuki,[1] Yuki Nakagawa,[1] Ryu Utsugi,[1] Masaki Abe,[1] Masayuki Tasaki,[1] Shinichiro Murayama,[1] Itsuhiro Takizawa,[1] Toshiki Tanikawa,[1] Kota Takahashi.[1] *[1]Division of Urology, Niigata University Graduate School of Medicine, Niigata, Niigata, Japan.*

Background Basiliximab is a chimeric monoclonal antibody(mAb) against CD25 (IL-2R alpha chain) which prevents circulating activated T cells for more than one month. However, the effect of double filtration plasmapheresis(DFPP) on its pharmacokinetics is still unknown. **Patients and Methods** We analyzed basiliximab sequential serum concentration(Basil.conc.) by ELISA in 10 kidney transplant recipients including 2 ABO-incompatible cases. 20mg of basiliximab was administered on the day 0 and 4 post-transplant together with cyclosporine or tacrolimus, MMF and steroid. **Results** Basil.conc. on day 0 post administration, day 4 pre- and post- administration, day 12, 19, 26, 33, 40, 47, 54 were measured. Mean ± SD of 8 cases without DFPP were, 6.3 ± 1.2, 2.4 ± 0.8, 8.8 ± 1.8, 3.4 ± 0.6, 2.6 ± 0.6, 1.6 ± 0.4, 1.1 ± 0.4, 0.8 ± 0.3, 0.5 ± 0.3, 0.3 ± 0.2 mcg/ml, respectively. Cmax was 8.8 ± 1.8 mcg/ml, Tmax was 4 ± 0 day and t1/2 was 11.0 ± 3.4 days in this group. Average basil.conc. were maintained over 0.2 mcg/ml more than 54 days after transplantation which is thought to be enough preventing circulating CD25 positive T cells less than 0.3%. In ABO incompatible cases, postoperative DFPP is sometimes done to remove anti-A or B antibodies. In one case, DFPP was performed on day3. Basil.conc. decreased from 8.8(day 0) to 0.5 (day 4 pre-)mcg/ml(5.7%) compared to those with natural decline in other 8 cases of 6.3 to 2.4mcg/ml (37.7%). Cmax was 8.8mcg/ml at day 0(Tmax) and t1/2 was 4.8 days, basil conc. was diminished below 0.2 mcg/ml after day 26 in this case. In another case, DFPP was done on day 9, basil.conc. pre- and post-DFPP were 5.1 to 2.0 mcg(38.5%). Cmax was 8.5 mcg/ml at day 4(Tmax) and t1/2 was 14 days. **Discussion and conclusion** This is the first report of the effect of DFPP on basiliximab pharmacokinetics in kidney transplantation. DFPP is directed to remove immunoglobulins, so removal rate of basiliximab is relatively high, at about more than 60% by only one session of treatment. It may be hypothesized that before achievement of basiliximab maximum saturation in the body by 2nd dose administration, DFPP more effectively remove basiliximab and affect the serum concentration and its clearance. Supplemental basiliximab should be considered after DFPP to maintain desired concentration to suppress IL-2R positive activated T cells in kidney transplantation.

Abstract# 786 **Poster Board #-Session: P42-II**

A SINGLE-CENTER EXPERIENCE WITH SIROLIMUS CONVERSION THERAPY IN RENAL ALLOGRAFT RECIPIENTS. Kristin L. Crowley, Karen L. Hardinger, Matthew Koch, Brent Miller, Graeme Mindel, Daniel Brennan. *[1]Transplantation, Barnes-Jewish Hospital at Washington University, St. Louis, MO.*

Sirolimus (SRL) is a macrocyclic triene antibiotic with immunosuppressive and antiproliferative properties approved for prophylaxis of acute rejection in kidney transplant recipients. There are few data regarding the use of SRL for conversion therapy. Previous studies conducted for conversion secondary to calcineurin-inhibitor toxicity have been small with a short period of follow-up (6 months). **Methods.** We performed a retrospective review of kidney transplant recipients at our center converted to SRL (n=45) for calcineurin inhibitor toxicity (n=14), chronic allograft nephropathy (n=13), increased serum creatinine (n=11), acute rejection (n=6), or other causes (n=1) to determine safety and efficacy of conversion. Twenty-four males and twenty-one females were reviewed (73% Caucasian) with a mean age of 44 years. Glomerular nephropathy (n=10), hypertension (n=9), diabetes mellitus (n=7), polycystic kidney disease(n=2), reflux (n=2), drug toxicity (n=3), rejection (n=1), and other cause (n=11) accounted for the diseases leading to transplant. Twenty-three patients (51.1%) underwent cadaveric kidney transplant. Mean follow-up post-transplant 5 ± 4 years and post-conversion to SRL 10.5 ± 7.2 months. **Results.** Mean serum creatinine at baseline 2.4 ± 0.7 mg/dL, at three months post-conversion 2.1 ± 0.7 mg/dL (p=0.04), at six months post-conversion 2.2 ± 0.9 mg/dL (P=NS), and at 12 months post-conversion 2.3 ± 0.9 mg/dL (p=NS). Four patients lost graft function and returned to dialysis. There was no change in hematocrit, white blood cell count, or platelets at three, six or twelve months. Mean total cholesterol did not change from baseline, 178.2 ± 52.5 mg/dL, versus 195.6 ± 54.3 mg/dL at twelve months (p=NS). Twenty patients (44.4%) were taking HMG-CoA reductase inhibitors at baseline compared to twenty-six patients (57.8%) at twelve months. Mean HgA1c, SRL dose, and SRL level did not change significantly from baseline. Mean steroid dose declined from 10.6 ± 11.7 mg at baseline to 7.3 ± 2.9 mg at twelve months post-conversion. Eight patients discontinued SRL therapy for increased serum creatinine (n=2), allergic reaction(n=3), elevated triglycerides (n=2), leukopenia (n=1) and fatigue (n=1). One patient was lost to follow-up. Conversion to SRL appears to be safe and associated with few adverse events in our patient population. It appears that regardless of the trends at three months and six months post-conversion, renal function tended to return to baseline at twelve months.

Abstract# 787 **Poster Board #-Session: P43-II**

A PILOT STUDY OF A CALCINEURIN-INHIBITOR FREE PROTOCOL FOR KIDNEY TRANSPLANT RECIPIENTS WITH DELAYED GRAFT FUNCTION OR MARGINAL DONOR KIDNEYS. David Shaffer,[1] William A. Nylander,[1] A. Tarik Kizilisik,[1] J. Harold Helderman,[2] Anthony Langone.[2] *[1]Division of Kidney and Pancreas Transplantation, Vanderbilt University Medical Center, Nashville, TN; [2]Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN.*

Purpose: The shortage of cadaver donors has prompted many centers to use kidneys from expanded or marginal donors (MDK), i.e. older donor age or donor history of hypertension or diabetes. MDK may be especially susceptible to nephrotoxicity due to calcineurin-inhibitor (CI) mediated vasoconstriction. Similarly, CI may exacerbate ischemic injury and delay recovery or impair long-term graft function in cases of delayed graft function (DGF). We conducted a pilot study of a CI-free protocol consisting of thymoglobulin, sirolimus (SRL), mycophenolate mofetil (MMF), and prednisone in recipients with DGF or MDK. **Methods:** 16 adult recipients of either MDK (donor age >55 or donor hypertension or diabetes) or with DGF were placed on a CI-free protocol consisting of thymoglobulin 1.5 mg/kg x 4 days, SRL 10 mg QD x 3 days, the 5 mg QD and adjusted to maintain trough levels 10-20 ng/ml, MMF 500 mg BID while on thymoglobulin, then 1 gm BID, and methylprednisolone 500 mg intra-op, then prednisone 30 mg QD tapered to 10 mg at 3 months. Biopsies were performed at 2 and 4 weeks for persistent DGF. **Results:** 16 recipients (13 CAD, 2 LRD, 1 LURD) were placed on this protocol. Inclusion criteria were DGF (8), donor age > 55 or donor hypertension or diabetes (4), or both DGF and donor age, hypertension, and diabetes (4). Mean follow-up is 243 days (range 422-14). Patient survival is 100%. Graft survival is currently 100% excluding 2 recipients still in DGF 14 days post-op. There were no cases of biopsy-proven acute rejection (AR). Mean creatinine is currently 1.4 (range 0.8-1.8) for all patients at least 3 mos post-op. Mean sirolimus level post loading dose was 11±6 ng/ml (range 2.8-28.1). Sirolimus-related complications included lymphocele requiring drainage (2) and wound seroma or infection (3). **Conclusions:** 1. A CI-free protocol with thymoglobulin and sirolimus results in very low rates of AR and primary non-function and excellent early patient and graft survival in recipients of MDK or with DGF. 2. A loading dose of sirolimus 10 mg x 3 days achieves therapeutic levels. 3. Bone marrow toxicity was easily managed with reduction in MMF although wound related complications remain a concern. 4. CI-free protocols may allow expansion of the kidney donor pool by encourageing utilization of MDK at high risk for DGF or CI-mediated nephrotoxicity.

Abstract# 788 **Poster Board #-Session: P44-II**

OPTIMAL IMMUNOSUPPRESSION WITH CYCLOSPORINE REQUIRES ASYMMETRICAL DOSING BASED ON INDIVIDUAL DIURNAL RHYTHMS. 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.*

Diurnal variation in cyclosporine(CsA) pharmacokinetics contributes to episodic deviations from the therapeutic window leading to increased risk of acute rejection and/or drug toxicity. Hypothethis: Asymmetrical dosing of CsA tailored to individual patients' metabolic rhythms would optimize efficacy and minimize toxicity. Methods: Beginning in December 1998, all patients undergoing kidney transplantation at our institution were prospectively treated with a diurnally split dose of CsA microemulsion given q12hrs(3.5mg/kg qAM, 3.0 mg/kg qPM). AM doses were adjusted to reach a daytime area under the concentration curve (AUC) of 7800 nghr/ml(utilizing 2hr and 6hr levels) and PM doses were adjusted to an AM trough of 300 ng/ml. Patients received high-dose steroids tapered to 20 mg prednisone by day 6 and to 7.5mg by 1 year. CsA was started within 48 hrs and mycophenolate mofetil (1000mg q12hr) was added on day 3 in most patients and continued for 3 months. One patient received antibody induction. All patients were followed for 1 year. Results: An average of eight 3-point profiles per patient were required during the first 3 months to maintain target CsA levels. Pharmacokinetic and functional data are summarized as mean ± SEM.

	1st Dose	Week 1	Month 1	Month 3	Month 6	Year 1
AM dose(mg/kg	3.5 ± .07	3.5 ± 0.1	3.3 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	1.9 ± 0.1
PM dose(mg/kg)	2.9 ± .06	2.6 ± 0.2	2.7 ± 0.2	2.1 ± 0.1	1.9 ± 0.1	1.6 ± 0.1
Trough(ng/ml)		317 ± 10	312 ± 8	285 ± 7	277 ± 8	214 ± 7
AUC(nghr/ml)		7709 ± 201	8584 ± 233	7543 ± 241	6671 ± 229	5680 ± 227
Creatinine(mg/dl)		4.50 ± 0.55	1.80 ± 0.15	1.66 ± 0.14	1.70 ± 0.13	1.54 ± 0.6

74 kidneys(76% cadaveric) were transplanted into 70 adult patients(4 double grafts). 49% of recipients were African-American, 59% were men, and 20% had hepatitis C. 77% had ≥3 HLA mismatches and 17% had PRA>20%. Biopsy for allograft dysfunction was required in 37% of patients during the first 12 months follow-up but only 1 patient each had evidence of rejection or significant toxicity. Patients continued to require increased CsA doses in the AM compared to the PM ($p<0.05$) throughout the study to maintain target levels. Conclusion: Diurnal dosing of CsA based on individual pharmacokinetic profiles optimizes CsA exposure thereby reducing the risk of rejection and toxicity after kidney transplantation.

Abstract# 789 **Poster Board #-Session: P45-II**

PREDNISONE DECREASES SENSITIVITY TO SIROLIMUS IN KIDNEY TRANSPLANT RECIPIENTS. Christine E. Chamberlain,[1] Allan D. Kirk,[2] Douglas Hale,[2] John Swanson,[2] Linda Cendales,[2] Roslyn Mannon.[2] *[1]Pharmacy, CC, NIH, Bethesda, MD; [2]Transplant Section, NIDDK, NIH, Bethesda, MD.*

Glucocorticosteroids induce the metabolism of calcineurin inhibitors (CNI). The same enzyme, CYP3A4, metabolizes Sirolimus and CNI. However, due to its long half-life and use in combination with cyclosporine there are limited data on the induction effects by oral steroids on sirolimus metabolism. We therefore examined sirolimus whole blood levels (HPLC-MS) in kidney transplant recipients (n=17) on sirolimus therapy during high to moderate doses of prednisone, and again when these same patients had been weaned to low to no prednisone. To correct for variations in sirolimus levels, dose and weight, we calculated sirolimus sensitivity (drug level/mg sirolimus dose/kg/day). All patients were on stable antimicrobial prophylaxis and maintenance immunosuppression to limit the potential effects of other enzyme inducers or inhibitors on the analysis. The mean of 3-4 drug levels were used around a stable dose of sirolimus and prednisone to reduce variability of the drug assay. Sirolimus sensitivity was examined in 17 transplant recipients who had stable doses of sirolimus and prednisone and corresponding sirolimus drug levels at Cpss (5-7 days after a dose change). Recipients were on prednisone for treatment of rejection and tapered or on a slow taper course post transplant. Steroid doses ranged from 0.2 to 2.7 mg/kg (median 0.3) during the high dose phase and 0 to 0.16mg/kg (median 0.12) during the low dose phase of analysis. Days post transplant ranged from 13-374 (median 48) versus 34-465 (median 138). The mean sirolimus sensitivity was 220ng/mg during high dose steroid therapy and increased to 346ng/mg during low dose therapy with a mean change of 126ng/mg. This represented a statistically significant increase ($p <0.015$) in sirolimus levels per dose in patients on 10 mg or less of prednisone. Prednisone decreased sirolimus levels most likely through induction of hepatic metabolism. These data indicate that sirolimus levels should be monitored closely during pulse steroids since drug levels may significantly decrease and during prednisone taper to minimize toxicity.

Abstract# 790 **Poster Board #-Session: P46-II**

EXPERIENCE WITH THYMOGLOBULIN (RATG) INDUCTION AND A SIROLIMUS (SRL)-BASED REGIMEN IN AFRICAN-AMERICAN (AA) RENAL ALLOGRAFT RECIPIENTS (RAR). A. Haririan,[1] K. Morawski,[2] J. Garnick,[3] D. Sillix,[1] S. Patel,[1] D. Granger,[2] M. West,[2] S. Gruber.[2] *[1]Department of Medicine; [2]Department of Surgery, Wayne State University School of Medicine; [3]Pharmacy, Harper University Hospital, Detroit, MI.*

AA RAR have been repeatedly shown to have worse graft survival compared to Caucasians, with higher rates of acute rejection (AR) and delayed graft function (DGF).

We present our experience with use of RATG induction and a SRL-based maintenance protocol in an attempt to reduce AR and improve graft outcome in this high-risk population. **Methods.** 54 adult AA were transplanted at our center from 7/19/01 to 7/24/02, with follow-up 8.9±3.4 months (mo). 14 received live-donor (LD) grafts. All RAR were induced with either Basiliximab (BSX) (Group I, n=30) or RATG (4-11 doses) (Group II, n=24), depending on donor source and recipient age, PRA, retransplant status, and presence of cardiovascular or infectious comorbidities. Maintenance agents included MMF 2 g and prednisone. Either SRL, in patients (pts) with slow graft function (SGF) or DGF (n=25), or tacrolimus (FK) in those with immediate graft function (IGF) (n=29) was added within 48 hours, with target trough levels 10-15 ng/ml for both. **Results.** Groups I and II were not statistically different with regard to age; BMI; cold ischemic time; % on SRL; DM; and % with DGF. Group II, by design, had more retransplants (3.3% vs 41.6%, P=0.001), higher PRA (peak 5.1±12% vs 43±41%, P<0.0001), and more years of ESRD (4.4±2.2 vs 7.9±3.3, P<0.0001). In Group I, 4 episodes of grade I AR occurred in 3 pts on SRL, one due to noncompliance and 3 due to inability to rapidly achieve target levels. 1 episode of grade II AR occurred in a noncompliant pt on FK. 3 RAR in Group I died from systemic infection, 2 following treatment of AR (Group I AR=13%; graft/patient survival=90%). There was no AR or graft/pt loss in Group II. S Cr at 1 and 6 mo were comparable in the two groups (1.7±0.6 vs 2.2±1.8, P=0.18; 2.6±3.6 vs. 2.5±3.2 mg/dl, P=0.9, respectively), despite a trend to more LD in Group I (P=0.08). Subgroup intention-to-treat analysis within Group II revealed comparable S Cr at 6 mo in pts who were on SRL (n=12) compared to FK (n=12), despite SGF/DGF in the SRL subgroup (2.0±0.7 vs. 1.6±0.3 mg/dl, P=0.16). **Conclusion.** Our results suggest that high-risk AA RAR who receive RATG compared with BSX induction have better graft and pt outcomes. Use of SRL in conjunction with RATG in pts with SGF or DGF effectively prevents AR and results in graft function ultimately comparable with that in pts with IGF on FK. However, when SRL is used along with BSX, rapid achievement of therapeutic levels is crucial.

Abstract# 791 Poster Board #-Session: P47-II

FIRST-YEAR CLINICAL DETERMINANTS OF TACROLIMUS PHARMACOKINETICS IN COMBINATION WITH MYCOPHENOLATE MOFETIL AND STEROIDS IN ONE HUNDRED *DE NOVO* RENAL ALLOGRAFT RECIPIENTS. Dirk R. J. Kuypers,[1] Kathleen Claes,[1] Pieter Evenepoel,[1] Bart Maes,[1] Yves Vanrenterghem.[1] *[1]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium.*

Despite the wide-spread use of Tacrolimus (T) as an effective immunosuppressive drug, little is known about the clinical determinants that influence drug pharmacokinetics (PK) in the long-term. We performed 500 AUC measurements for T (immunoassay) at 7 days, 6 weeks, 3, 6 and 12 months post-Tx in 100 *de novo* recipients on triple therapy with MMF and steroids. Model-independent PK parameters were determined (AUC_{0-12h}, Cmax, tmax, C_0, Cavgss, CLss). In addition, the intravenous (iv) clearance of T was determined from patients receiving continuous T infusion the first week post-Tx. An estimate for T bioavailability (F) was calculated using the iv and oral data. Clinical, demographic and transplantation-related candidate determinants were evaluated against PK parameters of T at 5 different time points after Tx using simple/multiple regression and multivariate analysis. Of approximately 50 variables in univariate analysis the following were withheld in multivariate analysis: age, sex, body weight, BSA, re-transplant, renal replacement therapy, donor sex and age, cold ischemia time, graft function, delayed graft function, hematocrit, albumin, lipids, steroid dose, MMF dose, hepatitis B or C, diarrhoea and liver disturbances. Results for day 7 are summarized (as e.g.) in table. Conclusion: Long-term clinical determinants of tacrolimus PK change over time in the crucial first year after Tx. Knowledge of the changing pattern of these determinants and their impact on clinical relevant PK parameters is required for tailoring tacrolimus therapy to the individual patient profile.

Determinants of tacrolimus PK parameters on day 7 post-Tx

	Dose (mg/d)	C_0 (ng/ml)	tmax (hr)	Cmax (ng/ml)	AUC_{0-12h} (ng*hr/ml)	CL (L/hr/kg)	F (percent)
Multiple regression analysis							
Sex (M/F)				0.07			
Donor sex (M/F)		0.06		0.06	0.04	-0.07	
Body weight (kg)	0.17						0.06
Delayed graft function (Y/N)			-0.06				
Creatinine clearance (ml/min)				-0.05	-0.04		0.09
Albumin (g/dl)							
HDL-cholesterol (mg/dl)		0.04	-0.08			-0.02	
Steroid dose (mg/kg/d)	0.18					-0.29	
Tacrolimus dose (mg/d)		0.07		0.24	0.22	0.55	
Diarrhoea (Y/N)	0.07						0.09
Hepatitis B or C (Y/N)	-0.05					0.08	
Hematocrit (%)						0.08	

Correlation factors for significant variables by stepwise and backward elimination (p<0.05)

Abstract# 792 Poster Board #-Session: P48-II

STEROID FREE MAINTENANCE IMMUNOSUPPRESSION FOR LIVING DONOR RENAL TRANSPLANTATION. Ronald M. Ferguson,[1] Mitchell L. Henry,[1] Amer Rajab.[1] *[1]Surgery/Transplantation, The Ohio State University, Columbus, OH.*

Long term corticosteroid use in renal transplant recipients leads to significant morbidity, mortality, and cost. Past attempts at steroid withdrawal following transplantation have met with mixed success. We instituted a protocol of steroid free maintenance immunosuppression (IS) following an immediate post-transplant four day rapid Prednisone taper and thymoglobulin induction. Maintenance IS consisted of Sirolimus and Neoral without corticosteroids. Target level monitoring of Rapamycin and Neoral were performed. Neoral was dosed to C-2 targets that were 2/3 of the "standard" Neoral dosing and Rapamycin trough level targets were 10 ng/ ml. Fifty mismatched living donor recipients were enrolled. Results: During the follow-up period, there have been no acute rejection episodes, no graft losses and one death due to encephalitis. No patients are currently receiving steroids. One patient has been converted from Neoral to Cellcept for nephrotoxicity and remains off steroids. The one, three, and six-month follow-up mean values are given below.

Mean Values

		Levels ng/ ml					
	Creatinine	RAPA	C-2	Platelet	WBC	Cholesterol	BP
1 Mo	1.51	14.4	1160	278	4.4	233	131/79
3 Mo	1.50	10.5	845	267	4.0	208	129/75
6 Mo	1.52	13.2	866	252	4.9	192	123/72

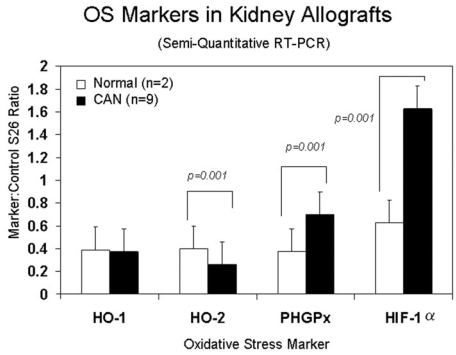
Mean post-transplant hospital discharge was 4.7 days. Thirty of the 50 patients have not been readmitted for any reason. Four have undergone transplant biopsy for renal dysfunction. None has shown acute rejection. Three of the fifty patients experienced wound problems that were successfully managed. There has been one case of mild CMV. These data provide promising evidence that maintenance steroid avoidance (as opposed to withdrawal) can lead to excellent results and yet maintain an extremely low incidence of acute rejection. This protocol is currently being expanded to include cadaveric donor renal transplants.

KIDNEY: ACUTE/CHRONIC REJECTION II

Abstract# 793 Poster Board #-Session: P49-II

INTRAGRAFT OXIDATIVE STRESS AND URINARY HYDROGEN PEROXIDE IN CHRONIC ALLOGRAFT NEPHROPATHY. Arjang Djamali,[1] Lynn Jacobson,[1] Rebecca Muehrer,[1] Jenifer Sprague,[1] Kenneth Waller,[2] Jonathan McAnulty,[2] Debra Hullett,[3] Bryan N. Becker.[1] *[1]Medicine, Nephrology Section, University of Wisconsin, Madison, Madison, WI; [2]Veterinary School, University of Wisconsin, Madison, Madison, WI; [3]Surgery, Division of Transplantation, University of Wisconsin, Madison, Madison, WI.*

Oxidative Stress (OS) has been invoked in both chronic kidney disease and chronic allograft nephropathy (CAN). However, little is known on its association with the pathogenesis of CAN. To directly address this question and to assess the effect of angiotensin receptor blockers on OS, we studied patients with clinical and pathological findings of CAN that were randomized to receive or not receive Losartan following the initial biopsy. The intragraft expression of 4 markers of OS was assessed by semiquantitative rt-PCR at the time of biopsy: heme oxygenase-1 (HO-1) for its reno-protective effects, phospholipid hydroperoxide glutathione peroxidase (PHGPX) as a scavenger of the cytotoxic hydrogen peroxide (H_2O_2), hypoxia-inducible factor-1α (HIF-1α) for its connection between hypoxemia and OS, and heme oxygenase-2 (HO-2) to determine its possible role as an anti-oxidant in CAN. Urinary OS was determined by H_2O_2 measurements using the Amplex Red Hydrogen Peroxide Assay (Molecular Probes) at the time of biopsy and 6 months after treatment. Semi-quantitative Rt-PCR results were expressed as a ratio of the OS marker to S26 control.



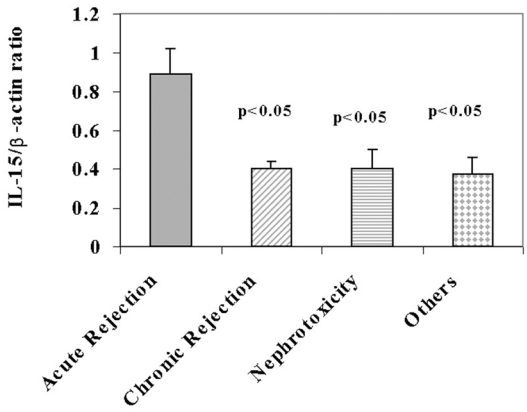
Patient	Urinary Hydrogen Peroxide in CAN: Urinary H2O2 (μM) at the time of Biopsy	Urinary H2O2 (μM) 6m after biopsy
Normal Functionning Transplant (n=1)	0.2	0.6
CAN+Losartan (n=5)	3.7±1.6	2.8±1.3
CAN without Losartan (n=4)	4.5±2.3	4.4±3.7

Our preliminary results demonstrate that (1) Oxidative stress is associated with CAN and chronic ischemia is the possible link between OS and CAN (2) The enhanced intragraft expression of PHGPX and the pattern of urinary H_2O_2 imply that this molecule might be a useful marker of OS in CAN (3) Losartan has potential anti-oxidant properties in CAN.

Abstract# 794 **Poster Board #-Session: P50-II**

INTRA-RENAL IL-15 mRNA EXPRESSION DIFFERENTIATES ACUTE REJECTION FROM NEPHROTOXICITY, CHRONIC REJECTION AND OTHER TRANSPLANT RELATED COMPLICATIONS. Ashwani K. Khanna,[1] Matthew Plummer,[1] Chrisopher Johnson,[2] Sundaram Hariharan.[1] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Surgery, Medical College of Wisconsin, Milwaukee, WI.*

Introduction: Despite emergence of newer anti-rejection therapies in renal transplantation, acute rejection remains a real problem. Acute rejection can potentially be the most powerful predictive factor for the later development of chronic rejection. There is need for better diagnosis of acute rejection and to distinguish acute rejection from other causes of renal dysfunction such as nephrotoxicity, chronic rejection, polyoma infection etc. IL-15 is a novel growth factor expressed and secreted by a variety of cells including renal cells and its exact role in renal transplantation is not clear. Methods: We analyzed intra-renal expression of IL-15 on a total of 30 biopsies (acute rejection n =6, chronic rejection n =6, nephrotoxicity n = 12 and others including membranous nephropathy, FSGS, recurrent DN and polyoma n =6). Patients received immunosuppressive treatment with either cyclosporine (n =15) or Tacrolimus (n=15). Using RT-PCR, intra-renal IL-15 mRNA expression was studied in renal biopsies from patients with histological diagnosis of acute rejection, chronic rejection CsA or Tac nephrotoxicity and others. Intra-renal mRNA expression of Results: IL-15 was significantly increased (figure) in-patients diagnosed with acute rejection (5 of 6) compared to chronic rejection, nephrotoxicity and than other recurrent diseases (4 of 24). There was no difference in the intra-renal of IL-15 mRNA expression in patients treated with Tacrolimus vs Cyclosporine (0.5 ± 0.09 vs 0.46 ± 0.09) Conclusion: This data demonstrates increased intra-renal mRNA expression of IL-15 in renal transplant recipients diagnosed with acute rejection compared to patients diagnosed with chronic rejection, nephrotoxicity and other transplant related disorders. The monitoring of renal biopsies for IL-15 mRNA has potential in identifying patients with acute rejection vs other causes of renal allograft dysfunction.



Abstract# 795 **Poster Board #-Session: P51-II**

IMPROVED OUTCOMES OF RENAL TRANSPLANTATION IN 122 AFRICAN-AMERICANS TREATED WITH A SIROLIMUS-CYCLOSPORINE REGIMEN: COMPARISON TO A CYCLOSPORINE-PREDNISONE REGIMEN AND TO CAUCASIANS. Hemanghsu Podder,[1] Lai Dejian,[1] Linda Schoenberg,[1] Richard Knight,[1] Stephan Katz,[1] Charles Van Buren,[1] Barry Kahan.[1] *[1]Div Immunology and Organ Transplant, Univ of Texas Medical School, Houston, TX.*

Objective: To assess the 3-year impact of addition of sirolimus (SRL) to a cyclosporine (CsA) based regimen in African-American (AA) and Caucasian (CAU) patients. **Methods:** Three groups of renal transplant recipients were treated contemporaneously: Group 1: AA—CsA-Prednisone (Pred; no SRL, n=43); Group 2: AA—SRL-CsA (n=122); Group 3: CAU—SRL-CsA (n=216). The mean (median) follow-up times were 68.3 (66.3), 39.2 (27.7), and 43.3 (31.9) months, respectively. Outcomes in Group 2 were compared to the other cohorts using log-rank and chi-square methods. **Results:** The demographic features were similar except for a greater proportion of living-donor recipients in the CsA-Pred group. Among SRL patients, CsA doses were reduced by over 50% compared to the CsA-Pred cohort (Group 1), and SRL concentrations (mean± SD) maintained at about 11ng/mL. The actuarial 3-year patient and graft survivals for the 3 groups were not significantly different by log-rank analysis. However, SRL reduced the cumulative incidence of acute rejection episodes (ARE): namely, 60.0, 22.0, and 23.0%, respectively (p<0.0001: Group 1 vs Group 2; p=0.90; Group 2 vs Group 3). Comparisons between AA and CAU patients receiving SRL-CsA showed no significant differences in overall or individual infection rates, including wound, urinary tract infections (UTI), pneumonia, cytomegalovirus (CMV), or aphthous ulcers. Group 2 AA displayed significantly fewer SRL-related side effects at any time during follow-up than Group 3 CAU recipients: joint pain (8.2 vs 17.6%; p=0.011) and diarrhea (30.3 vs 41.2%; p=0.03). Throughout the transplant course, AA showed a lower incidence than CAU in hypertriglyceridemia (89.3 vs 97.2%; p=0.003), but a similar incidence of hypercholesterolemia (94.3 vs 97.2%; p=NS), and treatment-induced diabetes (12.3 vs 6.5%; p=0.053). **Conclusion**: The SRL-CsA regimen was associated with a reduced incidence of ARE among AA, who tend to tolerate the drug regimen better than CAU.

Abstract# 796 **Poster Board #-Session: P52-II**

A RANDOMISED CONTROLLED TRIAL OF IMMUNOSUPPRESSION CONVERSION FOR PATIENTS WITH CHRONIC ALLOGRAFT NEPHROPATHY. John Stoves,[1] Charles G. Newstead,[1] Andrew J. Baczkowski,[2] Geoff Owens,[3] Marius Paraoan,[3] Russell G. Roberts,[4] Robin F. Jeffrey,[4] Abdul Q. Hammad.[3] *[1]Department of Renal Medicine, St James's University Hospital, Leeds, United Kingdom; [2]Department of Statistics, University of Leeds, Leeds, United Kingdom; [3]Department of Transplant Surgery, Royal Liverpool Hospital, Liverpool, United Kingdom; [4]Department of Renal Medicine, Bradford St Luke's Hospital, Bradford, United Kingdom.*

Objective. To assess the effect of immunosuppression conversion on progression of chronic allograft nephropathy (CAN) in adult cyclosporin (CsA)-treated renal transplant recipients (RTRs). *Inclusion criteria*: negatively sloping reciprocal of creatinine *vs* time (ROCT) plot over ≥ 6/12; no acute rejection (AR) for ≥ 3/12; normal transplant ultrasound/ Doppler; biopsy-proven CAN. *Exclusion criteria*: previous tacrolimus (FK506)/ mycophenolate mofetil (MMF); serum creatinine > 400 umol/l. **Intervention.** *A. MMF / reduced dose CsA*: MMF 500mg bd for azathioprine, increasing to 1g bd, CsA trough blood level (C_0) 75-100 ng/ml *B. FK506*: FK506 for CsA, C_0 5-10 ng/ml *C. Continuation of CsA-based regimen*. **Methods and main results.** A computer-generated randomisation sequence was used to allocate treatment to 42 patients in 2 UK transplant centres (7/99 - 6/02). Blood pressure (BP), GFR (99mTc-DTPA clearance), renal biochemistry and fasting lipids were monitored. 2 patients started dialysis prior to study completion (1 A, 1 B). 1 patient was intolerant of MMF. CsA dose was reduced by 24% (interquartile range (IQR) 14%-27%) for A. End-of-study CsA C_0 for A was 99 (IQR 90-113) ng/ml. The maintenance MMF dose was 1.5 (IQR 1.5-2) g/day. End-of-study FK506 C_0 for B was 7 (IQR 5-9) ng/ml. End-of-study CsA C_0 for C was 163 (IQR 145-215) ng/ml. Comparison of ROCT slopes before (-12/12 - 0) and after (0 - 6/12) intervention revealed a treatment advantage for A ($p < 0.05$). Comparison of the pre-study and 3/12 - 12/12 ROCT slopes (to isolate the early effect on graft function of CsA dose reduction) showed a non-significant difference between groups (A *vs* C, p=0.08). The GFR analysis (0 *vs* 6/12) also suggested a treatment advantage for A (p=0.05). Exclusion of patients with GFR < 20 ml/min/1.73m² gave a more significant result ($p < 0.05$). Intergroup differences in BP and lipids were not significant. No patients developed AR or diabetes mellitus. 7 patients reported gastrointestinal disturbance with MMF. MMF dose was reduced in 3 patients because of anaemia. **Conclusions.** There is a treatment advantage for MMF/ reduced dose CsA over FK506 and standard dose CsA in patients with CAN, at least in the short term. The CsA dose reduction component is likely to be of particular importance. Other findings suggest that early intervention is beneficial.

Abstract# 797 **Poster Board #-Session: P53-II**

PREDICTORS OF CHRONIC ALLOGRAFT NEPHROPATHY FOLLOWING RENAL TRANSPLANTATION. William Irish,[1] Beth Sherrill.[1] *[1]RTI-Health Solutions, Research Triangle Park, NC.*

INTRODUCTION: Chronic allograft nephropathy (CAN) is the principle cause of late renal allograft failure. Identification of factors associated with CAN may help to reveal mechanisms underlying this complication. **OBJECTIVE:** To identify factors associated with chronic allograft nephropathy separately for recipients of living and cadveric donors. **METHODS:** CAN was defined as graft loss beyond 6 months excluding deaths or loss due to acute rejection, recurrent disease, surgical complications, noncompliance, infection or thrombosis. Data from USRDS for primary renal transplants in adults between 1993-1998 was used. A competing risk analysis was performed using Cox proportional hazards models. Predictive models were stratified by transplant year and included donor and recipient characteristics plus pre- and post-transplant clinical variables including immunosuppression. **RESULTS:** CAN accounted for 75% of total graft failures (after 6 months) occurring in 15% (5638/36510) of transplants from cadaveric donors. Living donor transplants failed due to CAN in 10% of patients (1496/15040). Graft failure attributable to CAN occurred at a median time of 30 months post-transplant. These factors were predictive for CAN in all transplants: African-American recipient, pre-transplant dialysis, HLA mismatch, delayed graft function and acute rejection episodes. Facility size and recipient age were inversely associated with CAN. Patients with ESRD due to glomerular disease or polycystic kidneys were less likely to have CAN than were those with ESRD due to other causes. Use of maintenance Tacrolimus was also predictive of outcome from living donors. In transplants from living donors, increasing donor age was associated with an increased risk from CAN, whereas increasing age reduced the risk of CAN from cadaveric donors. Additional factors increased the risk of CAN for transplants from cadaveric donors: male recipient of female donor, African-American donor, donor hypertension, CMV+ donor/-recipient, cold ischemia time, pre-TX blood transfusion, comorbid diabetes, PVD or CVD, peak PR antibody, antibody induction. Recipients of cadaveric donors were less likely to have CAN if ESRD was due to diabetes, if donor organ was pre-treated, or if multiple organ were received. Use of maintenance MMF was shown to be protective for CAN. **CONCLUSION:** Factors associated with CAN are quite different for recipients of living vs. cadaveric organs. These differences could possibly suggest different etiologic mechanisms in these patient subgroups that may be immunologically based.

Abstract# 798 **Poster Board #-Session: P54-II**

ARE THE LESS FREQUENT ACUTE REJECTION EPISODES OF THE MODERN ERA ASSOCIATED WITH A GREATER RISK FOR GRAFT FAILURE? Rahul S. Koushik,[1] Abhinav Humar,[2] Arthur J. Matas,[2] Bertram L. Kasiske.[1] *[1]Department of Medicine, University of Minnesota, Minneapolis, MN; [2]Department of Surgery, University of Minnesota, Minneapolis, MN.*

Analysis of registry data has suggested that, as the incidence of acute rejection has declined, the relative risk of acute rejection associated for graft failure has increased (Transplantation 2000; 70:1098). We tested this hypothesis among 1st cadaveric (n=941) and 1st living donor (n=1329) transplants (TX) at a single center between January 1984 and July 2002. From 1994-1986 to 1999-2002, the 1-year actuarial incidence of acute rejection declined from 60% to 6% for cadaveric donor TX, and from 22% to 11% for living donor transplants. We performed separate Cox proportional hazards analyses for each era, adjusting for multiple patient and donor characteristics (not shown), to examine the impact of these less frequent acute rejections on graft survival. In each case, acute rejection was analyzed as a time-dependent covariate. Adjusted relative risk (RR) of the first acute rejection for graft failure:

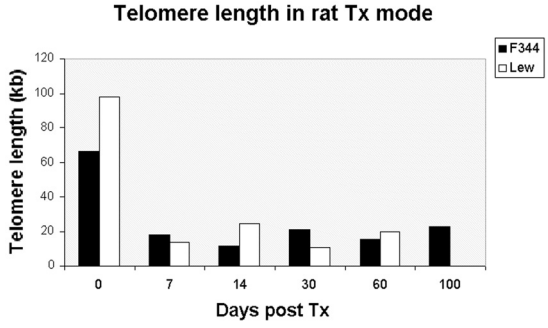
	First Cadaveric Donor TX		First Living Donor TX	
Era	GF/N	RR (95% Cl)	GF/N	RR (95% Cl)
84-86	143/186	3.20(2.22-4.61)	96/177	2.82(1.83-4.36)
87-89	104/144	2.55(1.63-3.97)	70/143	4.18(2.41-7.24)
90-92	97-156	1.79(1.11-2.90)	81/188	2.17(1.32-3.57)
93-95	79-154	2.64(1.67-4.44)	72/227	4.90(2.90-8.28)
96-98	55/158	1.91(0.91-4.01)	41/281	2.38(1.19-4.78)
99-02	18/143	26.1(5.11-133.3)	32/312	4.25(1.69-10.7)

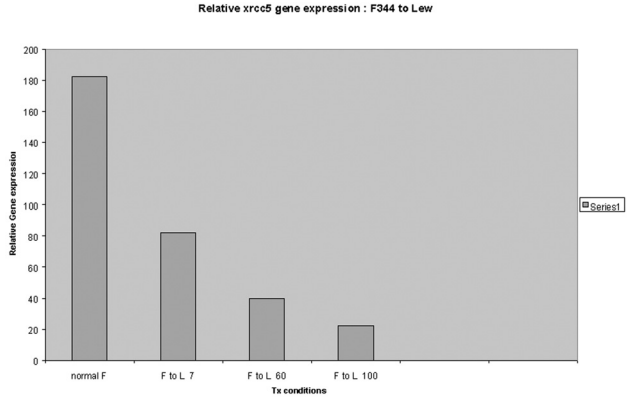
The effect of acute rejection was remarkably constant across all eras, with the possible exception of the most recent 1999-2002 era of cadaveric donor transplants. Most of the increased risk of acute rejection in this era was attributable to the risk of death-censored graft failure (RR= 29.6, 2.1-419). However, in this era there were only 18 graft failures (7 death-censored), so this isolated result should be treated with caution (note the wide confidence intervals). These data suggest that the less frequent acute rejections of the modern era may more be often associated with graft failure in cadaveric, but not living donor transplants.

Abstract# 799 **Poster Board #-Session: P55-II**

MASSIVE TELOMERE EROSION IN A RAT MODEL OF CHRONIC RENAL ALLOGRAFT REJECTION. Paul G. Shiels,[1] Claire E. Nolan,[1] Simone A. Joosten,[2] Vanessa van Ham,[2] Maria C. Borias,[2] Cees van Kooten,[2] Alan G. Jardine,[3] Leen C. Paul. *[1]Div. Cancer Sci. Mol. Pathol, Univ. Glasgow, Glasgow, United Kingdom; [2]Dept Nephrology, Leiden Univ. Med. Centre, Leiden, Netherlands; [3]Div. Cardiovascular Sci. and Medicine, Univ. Glasgow, Glasgow, United Kingdom.*

Introduction The pathogenesis of CAN remains to be fully determined. Both immune and non-immune processes are thought to be involved. Senescence is a possible non immune contributory factor. Cellular and physiological senescence are separate but overlapping entities.Accelerated senescence of both types results as a consequence of oxidative damage resulting from transplantation. This reduces the organ's capacity to withstand stress post-transplant and thus impact on the development of disease pathology. **Methods** Quantitative PCR and telomere measurement have been used to assess transplant related damage in grafts, in a rat model of chronic renal allograft rejection. **Results** We have demonstrated massive telomere loss (fig.1) and senescence associated gene expression in kidney allografts (fig 2). Telomere loss is coincident with as little as 45 minutes ischaemia.Furthermore, senescence associated beta galactosidase is expressed solely in the tubular epithelium of rejecting grafts, which has direct implications for graft function. **Conclusion** These data indicate that renal allografts are under oxidant stress at the time of transplantation. These findings suggest novel target areas for therapeutic intervention and possible means for a standard prognostic evaluation of grafts free from observer bias.





Abstract# 800 Poster Board #-Session: P56-II

INCREASING RECIPIENT AGE IS AN INDEPENDENT RISK FACTOR FOR CHRONIC GRAFT DETERIORATION: COMPARISON OF DOUBLE AND SINGLE RENAL ALLOGRAFTS IN AN EXPERIMENTAL MODEL. Christoph M. Heidenhain,[1] Andreas Pascher,[1] Kirstin Attrott,[1] Anja Reutzel-Selke,[1] Peter Neuhaus,[1] Stefan G. Tullius.[1] *[1]General, and Transplantation Surgery, Charite, Campus Virchow, Berlin, Berlin, Germany.*

Modifications of the immune response with increasing recipient age may alter graft outcome. In the current experiment we tested the influence of kidney mass (double and single renal allografts) and recipient age (3 and 18 months) on chronic renal allograft deterioration in a rat system. Single and double renal allografts from 3 months or 18 months old F-344 donors were grafted into 3 months or 18 months old bilaterally nephrectomizied Lewis recipients (n=5/group, CyA 1,5mg/kg/d x 10 d). All young recipients receiving single or double renal allografts from young or old donors survived the observation period of 180 days compared to a survival rate of 50% in old recipients independent from donor age (3 and 18 months) and renal mass (double and single kidneys). Proteinuria had significantly increased in parallel with recipient and donor age. However, all old recipients demonstrated significantly reduced renal function independent from donor age and renal mass compared to young recipients ($p< 0.05$). Morphological alterations by 180 days demonstrated significantly ($p< 0.01$) advanced glomerulo- and arteriosclerosis, tubular atrophy and fibrosis comparing double kidney grafts from young donors (2 ± 0.2 g) in old recipients (400 g ± 25 g) vs. single young kidneys (1± 0.1g) in young recipients (200 g ± 15 g). Furthermore, significantly increased cellular infiltrates were observed in older recipients ($p< 0.01$). Thus, increasing recipient age was associated with an acceleration of chronic graft deterioration. Recipient age represents an independent risk factor even in the presence of increased renal mass.

Experimental Model

	Young Recipient	Old Recipient
Single Young Graft	Y-Y	Y-O
Double Young Graft	YY-Y	YY-O
Single Old Graft	O-Y	O-O
Double Old Graft	OO-Y	OO-O

801 Poster Board #-Session: P57-II

IMPACT OF CYCLOSPORINE (CSA) EXPOSURE IN COMBINATION WITH SIROLIMUS (SRL) ON RENAL ALLOGRAFT OUTCOMES AT ONE YEAR. Barry D. Kahan,[1] Lai Dejian,[2] Linda Schoenberg,[1] Richard Knight,[1] Stephen Katz,[1] Charles Van Buren.[1] *[1]Division of Immunology and Organ Transplantation, University of Texas Medical School, Houston, TX; [2]University of Texas School of Public Health, Houston, TX.*

Objective: To assess the impact of the initial level of CsA exposure upon rejection incidence and serum creatinine (Scr) values at 1 year among sirolimus- (SRL-) treated patients. **Methods**: Four contemporaneous cohorts were compared for the incidence of acute rejection episodes (ARE, biopsy-proven ≤6 months), chronic rejection (CR; biopsy-confirmed ≤12 months), and progression of renal dysfunction, as defined by the difference between the 1-year and the nadir Scr. Group 1 received antilymphocyte antibodies plus SRL induction with delayed introduction of CsA at low dose (C_{av}<200ng/mL, n=54); Group 2, moderate CsA exposure (C_{av}>200ng/mL) plus SRL (n=180); Group 3, low CsA exposure (C_{av}<200ng/mL) plus SRL (n=157); and Group 4, full CsA exposure (C_{av} 550 tapering to 350ng/mL) with **no** SRL (n=118). SRL exposure was targeted to 10± 3ng/mL. The raw data of each patient were adjusted, using linear models accounting for average probabilities, to reflect the composite impact of 10 demographic, 10 clinical, and 15 laboratory features. **Results:** All SRL-treated patients (Groups 1, 2, and 3) displayed lower incidences of ARE than the no SRL (Group 4) cohort ($p<0.0001$). The ARE rate was lowest for Groups 1 (9.3%) and 2 (9.4%), moderate for Group 3 (20.6%; $p<0.01$), and highest for Group 4 (34.8%; $p<0.0001$). The association of CsA and SRL concentrations with ARE was described by a logistic function. The incidences of CR processes on histopathologic examination within 1 year—7.4%, 10.7%, 13.7%, and 15.2%, respectively (p=NS)—were not changed by adjustment for delayed graft function and HLA mismatch, the only 2 factors that correlated with this diagnosis (ANOVA; p=0.60). Nadir mean Scr values were 1.24, 1.36, 1.13, and 1.09, respectively, and the mean individual differences between 12 month and nadir were 0.30, 0.61, 0.40, and 0.51, respectively (p=0.06; Wilcoxon). When individual Scr values were adjusted for the impact of transplant source, recipient body mass index, gender, ethnicity, HLA mismatch, hemoglobin, SRL dose, and CsA C_2, Group 1 and Group 3 patients displayed significantly better preservation of renal function than Group 2 recipients (p=0.004). **Conclusion:** One year data show that in SRL-treated patients delaying introduction of low-exposure CsA under the cover of antilymphocyte antibody induction therapy minimizes subsequent deterioration of renal allograft function.

Abstract# 802 Poster Board #-Session: P58-II

PREDICTING LONG-TERM RENAL ALLOGRAFT OUTCOME: ROLE OF ALBUMINURIA. Vani Ram,[1] Mitzi Near,[1] Sasi Selvaraj,[1] Antonio Guasch.[1] *[1]Renal Division, Emory University, Atlanta, GA.*

Progressive renal insufficiency and graft loss develops in a significant number of renal allograft recipients. To characterize the role of albuminuria as a potential early marker of renal dysfunction in renal transplantation, we measured albumin excretion rate (AER), kidney function (creatininine clearance, Clcr, from the Cockroft-Gault formula) and other clinical parametes in a cohort of patients followed longitudinally for 3 years. Patient population: 492 adult renal transplant recipients (80% cadaveric, 20% living), median age 46 years (range 20-76), 67% male, 33% female, who were at least 3 months after transplantation and had yearly determination of AER were enrolled. Immunosuppresion consisted of prednisone+ calcineurin inhibitor+ mycophenolate mofetil in >98% of patients. Albuminuria (spot urine sample) was classified as: normoalbuminuria (<30 mg/g creatinine), microalbuminuria (30-300 mg/g cr) or macroalbuminuria (>300 mg/g cr). At baseline, normo-. micro- and macroalbuminuria were present in 51%, 41% and 8% of patients, respectively, and the degree of albuminuria was associated with progressive renal insufficiency (Clcr was 75±3, 69±4, and 55±5 ml/min, in normo-, micro- and macroalbuminuria, respectively, $p<0.05$). Median time post-transplantation was 2.5 years in macroalbuminuria vs 1.8 years in both normo- and microalbuminuria, $p<0.05$). At 3-year followup, of the normoalbuminuria patients at baseline, 24% had developd microalbuminuria and 5% macroalbuminuria, whereas in the baseline microalbuminuria group, 19% of patients had progressed to macroalbuminuria. Renal function correlated with the development of albuminuria: patients who maintained albuminuria had stable renal function (72±2 vs 71±2 ml/min, at baseline and 3 years, p=NS), whereas in microalbuminuria the change in Clcr over 3 years was 5 ml/min (p=0.12 vs normoalbuminuria), and in macroalbuminuria was 12 ml/min ($p<0.05$ vs micro- and normoalbuminuria). In summary: 1) the persistence of normoalbuminuria is associated with stable renal allograft function. 2) Microalbuminuria identifies a group at a higher risk for the development of macroalbuminuria. 3) Macroalbuminuria is associated with a rapid decline in renal allograft function. We conclude that albuminuria characterizes progressive renal insufficiency and may predict long-term outcome in renal transplantation.

Abstract# 803 Poster Board #-Session: P59-II

ANTI-HLA ANTIBODY EMERGENCE AFTER NONSPECIFIC ACTIVATION OF IMMUNE SYSTEM. Matthew Cooper,[1] Andrea A. Zachary,[2] Julie A. Graziani,[2] Robert A. Montgomery,[1] Mary S. Leffell.[2] *[1]Surgery, Johns Hopkins University, Baltimore, MD; [2]Medicine, Johns Hopkins University, Baltimore, MD.*

The presence of HLA-specific antibodies in patients awaiting transplantation has long been recognized as a potential barrier for organ acquisition. Levels of anti-HLA antibodies vary significantly when measured by changes in panel reactive antibody (PRA) determined by cytotoxicity or more sensitive ELISA methods. Pregnancy, transfusion and previous transplants are known to provoke anti-HLA antibodies; however, it is not established as to whether physiologic stresses (infection, trauma, or surgery) can induce antibody production. We present the data of 20 patients found to have a sudden rise in anti-HLA antibody following infection, transplant nephrectomy, and transfusion. Methods: Patients sera were screened for IgG, HLA-specific antibodies by ELISA using solubilized HLA class I and II antigens from multiple donors. Antibody specificity was determined in ELISA using a panel of defined phenotypes. A standard microcytotoxicity assay using a 60 member panel was used for further antibody characterization and PRA determination. Criterion for inclusion was an increase in ELISA optical density ratio (ODR) ≥ 5. Results: 12 patients awaiting renal transplant were found to have an increase in alloantibody within 1week-2 months following an infectious episode. While 2 of these 12 had previously been transplanted, no other potentially sensitizing events occured within this time period. Specificity was determined in 8 of 12 patients. 7 out of 8 demonstrated a polyclonal, multi-specific response while the 2 with previous transplant recalled donor-specific antibody (DSA). Transplant nephrectomy corresponded with a rise in alloantibody in 5 patients. 4 out of 5 developed DSA. Blood transfusion in 4 patients was accompanied by a sustained, multi-specific, anti-HLA antibody response. Mean increases in PRA were similar in all three groups. However, in 33% (4/12) of patients with infection, the increases in PRA were small (<10%) and might have been missed without the sensitivity of ELISA. Conclusion: Physiologic stress may provoke the emergence of anti-HLA antibody. This response following infection is often multi-specific and may be of shorter duration than that evoked by transfusion or nephrectomy. The magnitude, however, measured by ELISA as ODR is comparable. Among patients who have had previous transplants, donor specific antibodies are frequently recalled.