BOOK OF ABSTRACTS

LIVRE DES RÉSUMÉS

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Dr. Steven Paraskevas, Conference Chair
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BASIC SCIENCE

 # 392

APOPTOTIC ENDOTHELIAL CELL MICROENVIRONMENT, THROUGH MFG-E8 RELEASE, REPROGRAMS MACROPHAGES INTO PRO-FIBROTIC CELLS

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Chronic transplant vasculopathy (CTV) is an important cause of interstitial fibrosis and tubular atrophy in the renal allograft. It is characterized by the presence of apoptotic endothelial cells (EC), which generate a microenvironment that leads to the typical myointimal proliferation found in CTV. Macrophages (Mϕ) play an important role in CTV. However, the exact nature of the reprogramming induced by this microenvironment on Mϕ phenotype and potential mediators responsible are ill defined.
Human umbilical vein and murine EC were serum-starved for 4 h as a model of apoptotic EC [apoptotic serum-starved conditioned medium (SSC)]. Non-apoptotic SSC was generated where apoptosis was prevented by caspase-3 inhibition prior to starvation (caspase-3 inhibitor, SSC-DEVD vs SSC-DMSO control). A comparative proteomic approach were used to identify a secretome specific to apoptotic EC. To evaluate reprogramming, we stimulated different leukocyte subsets. Monocytes were harvested from healthy donors by Ficoll gradient and immunomagnetic selection and matured for 5-7 days to generate monocyte-derived Mϕ (HMϕ). HMϕ and murine bone marrow-derived Mϕ (MMϕ) were stimulated for 24h with SSC. Cells were harvested and supernatants kept for ELISA analysis to determine cytokine/chemokine production.
We identified that milk fat globule-epidermal growth factor 8 (MFG-E8) was secreted during apoptosis. Since MFG-E8 is important in Mϕ biology, we characterized it further. Immunoblotting of apoptotic EC revealed that intracellular MFG-E8 decreased overtime whilst its secretion was increased in SSC. Caspase-3 inhibition prevented MFG-E8 release. MMϕ produced more TGF-β, IL-10 and VEGF and less MIP-2 and MCP-1 when stimulated with SSC-DMSO compared to SSC-DEVD/SSC-ZVAD. HMϕ generated less IL-6, IL-8 and MCP-1 when exposed to SSC-DMSO. Elimination of MFG-E8 in SSC by immunoprecipitation and siRNA blunted this Mϕ reprogramming. Finally, our data suggest that SSC and MFG-E8 reprograms Mϕ through STAT-3 signaling.
This study constitutes the first description of the apoptotic-dependent release of MFG-E8 by EC. It also demonstrates the importance of the apoptotic microenvironment in the reprogramming of a regulatory, pro-fibrotic Mϕ phenotype. These results suggest an important contribution of Mϕ in the CTV-associated fibrosis.

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THE POTENTIAL OF NOVEL ORGANIC DYES TO PREVENT REJECTION OF PORCINE ISLETS IN MICE

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Background: A major limiting factor in the use of xenotransplantation for the treatment of Type 1 Diabetes Mellitus is cell mediated immune rejection. Prevention of xenograft rejection has been demonstrated by blocking both cell adhesion by interfering with the LFA-1 to ICAM-1 interaction using an anti-LFA-1 monclonal antibody (mAb), and by blocking the CD40-CD154 co-stimulatory interaction with another mAb. Anti-LFA-1 mAb is available clinically as a treatment for psoriasis, however, the anti-CD154 mAb has been shown to increase the risk of thrombo-embolic events in primate models, thereby halting clinical trials. An alternative to this mAb may be in the form of small inhibitory molecules (SIM), which are able to block protein-to-protein interactions by binding to small but high affinity areas. They are very specific and may therefore prevent undesirable side effects observed with anti-CD154 mAb. Suramin is an SIM that has been shown to block the CD40-CD154 interaction *in vitro*, however, no *in vivo* studies have confirmed this. A group of organic dyes share a similar molecular structure to suramin, including Direct Red 80 (DR80), and have also been shown to block this interaction *in vitro*. We hypothesize these organic dyes will inhibit the CD40-CD154 interaction, thereby preventing rejection of porcine islet xenografts, and will therefore be a viable alternative to the anti-CD154 mAb.
Methods: Islets from 3-day-old newborn pigs were isolated and purified using our standard protocol. Two thousand islet equivalents were transplanted under the kidney capsule of streptozotocin induced diabetic C57BL/6 mice. The mice were either left untreated, or treated with suramin or DR80 alone, or in combination with anti-LFA-1 mAb. Blood glucose levels were monitored twice weekly for 150 days post-transplantation. At this time, a survival nephrectomy was performed, and

the graft was assessed with immunohistochemistry for the presence of pancreatic beta cells and immune cells.
Results: Suramin demonstrated significant toxicity as 6/6 mice treated with suramin alone and 5/7 mice treated with suramin and anti-LFA-1 mAb required euthanasia. One of the 2 remaining mice achieved normoglycemia at 80 days post-transplant. DR80 was much less toxic, however all mice treated with DR80 as monotherapy remained hyperglycemic. Three of 5 mice treated with DR80 and anti-LFA-1 mAb have achieved normoglycemia at 150 days, and another 3/5 mice are trending towards normoglycemia at approximately 80-100 days post-transplant.
Conclusions: Suramin is a toxic therapy at the dose tested and therefore is not suitable for further *in vivo* testing. DR80 is a promising therapeutic agent and appears to prevent the rejection of porcine islets transplanted into mice when combined with anti-LFA-1 mAb.

#411

MINIMIZING DONOR ORGAN IMMUNOGENEICITY IN KIDNEY TRANSPLANTATION USING SIRNA

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Background: Immune rejection from donor antigen presenting cell (APC) plays an important role in graft acceptance in organ transplantation. We hypothesize that the process of RNA interference (RNAi) may be used to modify a graft so as to effectively suppress genes associated with maturation and singal of APC in order to impair the ability of APC to activate T cells. Such manipulation of immunogeneic gene expression may be performed by treatment of the organ ex vivo with short interfering RNA (siRNA) as part of the preservation procedure.
Methods: Kidneys were isolated from C57/BL6 mice, and perfused with 200 μl of HTK solution that contains 100 μg/ml siRNAs specifically targeting Rel B, CD40 and C3 genes respectively. The donor organs were perfused and preserved in the siRNA solution at 4 ^{0}C. After preservation in siRNA solution or control solution, kidneys were implanted into allogeneic Babl/C recipient whose both own kidneys had been removed, making the recipients surviving dependent upon the transplanted kidney graft. The protective effect of siRNA treatment was determined by renal function, histopathology and survival of grafts.
Results: siRNA was efficiently delivered into kidney organ through perfusion and preservation procedure as shown by the distribution of the fluorescent labelled siRNA. Gene expression of Rel B, CD40 and C 3 were significantly knocked down by siRNA solution. Perfusion/preservation of donor kidneys using siRNA solution remarkably improved renal function after transplantation with 2 times lower level of BUN and serum creatinine. The protective effect of siRNA was further demonstrated by improved histopathology, less monocytes infiltration. Furthermore, siRNA solution was capable of prolonging graft survival.
Conclusion: This is the first demonstration of that preservation of donor kidneys through RNAi strategy protects renal graft from direct-pathway initiated immune rejection in kidney transplantation.

#413

BLEBBISTATIN ATTENUATES COLLAGEN GEL CONTRACTILITY IN HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Background: Previous data in our lab suggest that human bone marrow derived mesenchymal stem cells (MSCs) spontaneously differentiate and adopt a myofibroblastic phenotype in culture. This may be due to the effect of matrix elasticity *in vitro*. Blebbistatin (BB), a specific inhibitor of non-muscle myosin II (NMM-II), may play a key role in modifying differentiation and proliferation *in vitro* as it abrogates the response of MSCs to matrix elasticity. We examined MSCs *in vitro* and compared their function to cultured human cardiac fibroblasts (CF).
Methods: The protocol received IRB approval. MSC and CF were isolated from patients undergoing open heart surgery, were cultured in standard conditions and plated onto collagen gel substrates. Cells were serum starved for 24 hours and treated with either TGF-β (10 ng/mL), BB (5 μM), BB (10 μM) or both for an additional 24 hours. The reduction in gel surface area was then analyzed and cell viability was assessed by Trypan blue exclusion.
Results: NMM-II was easily detected in MSCs and cardiac fibroblasts and expression increased over serial passage consistent with myofibroblast phenotype. MSC and CF contracted collagen gels, an effect that was enhanced with TGF-β treatment alone or attenuated by BB treatment alone. BB treatment alone enhanced cell survival

whereas TGF-β impaired cell survival. However BB treatment did not completely reverse the effects of TGF-β, even at higher doses.
Conclusions: MSC adopt a myofibroblast phenotype in culture. The have similar physiology to CF with respect to collagen gel contraction. Inhibition of NMM-II enhances survival and limits myofibroblast function, although TGF-β effect may employ an alternate myosin isoform. Examination of other myosin isoforms is warranted, as well as the ability of BB to prevent myofibroblast phenotype.

#414

MEVALONATE DEPLETION IMPAIRS SURVIVAL OF PRIMARY HUMAN MESENCHYMAL STEM CELLS THROUGH DEPLETION OF NF-KB

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Background: Circulating progenitor cells of bone marrow origin have been implicated in transplant cardiac allograft vasculopathy (CAV) and cardiac fibrosis. HMG-CoA reductase inhibitors ("statins") have been shown to slow the progression of CAV and improve patient survival, which was presumed to be as a result of altered lipid metabolism. We examined the effect of HMG-CoA reductase inhibition with atorvastatin on the viability of primary human bone marrow derived mesenchymal stem cells (MSC).
Methods: The protocol received IRB approval. MSC were isolated from the sternum of patients undergoing open heart surgery and were cultured in standard conditions. Cells were treated with atorvastatin 0.1, 1.0 or 10 μM for 48 or 96 hours. Cell viability was assessed using an optical MTT assay. NF-κB p65 expression was assessed by western blot. Rescue of NF- κB pathway function was achieved through overexpression of Ikk-β with an adenviral vector.
Results: Treatment of MSC with 0.1 - 10 μM atorvastatin resulted in progressively reduced cell viability which was associated with a progressive decline in NF-κB p65 expression. Viability could be rescued by coincubation with mevalonate or pretreatment with Ikk-β adenoviral vector.
Conclusions: Mevalonate depletion through HMG-CoA reductase inhibition impairs the viability of primary human MSC in culture through depletion of NF-κB p65. This represents an additional plieotropic effect of statins and may explain the beneficial effect of this therapy independent of its effect on lipid metabolism.

#417

THE ENDOTHELIAL APOPTOTIC BIOMARKER LG3 IS A NOVEL REGULATOR OF VASCULAR REMODELLING IN VIVO

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Rationale: Endothelial apoptosis is a key determinant of transplant vasculopathy (TV). Apoptotic endothelial cells (EC) release LG3, a C-terminal fragment of perlecan, and cathepsin-L, which may represent fingerprints of vascular injury of potential importance in neointima formation.
Objective: Our two goals were to determine whether circulating levels of LG3 and cathepsin-L, are increased in association with vascular injury of renal allografts, and to evaluate the impact of LG3 on vascular remodelling.
Methods: We conducted a case-control study to compare LG3 and cathepsin-L serum levels in human renal transplant patients with acute vascular rejection (AVR), tubulo-interstitial rejection (ATIR) and normal graft function. Orthotopic aorta transplantation between fully MHCmismatched mice was used to characterize the functional impact of LG3 on vascular remodelling.
Results and Conclusions: Cathepsin L and LG3 serum levels were increased in patients with AVR compared with normal allografts or ATIR. Patients in the lowest tertile for both mediators had significantly better long term graft survival compared with the 2nd and 3rd tertiles. In murine aortic allograft recipients, recombinant LG3 injections significantly increased neointima formation and decreased the number of CD31 positive EC within the allograft. Recombinant LG3 also induced a β1-integrin-dependent anti-apoptotic phenotype in smooth muscle cells in vitro. Collectively these results identify cathepsin L and LG3 as biomarkers of severe renal allograft injury and LG3 as a novel regulator of neointima formation.

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ANTIBODIES AGAINST THE LG3 FRAGMENT OF PERLECAN ARE NOVEL BIOMARKERS OF VASCULAR INJURY IN RENAL TRANSPLANT PATIENTS

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Vascular rejection (VR) is associated with endothelial apoptosis within glomerular and peritubular capillaries of renal allografts. We have previously shown that apoptotic endothelial cells triggers cathepsin L externalisation leading to matrix perlecan proteolysis and production of a cryptic C-terminal fragment (LG3).
Here, we hypothesized that an antibody response against the apoptosis-induced LG3 is raised during vascular injury and that anti-LG3 antibodies are serum markers of vascular rejection.
In order to evaluate the anti-LG3 antibodies response in a pure VR context, we used a murine model of orthotopic transplantation of a supra-renal aortic transplant between fully MHC mismatched Balb/c and C57Bl/6 recipients. Indeed, anti-LG3 titers were significantly elevated in mice that received a mismatched aortic allograft compared to their isogenic controls (n=8 for each group; 116±19 vs 28±16 Optical Density (OD)x1000; p=0.0358). We also found elevated anti-LG3 antibodies in two non-alloimmune models of vascular injury compared to controls: apolipoprotein E (ApoE)-deficient mice (n= 12 ApoE and 10 WT; 352±76 vs 106±19 ODx1000; p=0.0122), and femoral artery ligation (n=6 for each group; 96.9±3.2 vs 22.3±3.4 ODx1000; p=0.0032). This suggests that vascular injury, either immune or non-immune leads to enhanced anti-LG3 production.
We then performed a retrospective case-control study in human renal transplant patients to compare anti-LG3 titers in patients with acute VR (Acute T-cell mediated rejection Banff grade II or III, n=18) versus those with tubulointerstitial rejection (TIR) (Acute T-cell mediated rejection Banff grade I with negative C4d, n=18) or stable graft function (n=36). Human kidney transplant recipients who experienced acute VR had elevated anti-LG3 titers after transplantation when compared to subjects with TIR or stable graft function (median titers in ODx1000: AVR:159, TIR:100, normal graft function:96, $p<0.05$).
These data suggest that anti-LG3 antibodies are novel biomarkers of vascular injury, associated with VR in the context of renal transplantation.

#428

CD155 ON HUMAN VASCULAR ENDOTHELIAL CELLS ATTENUATES THE ACQUISITION OF EFFECTOR FUNCTIONS IN ACTIVATED CD8 T CELLS

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Vascular endothelial cells (ECs) control the activation of T cells in transplanted organs by acting as semi-professional antigen presenting cells. CD155 is a cell surface protein that has recently been shown to have immune regulatory properties. However, the role of CD155 on ECs in regulating T cell activation has not been studied. We have characterized the expression of CD155 on human ECs and examined its role in regulating the activation of T cells. CD155 was expressed on resting human umbilical vein and microvascular ECs, and was up-regulated in an IFNγ-dependent manner in vitro. This cell surface protein was also expressed exclusively in ECs lining the lumen of human arteries with transplant vasculopathy. When the function of CD155 was examined, antibody-mediated neutralization of CD155 did not affect CD8 T cell proliferation in response to stimulation with mitogen + ECs or allogeneic ECs. Surprisingly, neutralization of CD155 activity or siRNA-mediated inhibition of CD155 expression in ECs increased expression of IFNγ and granzyme B. This effect was specific for CD8 T cells as neutralization of CD155 did not affect IFNγ production from CD4 T cells activated by allogeneic ECs. Examination of IFNγ expression by intracellular cytokine staining indicated that CD155 neutralization did not affect the number of IFNγ-producing CD8 T cells in response to activation by ECs, but increased the level of this cytokine within the differentiated population. Taken together, these findings show that CD155 is an IFNγ-inducible immune regulatory protein on the surface of human ECs that attenuates the expression of effector molecules in CD8 T cells.

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INDUCTION OF IMMUNOLOGICAL TOLERANCE TO ALLOGENIC MYOBLAST USING A NON-MYELOABLATIVE APPROACH WITH ECDI-FIXED ALLOGENEIC SPLENOCYTE INFUSION

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The major challenge for myoblast transplantation (MT), a potential therapy for Duchenne muscular dystrophy, is to induce donor-specific tolerance to obviate the need for life-long immunosuppression. Tolerance to MT has been achieved in mice, but requires bone marrow transplantation (BMT) to induce mixed chimerism. The objective of this work is to develop a new protocol to induce immunological tolerance to MT by inducing mixed chimerism using a mild myeloablative protocol. Previous studies have shown that intra-venous injection of ECDI-fixed splenocytes can promote peripheral tolerance to islet cell transplantation. Dystrophic C57Bl10J mdx/mdx (H-2b) and Foxp3-GFP (H-2b) mice received BALB/c (H-2d) bone marrow after conditioning with Treosulfan (650 or 850 mg/kg on day -3,-2 and -1 relative to BMT) plus 10^8 ECDI-treated BALB/c splenocytes (H-2d) infused either into: a) peripheral vein (IVI; day -7, d1 and d7 relative to BMT); or b) the portal vein (PVI; day -7 and d1). No irradiation was given in either protocol. The preliminary results showed that all treated Mdx mice had increased levels of peripheral mixed chimerism, thirty days after BMT (from 1.25 to 14.65% for IVI and 3 to 9.75% for PVI). In addition, we observed a significant expansion of CD4+CD25+ Foxp3 regulatory T cells, in the spleen (83.81% vs.73%) and/or the PLNs (86% vs.74.55%) of the Foxp3-GFP mice treated with PVI protocol (d7). These results suggest a role for regulatory T cells in the potential tolerogenic effect of apoptotic cell infusion and indicate that this approach may be an effective and safe approach for generation of mixed chimerism and donor-specific stable tolerance.

#433

CHARACTERIZATION OF REGULATORY AND EFFECTOR T CELLS IN RENAL TRANSPLANT RECIPIENTS AFTER INDUCTION WITH THYMOGLOBULIN VS. BASILIXIMAB: AN INTERIM REPORT

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Introduction and Aims: ATG (Thymoglobulin) at low, non-depleting doses, promotes expansion and de novo generation of human regulatory T cells (Tregs) *in vitro*, but its effect *in vivo* is still unknown. We hypothesized that ATG given at standard induction doses may also expand Tregs *in vivo* in kidney transplant recipients.
Methods: 22 subjects enrolled in this ongoing, single-center, prospective, longitudinal observational study. Subjects received induction with usual dose of ATG (1.5 mg/kg/day x 5 days, n=11) or basiliximab (20 mg on days 0 and 4, n=11) and were maintained on Prograf and Cellcept. Blood was collected before (n=22) and at 3 (n=21), 6 (n=19) and 12 (n=7) months after transplant. Peripheral blood mononuclear cells were monitored by flow cytometry for the CD4+ subsets including total CD4+, 25+Foxp3+ (Tregs), 45RO+ (memory), 45RO+CCR7-62L- (effector memory) and 25hi45RA+31+ (recent thymic emigrant nTregs). Longitudinal statistical analysis of absolute cell counts and ratios were performed using non parametric paired tests and generalized estimating equations.
Results: ATG led to profound depletion of total CD4+ (pretransplant, 3, 6, 12mo: 0.60, 0.12, 0.15, 0.20 x10^9 cells / L; $p<0.01$), Tregs (0.026, 0.008, 0.009, 0.009; $p<0.01$) and CD4+ memory T cells (0.43, 0.09, 0.12, 0.10; $p<0.01$). In contrast, basiliximab did not cause significant depletion of total CD4+ (0.50, 0.49, 0.48, 0.47; $p=0.01$ vs ATG) or CD4+ memory T cells (0.37, 0.30, 0.27, 0.25; $p=0.02$ vs ATG), but intriguingly led to reduction in the Treg population (0.023, 0.011, 0.014, 0.013; $p=0.22$ vs ATG). Thus, the percentage of Tregs among total CD4+ T cells increased in the ATG (4.3, 6.8, 6.8, 8.0%), but decreased in the basiliximab group (4.7, 2.8, 2.9, 2.5; $p<0.04$ vs ATG). Over time, there was a trend towards a higher ratio of Tregs:Teffector memory cells with ATG (0.06, 0.10, 0.10, 0.21) compared to basiliximab (0.06, 0.05, 0.05, 0.07; $p=0.08$). There was no evidence of an increase in the thymic emigrant proportion within Tregs in either group.
Conclusions: These observations suggest that induction with ATG, but not basiliximab, leads to a relative Treg sparing, which results in an increase in the Tregs:Teffector

memory cells ratio. It further suggests that thymic output of nTregs is not affected by these drugs.

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MICROARRAY ANALYSIS OF BETA-CELL GENE EXPRESSION REVEALS THE PROINFLAMMATORY SIGNALING AND A SHIFT TOWARD AN IMMATURE CELL TYPE FOLLOWING HUMAN ISLET ISOLATION

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Introduction: Preserving functional β-cell mass is essential for successful islet transplantation. Beta-cell function and viability are undermined during islet isolation and may result in further cell loss and poor outcome. The aim of this study was to analyze the expression profiles of β-cells from the intact donor pancreas, isolated islets and cultured islets to understand changes induced by isolation and *in-vitro* culture.
Methods: Human pancreata were procured from multi-organ donors, and islet isolation was carried out using standard methods. Tissue samples from intact pancreas (n=8), freshly isolated islets (n=8) and islets cultured for 3 days (n=5) were frozen in OCT and sectioned. Beta-cells were obtained from these sections using laser capture microdissection, followed by RNA extraction and amplification. Amplified RNA was used for microarray analysis using Human WG-6 Expression BeadChip Kits. The slides were scanned using Illumina BeadArray Reader and expression data was analyzed by Flexarray, DAVID and Ingenuity Pathways Assist Software.
Results: Microarray data analysis showed that islet isolation and *in vitro* culture induced a large NF-kB mediated inflammatory response, characterized by upregulation of several cytokines, chemokines and their receptors. IL-8 was maximally induced by 3.6- fold and 56-fold in fresh and cultured islets respectively. Immunohistochemical studies confirmed IL-8 production by β-cells along with other cell types also. Many pancreas-specific transcription factors, Neurod1, MafA, MafB, Arx, Nkx2.2 and PBX1, were down-regulated suggesting loss of β-cell phenotype. Overexpression of Sox4, Sox9, and id2, the pancreatic progenitor cell-specific transcription factors, and vimentin, a mesenchymal cell marker, indicates de-differentiation of adult β-cells to a progenitor cell stage.
Conclusion: Islet isolation induces a strong inflammatory response which continues during *in vitro* culture. IL-8 and NF-kB emerged as prominent potential mediators of this response. Islet isolation also affects pancreatic transcription-factor networks and shifts the mature β-cell phenotype toward an immature or progenitor cell phenotype.

#437

PREVENTION OF LUNG ISCHEMIA REPERFUSION INJURY BY MESENCHYMAL STEM CELLS IN A MOUSE MODEL OF ORTHOTROPIC LUNG TRANSPLANTATION

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Ischemia reperfusion (I/R)-triggered pulmonary injury remains a major cause of graft failure in lung transplantation. Despite observed therapeutic benefits of mesenchymal stem cells (MSCs) in attenuating acute lung injury (ALI), their utility in preventing I/R injury in lung transplantation has not been elucidated. Our recent development of a mouse model of orthotropic lung transplantation, mimicking many salient features of clinical lung transplantation, has several advantages over conventional mouse lung I/R models using hilar clamping. Employing this orthotopic lung transplantation model, our current study was undertaken to test the ability of MSCs to attenuate lung I/R injury. Autologous bone marrow-derived MSCs were injected ($1x10^6$ or saline; i.v.) into C57BL/6 mice 2 hrs before receiving syngeneic left lung grafts. Transplantations were performed after 6-hrs cold ischemia time, with graft function being evaluated at 4, 24 and 72 hrs following reperfusion. As early as 4 hrs post-reperfusion, lung function as determined by arterial blood oxygenation (PaO_2/FiO_2, mmHg) was significantly improved in MSC-treated recipients compared to saline-treated controls. At 24-hrs, graft oxygenation capacity continued to improve in the MSC-treated group. By 72 hrs, oxygenation reached normal levels in the MSC-treated

group that was never achieved in controls. MSCs conferred significant protection to the transplanted lung, significantly reducing congestion, edema, hemorrhage, inflammation, intra-alveolar macrophages and microvascular permeability (wet/dry ratio, Evans blue extravasation) when compared with controls. Moreover, MSC treatment significantly diminished intragraft expression of various inflammatory cytokines (TNF-α, IL-6), Toll-like receptors (TLR4, TLR9), as well as cellular infiltration ($CD4^+$/$CD8^+$ T cells, neutrophils, macrophages), accompanied by increased anti-inflammatory TSG-6. TUNEL and activated-Caspase-3 staining demonstrated reduced numbers of intragraft apoptotic cells in the MSC-treated group. Interestingly MSC migration/trafficking to the lung graft occurred within 4 hrs post-reperfusion. We conclude that MSCs provide significant protection against lung I/R injury, and thus may be a novel strategy to improve outcomes after lung transplantation.

#438

DNT CELLS INDUCES LONG-TERM CARDIAC ALLOGRAFT SURVIVAL AND AUGMENTS RECIPIENT CD4+FOXP3+ TREG CELL ACCUMULATION

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Regulatory T (Treg) cells play an important role in the regulation of immune responses but whether Treg will induce tolerance in transplant recipients in the clinic remains unknown. Our previous studies have shown that $TCR\alpha\beta^+CD3^+CD4^-CD8^-NK1.1^-$(double negative, DN) T cells suppress T cell responses and prolong allograft survival in a single locus MHC-mismatched mouse model. In this study, we investigated the role of DNT cells in a more robust, fully MHC-mismatched BALB/c to C57BL/6 transplantation model, which may be more clinically relevant. Adoptive transfer of DNT cells in combination with short term rapamycin treatment (day 1- 9) induced long-term heart allograft survival (101 ± 31 vs. 39 ± 13 days rapamycin alone, $p<0.01$). Furthermore adoptive transfer DNT cells augmented CD4+Foxp3+ Treg cells accumulation in transplant recipients while depletion of $CD4^+$ Treg cells by anti-CD25 inhibited the effect of DNT cells on long-term graft survival (48 ± 12 days vs. 101 ± 31 days**,** $p<0.001$). In conclusion, DNT cells combined with short term immunosuppression can prolong allograft survival, which may be through the accumulation of $CD4^+Foxp3^+$ Treg cells in the recipient. Our results suggest that allograft tolerance may require the co-existence of different type Treg cell phenotypes which are affected by current Immunosuppression.

#440

IMPLICATION OF PI3K/MTOR SIGNALING IN STRESS-INDUCED MYOFIBROBLAST DIFFERENTIATION

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Microvascular rarefaction is a strong fibrogenic predictor in all types of solid organ transplants. Microvascular hypoperfusion leads to chronic deprivation in oxygen and growth factors. The accumulation of myofibroblasts within the devascularized interstitium leads to extra-cellular matrix accumulation and fibrosis. We propose that the stress response triggered by growth factor deprivation promotes differentiation of fibroblasts into myofibroblasts through the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) axis leading to long-lasting myofibroblast differentiation.

WI-38 human embryonic fibroblasts were exposed to medium free of serum and growth factors for up to 7 days.

Myofibroblast differentiation was evaluated by western blotting, PCR and immunofluorescence for: alpha-smooth muscle actin (αSMA), collagen I and stress fibre formation. PI3K/mTOR signaling was monitored by western blotting for phosphorylated and non-phosphorylated forms of downstream targets (p70S6K (mTORC1 target), Akt (PI3K and mTORC2 target). The functional impact of the PI3K/mTOR pathway in myofibroblast differentiation was evaluated with biochemical inhibitors (LY294002 for PI3K and rapamycin for mTOR).

Serum deprivation for 4 to 7 days increased αSMA protein and mRNA levels. Collagen I mRNA and stress fibre formation were increased in fibroblasts serum starved for 4 days. Akt phosphorylation at Ser473 decreased abruptly after initiation of serum starvation but was followed by re-phosphorylation after 2 days. PI3K inhibition with LY294002 blocked Akt re-phosphorylation and reduced αSMA and collagen I levels in serum starved-fibroblasts. The mTOR inhibitor rapamycin also reduced αSMA and collagen I expression. We then evaluated the importance of TGF-β signaling in this system by exposing fibroblasts to medium free of serum and growth factors in presence of a neutralising antibody against TGF-β1, 2 and 3 isoforms, or isotype-matched control. TGF-β neutralisation failed to reduce serum starvation-induced myofibroblast differentiation. In further support of a TGF-β-independent system, we found no evidence of SMAD-2 phosphorylation in fibroblasts serum starved for 1 or 3 days.

Collectively, these results suggest that growth factor deprivation induces myofibroblast differentiation through PI3K/mTOR-dependent and TGF-β-independent pathways. These results provide novel insights into fibrogenic mechanisms activated in conditions which, like microvascular rarefaction, are associated with reduced growth factor availability.

#448

NEW TOOLS IN GLYCONANOTECHNOLOGY FOR THE DETECTION AND CHARACTERIZATION OF ABO ANTIGEN-SPECIFIC B CELLS

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Introduction: Screening for ABO antigen-specific B cells will assist in management of ABO-incompatible organ transplantation. Flow cytometry assays to detect and characterize carbohydrate-specific B cells, however, are complicated by weak carbohydrate-antibody interactions, low frequency of specific B cells and non-specific cellular adhesion. Our current study focuses on exploiting glyconanotechnology tools for the development of a flow cytometry assay to detect and characterize ABO antigen-specific B cells.

Methods: Carbohydrate-coated microparticles were produced using multiple conjugation techniques to integrate both the anti-biofouling agent polyethylene glycol (PEG) and A, B or H antigens onto fluorescent silica microparticles. B cells were isolated from peripheral blood mononuclear cells of healthy human donors and incubated with particles in various ratios. B cell:microparticle conjugates were analyzed by flow cytometry.

Results: B cells readily bound to microparticles onto which PEG and antigens were added separately and dispersed throughout the particle. Non-specific binding was reduced from 17-35% to 2-3% by lowering the B cell:microparticle ratio from 1:1 to 100:1. Using microparticles onto which antigens were directly connected to the outer surface of longer PEG molecules resulted in substantial reduction of non-specific binding to <0.5%. Moreover, preliminary results demonstrated that the frequency of false-positive conjugates is further lowered to <0.1% by performing dual staining with carbohydrate-coated microparticles containing two different fluorescent dyes. ABO antigen-specific B cells were identified as cells that bound simultaneously to both fluorescent microparticles.

Conclusion: Reduction of non-specific binding by multiple conjugation techniques for construction of carbohydrate-coated fluorescent microparticles and the use of dual staining enable an effective flow cytometry assay to detect ABO antigen-specific B cells. This assay has the potential to increase the repertoire of tools available to both researchers and clinicians.

#454

DONOR PRECONDITIONING WITH CORM PROTECTS THE RENAL ALLOGRAFT

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Introduction and Objectives: We have previously shown that carbon monoxide releasing molecules (CORM) has anti-inflammatory properties in models of ischemia reperfusion injury (IRI) and isogeneic renal

transplantation. Herein, the ability of kidney donor treatment with CORM to prevent IRI in an allogeneic transplant model was assessed.
Methods: Brown Norway rats were transplanted into allogeneic Lewis rats after a 6 hr cold ischemic time. Rats were untreated, treated with a low dose of tacrolimus alone (0.2 mg/kg iv), pre-treated with 8 mg/kg IP CORM 18 hr prior to procurement, or treated with a combination of tacrolimus and CORM.
Results: Without immunotherapy, animals died within 3 days from uremia (creatinine 780±150 μmol/l) using this transplant model. In our experiment, a low dose of tacrolimus (0.2 mg/kg iv) was given to allograft recipients post-operatively. Control allografted animals did not receive CORM infusion. None of the tacrolimus-treated control animals survived beyond 12 days (Fig.1) and recipients had inferior graft function vs. CORM-treated animals at 7 days (creatinine 620±50 vs. 49±6 μmol/l; $p<0.001$). Excellent graft function was noted on day 70 in CORM treated animals (creatinine 52±9 μmol/l). Accordingly, histologic examination on day 10 showed lymphocytic infiltrate, glomerular congestion, and acute tubular necrosis in controls, whereas CORM treated animals had completely normal histology on day 10. By day 70, CORM treated animals demonstrated vascular lymphocytic infiltrates, despite having excellent renal function.
Conclusions: These results indicate that CORM preconditioning has profound effects upon allogeneic transplantation associated with reduction of transplant-relevant inflammation and damage.

#455

MIGRATION OF VSMC BY LG3 IN ACUTE VASCULAR REJECTION

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Acute vascular rejection (AVR) episodes are tightly associated with increased endothelial cell apoptosis. Prolonged EC apoptosis is associated with neointima formation characterized by migration of vascular smooth muscle cells towards the sub-endothelial area. We have previously shown that caspase-3 activation in endothelial cells fosters the extracellular translocation of cathepsin L. In turn the basement membrane proteoglycan perlecan is proteolysed, leading to the release of a C-terminal fragment LG3.

The scope of the present study was to assess whether LG3 impacts the pro-migratory activity of vascular smooth muscle cells (VSMC). To this end VSMC were exposed to recombinant LG3 (1 to 10 ug/ml) for up to 48 hours in vitro and migration was assessed by Wound Assay experiments. VSMC migration was significantly increased when incubated with LG3 compared to control (Control: 1.00±0,8423, LG3: 3.46±1,549,) (n=6). LG3 is known to interact with β1-integrins, which triggers downstream ERK1/2 activation. Hence we tested the functional importance of LG3-triggered ERK 1/2 activation in our system by exposing VSMC concomitantly to LG3 and either a selective MAPK inhibitor (PD98059), a MEK-1 inhibitor (UO126) or vehicle. Both inhibitors significantly reduced LG3-induced VSMC migration (Control: 1.00 ±0,8423, LG3: 3.46±1,549, LG3+PD98059: 1.168±0.9361, LG3+UO126: 0.5690±0.8774, LG3+vehicle: 2.336±1.410) (n=6). These results suggest that LG3 activates VSMC migration through ERK 1/2-dependent pathways. Increased migration of VSMC by LG3 is a novel pathway of importance for neointima formation.

#460

EPIDERMAL GROWTH FACTOR INDUCED MESENCHYMAL STEM CELLS PROMOTE RENAL FUNCTION RECOVERY IN EXPERIMENTAL ACUTE RENAL FAILURE MODEL IN MICE

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Acute renal failure (ARF) is a syndrome of rapidly declining of renal function. Ischemia-reperfusion (I/R) injury is the most common cause for this complication. Therefore, it is crucial to develop new therapies to improve renal function recovery in ARF. Multipotent mesenchymal stem cells (MSCs) have various effects, and possess special promise for renal repair.
In this study, using epidermal growth factor (EGF), an important factor on promoting angiogenesis and wound healing, stimulated MSC expansion *in vitro*, and evaluated whether infusion of EGF-induced-MSCs attenuates renal injury in experimental I/R induced ARF model in mice. All MSC samples were characterized for cell yield, proliferation capacity and phenotypes, and quantified by

colon formation *in vitro*. *In vivo*, expanded EGF induced MSCs were intravenously injected daily to C57 mice for 7 days, starting 3 days before I/R induction.
Our results showed that EGF induced-MSCs expressed positive mesenchymal markers and negative for hematopoietic markers. In EGF-MSC treated mice, serum creatinine (sCr), blood urea nitrogen (BUN) and urine creatinine (uCr) were found decreases compared to those in control mice. Treatment of EGF-MSC also down-regulated the levels of MCP-1, CXC, KC and MIP-2. The levels of VEGF and of transforming growth factor β (TGF-β) were found increased significantly in sera and ischemic renal tissues. Expression of caspase-3 and the number of apoptotic cells showed decreased in EGF induced-MSC treated mice. Macromorphological results displayed that wet weight of ischemic kidneys was lower in MSC treated mice than those in control mice. Renal histological changes were observed on day 3 after I/R. In control mice, the ischemic glomerular lufts showed the mass of collapse. The proximal tubules appeared frequently gross dilatations in which mostly contain protein casts. In most distal tubules, their epithelial cells were necrosis. While in MSC-treated mice, the number and severity of dilated proximal tubules were reduced with less protein casts, even though distal tubules necroses were also occasionally found. The number of collapsed glomeruli was decreased compare to those in control mice.
In conclusion, EGF-MSC cell therapy might promote renal function recovery that might be via inhibition of inflammation and apoptosis, and down regulation of CC and CXC chemokines in experimental ARF model. MSC infusion might provide new clinical application for renal failure in humans.

#465

SUPPLEMENTAL HYDROGEN SULPHIDE IMPROVES RENAL TRANSPLANT FUNCTION AND SURVIVAL FOLLOWING PROLONGED COLD STORAGE

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Introduction: Organ procurement is associated with significant ischemia and reperfusion injury (IRI) leading to increased rates of delayed graft function, acute rejection and early graft loss. Establishing novel methods to diminish IRI-induced tissue injury is paramount to preserving long-term renal graft survival. Hydrogen sulphide (H_2S) is a newly discovered, endogenous molecule that has recently been shown to minimize tissue injury. We aimed to characterize the protective role of H_2S in a murine model of renal transplantation (RTx).
Methods: Following bilateral native nephrectomy, Lewis rats underwent RTx with left kidneys obtained from syngeneic donors that were flushed, at the time of procurement, with 25 mL of either cold (4°C) University of Wisconsin (UW, Control) or cold UW + H_2S donor molecule (150 μM NaHS) solution and stored for 24 hours at 4°C in 50 mL of the same solution. Following RTx, metabolic cages were used to monitored various parameters of graft function until the time of death or 14 days; Sham operated rats were also followed. Renal grafts were then histologically assessed for cellular injury, apoptosis and markers of inflammation.
Results: H_2S treated kidneys showed marked improvement in turgor and color after RTx, compared to Controls. H_2S group also recovered rapidly from RTx and demonstrated increased activity, urine output, and survival (Figure 1) versus Control. Supplemental H_2S led to a decline in serum creatinine levels towards baseline (Sham) following RTx whereas levels remained high in Controls (Figure 2). Histologically, H_2S treated grafts revealed less glomerular/renal tubular injury and apoptosis compared to Control kidneys.
Conclusions: These finding are the first to report that supplemental H_2S has a protective role in transplant induced renal IRI and may have significant potential clinical implications.

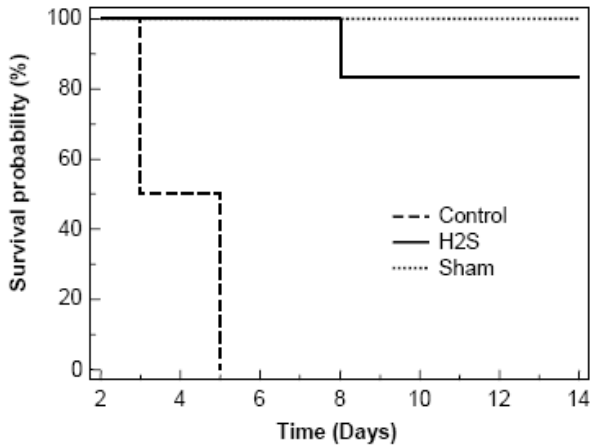

Figure 1. Effect of H_2S on survival

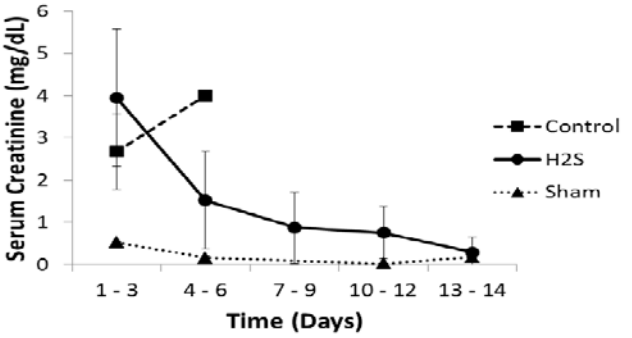

Figure 2. Effect of H_2S on serum creatinine

#469

LUNG PROGENITOR CELL PROFILES IN BRONCHIOLITIS OBLITERANS SYNDROME ARE MEDIATED BY CIRCULATING INFLAMMATORY AND STEM CELL-SPECIFIC FACTORS

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Purpose: Bone marrow-derived cells have been implicated in the pathobiology of many lung diseases including Bronchiolitis Obliterans Syndrome (BOS) after lung transplant. This study aimed to quantify both pro-fibrotic progenitors (fibrocytes) and Clara Cell Secretory Protein ($CCSP^+$) expressing epithelial progenitors. Cytokine mediators of cell mobilization or recruitment were also quantified in the hope of identifying cell and disease specific signals.

Methods: Bone marrow and blood was collected from end-stage BOS patients at re-transplant (n=7). Blood was also collected from clinic patients 1-10yrs post-transplant (n=48). $CCSP^+$ progenitors and CD45/Collagen-1^+ fibrocytes were quantified by flow cytometry. Plasma cytokines were quantified by Luminex-based magnetic suspension array.

Results: End-stage BOS patients had no change in the number of bone marrow CCSP+ cells, yet significantly fewer $CCSP^+$ cells were found in peripheral blood compared to lung donors (1.03±0.4% vs 2.78±0.5, p=0.01). Circulating fibrocytes were significantly increased in BOS patients (4.3±1.2% vs 0.86±0.1%, p<0.01). Of 17 candidate plasma cytokines examined, Stem Cell Growth Factor-β (SCGF-β) (p=0.02), Stem Cell Factor (SCF), Monokine Induced by γ-Interferon (MIG), and Macrophage Inhibitory Factor (MIF) (all p<0.01) were all increased in end-stage BOS. Fibrocyte numbers significantly correlated with SCGF-β levels (r^2=0.64, p=0.04).

Investigation of post-lung transplant patients classified as either stable (n=38) or BOS>1 (n=10) confirmed increased fibrocytes (0.81±0.2% vs.1.9±0.6%, p=0.03) and decreased $CCSP^+$ cells in BOS (1.5±0.2% vs.0.83±0.2%, p=0.11). The ratio of fibrocytes to $CCSP^+$ cells could differentiate BOS from stable patients (p=0.01). SCF plasma protein was increased in both stable and BOS compared to healthy controls, while MIF was increased only in BOS patients (all p<0.01). Circulating fibrocyte numbers significantly correlated with SDF-1 plasma levels in all post-transplant patients (r^2 = 0.18, p=0.01).

Cell surface phenotyping by flow cytometry confirmed expression of CD45, CD34, and CXCR4 on both $CCSP^+$ cells and fibrocytes. The expression level of these markers was not different between stable and BOS patients.

Conclusions: Progenitor cell profiles are altered in BOS, and can be used to distinguish stable from BOS patients. MIF may be an important mediator in this process. The mediators SCGF, SCF, and SDF-1 appear to have an important role in fibrocyte recruitment or maintenance in the context of chronic lung allograft dysfunction. Recruitment of progenitor cells by MIF or SDF-1 via CXCR4 may be important therapeutic target in this process.

 # 470

REDUCTION OF COLD ISCHEMIA-REPERFUSION INJURY BY GRAFT-EXPRESSING CLUSTERIN IN HEART TRANSPLANTATION

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Background: Cold ischemia-reperfusion injury (IRI) is a primary factor for graft dysfunction in organ transplantation. Clusterin (CLU) is a ubiquitous glycoprotein with cytoprotective activity. This study was designed to examine the impact of donor-expressing CLU on cold IRI.

Methods: Donor hearts from wild type C57BL/6J (H-2^b; B6 WT) versus CLU knockout C57BL/6J (H-2^b; B6 KO) mice were stored in saline at 4oC for 8 hours, followed by heterotopic transplantation to B6 WT mice. The function recovery of heart grafts was assessed by regular heart rate determination, and tissue injury was determined by

histological analyses and serum levels of creatine kinase (CK) and lactate dehydrase (LDH).
Results: Heart cells constitutively expressed CLU, and mature CLU protein was located in the endothelium as well as on the cell surface of cardiac myocytes. As compared to CLU deficient hearts WT hearts were more resistant to cold injury during cold preservation, and had a better function recovery from a prolonged cold preservation following transplantation. Superior graft function of CLU-expressing grafts was correlated with reduced neutrophil infiltration and reduced cardiac injury as evidenced by less apoptosis in histologic analyses. Further examination showed that ectopic expression of CLU in cultured myocytes benefited stability of cell membrane and prevented cell necrosis at cold temperature, and also prevented apoptosis induced by pro-inflammatory cytokines.
Conclusion: CLU expression renders donor hearts resistance to cold IRI in transplantation, suggesting that upregulation of CLU expression in donor hearts may have potential for prolonging heart graft viability in cold storage and following reperfusion.

#471

REDUCTION OF COLD INJURY OF DONOR ORGANS BY SUPPLEMENT OF RECOMBINANT CLUSTERIN PROTEIN IN COLD PRESERVATION SOLUTION

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Background: Cold preservation is a common method to protect donor organs from warm ischemia injury prior to transplantation, but itself induces cold injury and negatively impacts graft survival. Clusterin (CLU) is a major chaperonic glycoprotein in physiological fluids and has antiapoptotic activity. This study was designed to evaluate the effect of recombinant CLU protein (rCLU) on the prevention of cold injury in vitro and in vivo.
Methods: Human endothelial cell cultures were used as an in vitro model. Cardiac transplantation in mice was used as an in vivo model. Cell death in cultured endothelial cells was examined by lactate dehydrogenase (LDH) release assay and ethidium bromide stain, and cardiac transplant injury after prolonged cold storage was determined by transplant function recovery, histological analyses and creatine kinase levels in sera.
Results: Here, we demonstrated that supplement of rCLU to University of Wisconsin (UW) solution offers virtually complete protection of cultured human endothelial cells from cold-induced cell lysis or necrosis after 24-hour incubation at 4^{o}C, as evidenced by the fact that only a basal level of LDH release or ethidium bromide stained necrotic cells was detected in 1 mg/ml of rCLU-treated endothelial cells compared to those in bovine serum albumin (BSA)-treated controls. Further examination showed that rCLU in UW solution bound to the cell surface and enhanced membrane fluidity of cultured endothelial cells at 4^{o}C. The cytoprotective activity of rCLU was also demonstrated in a transplantation model, showing that donor hearts preserved in cold UW solution containing 1 mg/ml of rCLU exhibited reduced cardiac injury both at the end of cold storage and after reperfusion as compared to those of donor hearts treated with cold UW solution containing 1 mg/ml of BSA.
Conclusion: Cold preservation of donor organ using a solution containing rCLU may increase cell membrane fluidity by its chaperonic activity, resulting in protection of endothelial cells from cold-induced necrosis and organ transplants from cold ischemia-reperfusion injury. The results suggest that rCLU has potential in the improvement of cold preservation of donor organs.

#472

EFFECT OF RECIPIENT'S KIR GENES PROFILE AND THEIR INCOMPATIBILITY WITH DONOR'S HLA LIGANDS ON THE OUTCOME OF HUMAN RENAL TRANSPLANTATION: ASSOCIATION WITH INFLAMMATION AND TUBULITIS

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Introduction: Importance of Natural killer (NK) cells and the genetic regulation of their functioning is well documented for the defense against tumors, viral infection and outcomes of HCT but their importance in solid organ transplantation is not clear. The activation and inhibition of NK cells depend on the interaction of killer immunoglobulin like receptors (KIRs) with HLA class I molecules expressed on target cells. Here we assessed the impact of recipient KIR gene profile and their incompatibility with donor's HLA class I genes on the

occurrence of acute kidney allograft rejection and histological score indicative of allograft failure.
Methodology: A total of 218 kidney transplant recipients were genotyped for 16 KIR genes (6 activating KIR, 8 inhibitory KIR and 2 pseudogenes) by a multiplex KIR genotyping assay using Luminex based SSO method. Humans have various combinations of these 16 genes with two common haplotypes: Haplotype A is an inhibitory haplotype and Haplotype B is an activating haplotype. Incidence of acute rejection and eleven individual histological parameters scored in 6-12-months post-transplant surveillance biopsies were considered as clinical end points in the analysis. Each parameter was scored as 0 (normal), 1 (mild), 2 (moderate), or 3 (severe).
Results: Recipients carrying activating Haplotype B were found significantly associated with high scores (≥1) for inflammation (p=0.01, OR=3.4) and tubulitis (p=0.04, OR=2.6). The association with inflammation was stronger for patients carrying ≥3 activating KIR genes ((p=0.008, OR=3.8). Recipient KIR – donor HLA incompatibility for ≥2 KIR-HLA ligands also showed strong association with high scores of inflammation (p=0.007, OR=3.6) and tubulitis (p=0.02, OR=2.5).
Conclusion: The allogeneic interaction between recipient KIR and donor HLA antigens may constitute a risk of kidney allograft failure. NK cell mediated response may represent another potent mechanism of allo-immunity in solid organ transplantation.

#473

CAN THE PRE-TRANSPLANT NUMBER OF ENDOTHELIAL PROGENITOR CELLS PREDICT CHRONIC ALLOGRAFT FAILURE?

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Background: Chronic Allograft Failure (CAF) characterized by tubulointerstitial injury, endothelial dysfunction leading to vasculopathy and subsequent interstetial fibrosis is considered as the foremost cause of renal allograft failure. Endothelial progenitor cells (EPCs) represent a promising cellular target for the repair of the vascular damage. EPCs originate from the bone marrow, circulate in the peripheral blood, and play a crucial role in the repair or formation of blood vessels. In this prospective study, we aimed to determine the association of the pre-transplant count of circulating EPCs in kidney transplant patients with the incidence of rejection and various histological scores indicative of CAF.
Methods: A total of 33 renal transplant patients were recruited. Number of circulating EPCs in the peripheral blood were quantified by fluorescence-activated cell-sorting (FACS) analysis using flurochrome beads after staining with fluorochrome labeled antibodies directed against endothelial specific marker: VE-cadherin and progenitor/stem cell markers: CD34 and CD133. The EPCs were defined as $CD34^{pos}CD133^{pos}VE\text{-}Cadherin^{pos}$ cells. Incidence of histologically confirmed rejection (acute and/or chronic) and histological indicators for chronic allograft faillure: the combined grade of Interstitial Fibrosis and tubular atrophy (IF/TA) and Chronic Allograft Damage Index (CADI) scores were considered as clinical end points.
Results: The median absolute count of circulating EPCs is 37 cells /mL of the peripheral blood in pre-transplant specimens (range 9-102 cells/mL of blood). Higher counts of circulating EPCs in the pre-transplant blood specimen showed a clear trend of association with better graft outcome. The circulating EPC count was two-times higher in patients with IF/TA= 0 (median count=51 EPC/mL of blood) then patients with IF/TA≥1 (median count=22 EPC/mL of blood). Similarly pre-transplant circulating EPC counts in patients with no rejection (median EPC count=30 EPC/mL of blood) was less than that of patients with graft rejection (median EPC count=53 EPC/mL of blood). Althought statistical significance was not reached primarily due to limited number of samples, these results showed a trend of association.
Conclusions: Based on this data , we conclude that the pre-transplant count of circulating EPC may represent a predictor of renal allograft outcome. EPC may therefore signifies a potential target for the cell based repair to improve long term renal allograft function and prevent CAF.

#474

INNATE IMMUNE EFFECTORS ARE NECESSARY AND SUFFICIENT TO CAUSE EARLY POST TRANSPLANT FAILURE OF SMOOTH MUSCLE CELL RECOVERY WHICH PREDATES ALLOGRAFT VASCULOPATHY

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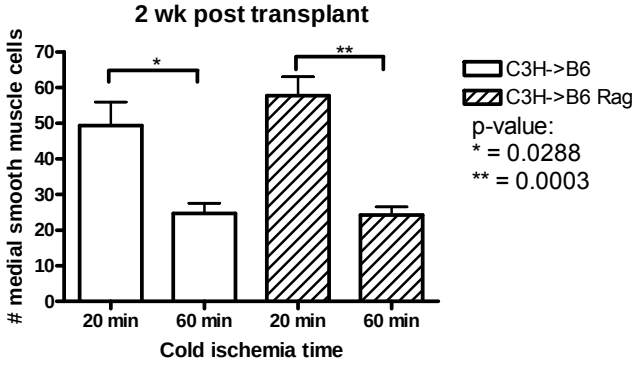
Background: We have previously shown that there is a significant influx of neutrophils early post ischemia reperfusion (IR) injury that correlates with impaired medial SMC recovery. We have also shown subsequent enhanced allograft vasculopathy in a mouse aorta transplant model exposed to prolonged cold IR. We hypothesized that the early effects in an allograft model postransplant are the result of innate immune elements that are nonspecifically activated, and enhanced by prolonged IR.

Materials and Methods: Aortic transplants were performed between fully disparate mouse strains under CyA immunosuppression (C3H/HeJ donors to C57BL/6 (B6; wildtype mice with intact innate and adaptive immunity or B6Rag-$1^{-/-}$ (Rag; immunodeficient mice with intact innate immunity but no adaptive immune effectors). The aortic grafts were subjected to usual (20min) or prolonged (60min) cold ischemia pre-transplant and then were harvested at 1wk and 2wk post-transplant, fixed in formaldehyde and sectioned for H&E (medial SMC quantification) and immunohistochemistry (macrophage (MO) and neutrophil (NO) infiltration).

Results: There was a surprising and significant NO influx at 1wk post transplant which was similar in magnitude regardless of the recipient strain. The median number of NO (per aortic cross section +/-sd) in Rag recipients of donor allografts exposed to 20min cold ischemia was 111.5+/-34.5 and in B6 recipients it was 90+/-52.4, P=0.2276). This infiltrate persisted to two weeks post transplant with trends to increased numbers of NO in grafts exposed to prolonged cold ischemia (Rag recipients of 20min ischemia grafts 24+/-13 vs 60min 36.5+/-15, P=0.1775; B6 recipients of 20min ischemia grafts 4+/-2.2 vs 60min 27+/-16, P=0.0022). There were similar numbers of infiltrating MO in the Rag vs B6 recipients of 60min ischemia grafts at 1 wk (P=0.2949) and at 2 wk (P=0.065). Medial SMC recovery was similarly impaired in aortic allografts exposed to prolonged IR regardless of whether the recipient had an intact adaptive immune response (B6 recipient) or not (Rag recipient) (Fig 1).

Conclusions: The failure of recovery of medial smooth muscle cells in murine aortic allografts occurs in the presence on an intact innate immune system, independent of any adaptive immune elements and is exacerbated by prolonged IR pre-transplant. The mechanism(s) by which the innate immune system interacts with the adaptive immune system to mediate enhanced allograft vasculopathy as a result of prolonged IR remains to be elucidated.

Figure 1.



 # 475

NORMOTHERMIC ACELLULAR EX VIVO LIVER PERFUSION (NEVLP) FOR THE STORAGE, ASSESSMENT, AND REPAIR OF MARGINAL LIVER GRAFTS.

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Livers retrieved after cardiac death (DCD) are often declined for transplantation because of the increased risk for graft failure and bile duct injury. The current cold static preservation technique is associated with a high risk of biliary complications and does not offer the opportunity to assess graft injury and function. We compared the novel acellular normothermic ex vivo liver perfusion with conventional cold static organ preservation for livers retrieved after cardiac death.

Methods: First, pig livers were perfused (n=5) for 12 hours in a cell free, oxygenated perfusion solution (STEEN), followed by 12hr perfusion with whole blood as a model of transplantation. ALT and histology were evaluated as parameters of the liver injury, urea synthesis, bile production and oxygen consumption were determined as marker of the liver function. In a second approach, pig livers either subjected to 1hr warm ischemia plus 12hrs cold storage in UW (n=5) or 1hr warm ischemia plus 4hr cold storage plus 8hr NEVLP. After 12hr organ

preservation the livers were perfused for 8hr in whole blood as a model of transplantation.
Results: 12hr acellular normothermic ex vivo liver perfusion followed by 12hr whole blood reperfusion was not associated with liver injury. Serum ALT (mean 27U/L) remained normal, and histology did not show any evidence of necrosis (<1%). Bile production (mean 3cc/hr), oxygen consumption (400mmHg), and BUN synthesis (1.8mmol/l) were within normal limits. In a second approach, NEVLP was compared to cold static storage in a DCD model using 1hr warm ischemia and 12hr preservation. NEVLP was associated with significantly decreased serum AST (26U/L vs 285U/L), decreased necrosis (10% vs 35%, $p<0.05$), and increased oxygen consumption (400mmHg vs 230mmHg, $p<0.05$). Cold static storage after DCD retrieval was associated with loss of peripheral arterial blood supply, while arterial blood flow was maintained in NEVLP preserved grafts. Accordingly, UW preserved grafts had massive biliary necrosis (90%), while bile ducts were normal in NEVLP preserved livers.
Conclusion: NEVLP allows prolonged organ storage with normal liver metabolism without inducing preservation injury. Bile duct injury after DCD liver retrieval develops in cold static, but not NEVLP preserved organs. NEVLP is a novel preservation technique for the assessment of marginal grafts.

#478

MANNOSE-LIPOSOME DIRECTED SIRNA SILENCING OF CD40 TO PREVENT CARDIAC ALLOGRAFT REJECTION

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Background: RNA interference may provide a novel therapy to prevent allograft rejection by blocking costimulatory molecules on antigen presenting cells (APC) that activate effector T cells or alter tolerance induction. As APC abundantly express mannose receptors, we hypothesized that mannose-liposome delivery of (siRNA) targeting CD40 may efficiently target APC to specifically block this co-stimulation pathway and prolong allogeneic heart graft survival.
Methods: Mannose-liposomes containing CD40 siRNA (Man-CD40) were prepared. Recipients (BALB/c) were treated with Man-CD40 (100μg siRNA/mouse), 3 and 7 days prior to heart transplantation, and 7, 14, 21 days after transplantation. Control groups included mice given mannose-liposomes with 'scrambled' siRNA, conventional liposomes with CD40 siRNA without mannose or CD40 siRNA alone. After siRNA treatment, recipients received a fully MHC-mismatched B6 heart.
Result: Mannose-liposomes specifically delivered CD40 siRNA to spleen and LN APCs and knocked down CD40 gene expression was detected by flow cytometry. Long term graft survival (>100 days) as determined by palpation, was achieved in 85% of the mice treated with Man-CD40. In contrast, allogenic hearts transplanted into control groups of recipients stopped beating within 7 to 20 days ($p<0.01$ for all). Real time PCR and flow cytometric analysis showed an approx. 3 times up-regulation of FoxP3 expression in spleen lymphocytes and a concurrent downregulation of CD40, CD80 and MHC II expression in splenic dendritic cells of Man-CD40 treated mice (35%-56% decrease vs control groups, $p<0.05$). DCs from Man-CD40-treated recipients promoted CD4+CD25+FoxP3+ regulatory T cell differentiation *in vitro*. Finally, histopathology of transplanted hearts demonstrated an overall reduction in lymphocyte interstitium infiltration, vascular obstruction, and edema in mice treated with Man-CD40.
Conclusion: Mannose-conjugated liposomes can augment delivery of siRNA to APCs and silence CD40. This approach for the first time describes targeted delivery using mannose liposomes to APC for costimulatory inhibition resulting in prolongation of allograft survival. Our results highlight the potential of this novel RNAi-based therapeutic strategy to in prevent allograft rejection.

#479

ALTERATIONS IN CD4+FOXP3+ REGULATORY T-CELL DIVERSITY AFTER INFANT HEART TRANSPLANTATION

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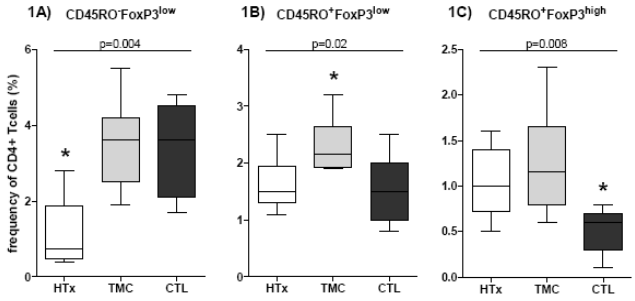
Introduction: $FOXP3^+$ regulatory T cells (T_{regs}) are active suppressors of antigen-activated immune reactivity. In infants, T_{regs} mainly have a naïve phenotype, which is considered to be beneficial for tolerance to both self and non-self antigens. It has been reported that graft survival after heart transplantation (HTx) in infants is higher than in

adults. Infant HTx recipients in our program usually undergo thymectomy, T-cell depletion and on-going immunosuppression, which may influence development and function of T_{regs}. In this study, we determined $FOXP3^+$ T-cell subsets in infants after HTx.

Methods: We analyzed peripheral blood samples of 1) HTx recipients (age at HTx (median (range)): 4 (1–7) mo; n=8). These pts had thymectomy and T-cell depletion at time of HTx and on-going immunosuppression; 2) thymectomized (TMC) non-HTx pts (age at TMC: 0.5 (0.2–5) mo; n=8). These pts had no T-cell depletion or immunosuppression; 3) non-HTx/non-TMC controls (CTL; n=7). Samples from HTx pts were taken 16 (13-25) mo after HTx. TMC pts and CTL were matched for age to the HTx pts (23 (12-27) mo, 19 (14-27) mo and 20 (16-32) mo, respectively). We determined expression of FOXP3, CD45RO and Ki-67 of $CD4^+$ T cells by flow cytometry.

Results: The frequency of $FOXP3^+$ cells within the $CD4^+$ T-cell population was significantly lower in HTx pts than in TMC pts and CTL (3.7% (2.6-5.9%) *vs.* 7.6% (5.3-9.0%) and 6.1% (3.4-7.2%), respectively; p=0.002). Combining FOXP3 with CD45RO expression showed that HTx pts had less $CD45RO^-FOXP3^{low}$ cells (naïve Tregs) than TMC pts and CTL (Fig 1A; p=0.004). The frequency of $CD45RO^+FOXP3^{low}$ cells (activated non-Tregs) was higher in TMC pts than in HTX pts and CTL (Fig 1B; p=0.02). Furthermore, HTX and TMC pts had more $CD45RO^+FOXP3^{high}$ cells (activated Tregs) than CTL (Fig 1C; p=0.008). Analysis of intracellular Ki-67 expression revealed that the frequency of proliferating cells within the $FOXP3^+$ cells was significantly higher in HTx pts than in TMC pts and CTL (30.5% (22.7-62.4%) *vs.* 18.3% (13.2-30.5%) and 15.2 (8.3-27.9%), respectively; p=0.003).

Conclusion: Frequencies of $FOXP3^+$ T-cell subsets in peripheral blood differs among infant HTx recipients, thymectomized non-HTx pts and controls, indicating that not only thymectomy, but also T-cell depletion and immunosuppressants impact development of peripheral regulatory T-cell populations in infants. Alterations in T_{regs} diversity under the persistent presence of alloantigens may promote development of alloantigen-specific T_{regs} contributing to better graft survival. Functional assays are underway to determine the suppressive function of T_{regs} after infant HTx.



#483

IL-2R BUT NOT CD80/86 BLOCKADE DOWNREGULATES B CELL-DEPENDENT T CELL ALLORESPONSE IN HIGHLY SENSITIZED KIDNEY TRANSPLANT PATIENTS

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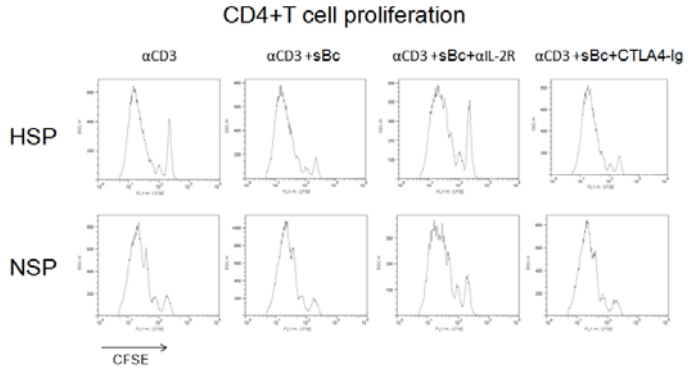
Background: Highly sensitized kidney transplant patients (HSP) are at increased risk of rejection. This may be due to an enhanced T cell alloresponse induced by B cell. We examined the effect of B-T cell interaction on T cell alloresponse in HSP.

Methods: PBMCs were isolated from 3 groups of patients: HSP (n=7, PRA>60%), non-sensitized patients (NSP, n=6, PRA=0-1%), and healthy controls (HC, n=7). HSP and NSP were hemodialysis-dependent and on the transplant list. CD4+T cells were labelled with CFSE, and plated for 5 days with anti-human CD3 antibody (αCD3) alone or with stimulated B cells (sBc, CD40L/IL-4). Then, 5 μg anti-human IL-2R antibody (αIL-2R) or CTLA-4-Ig was used to block B cell-dependent T cell alloresponse. Flow cytometry was performed after re-stimulation with PMA/ionomycin/brefeldin.

Results: Hemodialysis patients had increased CD4+T cell proliferation with αCD3 stimulation in comparison to HC (43.84% vs. 22.88%, p=0.02). Adding sBc further enhanced CD4+T cell proliferation significantly in HSP only (54.2% vs. 40.9%, p=0.02). The only significant difference in CD4+T cell subset proliferation was in TGF-β+CD4+T cells when comparing HSP to NSP (0.29% vs. 0.09%, p=0.02). There was no difference in IFN-γ+, IL-4+, IL-17+ or FoxP3+ CD4+T cell proliferation. IL-2R blockade significantly decreased CD4+T cell proliferation in the presence of sBc back to levels of αCD3 stimulation alone in HSP only (38.6% vs. 54.2%, p=0.01). This was

accompanied by an increase in IL-17+ (0.29% vs. 0.20%, p=0.04) and a decrease in FoxP3+ (0.56% vs. 1.13%, p=0.05) CD4+T cell proliferation. Use of CTLA-4-Ig (CD80/86 co-stimulatory blockade) had no effect on B cell-induced CD4+T cell proliferation.

Conclusion: T cell alloresponse is increased in hemodialysis patients compared to HC and is independent of sensitization status. In HSP, B cells further induced T cell alloresponse. Proliferation of TGF-β+CD4+T cells was higher in HSP and may be a regulatory response to B and T cell activation. B cell-dependent T cell alloresponse may be dependent on IL-2 signalling, and independent of CD80/86 co-stimulation. IL-2R blockade appears to shift CD4+T cell proliferation into IL-17+ and away from FoxP3+ subset. Use of IL-2R blockade may have a role in HSP.



#489

ADOPTIVE TRANSFER OF DNT CELLS FACILITATES MIXED CHIMERISM BY INDUCING ANTI-DONOR T CELL CLONAL DELETION AND SUPPRESSING NK CELL FUNCTION

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Establishment of immune tolerance and prevention of chronic rejection remain major goals in clinical transplantation. Mixed chimerism refers to a state in which allogeneic hematopoietic cells co-exist with recipient cells. This results in a state of tolerance towards both the donor and the host, thus avoiding chronic rejection and side effects of any drug treatment. Our previous study demonstrated that $\alpha\beta^{+}CD3^{+}CD4^{-}CD8^{-}NK1.1^{-}$(double negative, DN) T cells suppress NK cell-mediated bone marrow rejection in sub-lethally irradiated mice. In this study, we aimed to establish a mixed chimerism in a non-irradiation protocol. Adoptive transfer of donor-derived C57BL/6 DNT cells prior to C57BL/6 to BALB/c bone marrow transplantation (n=8) in combination of cyclophosphamide, but not cyclosporin, rapamycin, or FK506, helped establish stable mixed chimerism (40% at day 100, n=4-8 , $P<0.01$) and acceptance of B6 skin allografts but reject 3rd party C3H (H-2k) skin graft (>100 days vs. 11.2±2.2 days, $P<0.001$, n=4-8) . Adoptive transfer of CD4+ (n=4) and CD8+ T cells (n=4), but not DNT cells (n=8), induce graft-versus-host diseases in this regimen. DNT cells suppressed anti-donor T cell proliferation by approximately 88.0% compared to control mice ($p<0.05$) and TCRVβ clonal deletion was observed in DNT cell treated mice. Interestingly, we have found that DNT cells significantly suppressed NK cell mediated-C57BL/6 donor bone marrow rejection in CD4+ and CD8+ T cell-deficient BALB/c mice. The survival rate of donor-derived bone marrow cells significantly improved in DNT cell treated mice (the percentage of donor-derived cells in bone marrow increased from 0.06% to 1.02%). Taken together, our results suggest that adoptive transfer of DNT cells can control both adoptive and innate immunity and promote a stable mixed chimerism and donor-specific tolerance and does not require ablative bone marrow irradiation. These data demonstrate the diverse function of DNT cells and a potentially novel therapeutic use in transplant tolerance induction.

#490

AIRWAY PRESSURE AND COMPLIANCE IN THE EVALUATION OF DONOR LUNG INJURY DURING PROTECTIVE EX VIVO LUNG PERFUSION IN A PORCINE BRAIN DEATH MODEL

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Purpose: Use of ex vivo lung perfusion (EVLP) for evaluation requires accurate and preferably early detection of lung injury during perfusion. We noticed that changes in compliance and airway pressure often precede a fall in the delta pO_2 in rejected injured human donor lungs during normothermic acellular lung protective EVLP. The aim of this study was to confirm this and to explore whether these changes correlated with post-transplant outcomes in a porcine model.
Methods: Rejected human donor lungs (n=2), pig lungs injured by 10h of brain death and 24h of cold ischemia, and normal pig lungs were perfused for 12h (n=5/group). Delta pO_2, lung compliance, airway pressures, and pulmonary vascular resistance (PVR) were measured hourly during EVLP. The left lungs were transplanted in the pig experiments.
Results: In severely injured human lungs, compliance dropped (-20±2mL/cmH_2O from start, mean±SEM) and airway pressure increased (7.5±0.5cm H_2O from start) prior to a fall in pO_2 (596±15mmHg to 378±40). In our pig model, pO_2 remained stable during EVLP in both injured and control groups (526.6 mmHg±21.2 vs 536.3±7.4, respectively); however, in the injured group, compliance decreased from 31.0±5.6 at 1h to 11.0±0.4 mL/cm H_2O at 12h EVLP and peak airway pressure increased from 14.0±1.7 to 27.4±2.3 cm H_2O. PVR started high in injured lungs (1200±207dyn.s.cm-5) then dropped to near control levels (480±84). Control groups had stable compliance, airway pressures, and PVR. Post transplant, the injured group had a poor oxygenation (PaO_2=85.6±9.8mmHg) but the control group had good post-transplant PaO_2 (569.8±25.7).
Conclusion: While poor pO_2 is classically indicative of lung injury, our study suggests that decreases in lung compliance and increases in airway pressure can be earlier signs of injury during EVLP that predict post-transplant function. This data suggests that transplantation should be performed only when all three of these physiological parameters are stable or improve over at least 4 hours of EVLP.

#491

B CELLS ARE ESSENTIAL FOR SUSTAINING THE LATER PHASE OF RENAL ALLOGRAFT REJECTION IN MICE

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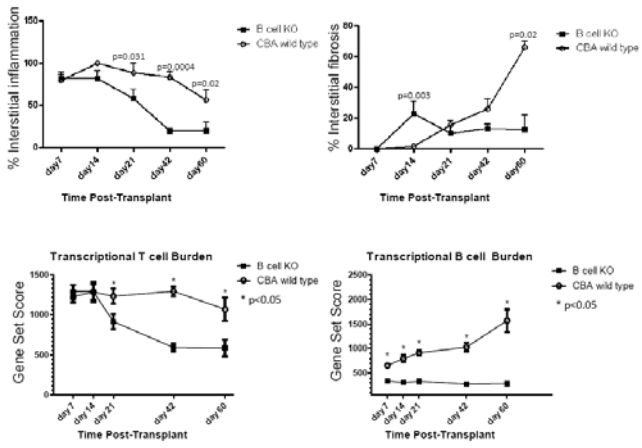
In human renal allografts, B cells accumulate in areas with interstitial fibrosis and tubular atrophy (IFTA), and B cell infiltrates increase with time post-transplant. However, the role of B cells in allograft rejection is not fully understood.

We analyzed graft rejection of CBA (H-2k) kidneys in Igh-$6^{-/-}$ (H-2d, B cell deficient) or wild-type hosts in a non-life supporting model. Transplants were harvested on day 7, 14, 21, 42, and 60 (total n=100), and investigated by histology and NimbleGen microarrays.

At the early time points (day 7 and 14) there were no differences in the histological and molecular phenotype in the transplants from the control and B cell deficient recipients. However, as expected, the expression of B cell associated transcripts were absent in the B cell deficient mice but continuously increased with time in the wild type allografts. By day 21 interstitial infiltrate and tubulitis were decreased in B cell deficient allografts along with decreases in the T cell and macrophage burdens, gamma-interferon effects, and injury as assessed by transcript expression ($p<0.05$). Histological and molecular signs of cellular rejection remained decreased through day 60 in B cell deficient hosts compared to wild-type. This resulted in significantly less interstitial fibrosis on day 60 ($p=0.02$). [Figure1]

The results indicate that host B cells have little influence on early cellular rejection of mouse renal allografts. However, maintenance of the inflammatory response and the onset of chronic injury, resulting in parenchyma dedifferentiation, requires B cells.

Figure 1



#494

COMBINATION OF NOVEL ANTI-CD45RB AND ANTI-CD40 CHIMERIC ANTIBODIES PROLONGS RENAL ALLOGRAFT SURVIVAL IN CYNOMOLGUS MONKEYS

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Peripheral tolerance in primates remains elusive. Based on previous results in which a human-mouse chimeric α-CD45RB mAb (6G3) modestly prolonged renal transplant graft survival (GS) in cynomolgus monkeys (cynos), we examined a combination of newly created rhesus-mouse chimeric α-CD45RB (r6G3) and α-CD40 mAbs. Cyno renal transplant recipients with ≥1 haplotype (MAMU) mismatches were treated with α-CD45RB (r6G3-IgG1 or r6G3-IgG4 given as 10→5 mg/kg iv on d-1,0,7, then 2x/wk till d29) plus α-CD40 (mouse α-human 3A8 or mouse α -rhesus 2C10-IgG4 dosed at 20→5 mg/kg iv, d-1,0,5,7, then 2x/wk till d58). Recipients treated with r6G3-IgG4 and α-CD40 (3A8) had 5d and 47d GS (AMR/CMR). Adding a short course of rapamycin (1 mg/kg po, POD 0-10) may prolong GS (69d; AMR/CMR). Treatment with 6G3-IgG1plus α-CD40 (3A8) resulted in GS of 14d (AMR) and >240d. Cynos treated with 3A8 and α-CD45RB developed adverse events not seen previously in cynos treated with α -CD45RB alone, including vomiting (n=4), altered consciousness (n=2), early unexplained death (n=2), and rise in creat. (n=1). Symptoms occurred as early as the first 1-2 doses, raising the possibility of a cytokine release effect (now under evaluation). Notably, the one long-term survivor had no side effects. Given this potential efficacy, a new α -rhesus CD40 mAb (2C10-IgG4) was combined with r6G3-IgG1. Both recipients exhibit >70d GS with normal creat. (64-102 mmol/L) and no adverse effects. In testing potential immunological mechanisms, both α-CD45RB isotypes induced a dramatic shift in CD45RB isoform expression on T cells to the lower Mr isoforms and CTLA-4 levels were increased (2-3.6-fold *vs.* Pre-Tx). Notably, expression of CD40, and CD80 and CD23 activation markers on B cells was reduced while receiving α-CD40, without B cell depletion. DCs also expressed lower levels of CD40 during the early PODs. Our results suggest that α-CD45RB r6G3-IgG1 is more effective than r6G3-IgG4 and that α-CD40 2C10 avoids adverse reactions that may limit efficacy of 3A8. The combination of α-CD45RB and α-CD40 can induce long-term GS which may be enhanced by rapamycin. The ability of these novel chimeric mAbs to down-regulate T and B cell activation holds significant promise for future clinical application.

#495

NEONATALLY-TOLERIZED MICE THAT ACCEPT THIRD-PARTY CARDIAC ALLOGRAFTS SHOW NO EVIDENCE OF GENERALIZED IMMUNOSUPPRESSION

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Introduction: In a mouse model of neonatal tolerance to cardiac allografts, shown previously to be mediated by regulatory T cells, C3H/He (C3H; H-2^k) neonates injected with fetal liver cells (FLC) from BALB/c (BALB; H-2^d) mice subsequently accept heart grafts from BALB donors and also accept third-party grafts from C57BL/6 (B6; H-2^b) donors; skin allografts are rejected. Acceptance of third-party grafts could be due to relative global suppression of immune responses, possibly caused by the neonatal FLC treatment. If this is correct, FLC-treated mice would be expected to mount reduced responses to other immunologic stimuli. Herein we investigated the immune response of FLC-treated versus untreated mice after protein vaccination and after re-exposure to a chemical allergen.

Methods: FLC-treated (*n*=14) and untreated (*n*=10) C3H mice were challenged as adults using a delayed-type hypersensitivity (DTH) protocol in which the chemical allergen oxazolone was applied to the flank on two consecutive days and then re-applied to the inside of one ear five days later. Ear swelling was assessed 24, 48, and 72 hours after re-exposure. In other experiments, FLC-treated (*n*=12) and untreated (*n*=12) C3H mice were vaccinated with diphtheria (DT) and tetanus (TT) toxoids, with two subcutaneous injections four weeks apart. Two weeks after the second immunization, mice were assessed for humoral (serum antibodies) and cellular (cytokine production and proliferation) responses against the toxoids.

Results: The DTH study revealed no significant differences in ear-swelling between FLC-treated and

untreated mice. Preliminary findings from the vaccination study showed that splenocytes from FLC-treated and untreated mice were equivalent in proliferating and producing IFN-γ and IL-5 upon culture with specific (DT/TT) or non-specific (Con A mitogen) stimulatory agents.
Conclusion: These findings suggest that acceptance of third-party cardiac allografts in adult mice treated as neonates with FLC is not due to a state of generalized immunosuppression.

#497

A CELL PENETRATING HEME OXYGENASE TO IMPROVE THE RESISTANCE OF STEATOTIC LIVERS TO REPERFUSION INJURY FOLLOWING TRANSPLANTATION

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Objective: The development of a cell penetrating heme oxygenase 1 (HO1) protein for treatment steatotic livers *ex vivo* prior to transplantation to potentially improve their resistance to reperfusion injury.
Methods: Cell penetrating enhanced green fluorescent protein (CPP-EGFP) and HO1 (CPP-HO1) clones were generated by PCR amplification of EGFP and HO1 cDNA using primers containing the sequence for an upstream cell penetrating peptide (CPP). PCR products were cloned into an expression vector; the resultant clones transformed into *E. coli;* and protein synthesis induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG). Proteins were purified using nickel agarose columns and identified by western blot analysis. HO1 function was confirmed by spectrophotometric quantification of bilirubin production by CPP-HO1 treated HEK293T cells supplemented with hemin, biliverdin reductase and the necessary cofactors. Cell penetrating ability was confirmed by immunohistochemical and fluorescence microscopic analysis of treated cells for the presence of intracellular CPP-EGFP and CPP-HO1.
Results: Sequence analysis confirmed the successful generation of CPP-EGFP and CPP-HO1 clones. Induction of protein expression resulted in a colour change of the media and bacterial pellet of CPP-HO1 expressing *E. coli* from beige to green, due to production of biliverdin. Western blot analysis confirmed the successful purification of both CPP-EGFP and CPP-HO1. CPP-EGFP and CPP-HO1 were found to efficiently penetrate hepatoma cells *in vitro*. CPP-HO1 was found to penetrate HEK293Tcells, and extracts from treated cells catalyzed the breakdown of heme into bilirubin, whereas untreated cells did not.
Conclusions: We have successfully generated a functional heme oxygenase protein that is able to penetrate cells *in vitro*. Further work will determine if 1) CPP-HO1 can provide cellular protection in an *in vitro* model of ischemia/reperfusion injury 2) if CPP-HO1 is able to penetrate the hepatocytes of *ex vivo* treated livers and 3) whether the treatment of steatotic livers with CPP-HO1 protects these livers from reperfusion injury.

#498

NOD NK CELLS RESIST ALLOGENEIC CHIMERISM THAT CAN BE DAMPENED BY RAPAMYCIN

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Mixed hematopoietic chimerism is difficult to induce and maintain in the non-obese diabetic (NOD) mouse model of human type-one diabetes. This difficulty lies largely in the inherent tolerance resistance mounted by the NOD immune system. Although the contribution of adaptive alloimmunity to tolerance resistance is well recognized, innate alloimmunity of natural killer (NK) cells is potentially relevant, as NK cells can resist hematopoietic cell engraftment. Their role in chimerism induction on an autoimmune background, however, has not been investigated. Perhaps, NOD NK cells have been overlooked due to their defective cytotoxic killing of many different NK sensitive tumour cell lines, and defective cytokine synthesis. In contrast to their previously described defective phenotype, in this study, we demonstrated the rejection of allogeneic chimerism by NOD NK cells in the presence or absence of adaptive immunity. Administration of anti-asialo GM1 to deplete NK cells, using F1 hybrid donor cells, or giving mega-doses of donor cells to overcome NK mediated killing effectively prevented rejection. We further observed that rapamycin dampened allogeneic resistance by NOD NK cells, as significantly less killing of allogeneic cells occurred in recipients of rapamycin than controls. This suppressive effect was also apparent in B6 NK cells. Rapamycin, however, was significantly less effective than antibody depletion. We concluded that NOD NK cells are

capable of rejecting allogeneic chimerism. Moreover, rapamycin, in combination with other agents but not as a standalone agent, may be used to overcome the NK cell barrier.

#499

CHARACTERISATION OF THE INTRACELLULAR SIGNALLING CASCADE IN ADULT B-CELLS

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Introduction: The immature immune system in early childhood has an impaired ability to respond to T-cell independent antigens, including blood-group polysaccharides. This irresponsiveness towards polysaccharides allows for successful blood group incompatible heart transplants in children, mostly leading to persistent, donor-specific blood group tolerance. We hypothesized that the signalling through the B-cell co-receptor CD21 in response to the complement component C3d is crucial in polysaccharide response and investigated B-cell signalling *in vitro*.
Methods: Adult peripheral blood mononuclear lymphocytes were stimulated with anti-human IgM antibodies, with or without the addition of C3d. The cells were fixed at various time points after stimulation, permeabilised, and stained with fluorescent antibodies specific for phosphorylated Syk and Akt, intracellular signal transduction proteins. The overall kinetics of signal transduction through the B-cell receptor and B-cell co-receptor was characterised using flow cytometry.
Results: The rate of Syk phosphorylation increased during IgM and C3d stimulation compared to IgM sitmulation alone. Similarly, Akt phosphorylation peaked with the early Syk peak during IgM and C3d stimulation, and gave a secondary peak corresponding to the phosphorylation rate observed for IgM stimulation alone. Stimulation with C3d alone did not induce Akt or Syk phosphorylation.
Discussion: Stimulation of adult B-cells through the B-cell receptor and co-receptor enhances signalling component phosphorylation compared to B-cell receptor stimulation alone, while stimulation of the co-receptor alone does not induce signalling. Our future experiments seek to explain infant B-cell irresponsiveness to polysaccharide antigens on the basis of the signalling cascade through the B-cell co-receptor, CD21. Immaturity of B-cell signalling in childhood may contribute to blood group tolerance following ABO-incompatible transplantation and therefore provide therapeutic targets to induce similar responses in adults.

#504

GLYCYRRHIZIC ACID (GZA) CAN BLOCK HMGB1 MEDIATED TUBULAR EPITHELIAL CELL (TEC) INJURY AND NK CELL ACTIVATION FOLLOWING RENAL IRI

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Kidney ischemia reperfusion injury (IRI) leads to the death of tubular epithelial cells (TEC). The release of High Mobility Group Box-1 (HMGB1) and other damage associated molecular pattern (DAMP) promotes inflammation and injury of viable TEC. Therapies to block HMGB1 are currently limited to neutralizing antibodies. Glycyrrhizic acid (GZA) is a functional inhibitor of HMGB1 but its ability to attenuate HMGB1 mediated injury of viable TEC remains unknown. In vitro, TEC lines (NG1.1) subjected to hypoxia (2-5% oxygen for 20 min) underwent death and progressively released HMGB1 into the supernatant up to 24 hours (no treatment density ratio: 0.574 vs. 24h post hypoxia density ratio: 8.876). Exposure of hypoxic TEC to GZA reduced cell death by 20% (co-positive Annexin-V/PI by FACS) after 24 hours in as compared to the untreated hypoxic TEC. Conditioned media from hypoxia exposed TEC induced TEC death when added to viable monolayers and Annexin V/PI positivity increased compared to control untreated TEC. (media control: 36.1±4.4% vs. conditioned media: 51.25±9.75%, $p=0.06$, $n=3$). MCP-1 is a chemoattractant for NK cells, which can kill TEC in vitro and following IRI., Using quantitative PCR, hypoxia induced a 9 fold increase in TEC expression of MCP-1 mRNA expression at 24 hours (no treatment: 1±0 vs. 24h post hypoxia: 9.316±2.85, $p<0.05$, $n=3$). MCP-1 expression was inhibited by GZA in a dose dependent manner to levels observed in naive TEC. As activated NK cells have greater capacity to kill TEC, we tested if HMGB1 can directly activate NK cells to promote further injury. Purified NK cells exposed to endotoxin free HMGB1 were activated with resulting 2.5 fold increase in IFN-γ (naïve: 1±0 vs. HMGB1 treatment: 2.507±0.516, $p<0.05$, $n=3$) and Granzyme B (naïve= 1±0 vs. HMGB1 treatment: 2.433±0.582, $p<0.05$, $n=3$) mRNA compared to no treatment. We then tested the effect of HMGB1 neutralization by GZA in vivo. Uni-nephrectomized C57BL/6J mice were subjected to clamping of the remaining renal pedicle for 45 min to induce IRI. HMGB1 protein, shown by Western blot, increased progressively in kidneys 24 hours post ischemia (naïve density ratio: 0.953±0.707 vs. 24h post IRI density ratio: 5.368±0.239, $p<0.001$, $n=3$). HMGB1 was diffusely detected in sections

by immuno-histochemistry, including tubular lumen. GZA treatment of mice reduced IRI at 48h and improved function (creatinine without GZA: 120±35µmol/L vs. with GZA: 31.4±5 µmol/L, $p<0.05$, n=5). GZA reduced tubular necrosis and neutrophil infiltration by blinded histological assessment. These data demonstrate for the first time that HMGB1 released following hypoxic kidney injury can propagate renal injury by altering the survival of TEC as well as attracting and activating NK cells, which in turn may mediate further injury. Inhibition of HMGB1 release by TEC or blocking HMGB1 interaction with TEC may provide a new therapeutic strategy to limit inflammatory renal injury following IRI and transplantation.

#505

THE EFFECTS OF MOLECULES CORM-3 AND H2S ON RENAL PROTECTION DURING PULSATILE PERFUSION

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Background: Studies on small animal models have shown that through their vasodilatory and antiapoptotic properties, carbon monoxide releasing molecules (CORM)-3 and hydrogen sulfide (H2S) can protect kidneys during prolonged periods of cold storage. This study expands on previous work by assessing the effect of CORM-3 and H2S on pump resistance, cell injury, and apoptosis in the cold perfused porcine kidney.
Methods: Ten kidneys were procured from domestic farm pigs, flushed with heparinized solution after 5 minutes of warm ischemia, and placed on the Lifeport perfusion pump at 4°C for 48 hours. The kidneys were randomly assigned to one of four treatment groups: control cold storage (n=2), control cold pulsatile perfusion (n=2), 100 µM CORM-3 pulsatile perfusion (n=4), or H2S pulsatile perfusion (n=2). Perfusion flow and resistance were recorded, and the kidneys were stained for TUNEL and histology was assessed.
Results: Compared with control kidneys, pulsatile perfusion with H2S showed decreased $TUNEL^+$ cells (4.5/10 hpf) versus control kidneys (10.5/10 hpf) (p=0.007), and perfusion with CORM-3 showed a decrease in glomerular necrosis. Mean vascular resistance was lower in both CORM-3 and H2S kidneys versus control kidneys (p=0.0003 and p=0.0008). Accordingly, mean flow was higher in the H2S group versus control (p=0.009) at 1 hr. Even by 47 hr, perfusion parameters were superior in H2S-treated kidneys (p = 0.003).
Conclusions: This preclinical pilot study shows that both CORM-3 and H2S play a role in decreasing cell injury and improving perfusion parameters in kidneys undergoing cold pulsatile perfusion. This provides rationale to assess both agents in combination and to assess the ability of these small molecules to protect the graft against storage injury in porcine transplant models.

#510

A SUBCUTANEOUS CELL POUCH™ AS AN ALTERNATIVE TO INTRAPORTAL INFUSION OF ISLETS OF LANGERHANS TO RESTORE CARBOHYDRATE CONTROL IN THE DIABETIC RECIPIENT

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Islet transplantation *via* intrahepatic portal infusion has shown promise as a treatment for type-1 diabetic patients; however, this procedure has inherent risks including, increased portal pressure, thrombosis, hepatic steatosis and hemorrhage. An alternative site of islet engraftment avoiding direct contact with systemic blood would be an attractive improvement to intraportal infusion. This study examines the efficacy of Sernova's pre-vascularized Cell Pouch™ (CP) as a surrogate, subcutaneous islet transplantation site in a porcine islet autograft model.

Eight week old Yorkshire-Landrace pigs were implanted 4-8 weeks prior to 90% pancreatectomy and islet isolation. To ensure a stringent hyperglycemic state, pigs were dosed intravenously with 150mg/kg streptozotocin post-pancreatectomy. The isolated immature islets were transplanted five days post-isolation allowing recovery from the pancreatectomy and confirmation of the diabetic state. Graft function was assessed through biweekly blood glucose and weight measurements, as well as monthly intravenous glucose tolerance tests (IVGTTs).

The CP incorporated with tissue and angiogenesis formed an endocrine organ-like structure. The islet grafts with the CP showed good long-term function which correlated strongly to islet viability and transplant dose (IEQ/kg) with 4 of 7 animals restoring glucose control.

Functional islet grafts secreted a significant amount of C-peptide compared to basal levels following an IVGTT; while non-functional transplants failed to respond to a glucose challenge as evident through a lack of C-peptide secretion. Within the CP, positive insulin and microvessel histological staining and post long-term glucose control further corroborates the endocrine organ-like structure established with the Cell Pouch™. Following CP removal, a significant deterioration in glucose disappearance rates, blood glucose area under curve and C-peptide levels occurred. The minimal invasiveness, positive long-term function, promotion of angiogenesis, ease of explant and potential islet sparing properties of this CP in a stringent large animal model supports the Cell Pouch™ for potential use in clinical islet transplantation.

#512

ISLETS TRANSPLANTED INTO A PREVASCULARIZED SUBCUTANEOUS CELL POUCH™ DEMONSTRATES LONG-TERM EFFICACY MEASURED BY C-PEPTIDE

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Intahepatic portal infusion of pancreatic islets is the standard of care in restoring carbohydrate control in transplanted type-1 diabetics. This procedure is associated with risks including increased portal pressure, thrombosis, immunogenic and non-immunogenic reactions. Reactions include the instant blood-mediated inflammatory reaction (IBMIR), which leads to rapid islet loss upon infusion into portal circulation. A product providing an alternative site of islet engraftment avoiding direct contact of islets with systemic blood and capable of secreting necessary factors into the circulation controlling glycaemic levels, would be an attractive improvement to intraportal infusion.

Using a porcine autograft model, this study examined the efficacy of Sernova's pre-vascularized, subcutaneous proprietary Cell Pouch™ as an alternative site for natural islet engraftment. To assess islet graft function post-infusion into the Cell Pouch™, intravenous glucose tolerance tests (IVGTTs) were conducted prior to transplant (baseline), at various time points post-transplant (4-12 weeks) and following Cell Pouch™ removal.

Islet transplant recipients exhibited significant C-peptide release compared to basal levels following an IVGTT (Basal: 0.147 ± 0.023 ng/ml vs Peak: 0.338 ± 0.070 ng/ml; n=4, p=0.0181), in comparison to non-pancreatectomized (baseline) subjects (Basal: 0.236 ± 0.059 ng/ml vs Peak: 0.562 ± 0.147 ng/ml; n=4, p=0.0274). Post Cell Pouch™ removal, C-peptide release following dextrose infusion was abolished (Basal: 0.246 ± 0.054 ng/ml vs. Peak: 0.268 ± 0.057 ng/ml; n=4, p=0.1144). This mirrored the hyperglycaemic state in diabetic control animals. These findings strongly support the concept that Sernova's subcutaneous Cell Pouch™ is efficacious in detecting and responding to glycaemic levels within the systemic circulation.

#514

THE ESTABLISHMENT OF A STRINGENT LARGE ANIMAL MODEL OF INSULIN-DEPENDENT DIABETES

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Various small animal insulin-dependent diabetes models have been well established. The induction of diabetes in large animal models has been less consistent. To test the efficacy of islet transplantation in the Cell Pouch™, a stringent porcine islet autograft diabetic model was established. To facilitate adequate islet yields while maintaining marginal exocrine function and minimizing surgical complications, a 90% pancreatectomy was performed preserving the pancreaticoduodenal arcade. Nevertheless, glycaemic control was maintained, (3.3 ± 0.6 days, n=3) as evident by euglycaemic non-fasting blood glucose levels (4.9 ±1.3 mmol/L n=3). To ensure a rigorous model of diabetes, pigs were dosed intraveneously immediately following the pancreatectomy with 150 mg/kg of streptozotocin (STZ), which ensured a stringent hyperglycemic state. These animals became hyperglycemic (18.0 ± 4.4mmol/L, n=9) within days (2.0 ± 0.9 days) of pancreatectomy and STZ administration. This porcine model of diabetes induction, created by marginal pancreatectomy and STZ dosing is a dependable method of assessing the efficacy of insulin producing cells in auto-, allo- and xeno-transplantation. Efficacy was demonstrated for islets transplanted into the Sernova subcutaneous Cell Pouch™ for three months and following Cell Pouch™ removal, the diabetic state returned reinforcing this robust model of diabetes.

#521

THE UTILITY OF AN IN-TUBE INTERFERON GAMMA RELEASE ASSAY (QUANTIFERON-HCV) FOR ASSESSMENT OF THE SEVERITY OF RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION

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Introduction: Strong T-cell responses have been associated with successful host response to Hepatitis C (HCV) infection, and a robust specific and global immune response favourably impacts the clinical course of recurrent HCV after liver transplantation (LT). Quantiferon tests are in-tube interferon-gamma release assays (IGRAs), which have been successfully developed for tuberculosis and CMV infection. This prospective study reports the development and proof-of-concept assessment of an IGRA for assessment of the severity of HCV infection post-LT.

Patients and Methods: A pool of HCV-specific peptides of known CD4 or CD8 affinity, representing all major HCV proteins were identified and synthesized. One millilitre whole-blood aliquots from study subjects were stimulated with HCV-specific peptide pools, and mitogen (phytohemagglutinin), and incubated for 24 hours at 37 degrees Celsius. The supernatant was then removed and studied with a quantitative interferon-gamma ELISA, to assess the elicited interferon-gamma (IFN-G) response to stimulation.

Results were correlated with allograft biopsies or fibroscan results when available. Statistical analysis was performed using Mann-Whitney 'u' tests with a 'p' value of<0.05 being considered significant.

Results: Twenty-two patients with recurrent HCV post-LT were studied. Patients with advanced allograft disease (fibrosis score≥2 on liver biopsy) had significantly lower CD4 and absolute mitogen stimulation values than patients with less severe recurrent HCV(. CD4 specific IFN response 0.02 ± 0.01 vs. 0.13 ± 0.08, p=0.05; mitogen IFN response 14.4 ± 2.6 vs. 27.6 ±3.6; p=0.019)[Figure-1]

Conclusion: Quantiferon-HCV provides an inexpensive and robust assessment of HCV-specific cellular response without the requirement of lymphocyte isolation. Significantly lower specific and global immune responses were seen in patients with advanced recurrent HCV post-LT. Interferon-gamma release after mitogen stimulation using the Quantiferon assay may serve as a surrogate for global immune response in patients with recurrent HCV infection post-LT, and therefore help in distinguishing recurrent HCV from ACR. Low CD4 or mitogen scores may help identify patients at risk of aggressive recurrent disease and select patients who could benefit from early anti-HCV treatment or modification of immunosuppression.

[Figure-1]

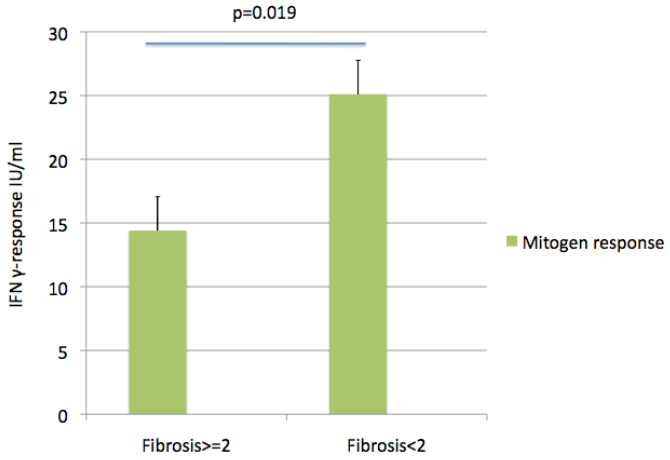

Figure showing differences in Quantiferon mitogen response in patients with advanced allograft fibrosis (≥2) and fibrosis < 2 in recurrent HCV post-LT

CLINICAL

#391

COMPLICATIONS OF URETEROVESICAL ANASTOMOSIS IN ADULT RENAL TRANSPLANTATION: COMPARISON OF THE LICH-GREGOIRE AND THE TAGUCHI TECHNIQUES.

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Background: Our aim is 1) to identify the incidence of urological complications in adult renal transplantation comparing two different ureterovesical anastomosis techniques, the Taguchi (T) and Lich-Gregoire (LG). To evaluate the relation between urological complications and first graft rejection or loss.

Methods: Retrospective analysis of adult renal transplants performed at the MUHC between 2000-2009. Excluded: multi-organ transplants, re-do transplants, variant ureteric anastomosis and patients received grafts from UNOS ECD. 372 patients were analyzed. 209 patients (56%) in the T group and 163 patients (44%) in the LG group. Fisher's exact test was used to compare the groups for urological complications. A multivariate analysis was performed to identify factors associated with graft rejection and death.
Results: 21 patients developed a urinary leak or stricture. Hematuria requiring intervention developed in 55 patients. A higher incidence of complicated hematuria in the T group when compared to the LG group (37 vs.18, p=0.04)). No differences in other ureteric complications between the 2 groups. Delayed graft function OR= 3.4 (95% CI=1.8-6.3) and grafts from a deceased donors OR= 2.2 (95% CI=1.1-4.5) are factors predictive of graft loss. Factors predictive of first episode of rejection include delayed graft function OR = 2.4 (95% CI= 1.3-4.4), and the development of ureteric stricture OR = 3.9 (95% CI=1.8-8.7).
Conclusion: Both techniques can be used interchangeably for adult renal transplantation. T technique is associated with a greater risk of hematuria. Ureteric strictures do shorten the time to first graft rejection. These results overall favor the LG technique.

#393

THE MODELS FOR END-STAGE LIVER DISEASE ACCURATELY PREDICT 90-DAY LIVER TRANSPLANT WAIT-LIST MORTALITY IN ATLANTIC CANADA

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Objective: If the Model for End-stage Liver Disease (MELD) is to be adopted in Canada as a component of the national system for allocation of deceased donor liver allografts, it is imperative that this prognostic model be validated in Canadian liver transplant (LT) candidates. The objective of this study was to determine the generalizability of the predictions for 90-day mortality generated by the MELD and the serum sodium augmented MELD (MELDNa) to Atlantic Canadian adults with End-Stage Liver Disease (ESLD) awaiting LT.
Methods: The predictive accuracy of the MELD and the MELDNa was evaluated by measurement of the discrimination and calibration of the respective models' estimates for the occurrence of 90-day mortality in a consecutive cohort of adult LT candidates with ESLD accrued over a five year period. Accuracy of discrimination was measured by the area under the Receiver Operating Characteristic (ROC) curve. Calibration accuracy was evaluated by comparing the observed to model estimated incidences of 90-day wait-list failure for the total cohort and within quantiles-of-risk.
Results: The area under the ROC curve for the MELD was 0.887 (95%CI: 0.705-0.978), consistent with very good accuracy of discrimination. The area under the ROC curve for the MELDNa was 0.848 (95%CI: 0.681-0.965). The observed incidence of 90-day wait-list mortality in the validation cohort was 7.9%, which did not differ significantly from the MELD estimate of 6.6% (95%CI: 4.9-8.4) ($p = 0.177$), or the MELDNa estimate of 5.8% (95%CI: 3.5 – 8.0) ($p = 0.065$). Global goodness-of-fit testing found no evidence of significant lack-of-fit for either model (MELD Hosmer-Lemeshow $\chi^2(3) = 2.941$, $p = 0.401$, MELDNa $\chi^2(3) = 2.895$, $p = 0.414$).
Conclusions: Both the MELD and the MELDNa accurately predicted the occurrence of 90-day wait-list mortality in this cohort. The MELD prognostic models are generalizable to Atlantic Canadians with ESLD awaiting liver transplantation.

#396

CMV transmission in clinical islet transplantation and the impact of T depleting induction

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Background: The incidence, risk factors and clinical consequences of cytomegalovirus (CMV) is largely unknown in islet transplant (IT)
Methods: This study investigated the epidemiology of CMV infection and factors associated with transmission, reactivation or disease in 125 patients receiving 274 ITs.
Results: The donor(D)/ recipient(R) serostatus was D+/R- 30.7%,D+/R+ 27.4%,D-/R+ 13.1% and D-/R- 28.8% of procedures.Prophylaxis was used in 69% of transplants;however,14/125 patients(11.2%) experienced infection. Six patients seroconverted post-transplant and 8 other patients developed positive viremia. Median peak viral loads in these patients were 1755 copies/ml(range 625 – 9,100,000).The main risk factor for viremia was the

use of lymphocyte depleting induction($p<0.001$) and viremia was more likely to be found in R+ patients vs.D+/R- patients($p=0.06$).Transmission from IT(seroconversion or viremia) occurred in 8/84 of D+/R- transplant procedures(9.5%).Only 2 cases of CMV disease occurred.

Conclusion: CMV transmission can occur after IT although with low incidence compared to solid organ transplantation.However,the risk for symptomatic CMV disease is very low. The use of lymphocyte depleting therapies is the primary risk factor for the development of viremia.

#404

PULSATILE RENAL PERFUSION IMPROVES DOPPLER INDICES OF INTRA-RENAL BLOOD FLOW AND ALLOGRAFT FUNCTION IN KIDNEYS OBTAINED FROM DONORS AFTER CARDIAC DEATH

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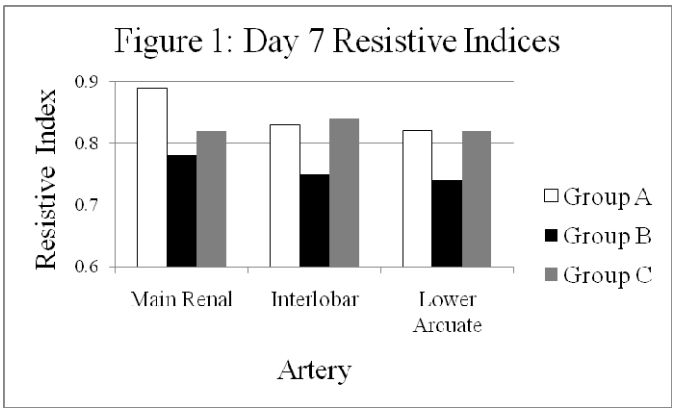
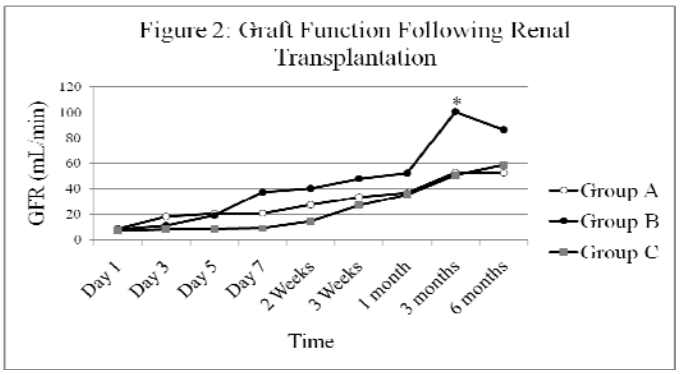
Introduction: Increasing evidence suggests that pulsatile-perfusion (PP) of transplanted kidneys (TX) may improve long-term renal allograft function compared to cold storage alone; whether this applies to kidneys obtained from donors after cardiac death (DCD) remains unknown. We examined whether PP improved DCD renal allograft survival compared to kidneys preserved in cold storage. In addition, we evaluated whether Doppler perfusion indices were predictive of long term graft function and survival.

Methods: We identified 22 patients who received DCD TX. Group A (n=6) received the first of a pair of kidneys, Group B (n=6) received the second of the pair after it was placed on PP while its mate (Group A) was transplanted. Group C (n=10) consisted of a matched cohort of recipients who had also received the second of a pair of donor kidneys without PP storage. Patient and donor demographics were collected, Doppler ultrasound was done on day 7 and resistive indices (RI) calculated, GFR was determined using the MDRD for 6 months after TX.

Results: Group B had the longest cold ischemic times (854 min) compared to Group A (567 min, $p = 0.039$) and Group C (563 min, $p= 0.010$). Despite this, Group B had the lowest Doppler RI's following TX compared to both Groups A and C which showed similar Doppler indices (Figure 1, $p = 0.035$). GFR trended to be better in Group B compared to Groups A and C throughout the follow-up period, becoming significant at 3 months post-TX (Figure 2).

Conclusions: Doppler ultrasound indices may be a predictor of long-term graft function in DCD kidney transplants. Pulsatile perfusion of DCD kidneys appears to positively impact long-term allograft function compared to static cold storage.





#406

INCIDENCE, RISK FACTORS AND OUTCOMES OF NEUTROPENIA IN RENAL TRANSPLANT RECIPIENTS: FOCUS ON A STEROID FREE PROTOCOL

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Background: Following renal transplantation, neutropenia occurs in a reported 28-58% of patients on standard immunosuppressive protocols of calcineurin inhibitor (CNI), mycophenolate mofetil (MMF) and a corticosteroid. Neutropenia and its treatment may have detrimental consequences such as increased incidence of infection and

acute rejection. In our center, a steroid free protocol is used in low immunologic risk renal transplant recipients. There are currently no published reports characterizing the rates and impact of neutropenia in a steroid free renal transplant population.
Methods: Single center, retrospective chart review of all adult kidney transplant recipients under the steroid free regimen from January 2004 to December 2008. Data were collected for 1 year post-transplant.
Results: Of 202 renal transplant recipients included and maintained on a steroid free regimen, 107 (53%) experienced at least one episode of neutropenia during the 1 year follow-up. Mean onset of neutropenia was 111 days following transplant and mean duration of neutropenic episodes was 27 days. Significant risk factors for neutropenia on univariate analysis were cytomegalovirus (CMV) viremia, CMV serology mismatch and valganciclovir prophylaxis. On multivariate analysis, none of these parameters remained statistically significant. MMF doses were significantly lower in neutropenic patients at 3, 6 and 12 months ($p<0.001$). The incidence of acute rejection, graft failure, and patient mortality at 1 year were similar in neutropenic and non-neutropenic patients. There was no difference in renal function measured by serum creatinine between neutropenic and non-neutropenic patients at six (126 vs. 124 µmol/L) and twelve months (131 vs. 121 µmol/L) respectively.
Conclusions: Neutropenia was common in our steroid free patient population and similar to that reported in the literature for steroid-based regimens. The etiology of neutropenia appears to be multifactorial, and did not significantly affect graft or patient outcomes at one year post transplant, despite significantly lower MMF dosing in the neutropenic cohort.

#407

CAN LONG-TERM VENTRICULAR ASSIST DEVICES BE SAFELY IMPLANTED IN LOW-VOLUME NON-HEART TRANSPLANT CENTERS

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Background: Mechanical circulatory support (MCS) using an implantable ventricular assist device (VAD) has been established as an appropriate therapy for bridge to transplant (BTT) or transplant candidacy (BTC) in patients with advanced heart failure. Previous studies have suggested outcomes following VAD implantation may be dependent on institutional procedural volume. We report the characteristics, treatment, and outcomes of patients receiving an implantable VAD at our center to determine if MCS programs can be safely established at tertiary care, low-volume, non-heart transplant centers in Canada.
Methods: We conducted a retrospective review of all patients who received an implantable VAD over a two-year period (2008-2010) following inception of the heart failure program at our Canadian center, where there is access to a variety of MCS solutions.
Results: During the study period, 41 patients received MCS and 11 were supported with an implantable VAD (mean age 52 years, 82% male) as BTT in 64% and BTC in 36%. Indication for device placement was post-cardiotomy in 36 %, ischemic cardiomyopathy in 36%, and non-ischemic cardiomyopathy in 28%. The majority of patients (64%) were INTERMACS category I, while 27% were category II, and 9% were category III. Prior to VAD placement all patients required at least 1 inotropic medication, 64% had suffered a cardiac arrest, 64% required an intra-aortic balloon pump, and 72% required mechanical ventilation. Biventricular support was required temporarily in 36 % of patients. ICU and 180-day survival were 100%. Median ICU and hospital length of stay were 11 and 77 days respectively. Survival on continued support or to transplantation was 82%, with a mean duration of support of 221 days. At outpatient follow-up all surviving patients were NYHA class I or II.
Interpretation: Successful MCS programs can be safely and effectively established at low-volume non-heart transplant centers with survival rates comparable to INTERMACS registry data and high volume single centers, when managed by a comprehensive MCS team.

#408

IMPACT OF CHANGES IN NUTRITIONAL STATUS ON POSTOPERATIVE OUTCOMES IN LUNG TRANSPLANT RECIPIENTS

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Background: The nutritional status before transplantation is an important component of the patient's general condition and can contribute to the post-transplant morbidity and mortality. It well established that the nutritional status represented mainly by BMI is a predictor of success in lung transplantation. This study aims to assess the impact of nutritional status and its evolution

while awaiting a lung transplant on the post-operative mortality and morbidity.
Methods: 209 consecutive cases of lung transplantation performed between 2000 and 2007 were reviewed. The study variables include: operative mortality, length of ICU and hospital stay, rate of post-operative complications, intake of protein and energy, biochemical parameters and weight changes during the waiting period.
Results: The risk of death increased with increasing BMI strata with a relative risk of death during the hospital stay of 3,31 (IC95% 1,19-9,26) for BMI 25-29.9 and 8,83 (IC95% 2,98-26,18) for BMI ≥ 30 with a worse postoperative outcome in terms of surgical complications (p=0,003), length of stay in intensive care unit (p=0,031) and length of hospital stay (p<0,001) for patients with BMI ≥ 30 compared with patients of normal weight. Patients in whom the BMI evolved inadequately during the waiting period experienced a prolonged hospital stay (p=0,015). Patients whose intake was suboptimal in the pre-transplant period have also a prolonged hospital stay (p=0,002) with more infectious (p=0,038), digestives (p=0,003) and surgical (p=0,029) complications but no detectable impact on the mortality.
Conclusion: Our results suggest that obesity and overweight, inadequate changes of BMI during the waiting period and suboptimal protein-energy intake negatively affect the outcomes in lung transplant recipients.

#415

SUCCESSFUL TOLERANCE INDUCTION WITH A POST KIDNEYTRANSPLANTATION (TX) REGIMEN OF TOTAL LYMPHOID IRRADIATION (TLI), ANTITHYMOCYTE GLOBULIN (ATG) AND DONOR PURIFIED CD34 PROGENITOR CELLS IN HLA-MATCHED RECIPIENTS

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Immune tolerance and persistent mixed chimerism can be achieved reproducibly after combined organ and hematopoietic cell Tx in mice conditioned with TLI and ATG. We studied the safety and reproducibility of this approach in clinical Tx.
Methods: Fifteen patients (pts), 6 men and 9 women, age range 22 to 61 years, have undergone HLA-matched living related kidney Tx followed by conditioning with TLI (800-1200cGy) and ATG. This was followed by infusion of donor CD34+ hematopoietic progenitor cells and T cells. Criteria for withdrawal of cyclosporine monotherapy at least 6 months after Tx were stable chimerism for a minimum of 6 months, and absence of rejection and graft versus host disease (GVHD).
Results: All 15 pts had excellent kidney graft function, with serum creatinine range 0.8 to 1.3 mg/dL, at last observation 2 to 62 mo (median= 33) following Tx. Length of stay for transplant hospitalization was 4 to 7 days (median=5). Four pts required readmission within the first year, 1 each for neutropenic fever, ureteral stricture, pyelonephritis and acute rejection. Three pts developed varicella zoster, and one primary CMV infection. None had GVHD. Three pts had reversible acute rejection episodes, and one had immediately recurrent focal segmental glomerulosclerosis; all 4 remain on immunosuppression. Eight pts have been completely withdrawn from anti-rejection drugs for 6 mo to 3 years (median=23 mo). No pt developed rejection after successful withdrawal. Surveillance biopsy 1 year after withdrawal showed no acute or chronic rejection (5/5 pts). Patients 13,14 and 15 have stable chimerism at 2 to 4 mo post Tx. The % of NK T cells among total Tcells increased significantly early post conditioning regimen. The Tregs/naïve CD4 T cell ratio increased > 10 fold in the majority of pts during the first 6 mo post Tx. Donor unresponsiveness in mixed lymphocyte culture was demonstrated in 4 /4 pts after withdrawal while 2/2 pts who failed withdrawal remained responsive to their donor. All responded to third party.
Conclusion: This post Tx conditioning regimen promoted the safe development of mixed chimerism and tolerance in a majority of HLA-matched kidney Tx recipients.

#426

FIRST CANADIAN EXPERIENCE WITH SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANT WITH ORGANS PROCURED FROM DONATION AFTER CARDIAC DEATH

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Objective: To help expand the donor pool here in Canada, organs from donation after cardiac death (DCD) donors are becoming increasingly utilized. Use of DCD organs for renal and liver transplant have been accepted with good outcomes across most Canadian transplant programs. However, acceptance of DCD pancreas for transplant has been limited, due to increased sensitivity to warm ischemic

injury and vascular thrombosis. To date, only two DCD pancreas transplants have been performed in Canada.

Patients and Methods: The SPK transplants were performed 3 and 18 months ago. Although donor selection has been limited to donors under the age of 35 years (20, 35 years), procurement and transplant techniques were identical to those used in neurologic death donors. Mean warm ischemic time for the pancreas was 24 minutes, while cold ischemic times for the pancreas were 6 and 8 hours. Immunotherapy included thymoglobulin induction, with tacrolimus, cellcept and prednisone maintenance therapy.

Results: Both recipients demonstrated immediate pancreas transplant function without any insulin requirement following transplant. However, both recipients had delayed renal graft function. Hospital stay was 11 days and 20 days for the two recipients. One patient required drainage of a peripancreatic abscess. Currently, both recipients have excellent renal function with serum creatinine values below 120 mg/dl and HbA1c levels under 5.5%. At 3 months and 18 months post-operatively, both patients are normoglycemic and off all diabetic medications.

Conclusion: Use of DCD organs for transplant represents a feasible option to expand the donor pool. Initial experience with DCD pancreas transplants demonstrate excellent initial and intermediate graft function. The long-term function of DCD pancreas transplants require continued assessment.

#427

ALOPECIA DEVELOPING AFTER SUCCESSFUL CLINICAL ISLET TRANSPLANTATION

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Successful clinical islet transplantation (CIT) for type 1 diabetes (T1DM) requires control of both allo- and auto-immunity. The development of the autoimmune condition alopecia areata following successful CIT is therefore unexpected. We report three cases of alopecia which developed after successful CIT.

Three Caucasian, female recipients (age 45-52 years) with longstanding T1DM complicated by severe hypoglycemia and/or glycemic lability underwent CIT in 2001/2002, achieving insulin independence after either a single (n=1, 3801 IE/kg) or two islet infusions (n=2, 14,657 and 18,105 IE/kg). Induction consisted of daclizumab with sirolimus and tacrolimus for maintenance. Each received a further islet infusion between 3 and 5 years later. Thymoglobulin and etanercept were used for induction in the two subjects receiving third infusions and maintenance immunosuppression changed to tacrolimus and mycophenolate mofetil in all.

Alopecia developed in all subjects 7 - 8 years after initial infusions and between 10 months and 4 years after the most recent islet infusion. The extent of alopecia ranged alopecia areata with 5 bald patches to alopecia totalis. Tacrolimus levels were elevated on at least one occasion close to the time alopecia was reported. The diagnosis of alopecia was confirmed by consultant dermatologists and treated with either a contact sensitizer (anthralin) or intra-lesional cortisone. Tacrolimus was continued in 2 cases (one as monotherapy) while cyclosporine replaced tacrolimus in the third. The progression of alopecia has either been halted or reversed in all cases. These three cases represent a crude incidence of <2.5% over 5 years compared with a prevalence of alopecia in islet transplant candidates (pre-transplant) of <1%.

Although alopecia is associated with T1DM, its appearance after CIT suggests a role for anti-TNF drugs, lymphodepletion or tacrolimus. Further research is required to identify causal factors.

#429

IMPACT OF RESTRUCTURING AN ORGAN DONATION PROGRAM ON OVERALL DONATION AND LUNG TRANSPLANTATION

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Historically poor organ donation (OD) and conversion rates (CR) prompted a restructuring of OD activities in Manitoba in 2006. Transplant Manitoba Gift of Life (TMGOL), an organ procurement organization independent of any other transplant activity, was created. Its mandate was to increase organ donation by actively engaging all stakeholders in the health care system and the general public in a systematic and structured donor awareness education initiative. Additional staff and protocols for the care of potential donors were but in place to support all sites. A comprehensive audit process was also introduced to make all stakeholders aware of OD and the potential for improvement. Two year retrospective data (2004/5) is compared to the next 4 years (2005/9) of operation of the TMGOL. The number of OD referrals increased from 23 to 29.5per year. The audit of all deaths within the health region uncovered an additional 25.6 potential donors per year that were not referred to

TMGOL. This 53% referral rate was comparable to the retrospective cohort. The conversion rate (actual/referred) for OD was 49% compared to a historical rate of 29%. Lung donation was realized in 46% of referred donors compared to 30% and there was a steady improvement over time. The OD rate for the Province of Manitoba over the last 4 years was 11.7 per million and the trend over time was also one of improvement. Conclusion: 1. About half of potential donors are still not referred for assessment. 2. We saw an improvement in absolute numbers of actual donors and conversion rates with the introduction of TMGOL. 3. The rate of lung donation also improved with the introduction of TMGOL.

#430

THE USE OF TRANSFERRIN RECEPTOR FERRITIN INDEX TO DIAGNOSE IRON DEFICIENCY IN PEDIATRIC RENAL TRANSPLANT PATIENTS

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Introduction: Serum ferritin and transferrin saturation (TSAT) are used to diagnose iron deficiency (ID) but are known to be affected by concomitant inflammation. As children with chronic kidney disease post kidney transplantation (Tx) often have low grade inflammation, these markers may not be reliable. A ratio of serum soluble transferrin receptor (sTfR) to log ferritin has been proposed as a new marker of ID and is supposed to be less dependent on inflammation.
Objectives: The objective of this study was to assess the prevalence of ID in our patients post Tx using various markers including sTfR/log ferritin. We hypothesized that the sTfR/log ferritin index would be more sensitive to detect ID in patients post Tx compared to currently used KDOQI criteria (ferritin <100 µg/L and TSAT<20%).
Methods: We performed a retrospective chart review of all renal Tx patients (n=25) currently followed at our institution. All available serum ferritin, TSAT and sTfR values were collected from the post transplant follow-up period in each patient; an average value per patient was used for analysis. ID was determined based on KDOQI criteria and sTfR/log ferritin index >0.6*.
Results: Complete data was obtained on 21 children (11 males, aged 1.6 to 16.7 years at Tx, followed for an average of 2.4±1.3 yrs). Median Schwartz GFR was 63.18 mL/min/1.73 m^2 (range 34 – 92); 81% of patients received iron supplementation. Based on KDOQI guidelines only 5/ 21 children (24%) were found to be iron deficient. However based on sTfR/log ferritin index 16/21 (76%) of patients were iron deficient (Fischer exact test p=0.0017). Mean ± SD sTfR/log ferritin index was 0.88±0.29 (iron deficiency if sTfR/log ferritin index ≥0.6).
Conclusions: The prevalence of iron deficiency was significantly higher when using the sTfR/log ferritin index. It seems to be a more sensitive marker of iron deficiency in renal transplant patients.

*Horl, Walter. J Am Soc Nephrol 200718: 382-393

#431

DETERMINANTS OF TREATMENT FAILURE IN RENAL TRANSPLANT PATIENTS WHO ARE CONVERTED FROM A CALCINEURIN INHIBITOR TO RAPAMYCIN

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Introduction: Our aim was to identify the factors that predict treatment failure after conversion from a CNI-based to a rapamycin-based regimen, to improve patient selection for this strategy.
Methods: We performed a retrospective cohort study among transplant recipients who received a kidney graft in 2 Canadian transplant centers and who were transferred from CNIs to rapamycin. We performed multivariate linear and logistic regression analyses to identify the determinants of treatment failure, defined as worsening graft function or the development of significant proteinuria (levels ≥3g/L on urine analysis).
Results: Between July 2001 and May 2007, 193 patients were converted from a CNI-based to a rapamycin-based immunosuppression regimen. One year after conversion, average graft function was stable (change in GFR: 0 ml/min/1.73m^2, 95% confidence interval -0.03, 0.03). In a multivariate model, a deterioration in kidney graft function over the first year was explained by GFR at the time of conversion (ß: -1.5 ml/min/1.73m^2 per each 10 ml/min/1.73m^2 decrease, 95% CI -2.7, -0.3) and the presence of proteinuria ≥1g/L at conversion (ß: -8.6 ml/min/1.73m^2, 95% CI -14.77, -2.37). Recipient age, gender, center, transplant date, motive for conversion, time to conversion, the CNI used before conversion, and previous rejection were not associated with worsening

graft function. Only 5.7% of patients developed overt proteinuria in the year after conversion. Proteinuria ≥1g/L at conversion was strongly associated with the later development of significant proteinuria (odds ratio (OR): 6.15, 95% CI 1.71-22.70). There was a trend for an association between longer time between transplantation and conversion and the development of significant proteinuria (OR: 1.11 per 1-year increase, 95% CI 1.00, 1.23).

Conclusion: Our results support excluding patients with impaired renal function and pre-existing proteinuria when conversion from CNIs to rapamycin is contemplated, due to their greater risk of adverse graft outcomes.

#435

LONG TERM VENTRICULAR ASSIST DEVICES, EXPERIENCE OF A SINGLE ACADEMIC CENTER

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Background: There have been recent improvements in technology and a rapid growth in Left ventricular assist devices (LVAD) utilization; and several recent landmark clinical trials. LVADs now account for 1/3 to 1/2 of transplant patients in many centers. It is important to examine real world clinical experience with LVADs in Canada.

Methodology: Retrospective chart review of consecutive patients; who received long-term ventricular assist devices (VADs) as a bridge to heart transplantation in a single centre 2004-2010. Peri-operative morbidity and mortality, and survival to transplant or ongoing support were examined.

Results: 40 VADs were implanted in 39patients; 12 received Thoratec PVADs (including 1 BIVAD), 4 received a Thoratec IVAD, and 23 patients received 24Heartmate II (HMII) LVADs. Mean age of 51(+/-6), range 15-71, 77% male, 32 % ischemic cardiomyopathy, 55% dilated cardiomyopathy, 65% with pulmonary hypertension. The majority of patients were hospitalized prior to surgery (90%), preoperative IABP in 24%, pre-op intubation in 13%, 22% had undergone previous cardiac surgery (redos).

Overall 30-day survival was 93% and not different between VAD types. Median ICU and hospital stay were 10 days (+/- 9); and 63 days (+/- 25), respectively. 33% re-op for bleeding in the HMII group compared to 44% in the PVAD group. 25 % G.I bleeding in the HMII compared to none in the PVAD/IVAD group. 8 % of patients developed stroke. . 3 patients (13 %) of the HMII group developed CVA (2 ischemic and 1 hemorrhagic) but survived to discharge and are still on VAD support.

3 patients (19%) of the PVAD/IVAD group died on support before transplant compared to 5 patients (21%) in the HMII group. The most common cause of death in the PVAD/IVAD group was Hemorrhagic Stroke while in the HMII group it was Multi Organ Failure (MOF).

20 patients (50%) have been successfully transplanted with an average time on LVAD support to transplant of 5.2 months (+/- 4.9). 12 patients (30%) are still on LVAD support with an average time on LVAD support of 14.2 months (+/- 13.3); 3 patients are no longer transplant candidates and are destination therapy cases. 1 patient was successfully explanted (PVAD) at 11 months (Post-Partum Cardiomyopathy).

Survival to transplant or ongoing support was 70 % at 1 yr.

Conclusion: Differences between PVAD and HMII showed clear separation between in-hospital outcomes and fallow up. Stroke was the cause of death in the PVAD/IVAD group during fallow up (i.e: after discharge) while in the HMII group none of the patients developed stroke after discharge. The utilization of LVADs has increased over the past 7 years. Outcomes continue to improve despite the increasing age and complexity of the patients. Peri-operative mortality and long- term survival to transplant or ongoing support are equivalent to those reported in recent clinical trials.

#436

THE IMPORTANCE OF PRA IN HLA IDENTICAL KIDNEY TRANSPLANT RECIPIENTS

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Introduction: In HLA identical kidney transplant recipients, the importance of non-donor specific anti-HLA antibodies is unclear. In this analysis, we describe the immunosuppressive treatment and outcomes of HLA identical kidney transplant recipients with different levels of anti-HLA antibody as determined by panel reactive antibody (PRA).

Methods: We studied 13,454 zero HLA-A, B, DR mismatched adult (≥ 18 years) kidney-only transplant recipients in the

USRDS between 1995 and 2007. Cox multivariate regression was performed for the outcomes of acute rejection (AR), during the first post transplant year, and graft loss with adjustment for year of transplantation, immunosuppression, donor/recipient age, donor/recipient gender, recipient ethnicity, diabetes, pre-transplant dialysis exposure, delayed graft function, and donor source.

Results: The use of depleting and non-depleting antibody induction increased during the study period from 26% and 4% between 1995-1998 to 39% and 30% respectively between 2003-2007. Induction (both depleting and non-depleting) was more frequent in patients with higher PRA: induction was used in 45%, 45%, 57%, and 63% of recipients with PRA 0%, 1-29%, 30-79% and >=80% respectively ($p < 0.0001$).

The incidence of AR during the first year was 13%, 14%, 11% and 13% in recipients with PRA of 0%, 1-29%, 30-79% and ≥80% ($p = 0.01$). In multivariate analysis, there was no significant association between PRA and risk of AR (Table).

Patients with peak PRA ≥ 80% had increased risk of graft loss from any cause (Table), and death censored graft loss (data not shown). However, in analyses stratified by use of induction agent, high PRA patients who received depleting/non-depleting antibody induction were not at increased risk of graft loss from any cause (Table), or death censored graft loss (data not shown).

Conclusion: We conclude that high PRA (≥80%) zero mismatched kidney transplant recipients are at increased risk for graft loss despite not having an increased risk of clinical AR. The risk of graft loss in these patients was not evident in those who received antibody induction. These findings suggest that high levels of non-DSA antibodies may be a risk factor for graft loss in HLA identical recipients, and that these patients may benefit from antibody induction.

Adjusted Risk				
	PRA 0% n=6855	PRA 1-29 % n=4228	PRA 30-79 % n=1230	PRA ≥ 80 % n=1141
Acute Rejection	1.00	1.08 (0.96,1.21)	0.99 (0.80,1.22)	1.18 (0.96,1.47)
Graft Loss from any cause	1.00	1.01 (0.94,1.09)	1.04 (0.92,1.18)	1.25 (1.11,1.41)
Adjusted Risk All Cause Graft Loss - Stratified by Induction				
	PRA 0 %	PRA 1-29 %	PRA 30-79 %	PRA ≥ 80 %
Depleting Antibody n=3278	1.00	0.95 (0.81,1.12)	0.98 (0.75,1.29)	1.15 (0.90,1.48)
Non-depleting Antibody n=3109	1.00	1.10 (0.93,1.30)	1.11 (0.84,1.49)	1.21 (0.89,1.62)
No Induction n=7067	1.00	0.94 (0.85,1.03)	1.15 (0.97,1.36)	1.28 (1.07,1.53)

#439

IMPROVING SURVIVAL WITH DOUBLE LUNG TRANSPLANTATION FOR PULMONARY FIBROSIS

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Background: Pulmonary Fibrosis (PF) is the second most common reason for lung transplantation (LT), yet survival is poorest when compared to other disease conditions. PF accounts for 28% of LTs at our centre and we examined our outcomes for these patients.

Method: We retrospectively analysed the outcomes for all LTs in our centre between 1989 and August 2010. Patients with PF were compared to all other LT patients and the International Society for Heart and Lung Transplantation (ISHLT) 2010 Registry Data.

Results: Of 427 LT patients, 93 were diagnosed with PF. Postoperatively, the total number of rejections, malignancies and infections were significantly lower in the PF group. Postoperative FEV1 and FVC were comparable between groups, but PF group performed poorer in the 6-minute Walk Test. Overall mean survival was lower in the PF group; however actuarial survival at 5 years was better than the ISHLT Registry Data (59% vs. 44%). Survival was significantly better for PF patients who received double LTs ($p=0.003$), while only 3 of 16 single LT patients are still living.

Conclusions: PF patients are a sicker more challenging group for LT with decreased survival compared to non-PF patients. Double lung transplantation provided significantly improved survival and results continue to improve with a 59% 5 year survival rate.

Table 1: Patient demographics and postoperative outcomes

	No PF (n=334)	PF (n=93)	p value
Age	47 ± 13	57 ± 7	<0.001
Gender, M/F	56% / 44%	82% / 18%	<0.001
Single / Double LTx	21% / 79%	17% / 83%	0.425
CPB Time (min)	230 ± 69	258 ± 55	0.003
Intubation Time (hours)	144 ± 226	260 ± 311	<0.001
ICU Stay (days)	10 ± 12	15 ± 14	0.001
Hospital Stay (days)	38 ± 33	52 ± 53	0.001
Total Rejections	1.15 ± 1.8	0.65 ± 1.1	0.010
Total Malignancies	0.26 ± 0.7	0.51 ± 1.0	0.007
Total Infections	2.67 ± 3.1	1.97 ± 2.5	0.046
Total CMV Infections	0.66 ± 1.5	0.30 ± 0.8	0.022

#441

PHARMACOKINETICS OF GANCICLOVIR IN SOLID ORGAN TRANSPLANT RECIPIENTS ON HIGH FLUX-HIGH EFFICIENCY HEMODIALYSIS

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Background: Ganciclovir and its oral prodrug valganciclovir are antiviral agents used in the prevention and treatment of CMV infection in solid organ transplant recipients (SOTR). Optimal dosing in patients with kidney failure on high flux-high efficiency hemodialysis (HD) is unclear, however underdosing may lead to treatment failure or the development of ganciclovir-resistant CMV strains. Current dosing recommendations are based on limited, outdated literature suggesting 50% ganciclovir removal by older less efficient HD methods.
Objectives: The primary objective was to determine the percentage ganciclovir removal with current HD methods. Secondary objectives included calculation of elimination half life (t½) and assessment of clinical outcomes.
Methods: This was a prospective, observational, single centre study conducted from Nov 2009 to Apr 2010. Eligible patients were adult SOTR admitted to the Transplant unit or ICU on HD and receiving intravenous ganciclovir or oral valganciclovir for CMV treatment or prophylaxis. Six blood samples per subject were drawn over a period of approximately 24 hours and ganciclovir levels were determined by HPLC. Plasma concentration versus time curves were generated and percentage ganciclovir removal and other pharmacokinetic parameters calculated. Clinical outcomes to the end of the study period were assessed through review of patient charts.
Results: Five SOTR were enrolled, 2 on ganciclovir for CMV treatment and 3 on valganciclovir prophylaxis. One patient had CKD and 4 had acute renal dysfunction. Dialysis sessions were 3 to 4 hours duration. The percentage ganciclovir removal by HD ranged from 50.7 to 97.1%. Off dialysis, t½ was prolonged and ranged from 14.4 to 160 hours in the CKD patient. Calculated t½ on HD ranged between 0.6 to 3.1 hours. CMV viremia cleared in both patients receiving treatment, while breakthrough CMV infection occurred in one patient on prophylaxis, likely due to nonadherence.
Conclusion: In this study, observed removal of ganciclovir on high flux-high efficiency HD was greater than previously reported. Clinical implications of this finding are unclear. Further study in a larger cohort of patients with additional blood sampling is warranted.

#442

ASSESSING HEALTH-RELATED QUALITY OF LIFE IN LUNG TRANSPLANT RECIPIENTS USING THE HEALTH UTILITY INDEX (HUI3) MEASURE

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Purpose: Transplantation has a profound effect on health-related quality of life (HRQL). The Health Utility Index Mark 3, HUI3, is a preference-based measure of HRQL that captures the risk inherent in lung transplantation, as patients undergo a life-threatening experience in order to improve their health status. We stratified lung transplant recipients by underlying diagnoses and compare and identify determinants of health status in lung transplant recipients.
Methods: Consecutive lung transplant recipients attending the lung transplant outpatient clinic in a tertiary institution completed the 15-item HUI questionnaire on a touch-screen computer at every visit during a two years period. The HUI3 covers a range of severity and co-morbidities and assesses a full range of health among patients. Overall HUI3 scores are on a scale in which dead = 0.00 and perfect health = 1.00; and disabilities categories range from no disability = 1 to severe disability < 0.70. Descriptive statistics were used to compare patients' health status. Random-effect models with time since transplant as a random variable and age, gender, underlying diagnoses, infections and broncholitis obliterans syndrome (BOS) as

fixed variables, were built to identify determinants of health status.
Results: Two hundred and fourteen lung transplant recipients of whom 61% were male with a mean age of 52 (19-75) years were recruited in the study. After two years of transplantation, Chronic Obstructive Pulmonary Disease (COPD) and Cystic Fibrosis (CF) patients displayed moderate disability (mean overall HUI3 scores of 0.70 and 0.74 respectively). Whereas, Pulmonary Fibrosis (PF) and Pulmonary Arterial Hypertension (PAH) patients displayed severe disability (mean overall HUI3 scores of 0.68 and 0.65 respectively). Ambulation, cognition, emotion and pain were the most affected single-attributes across diagnoses. Patients with PF had the worst emotion and cognition levels. The percentage of women in the severe category was higher than men (39% versus 28%).
Within two years post-transplant, 65 patients developed BOS but only 16 died (10 women and 6 men). Random-effect models confirmed that development of BOS was the main determinant of health status ($p<0.05$).
Conclusion: The HUI3 allowed us to compare patients' HRQL and identify determinants of health among lung transplant recipients with different underlying diseases. Description of patients' HRQL among different diagnoses groups can be used by clinicians to assess individualized patient care. BOS continue to be the main determinant of health status in lung transplant recipients.

#443

INCORPORATING MEASURES OF HEALTH-RELATED QUALITY OF LIFE (HRQL) INTO THE ROUTINE CLINICAL CARE OF LUNG-TRANSPLANT PATIENTS

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Purpose: Results from an earlier randomized controlled clinical trial indicated that the routine use of HRQL measures affected patient management. As a consequence the clinic decided to incorporate the use the Health Utilities Index System (HUI) and the Chronic Respiratory Questionnaire (CRQ) into standard care for lung-transplant patients. We hypothesized that the use of HRQL measures: 1) would reduce the duration of clinical encounters; 2) that patients would find completing the HRQL questionnaires at every clinic visit satisfactory; 3) and that clinicians would find the innovation useful.
Methods: Consecutive lung transplant patients visiting the out-patient clinic, University of Alberta Hospital, completed the Chronic Respiratory Questionnaire (CRQ) and the Health Utilities Index (HUI) on touch-screen computer. Information on the patient's HRQL was made available to the members of the transplant team prior to the encounter with the patient. In every consultation room a clock was placed on the desk and activated at the commencement and termination of the patient-clinician encounter. At the end of every visit, clinicians completed a questionnaire on the usefulness of having HRQL information available. At 6-months patients completed a survey on their experiences.
Results: The patient sample consisted of 172 lung transplant with a mean (standard deviation) age of 52 (13.3) years old; 47% were female. The eight members of the transplant team (four pulmunologists, three nurses, and one pharmacist) that had an average of 9 years of practice in pulmunology completed the questionnaires. The mean duration of patient-clinician encounters in minutes was 15.15 (4.52) significantly shorter than the control cohort 17.30 (12.14). Ninety eight percent of patients were happy to complete the CRQ and HUI at each clinic visit. Ninety-one percent of clinicians were completely satisfied with the intervention.
Conclusions: The incorporation of HRQL assessments in the routine clinical care of lung transplant patients resulted in a reduction of the duration of patient-clinician encounters. The experience was well accepted by patients and clinicians. The routine use of HRQL assessments in lung transplant patients has become standard practice.

#445

USING PATIENT-CENTRED CARE TO ENHANCE ADHERENCE TO MEDICATION AND EXERCISE IN LUNG TRANSPLANTATION: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Purpose: Previous studies suggest that the use of patient-reported outcome (PROs) measures in routine clinical care enhances patient centred-care and improves adherence to the advice of clinicians. This randomized controlled clinical trial examined the usefulness of including PROs in the routine clinical care of lung-transplant patients. We hypothesized that the inclusion of PROs in routine clinical

care would improve patient adherence to medication and exercise.

Methods: The study was conducted at the out-patient clinic of a tertiary institution. Patients were randomly assigned to intervention (completion of Health Utilities Index (HUI) questionnaire, on touch-screen computer with feedback to clinicians) and control group (completion of HUI without feedback). Feedback involved a graphical representation included in patients' chart. HUI includes 9 domains and health, ranges within each domain from no problem through severe problem, and captures the effects of co-morbidities. At the beginning and at the end of the study, patients completed Morisky's scale and Godin's measure. The Morisky scale contains four questions about medication use patterns. Higher scores reveal lower adherence. Godin's assesses daily activities and exercise in three categories of exercise: mild, moderate and strenuous. Each category includes the number of times and the duration in minutes that the individual exercised. ANCOVA was conducted to adjust for baseline difference in scores.

Results: Two hundred and thirteen patients with a mean age of 53 years were randomized (108 to intervention and 105 to control groups). At the end of our study, there was a trend toward greater adherence to medications among patients in the intervention group, although the difference was not statistically significant between the groups after adjusting for baseline scores ($p = 0.16$). Patients were more adherent to exercise and mild exercise duration increased in the intervention group but there were no statistically significant differences in mean time per week and duration of mild, moderate and strenuous exercise between the groups at the end of the study ($p = 0.13$; $p = 0.19$; $p = 0.50$; $p = 0.36$; time, $p = 0.63$; duration, $p = 0.35$ respectively).

Conclusions: We were unable to detect effects of using PROs on patient adherence during the follow-up period of this study.

#447

LONG TERM IMMUNE RESPONSES TO PANDEMIC H1N1 INFECTION IN TRANSPLANT RECIPIENTS

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Introduction: In organ transplant recipients it is unknown if natural infection with influenza confers protection from re-infection with the same strain during the next influenza season. Pandemic H1N1 (pH1N1) caused a spectrum of disease in transplant patients during the 2009 influenza season. Exogenous immunosuppression may have impaired cellular and humoral responses to influenza infection. The purpose of this study was to determine if natural infection with pH1N1 in 2009 resulted in a sustained immunologic response, and therefore protection from re-infection in the current flu season.

Methods: SOT recipients with microbiologically proven pH1N1 infection in 2009/2010 underwent serologic and cell-mediated immunity (CMI) testing for pH1N1 prior to the onset of the 2010/2011influenza season. Serology for pH1N1 (A/California/07/2009-like) was performed using hemagglutination inhibition assay (HAI). Concurrent testing for A/Brisbane/59/07 (H1N1) was done to rule-out cross-reacting antibody. A protective titer was defined as $\geq$ 1:40. CMI testing was measured with a flow-cytometry based assay using peripheral blood mononuclear cells stimulated with live influenza A/California/4/2009 (H1N1) to determine IFN-γ response by CD8+ T-cells.

Results: We analyzed 22 adult patients with previous pH1N1 infection including lung (n=7), kidney (n=5), heart (n=3), liver (n=3), and other transplants (n=4). Median time from transplant to infection was 4.5 years (range 0.3 - 18.7). Follow up HAI on all 22 patients and CMI on 5 patients was done at a median of 222.5 days (range 173 – 463) after infection. Despite microbiologically proven pH1N1 during the 2009 influenza season, only 10/22 (45.5%) had a positive serology at follow up. Of these, three patients were excluded from HAI analysis due to positive cross-reactive antibody to A/Brisbane leaving 7/19 (36.8%) patients with true positive serology. Thoracic transplant recipients were less likely to have a sustained humoral response compared to other types of transplant (11.1% vs. 60.0%; p=0.057). Positive serology was associated with a shorter time from infection to serologic measurement (224 ± 65.3 vs. 273 ± 77.2 days; p=0.047). Factors such as time from transplant, immunosuppression, early or late antiviral therapy, the presence of lymphopenia and previous influenza vaccination were not predictive of sustained humoral response. Of the 5 patients that underwent CMI testing, 2/5 had a positive CD8+T-cell IFN-γ response. In this subset, the CD8 response correlated with serologic response.

Conclusion: Our results demonstrate that a substantial proportion of transplant patients with previous pH1N1 infection lack both long-term humoral and cellular immune responses to pH1N1. These patients are likely at risk for re-infection and preventative strategies would be appropriate.

#449

ABO-INCOMPATIBLE HEART TRANSPLANTATION IN CHILDREN - HOW FAR CAN WE GO? A MULTICENTER SURVEY.

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ABO-incompatible heart transplantation in children - how far can we go? A Multicenter Survey.

Introduction: Since 1995 intentional blood group incompatible (ABOi) heart transplantation (Tx) in early childhood was introduced in several centers. Safety limits of this approach remain undetermined. We present a multicenter survey addressing clinical and immunological aspects to determine experience boundaries.

Methods: Following ethics approval a standardized survey was conducted in 6 major centers for pediatric Tx in Europe and North America requesting data pre- and post-Tx. Data were collected anonymously and statistically analysed.

Results: 58 ABOi Tx were performed in 57 patients. Median age at Tx was 6.8 mo (0.03 - 90), median post-Tx follow-up 38.7 mo (0.46 – 117), accumulating 187 patient-years. Gender was equally distributed. Pre-Tx mechanical support was used in 47%. ABOi combinations included donors of all blood groups: A (25), B (18), AB (15). Median anti-donor antibody (ab) peak titres pre-Tx were 1:8 (0- 1:64) for anti-A and 1:4 (0-1:32) for anti-B. Titres against the donor blood type tended to be lower post- than pre-Tx (p=0.09) but higher against third party in blood group O recipients. Induction was performed with ATG in 61%, anti-CD25ab in 32%, 7% received no induction. 4 episodes of acute cellular rejection (≥2R) 7 of ab-mediated rejection (AMR) were documented. 5 pts received ab removing treatment in the post-Tx phase either pre-emptively or due to AMR. 1 pt developed severe graft vasculopathy 5 years post-Tx. All patients received calcineurin Inhibitors, 62% mycophenolate, 10% azathioprine, 2% everolimus, 24% steroids. Freedom from death or re-Tx was 100%/96%/69% at 1/5/10 years. None of the 4 graft losses was attributed to AMR and neither pre- nor post-Tx donor specific blood group antibodies were different in patients with graft loss.

Conclusions: Successful ABOi HTx can be performed at older age and with higher pre-Tx ab titres than previously assumed. Successful ABOi-Tx is possible without ATG induction or intensified maintenance immunosuppression. Severe rejection and graft vasculopathy are rare. Low post-Tx titres indicate accommodation or tolerance towards the donor blood group, or elements of both.

#450

SHOULD LOW GFR PRECLUDE CONSIDERATION FOR PANCREAS AFTER KIDNEY TRANSPLANT (PAK)?

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Background: The level of kidney allograft function is an important determinant of eligibility for pancreas after kidney transplantation (PAK).

Methods: We determined the association of estimated glomerular filtration rate (eGFR) immediately prior to pancreas transplantation with kidney allograft survival after pancreas transplantation among 2,776 PAK recipients in the United States between 1987-2007.

Results: The median eGFR prior to pancreas transplantation was 57ml/min (95% CI, 30, 93ml/min) and PAK recipients had stable kidney function prior to pancreas transplantation.

In a multilevel mixed effect model, the annualized change in GFR prior to pancreas transplantation was only -0.07 ml/min/year, (95% CI, -0.42, – 0.28).

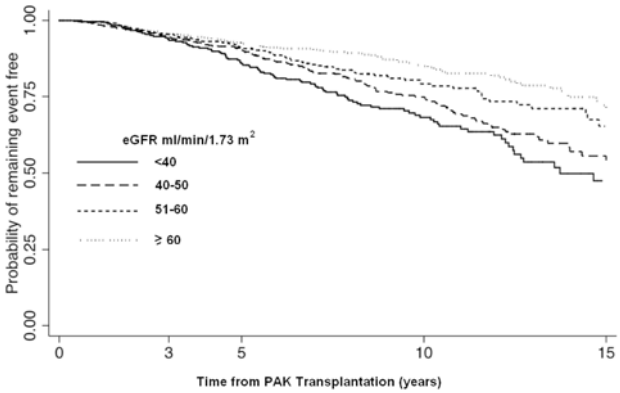
The figure (see figure 1) shows that although the eGFR prior to PAK was associated with long-term kidney allograft survival after PAK, there was no impact until three years after PAK and patients with eGFR <40 ml/min/1.73m^2 prior to PAK, still had excellent long-term kidney allograft survival (69% at 10 years).

Kidney allograft function remained stable after pancreas transplantation (annualized change in GFR-1.54 ml/min/year, (95% CI, -1.73, -1.36) and there was no association with the baseline GFR prior to pancreas transplantation.

Conclusion: Among patients selected for PAK based on their pre-pancreas level of kidney function and stability, GFR has limited impact on long-term kidney survival.

Other indicators of pre-PAK kidney allograft health should be studied to inform patient selection for PAK.

Figure 1: Kidney allograft survival in patients according to level of kidney allograft function prior to pancreas after kidney (PAK) transplantation



#451

EVALUATING THE RELATIONSHIP BETWEEN POST-OPERATIVE SERUM TACROLIMUS CONCENTRATIONS FOLLOWING RENAL TRANSPLANTATION AND DELAYED GRAFT FUNCTION: THE DEGRAFTS STUDY.

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Background: Delayed graft function (DGF) is a particularly significant complication of renal transplants. Despite its associated nephrotoxicity, tacrolimus is the main immunosuppressant in most immunosuppressive protocols following kidney transplantation. To our knowledge, no published study has evaluated the relationship between supratherapeutic serum tacrolimus concentrations and DGF.
Objective: This study aimed to determine if supratherapeutic mean serum tacrolimus concentrations in the first seven days following kidney transplantation are associated with DGF in kidney graft recipients.
Methodology: In this multicenter retrospective study, the primary outcome was the difference in DGF incidence between patients with mean serum tacrolimus concentrations > 12 ng/mL and patients with mean serum tacrolimus concentrations ≤ 12 ng/mL. Risk factors of developing DGF as well as inducing supratherapeutic serum tacrolimus concentrations were also assessed as secondary outcomes.
Results: 369 patients were included in the study, out of which 82 developed DGF. Mean serum tacrolimus concentrations were not found to correlate with an increase in DGF incidence. Body mass index (BMI) (OR : 1.20; 95% CI : 1.12 – 1.30), the number of days on a nephrotoxic medication (OR : 1.44; 95% CI : 1.14 – 1.81) as well as the DGF nomogram score (OR : 1.08; 95% CI : 1.05 – 1.11) were found to be risk factors for DGF, whereas pre-transplant dialysis was shown to decrease the risk of DGF. It was also found that the presence of at least one nephrotoxic agent increased the risk of DGF with an OR of 4.96 (95% CI : 2.49 – 9.89, $p < 0.001$).
Conclusion: Mean serum tacrolimus concentrations > 12 ng/ml are not correlated to an increase in DGF. Risk factors of DGF are the BMI, the number of days on a nephrotoxic medication and the DGF nomogram score. A noteworthy result of this study is the significant increase in the risk of DGF following renal transplantation when at least one other nephrotoxic agent is present.

#453

A SURVEY OF STRATEGIES FOR SAFE LIVING AMONG LUNG TRANSPLANT RECIPIENTS

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Introduction: Current guidelines for safe living strategies outline simple measures for the prevention of serious infectious complications. After lung transplantation, direct exposure of the allograft to the environment and potent immunosuppression amplify the importance of adherence to these strategies. However, there is no literature assessing patient compliance with safe-living practices. We conducted a survey of lung transplant patients to further define this.
Methods: Adult lung transplant patients were asked to complete the survey at a clinic visit or by telephone

administered by trained personnel. The survey consisted primarily of questions on a 5-point Likert scale and included the following categories: handwashing, gardening, respiratory infections, food and water safety, animal contact, travel, occupation.

Results: We surveyed 150 transplant recipients. Mean age was 54.5 ± 13.4 years and mean time post-transplant was 4.6 ± 3.7 years. Transplant types were double lung (n=128), single lung (n=13), heart/lung (n=9). Handwashing as a way to prevent infection (assessed in multiple scenarios) was practiced always or almost always by 135/150 (90.0%). Of those that worked with soil/garden, 50/71 (70.4%) never wore a mask although 30/71 (42.3%) always wore gloves. Precautions related to other environmental exposures (ill contacts, construction sites, crowded areas) and to food and water safety were variable and several areas for potential education were identified. Pet ownership was common (52%) but specific precautions related to handling of pets were practiced by most. One-third of patients continued to work after transplant but of these 61.2% had modified their occupation often due to perceived infectious risks. The majority of patients were always compliant with influenza vaccination (92.7%) although some (4.7%) had never received vaccine. Only 10.7% patients had travelled to a developing country after transplant. Gender and time from transplant did not influence safe living practices. However, lung transplant recipients less than 40 years old were less compliant with safety measures. For example, this group was less likely to wash hands after contact with plants/soil (p=0.007) or pets (p=0.05) and less likely to wear a mask when visiting someone ill (p=0.049).

Conclusions: This study provides important insight into safe living practices following lung transplantation. It provides information on specific areas and sub-groups of patients that could be targeted for enhanced education with potential significant clinical benefit.

#457

A CLINICAL REVIEW OF SHORT TERM VENTRICULAR ASSIST DEVICE (VAD) USE

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Background: Short-term VADs are used for the highest risk cardiogenic shock patients, often as a bridge to decision. Current reviews of short-term VAD utilization report 60-75% in-hospital mortality. This study aims to review our experience with a newer short term VAD.

Methods: All consecutive short-term VAD (Levitronix ©) implanted between 2007 and 2010 were reviewed. Pre-operative, operative, and post-operative patient data were recorded and analyzed. The outcomes of interest were survival to explantation and survival to discharge.

Results: From 2007 to September 2010, 3084 cardiac surgery cases were performed and 34 (1.10%) of cases required short-term VAD support. Indications for VAD support were: primary cardiogenic shock (7/34), post-cardiotomy cardiogenic shock (23/34), or post transplant graft dysfunction (4/34). All patients were failing despite high-dose inotropes and/or IABP support. Survival to explantation was 13/34 (38%), survival to discharge was 10/34 (29%). In-hospital mortality occurred in 5/7 (71%) primary cardiogenic shock patients, in 13/23 (56%) post-cardiotomy patients, and in 3/4 (75%) post transplant patients. In the post cardiotomy group, there was no mortality difference in patients who received intra-op VADs vs. post-op VADs. Average time to explantation was 11 days; death, 6 days; and discharge, 90 days. Post-operative bleeding occurred in 11 patients, who all returned to the OR for tamponade, 1 patient returned to OR for cannula repositioning and another returned for device removal due to sepsis. 1 patient died of stroke and another experienced TIA. Average VAD flows were 5L/min. There were no device malfunctions, hemolysis, or thrombotic events.

Conclusion: In this group of high risk, critically ill patients, the Levitronix © short-term VAD offered reliable hemodynamic support. Ease of implantation and post-operative management make this device a good treatment option for patients who are failing on IABP and high-dose inotropes. Despite this, overall survival remains limited. The in-hospital mortality rate is 62%, likely due to the pre-VAD clinical status of patients and perhaps to delayed decision making for VAD support.

#458

SEQUENTIAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOLLOWING SOLID ORGAN TRANSPLANTATION IN CHILDREN, FEASABILITY AND OUTCOMES IN A SINGLE CENTRE.

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Introduction: Hematopoietic stem-cell transplantation (HSCT) following solid organ transplant (SOT) has been described in adults, primarily as case reports. We sought to describe the 10 year experience at a large Canadian pediatric tertiary care center of the use of allogeneic HSCT following SOT.
Methods: Outcomes of pediatric recipients of allogeneic HSCT following SOT between 2000- 2010 at were reviewed. Indications, conditioning regimen, donor type, morbidity and mortality were described.
Results: Four children (median age 9.5-yrs (range 1.75-14yrs)) received allogeneic HSCT following SOT (table 1). Two of the patients had received heart transplants and 2 patients had undergone liver transplantation. Indications for allogeneic HSCT were: T-cell lymphoma/ PTLD in the 2 patients post heart transplant and severe aplastic anemia (SAA) in the 2 patients post liver transplant. The mean time between SOT and HSCT was 4.2 yrs (range: 1.8-7.8yrs). All patients engrafted after HSCT. All 4 patients died in a range of 37days to 1year after HSCT. Causes of death were multi-organ failure (1 pt), infection and MOF (2 pts) and solid organ rejection (1 pt). Though 3 patients survived beyond day+100, multiple infectious complications occurred including ebstein-barr virus (EBV) reactivation, adeno-virus infection, and gram-negative sepsis. The two patients with prior liver transplantation had liver complications during HSCT. One had veno-occlusive disease and one had portal hypertension and liver failure. One patient EBV positive B cell PTLD post HSCT. The 2 patients transplanted for lymphoma did not have evidence of recurrence at 7 months and 1 year after HSCT.
Discussion and conclusion: Though feasibilty has been shown , we conclude that allogeneic HSCT follwing SOT is a high risk procedure that resulted in severe morbidity and mortality in children and other therapeutic options should be explored.

Table 1:

Patient #	Solid organ transplant	Age at SOT	Indication for HSCT	Age at HSCT	Stem-cell source	Conditioning regimen	Complications	Outcome
1	Heart-for hypoplastic left heart	3months	PTLD/Tcell lymphoma	21m	Cord 4/6 mm	Busulfan Cyclophosphamide Melphalan Alemtuzumab	Gram negative bacteremia	Died 1-y post HSCT from heart rejection
2	Heart-for hypoplastic left heart	2months	PTLD/Tcell lymphoma	8yr	Cord 4/6 mm	Cyclophosphamide Total-body-irradiation thymoglobulin	1. EBV-positive B-cell PTLD 2. HSV - reactivation 3. Influenza A pneumonia 4. Adenovirus pneumonia	Died at 7 months post HSCT from infection
3	Orthotropic liver –for fulminant liver failure	8-yo	SAA	14-yo	Haplo-identical	Melphalan Fludarabine Thiotepa Antithymocyte-globulin	1.Veno-occlusive disease 2. Adenovirus infection 3. gram positive sepsis	Died at 6 month post HSCT -from infection and MOF
4	Orthotropic liver –for fulminant liver failure	9-yo	SAA	11-yo	10/10 living unrelated donor	Melphalan Fludarabine Antithymocyte-globulin	Acute liver failure Renal failure	Died at day +37- from MOF

#462

CD4, CD8, TREG AND TH17 KINETICS IN SOLID ORGAN TRANSPLANT PATIENTS WITH CMV INFECTION

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Background: Treg and Th17 cell response play important roles in transplant rejection and tolerance but have not been assessed in patients with CMV. We hypothesized that CMV reactivation may be accompanied by significant alterations in Treg and Th17 cell populations. The present study aimed to assess different T-cell populations in patients with CMV reactivation.
Methods: We prospectively evaluated 30 transplant patients with CMV viremia. CMV-specific CD4 and CD8, total Treg and Th17 cell percentages were evaluated at baseline in all patients and then longitudinally in patients receiving treatment using flow cytometry with surface marker and intracellular staining. Results were assessed in the context of viral load, viral clearance kinetics and recurrence.

Results: Two subsets of patients were included. The first had CMV disease/high-level viremia (n=20) while the second had spontaneous viral clearance (n=10). Median baseline viral loads were 41,575 and 1,135 copies/ml respectively (p=<0.001). CD4 and CD8 response did not differ between subgroups. However, in treated patients, higher baseline CD4 or CD8 T-cell responses were associated with more rapid rates of viral clearance after initiating antiviral therapy (correlation with slope of decay curve: p=0.014 for CD4; p=0.002 for CD8). Baseline Treg percentages (all 30 patients) ranged from 0.4 to 10.70%. Treg percentages were significantly higher in patients with CMV disease/high-level viremia compared with those that spontaneously cleared virus (median 2.05 vs 1.25; p=0.01). High baseline Treg percentages were associated with higher viral loads (p=0.01). Baseline Th17 response ranged from 0.6-9.4% and were strongly correlated with Treg responses (p<0.001). Longitudinal changes in T-cell subsets over time were complex displaying no single pattern of change. Some patients had substantial changes over time particularly in Treg percentages (range 6.25 fold-decline to a 2.5 fold increase over the course of treatment). In an analysis of virologic relapse, high baseline Treg percentages were associated with an increased likelihood of relapse (p=0.06).
Conclusions: This study provides novel insight into Treg and Th17 dynamics in patients with CMV reactivation. Treg and Th17 percentages increased in parallel in patients with high viral loads and substantial difference were observed in patients requiring treatment vs. those with spontaneous viral clearance.

#463

SERUM FGF-23 LEVELS PREDICT MORTALITY AND ALLOGRAFT LOSS IN KIDNEY TRANSPLANT RECIPIENTS

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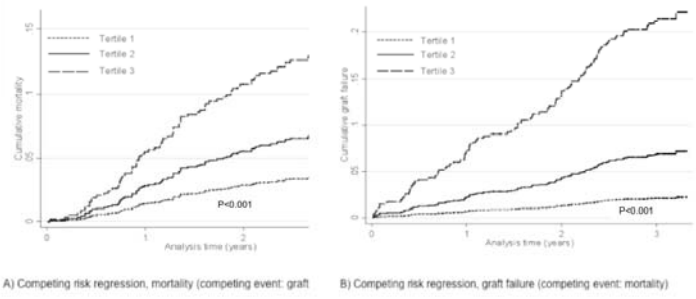
Background: Large epidemiologic studies demonstrated significant associations between increased levels of serum FGF-23 and adverse clinical outcomes, including more rapid progression of kidney disease, cardiovascular disease, and death. No published studies examined the association between FGF-23 and clinical outcomes in kidney transplant (Tx) recipients.
Methods: We collected socio-demographic parameters, medical and transplant history and laboratory data at baseline from 984 stable prevalent Tx recipients (mean age 51±13 years, 57% males, mean eGFR 51±21 ml/min/1.73m2, median Tx vintage 72 months [interquartile range 74 mo] and 21% diabetics). Serum FGF-23 was measured using a C-terminal enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). To assess if serum FGF-23 predicts the risk of all-cause mortality and of death censored graft loss we used semiparametric competing risks regression analysis.
Results: During the 37 months follow-up, 87 subjects died and 101 patients returned to dialysis. Both mortality and death-censored allograft loss was significantly higher in patients in the higher tertiles of baseline FGF-23 (Figure A and B). Ln-transformed serum FGF-23 was independently associated with mortality ($HR_{1\ increase}$ = 1.78; 95% CI: 1.39-2.28) and of death censored graft loss ($HR_{1\ increase}$ = 1.60; 95% CI: 1.26-2.03) in multivariable-adjusted models. In the multivariable models, the highest tertile of FGF-23 was independently associated with a 2.29 (95% CI 1.15, 4.55) HR of mortality, and a 2.77 (95% CI 1.29, 5.92) HR of death-censored allograft loss.
Conclusions: High FGF-23 levels are independently associated with increased risk of mortality and death-censored graft loss in prevalent kidney transplant recipients.

Figure: Tertiles of FGF-23 and clinical outcome



A) Competing risk regression, mortality (competing event: graft B) Competing risk regression, graft failure (competing event: mortality)

#464

CIRCULATING ANGIOPOIETIN-2 LEVELS AND MORTALITY IN KIDNEY TRANSPLANT RECIPIENTS

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Introduction: Endothelial activation and dysfunction represent early events in the pathogenesis of arteriosclerosis. Angiopoietin 2 (Angpt2) impairs endothelial function by preventing Ang-1 from binding to their common endothelial-specific receptor Tie2. We have recently shown that Angpt2 is increased in essential hypertension, increases with the progression of chronic kidney disease and correlates with arteriosclerotic burden in dialysis patients. Here we examined whether circulating Angpt2 levels also predict mortality and/or graft-loss in renal transplant recipients.
Methods: For this case control study we selected 130 kidney transplant recipients who died (n=60) or returned to dialysis (n=70), as well as 130 age and gender-matched kidney transplant recipients without an event (controls) from a total of 993 kidney transplant recipients followed prospectively at a single transplant center for a median of 2.3 years. Serum Angpt2 at baseline was measured by in-house immuno-luminometric assays (ILMA) by a blinded investigator. Association of baseline Ang2 levels with all-cause mortality and wit graft failure was examined in competing risk regression analyses.
Results: Median Angpt2 concentrations were significantly higher in patients who died and/or returned to dialysis (median [interquartile range – IQR] 4.0 [2.5-6.0] ng/mL) as compared to patients who did not suffer from any event during the study period (2.8 [2.0-3.7] ng/mL; $P < 0.001$). Ln (natural log) Angpt2 levels correlated positively with C-reactive protein levels (r= 0.380, $P < 0.001$), and the Charlson Comorbidity Index (r= 0.213, $P < 0.001$), and were inversely associated with eGFR (r=-0.322, $P<0.001$) hemoglobin (r= -0.276, $P < 0.001$) and serum albumin concentrations (r= -0.372, $P < 0.001$). In multivariate analyses baseline Angpt2 levels independently predicted mortality (multivariable adjusted hazard ratio associated with one log unit higher Ang2 level: 2.30 (95% confidence interval: 1.20-4.40) p=0.012). Angpt2 levels were not associated with graft loss (p=0.7).
Conclusion: Angpt2 is elevated in kidney transplant recipients, particularly those with higher co-morbidities and micro-inflammation, possibly reflecting pronounced endothelial activation and dysfunction. Circulating Angpt2 is also a strong and independent predictor of mortality in stable kidney transplant recipients.

#466

SERUM ERYTHROPOIETIN LEVEL AND MORTALITY IN KIDNEY TRANSPLANT RECIPIENTS

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Background: Post-transplant anemia is frequently reported in kidney transplant recipients and is associated with worse patient survival. Resistance to serum erythropoietin, similarly to resistance to erythropoesis stimulating agents, is reportedly associated with clinical outcome in patients with end stage renal disease. We examined the association between serum erythropoietin levels and mortality among kidney transplanted patients.
Methods: We collected socio-demographic, clinical, medical and transplant history and laboratory data at baseline in 886 prevalent kidney transplant recipients (mean age 51±13 [SD] years, 60% men, 21% diabetics). A solid-phase chemiluminescent immunometric assay was used to measure serum erythropoietin. Cox proportional hazards regression was employed to model the association between baseline serum erythropoietin levels and all-cause mortality risk.
Results: During the median 39 months follow-up, 99 subjects died. The median serum erythropoietin level was 10.85 U/l (IQR: 7.6-15 U/l) and hemoglobin was 137±16

g/l. The mortality rate was significantly higher in patients with higher erythropoietin levels (crude mortality rate [95%CI] in the highest to lowest erythropoietin tertiles were 51.7 [38.6–69.3], 35.5 [25-50] and 24.0 [15.8-36.4] per 1,000 patient-years, respectively (p=0.008)). In fully adjusted Cox models each SD higher serum erythropoietin level significantly predicted all-cause mortality: $HR_{1\ SD\ increase}$ 1.28; (95% CI: 1.02-1.62). Furthermore, serum erythropoietin predicted mortality in all analyzed subgroups.

Conclusions: In this sample of stable prevalent kidney transplant recipients, higher serum erythropoietin levels were associated with increased mortality after adjustment for important risk markers.

#467

PORTAL VEIN THROMBOSIS IS A PREVENTABLE COMPLICATION IN CLINICAL ISLET TRANSPLANTATION - RELATIONSHIP OF HIGH PACKED CELL VOLUME, PORTAL HYPERTENSION AND RISK OF PORTAL THROMBOSIS

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Introduction: Islet transplantation improves glycemic control in Type1 diabetes complicated by refractory hypoglycemia. Percutaneous transhepatic portal access avoids surgery, but is rarely associated with bleeding or portal vein thrombosis. Herein, we evaluate factors affecting portal pressure and risk factors of portal vein thrombosis post islet transplantation.

Methods: We reviewed records of 278 intraportal islet transplant procedures in 127 patients (mean 2.19 infusions/patient). Portal venous pressure (mmHg) was measured by using a pressure transducer before and after completion of islet infusion. A doppler ultrasound was performed in 24 hours post transplantation for all cases to assess the complications such as portal vein thrombosis, hematoma or bleeding routinely.

Results: The mean islet mass was 407,221 IE (5,908 IE/kg) with mean packed cell volume of 4.1 mL (range: 1.5 - 7.9). Institution of therapeutic heparinization, effective catheter tract ablation with Avitene paste, and limiting packed cell volume to < 5 mL has completely prevented this complication in 101 islet transplant procedures over the past 4.3 years. Univariate analysis revealed that standard liver volume correlated negatively with portal pressure rise (r=-0.257, P<0.01), with a larger liver volume experiencing less perturbation in portal pressure. Packed cell volume correlated positively with elevated portal pressure (r=0.463, P<0.01). Ten patients (3.6%) developed partial thrombosis of the intrahepatic portal vein (none since August 2006). Univariate analysis revealed that both portal pressure elevation (r=0.256, P<0.0001) and high packed cell volume (r=0.161, P<0.01) were risk factors for thrombosis. Packed cell volume <5.5 mL (sensitivity 50%, specificity 84.5%) and portal pressure rise <4.5 mmHg (sensitivity 70%, specificity 73.2%) were founded to be cut offs to prevent portal vein thrombosis.

Conclusions: Portal thrombosis is a preventable complication in clinical islet transplantation, provided therapeutic anticoagulation is maintained, and packed cell volume is limited to <5 mL.

#468

THE IMPACT OF PANCREAS ON CALCULATED CARDIAC RISK IN SIMULTANEOUS KIDNEY PANCREAS TRANSPLANTATION

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Introduction: It is not clear whether pancreas transplantation in type I diabetics with renal impairment improves overall patient health and survival. The aim of this study was to look at the impact of pancreas transplant on simultaneous kidney pancreas (SPK) recipients using the calculated ten year cardiac risk of patients using the Framingham Cardiac Risk Calculator.

Methods: A retrospective review was performed on all SPK recipients from a single centre between 2007 to present. The Framingham 10-year risk for general cardiac disease was calculated for all patients pre-operatively and compared to their calculated cardiac risk one year post-operatively. We also compared each individual cardiac risk factor separately to decipher which factors conferred the most significant influence on cardiac risk reduction (blood pressure, cholesterol, glycemic control). Also, we compared the calculated cardiac risk of the SPKs to a cohort of patients with type I diabetes receiving a solitary kidney (SK) transplant.

Results: Thirty-six consecutive SPK patients and 10 SK recipients with type I diabetes were analyzed. Using the Framingham Cardiac Risk Calculator, the mean pre-operative risk for the SPK group was 12.4±9.0% compared with a mean risk of 5.3±4.3% 1 year post-operatively (p = 0.0002). When analyzing the SK group, there was no statistical improvement in the calculated cardiac risk from pre-operative vs.post-operative period. One year post-operatively, 86% of SPK patients were normoglycemic and decreased their overall number of antihypertensive medications by a mean of at least one agent. Also, when

comparing the SPK group with the SK group 1 year post-operatively, the SPK group had a significantly lower LDL and total cholesterol (p=0.0007) and significantly lower HbA1c (mean 5.39%, p=0.0003). However, there was no statistical difference in systolic blood pressure or overall renal function.
Conclusion: Overall, SPK transplantation has a positive impact on patient health according to significant improvement in their calculated cardiac risk one year post-operatively. Resulting impact of pancreas transplantation on cardiovascular complications including myocardial infarction, stroke and death continue to be studied.

#476

THE IMPACT OF MELD-BASED LIVER ALLOCATION ON THE QUÉBEC LIVER TRANSPLANT WAIT LIST

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Background: MELD-based liver allocation and the impact on patients on the waitlist have been widely demonstrated. In Quebec, a MELD-based liver allocation protocol was implemented in July 2009. Points are given for liver failure (MELD-IH), for hepatocellular carcinoma (MELD-CHC) based on the number and size of tumor(s), with additional points for larger tumors (up to 5cm) or more numerous tumors (up to 3). Pediatric cases, as well as other special cases are evaluated on a case-by-case basis (MELD-DER) and an arbitrary score is assigned.
Objective: Assess the effect of implementing a MELD-based liver allocation protocol on the Quebec liver transplant waitlist.
Methods: Data on the new allocation protocol, from its implementation in July 2009 to October 2010, was assessed and compared to data from April 2008 to July 2009, the 15-month period prior to the new allocation protocol. Data analyzed included the number of liver transplants by diagnosis (HCC, MELD-CHC vs. liver failure, MELD-IH), a comparison between average MELD and patient status at the time of transplantation, and wait time. The number of patients on the waitlist, the number of patients withdrawn from the list, as well as deaths on the waitlist was also analyzed.
Results: The results show little change in the number of HCC vs. liver failure patients transplanted since the new allocation system was implemented (HCC before/after 24/25, and liver failure before/after 72/71). MELD-DER patients represent 15.4% of all MELD scores. Urgent status patients (Status 3F, 4, 4F) decreased from 17 to 13. The mean wait time for transplanted patients decreased from 220.9 days to 179.3 days. 62.7% of transplanted patients had MELD scores between 21 and 30, while 47.3% had MELD score between 11 and 20. The number of patients on the waitlist decreased from 186 to 154, as a result of definitive withdrawals prior to the implementation of the new system. 21 patients died while on the waitlist in both periods.
Conclusion: The implementation of a MELD-based liver allocation protocol in Quebec has had a positive impact on the liver transplant wait list by successfully decreasing wait-time for liver transplantation without increasing the number of deaths on the wait-list.

#477

A RETROSPECTIVE ANALYSIS OF NUTRITIONAL PREDICTORS OF POST TRANSPLANT MORBIDITY AND GROWTH IN PEDIATRIC LIVER TRANSPLANT.

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Liver transplantation (LT) is life-saving therapy for children with end-stage liver disease, offering an excellent opportunity for a good quality of life. Pre and post-transplant nutritional status are determinants of survival rates, quality of life and growth after LT. Malnourished patients have twice the infection rate, higher rates of surgical complications, re-tranplantation and mortality compared to adequately nourished patients. Survival rates, quality of life and growth after LT are partly dependent on pre and post-transplant nutritional status . Better understanding of factors associated with poor nutritional status and outcomes is needed. The goals of this study therefore were: 1) to retrospectively determine the preoperative nutritional parameters that are predictive of post-operative outcomes in a pediatric LT population; 2) to identify nutritional factors and disease states that are predictive of growth after LT. A retrospective chart review of patients who had a LT between January 1999

and Dec 2008 at a Pediatric Liver Transplant Centre was conducted. Data were collected on post transplant outcomes including length of stay, infections and surgical complications as well as nutritional predictors of outcome. Preliminary outcome data on 20 of 130 patients have been analyzes. Mean age of translpant reciepients was 3.7 yrs, and were at least 2 years post LT. The average length of hospital stay was 39 days with an average of 4 days spent in the ICU. Patients suffered an average of 2 infectious complications and 1 surgical complication requiring readmission to ICU. There was an average of 1.3 readmissions during the first year following LT. This preliminary data suggest our patients undergo prolonged hospitalization and recovery time. Analysis of the entire data set should provide information on the nutritional factors that will improve these outcomes.

#480

CYTOMEGALOVIRUS CELL MEDIATED IMMUNITY MONITORING IN PATIENTS WITH LOW LEVEL CMV REACTIVATION

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Background: A CD8+ T-cell response to Cytomegalovirus (CMV) after antiviral prophylaxis has been associated with a decrease in the incidence of CMV disease. There are limited data on the clinical utility of cell-mediated immunity (CMI) testing at the onset of CMV viremia. We sought to determine whether a CMI response could predict the course of low level CMV viremia in a cohort of transplant recipients.
Methods: We conducted a prospective study of adult organ transplant recipients who developed low level viremia by real-time plasma CMV PCR, below the threshold for pre-emptive therapy (set at 15,000 copies/mL). Patients were enrolled at the onset of viremia, were asymptomatic and not receiving antiviral therapy. All patients had CMI measured at baseline, 7, and 14 days using the QuantiFERON-CMV® assay (Cellestis Inc.) and were followed for 3 months for progression of CMV viremia by weekly CMV PCR.
Results: We enrolled 34 patients; of these, 2 were excluded due to death and inadequate sample collection respectively. For the 32 patients analyzed, mean age was 56±10.5 years. Transplant types were kidney (n=17), lung (n=6), liver (n=4), heart (n=2), and combined (n=3). 6/32 patients were CMV D+/R- and 26/32 were R+. Median time from transplant was 3.35 (range 0.33 - 235) months. Median viral load at enrolment was 1,162 (range 500 - 7,600) copies/mL. Nine patients were given antiviral therapy during the follow-up period for symptomatic disease (3/9) or exceeding the pre-emptive threshold (6/9). A CMI response (IFN-γ cutoff value ≥0.2 IU/mL) was present in 22/31 at baseline, day 7 (24/32), and day 14 (18/29). Patients with CMV CMI response at the onset of viremia were significantly less likely to require antiviral therapy (13.6% vs. 71.4% for non-responders; p=0.008). The rate of spontaneous clearance of viremia in those with a positive CMI was 86.4% vs 28.6% in those with a negative CMI (p=0.008). The use of a lower cutoff value to define a CMV-specific response (≥0.1 IU/ml) in this population slightly improved the sensitivity of the test for detection of spontaneous clearance of viremia (90.5% for IFN-γ≥0.1 IU/mL vs. 85.7% for IFN-γ≥0.2 IU/mL), without changing specificity (62.5%).
Conclusion: CMI assessment in patients with low level CMV viremia may be useful to predict the need for antiviral therapy. The specificity of this test may limit its use as a single tool for monitoring of these patients.

#481

NEUROLOGICAL DEATH DIAGNOSIS: DOES GOOD PRACTICE FOLLOW RECOMMENDATIONS?

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Considerations: The neurological death diagnosis (NDD) is a medical procedure for which strict recommendations were published in 2003. However, some studies show variability in the application of these criteria. In order to standardize the NDD procedure among clinicians, a dedicated worksheet was developed and distributed to all Quebec hospitals in 2002 by Québec-Transplant.
Objectives .1. Verify how this standardized form was used by clinicians. 2.Analyse if clinicians are following CCDT recommendations
Methods: A retrospective study of all cases of organ donors in the Eastern part of the province of Quebec (219) was conducted for the years 2005 to 2009. The analysis was made from the NDD standardized sheet, medical notes and consultations.
Results: Donors were male in 55% of cases with a mean age of 51 years. The main cause of death was stroke (63%), followed by head trauma (21.9%). The full neurological examination was performed in 70.3% for the first NDD and 75.5% for the second exam. Oculo-cephalic or oculo-vestibular reflexes are most often missing during the

examination (32.3% and 26.4%). The apnea test was completed respectively in 88.6% and 79.6%. The main cause of the failure to achieve the apnea test on the 1rst NDD is hemodynamic instability (32%). For the second NDD, it is linked to a conclusive angiography performed during the first NDD (41.9%). The cessation of the apnea test is mainly related to desaturation. The suggested starting values for the apnea test were generally met. The most common complementary test is the four vessels angiography (79.2% for the first NDD and 91.7% for the second NDD). The main reasons for performing an ancillary test for the first NDD, is hemodynamic instability / desaturation (22.6%) and impossibility to perform a complete neurological examination (20.8%). On the second NDD, it is mainly related to the presence of reflex or spontaneous movements. The standardized sheet was used in 88.4% of cases for both clinical examinations.
Conclusion: The study confirmed that the standardized form for the NDD is used in a great percentage of time and the CCDT recommendations are well documented on this worksheet.

#482

CYTOKINE EXPRESSION PROFILLING AND PREDICTION OF PROGRESSION OF CYTOMEGALOVIRUS VIREMIA IN TRANSPLANT RECIPIENTS

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Background: An imbalance of the Th1/Th2 axis of the cellular immune response, with a relative increase of Th2 cytokines may play a role in the pathogenesis of CMV infection in transplant recipients. Th17 T cell responses may also play a role in controlling CMV viremia. We aimed to comprehensively characterize the expression of Th1, Th2 and Th17 cytokines in the blood of patients with low level viremia.
Methods: We prospectively studied transplant patients with asymptomatic, low level CMV viremia. Whole blood was stimulated with 21 CMV HLA-restricted peptides (from QuantiFERON-CMV test) and plasma was tested for a panel of 65 cytokines/chemokines as quantified by a Luminex bead array assay. Patients were monitored with weekly viral load testing and started antiviral therapy when necessary.
Results: We analyzed 32 patient samples. The majority 61/65 (93.8%) of cytokines/chemokines were detectable in >60% of samples (4 cytokines (IL-3, IL-9, IL-21, Eotaxin-3) detected in <10% of samples.) The proinflammatory cytokine IL-8 (median 4,129 pg/ml) was amongst the most abundant, followed by IL-6 (median 375 pg/ml), IFN-γ (median 41 pg/ml) and TNF-α (median 41 pg/ml). The following Th2 cytokines were amongst those whose levels were the lowest to be documented: IL-5 (median 0.48 pg/ml), IL-13 (median 2.54 pg/ml) and IL-4 (median 11.15 pg/ml). The ratio given by the sum of Th1 cytokines IFN-γ, TNF-α, TNF-β and IL-2 divided by the sum of the Th2 cytokines IL-4, IL-5, IL-13 and IL-10 (i.e. Th1:Th2 ratio) in patients that cleared viremia spontaneously was significantly higher than in patients that progressed (Th1:Th2 ratio of 15.76 vs. 2.36 respectively; p=0.015). The Th1:Th2 ratio showed better performance as a potential test for prediction of spontaneous clearance of viremia (AUC 0.821 CI 0.676 − 0.967; p=0.005) when compared to IFN-γ detection alone (AUC 0.754, CI 0.578 − 0.929; p=0.028). Cytokines necessary for Th17 differentiation and maintenance, such as IL-6 and IL-23 (median 77.1 pg/ml) were elevated. However, Th17 cytokines IL-17 (median 3.44 pg/ml) and of IL-21 (median 18.37 pg/ml) were low.
Conclusion: Broad detection of cytokines in patients with CMV reactivation, suggests that Th1/Th2 imbalances may be important for determining the natural history of viral replication. Stimulation of whole blood with CMV peptides elicits a robust Th1 cytokine expression, which is augmented in patients that achieve spontaneous clearance of low level viremia.

#484

PROFILING HOST AND VIRAL MICRORNA RESPONSES TO CYTOMEGALOVIRUS VIREMIA IN TRANSPLANT RECIPIENTS

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Background: MicroRNAs (miRNAs) are small (18-22 nt) RNAs that inhibit target mRNA translation and are important regulators of gene expression. The human microRNAome contains almost 1000 miRNAs and CMV itself has 17 known miRNAs. The in-vivo host and viral miRNA response to CMV reactivation has not been previously assessed.
Methods: Human and CMV miRNA microarray was performed in blood leukocytes from kidney, liver and heart

recipients with CMV viremia (n=18) or no CMV infection (n=7). A minimum 2-fold difference in miRNA levels between groups was considered relevant and a Bonferroni-corrected Oneway ANOVA p<0.05 statistically significant. RT-qPCR was used for validation.

Results: Two groups could be defined according to their viremia levels: HIGH (median 44,500 copies/ml) and LOW (median 980 copies/ml) (p=0.003) and subsequent analysis took in account these CMV viremic groups and an aviremic group (i.e. CTRL). The comparison of LOW, HIGH and CTRL (n=25) revealed 2 downregulated (miR-379 p=0.017 and miR-185* p=0.042) and 1 upregulated (miR-1244; p=0.002) human miRNAs in the groups with CMV infection. The hcmv-miR-UL22A expression was 2.51-fold higher in HIGH than LOW (p=0.001). The miRNA expression in only kidney recipients showed a set of 4 downregulated (miR-30b* p=0.044; miR-769-5p p=0.041; miR-326 p=0.020 and miR-185* p=0.003) and 1 upregulated (miR-1244 p=0.007) human miRNAs in HIGH and LOW, along with a 2.52-fold higher expression of hcmv-miR-UL22A in HIGH than LOW (p=0.024; 2.45-fold higher in HIGH by RT-qPCR). Surprisingly, the expression of 16 other CMV miRNAs between HIGH and LOW did not reach statistically/biologically significant differences, suggesting that CMV miRNA expression is not proportional to the viral load. In both analysis, the internal comparison between CMV groups showed that HIGH had a 2 to 5-fold more intense down/upregulation of the human miRNAs as compared to LOW. Computational algorithms defined several potential candidate gene targets both in the virus and in the host.

Conclusions: CMV infection in transplant recipients results in marked miRNA expression changes in both the virus and the host microRNA profiles that are more intense the higher viremia is. Given their usual inverse relationship with gene expression, both miRNA-related de-repression and repression of genes may play a role in CMV pathophysiology.

#486

PHENOTYPE ANALYSIS OF T-CELL SUBSETS IN LONG-TERM LIVER TRANSPLANT PATIENTS ON MAINTENANCE ANTI-CD25 MONOCLONAL ANTIBODIES VS. CALCINEURIN INHIBITORS AND SIROLIMUS

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Background: We previously described the use of anti-CD25 monoclonal antibodies (mAb) for maintenance immunosuppression to replace calcineurin inhibitors (CNI) in liver transplant (LTx) pts with chronic kidney disease.

Purpose: To determine the impact of maintenance anti-CD25 mAb immunosuppression vs. CNI and sirolimus (SRL) on T-cell subsets in long-term LTx pts with chronic kidney disease.

Methods: This was a cross-sectional study in stable (no evidence of infection or acute rejection) adult LTx pts (>1-yr post-LTx), including 7 pts on CNI (7 on mycophenolic acid (MPA)), 6 pts on SRL (5 on MPA) and 8 pts on anti-CD25 mAb (6 on MPA). Pts on anti-CD25 mAb received daclizumab 1.5 mg/kg q.2 months, keeping the CD25 saturation rate (measured on peripheral CD4+ cells by flow cytometry) >98%. T-regs were analyzed in peripheral blood using flow cytometry.

Results: Results are shown in Table 1.

Table 1. T-cell subsets

	CNI	SRL	Anti-CD25 mAb	Healthy controls
CD3+ (%)	49±14[a]	57±14	55±15	71±10
CD8+ (%)	40±14	39±10	34±12	36±9
CD4+ (%)	36±7.5	36±10	36±12	46±13
CD4+FoxP3+ (%)	6.4±2	13±5[b]	7±2	9.5±4.9
CD4+CD25+CD127Low (%)	4.9±1.4[c]	8.7±4	1.2±1.4[d]	6.4±0.8
FoxP3+ in CD4+CD25+CD127Low (%)	66±11[e]	82.5±4	73±8.5	81±5

[a]P<0.05 vs. healthy controls, [b]P<0.05 vs. CNI and anti-CD25 mAb, [c]P<0.05 vs. SRL and anti-CD25 mAb P<0.05, [d]P<0.001 vs. SRL and healthy controls, [e]P<0.001 vs. SRL and healthy controls.

Conclusion: Stable long-term LTx pts on maintenance anti-CD25 mAb have lower CD4+FoxP3+ cells vs. SRL and lower CD4+CD25+CD127Low cells vs. SRL and CNI, without any evidence of acute rejection. Further studies are necessary to determine the relationship between peripheral blood and biopsy sample T-cell subsets.

#487

PRE OPERATIVE IMMUKNOW? CYLEX ASSAY PREDICTS REJECTION RISK IN KIDNEY TRANSPLANT PATIENTS

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Introduction and Objectives: Currently, immunotherapy is titrated based on toxicity and drug levels, independent of the true immune state. The ImmuKnow® assay measures cell-mediated immunity by quantifying ATP release from

CD4+ T-cells in peripheral blood. Theoretically, patients with lower levels are predisposed to infection/malignancy, while higher values are associated with rejection. Herein we hypothesized that this assay could predict complications associated with over/under-immunosuppression in kidney transplant (KT) patients.
Methods: Sixty seven patients undergoing KT were recruited prospectively and had ATP levels measured pre-operatively, and at specified intervals over 2 months. Clinicians were blinded to ATP levels. Clinical events including rejection, infection/cancer were documented with a median follow-up of 21 months (3-35). Parameters including absolute ATP levels and changes in ATP patterns (slopes, delta) were analyzed. Association between ATP parameters and clinical outcomes were compared using the likelihood ratio test and Kaplan Meier curves.
Results: Absolute ATP values post-operatively had poor predictive value with regards to rejection or infection/malignancy. As well, changes in ATP values were poorly associated with complications. Importantly, patients with pre-transplant ATP values ≤300 ng/ml had significantly less rejection episodes vs. those with ATP values >300 ng/ml (($p<0.0001$, Figure). As well, the ≤300 ng/ml group also had significantly delayed time to rejection event vs. the >300 ng/ml group ($p<0.05$). When pre-transplant ATP values were further divided roughly into tertiles, with values <250, 250-350, and >350 ng/ml, the patients in the lowest tertile had far fewer rejections than the remaining groups. There were no significant differences in gender, age, etiology of renal failure, immunotherapy, or sensitization risk (%PRA, HLA match) between these two groups.
Conclusions: For the first time, we have evidence that a pre-operative ImmuKnow® level can stratify KT patients into low/high risk groups for rejection. Future studies used to assess the utility of this assay to design individualized immunosuppressive regimens are required.

#488

PHENOTYPE ANALYSIS OF T-CELL SUBSETS IN LONG-TERM HEART TRANSPLANT PATIENTS ON MAINTENANCE ANTI-CD25 MONOCLONAL ANTIBODIES VS. CALCINEURIN INHIBITORS AND SIROLIMUS

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Background: We previously described the use of anti-CD25 monoclonal antibodies (mAb) for maintenance immunosuppression to replace calcineurin inhibitors (CNI) in heart transplant (HTx) pts with chronic kidney disease.
Purpose: To determine the impact of maintenance anti-CD25 mAb immunosuppression vs. CNI and sirolimus (SRL) on T-cell subsets in long-term HTx pts with chronic kidney disease.
Methods: This was a cross-sectional study in stable (no evidence of infection or acute rejection) adult HTx pts (>1-yr post-HTx), including 9 pts on CNI (9 on mycophenolic acid (MPA)), 6 pts on SRL (6 on MPA) and 9 pts on anti-CD25 mAb (7 on MPA and 2 on SRL). Pts on anti-CD25 mAb received daclizumab 1.5 mg/kg q.2 months, keeping the CD25 saturation rate (measured on peripheral CD4+ cells by flow cytometry) >98%. Th-1, Th-2, Th-17 and T-regs were analyzed in peripheral blood using flow cytometry.
Results: Results are shown in Table 1.

Table 1. T-cell subsets

	CNI	SRL	Anti-CD25 mAb	Healthy controls
CD3+ (%)	55±13	59±16	57.5±14	71±10
CD8+ (%)	31±16	31±13	38±18	36±9
CD4+ (%)	57±19	48±15	39±19	46±13
CD4+FoxP3+ (%)	7±1.6	11±3	8±2	9.5±4.9
CD4+CD25+CD127Low (%)	4.4±1.9	6.4±2	1.9±2[a]	6.4±0.8
FoxP3+ in CD4+CD25+CD127Low (%)	73±5.4[b]	86±6	77±5.7[c]	81±5

[a]$P<0.001$ vs. SRL and healthy controls and $P<0.05$ vs. CNI, [b]$P<0.001$ vs. SRL and $P<0.05$ vs. healthy controls, [c]$P<0.05$ vs. SRL

Conclusion: Long-term HTx pts on maintenance anti-CD25 mAb have lower CD4+CD25+CD127Low cells vs. SRL and CNI, and lower FoxP3+ in CD4+CD25+CD127Low vs. SRL treated pts without any evidence of acute rejection. The absence of acute rejection suggests that further studies are required to assess the relationship between peripheral blood and endomyocardial biopsy T-cell subsets.

#492

CAN WE IMPROVE BK VIRUS PROGNOSIS?

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Introduction and Aims: BK virus (BKV) is a serious problem in renal transplantation. 10 to 20% of kidney recipients will develop BK viremia and about 5% will progress to BK nephropathy with an estimated graft loss of 8 to 13 / 100 patient-years. The aim of this report was to evaluate the impact of two protocols on BK nephropathy (BKN) development, serial viral loads, graft function and survival.
Method: 293 subjects transplanted between January 2005 and January 2010 were included in this observational, longitudinal retrospective study. Until June 2007 (first period), screening was done bimonthly for 2 years and annually thereafter until year 5. Upon detection of a sustained viremia above 10^4/mL, graft biopsy was performed and BKN was treated by a reduction of all immunosuppressants or a switch from MMF to Leflunomide. After June 2007 (second period), screening was done monthly for 2 years. Any sustained viremia was treated by a reduction/discontinuation of MMF. Biopsy was performed only for graft dysfunction or important viremia and Leflunomide was used only if acute rejection was found concurrently with BKN. Statistical analysis between periods was conducted using Fisher's and log rank tests and mixed models.
Results: BK viremia developed in 29/143 subjects (20%) in the first period compared to 41/150 (27%) in the second period (p=0.17). Among the biopsied subjects (first vs. second period: 19/29 vs. 20/41; p=0.22), the incidence of BKN was significantly less in the second period (68 vs. 25%; p<0.01). Viral clearance was also higher and earlier in this group (p=0.017). In a longitudinal model of serum creatinine adjusted for recipient and donor's age and gender, graft function was better in the second period for all time points studied (all p<0.01). Within viremic patients, 2 graft losses were seen (incidence of 1.3 / 100 patient-years post transplant). Three acute rejections and three borderline rejections were observed after modification of immunosupression.
Conclusions: Strict monitoring and prompt reduction/discontinuation in MMF is an effective method to achieve a more efficient reduction in BK viral loads and seems to favour a better renal function.

#493

PRELIMINARY RESULTS OF RANDOMIZED CONTROLLED TRIAL ASSESSING THE IMPACT OF VERAPAMIL IN PULSATILE PERFUSION SOLUTION

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We have previously shown that cold storage perfusate flush with erapamil improves renal allograft function. As well, there is evidence that pulsatile perfusion improves renal allograft function vs. cold storage. Herein, we examine the impact of verapamil supplementation to pulsatile perfusion solution.
Thus far, a total of 20 kidneys were randomized to storage using 5 mg verapamil instillation into pulsatile perfusion solution vs. storage with standard pulsatile perfusion solution alone (Lifeport). Using paired kidney analysis model, in which one kidney from each donor was treated with control and the other with verapamil, vascular resistance, urine output, delayed graft function (DGF) rate, GFR (MDRD), and graft survival was analyzed. As well, complications including hypotension and rejection were recorded as well.
Recipient demographics were similar between treatment groups. Although verapamil reduced the early vascular resistance and increased flow within the pumped kidneys, differences were not detectable within 1 hr of pulsatile perfusion therapy. As well, although urine output was higher in the verapamil group vs. control (3.7L vs. 2.6L/24hr), DGF and slow graft function rates were similar between groups. Accordingly, 1 week GFR was superior in the verapamil group (63.1 vs. 47.9 umol/l; p<0.01) as well as 1 year post-operatively (84.1 vs. 68.9; p<0.05). Overall graft and patient survival as well as complication rates were similar between groups.
Although numbers are small, these favorable results support continued evaluation of verapamil supplementation to pulsatile perfusion solutions. We continue to accrue patients and follow long-term outcomes.

#500

LOWER TIME-DEPENDENT MMF DOSE IS ASSOCIATED WITH DEATH CENSORED GRAFT LOSS AFTER KIDNEY TRANSPLANTATION

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Background: Reduction of mycophenolate mofetil (MMF) dose has been shown to predict graft loss in registry

analyses. In those analyses, however, MMF dose reduction was considered as categorical variable or dose at a certain time point. We are aware of one study, which looked at time-dependent MMF dose and the risk of acute rejection.
Purpose: To determine whether time-dependent MMF dose after renal transplantation was associated with increased risk of subsequent death censored graft loss.
Methods: Data was analyzed from 700 patients (51±13 yr-old, 64% men, 32% diabetics) who received a renal transplant between January 1997 and December 2009. The patients were discharged home on MMF. Cox regression was used to model MMF dose as a time-dependent variable, with time to death censored graft loss as the primary outcome.
Results: 92 patients experienced death censored graft loss during the follow-up period (median follow-up 2159 days [range: 337-5070]). Each 250 mg lower time-dependent MMF dose (a measure of MMF exposure over the follow-up period) was a significant predictor of death censored graft loss (HR 1.12, 95% CI 1.02-1.12, p=0.019) even after adjustment for recipient age, gender, baseline diabetes, hypertension, smoking status, type of donor (deceased or living; standard vs. expanded criteria), number of HLA mismatches, the presence of acute rejection or delayed graft function.
Conclusion: Lower time-dependent MMF dose is a significant predictor of death censored graft loss after renal transplantation. Further studies should assess the impact of MPA therapeutic drug monitoring in renal transplant recipients requiring MMF dose reduction.

#501

LONG-TERM FUNCTION AND CLINICAL OUTCOME OF PEDIATRIC KIDNEYS TRANSPLANTED TO ADULTS: A SINGLE-CENTER EXPERIENCE

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Introduction and Objective: There is an increasing discrepancy between supply and demand in renal transplantation. Utilization of pediatric deceased donor kidneys has been introduced to address this problematic. In this study, we reviewed cases of en bloc and single pediatric kidneys transplanted to adults and evaluated allograft long-term function and survival.
Methods: From April 1990 through June 2009, 61 adult recipients received deceased donor renal transplants from donors aged 10 years or less in a single center. Twelve cases were performed with en bloc kidneys (EBK) and 49 cases were done with single separated allograft (SK). Graft outcomes from these pediatric donors were reviewed for complications, long-term renal function and survival. We compared all parameters with a cohort of 900 adult kidney donors transplanted in the same institution (living donors, standard or extended criteria donors (ECD)).
Results: Dual pediatric donors were on average younger (4 vs 7 years) and weighted less (15 vs 27 kg) than single pediatric donors. Single pediatric donors were associated with a significantly higher rate of renal artery stenosis (TRAS) comparatively to adult donors (18.0 vs 1.4, 4.5 and 1.3%). Acute rejection, thrombosis, urinary leak and ureteral stricture were similar between all groups. Concerning estimated glomerular filtration rate (eGFR), single pediatric donors were comparable to other groups and superior to ECD at 3, 12, 36 and 60 months. Dual pediatric donors demonstrated an increasing eGFR from 61 ml/min/1.73 m^2 at 3 months to 83 ml/min/1.73 m^2 at 60 months. They were associated with a significantly higher eGFR compared to standard, extended and living donors at 36 months (83 vs 49-63 ml/min/1.73 m^2). Graft survival was comparable in all transplant groups, but living donors and dual pediatric donors showed a trend for a better graft survival.
Conclusions: Pedia tric donor kidney transplant is a viable option to address shortage of organs. EBK transplant allowed utilization of small donors with excellent outcomes. Despite a higher rate of TRAS, SK transplant achieved good function and survival comparable to adult standard deceased and living donors, with the potential advantage of giving access to transplantation to more listed-patients. This article did not assess the optimal selection criteria that should be used to decide between EBK and SK. Future studies should try answering this important question.

#502

CD4 T HELPER CELL PROFILING IN HEART TRANSPLANT RECIPIENTS: COMPARISON BETWEEN CNI-, RAPAMYCIN- AND ANTI-CD25 MAB-BASED IMMUNOSUPPRESSION

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Background: Anti-CD25 monoclonal antibodies (mAb) have been shown to reduce the incidence of acute rejection in solid organ transplantation (Tx). We have used anti-CD25 mAb or rapamycin for maintenance immunosuppression as a substitute to CNI in pts with chronic kidney disease (CKD).

Purpose: To determine whether there is a difference in Th1, Th2 and Th17 between heart Tx pts on maintenance CNI-, rapamycin- and anti-CD25 mAb-based immunosuppression.
Methods: Four groups were compared. Group 1 (n=11) included heart Tx pts who were converted from CNI to CD25 mAb (daclizumab) for >6 months due to CKD; Group 2 (n=12) included heart Tx pts on CNI; Group 3 (n=5) included heart Tx pts on rapamycin and Group 4 (n=6) included healthy control. All pts on CNI were on mycophenolic acid (MPA) (11 on mycophenolate mofetil (MMF) and 1 on mycophenolic sodium (MPS)). All pts on rapamycin were on MMF. Pts on anti-CD25 mAb were on either MPA or rapamycin (3 on rapamycin, 2 on MPS and 6 on MMF). None of the pts experienced acute rejection. Peripheral blood mononuclear cells were isolated on density-gradient, and cultured with PMA, ionomycin and brefeldin for 6 hours. Cell surface staining with anti-human CD4 (FITC) and intracellular staining with anti-human IFN-γ, IL-4 and IL-17A antibodies (all PE) was then performed for flow cytometry analysis of Th1, Th2 and Th17 respectively.
Results: Th1 and Th17 cells as a proportion of CD4+ T helper cells were lower on CNI (n=4) vs. anti-CD25 mAb (n=6) and healthy controls (n=6). No differences were observed between groups for proportion of Th2 cells.
Conclusion: These results suggest heart Tx pts on CNI have a depressed Th1 and Th17 population as a proportion of CD4+ T helper cells. Pts on anti-CD25 mAb have Th1 and Th17 populations similar to that of healthy controls. This may have implications for risk of infection, malignancy and acute rejection in heart Tx pts.

#503

CD4 T HELPER CELL PROFILING IN LIVER TRANSPLANT RECIPIENTS: COMPARISON BETWEEN CNI-, RAPAMYCIN- AND ANTI-CD25 MAB-BASED IMMUNOSUPPRESSION

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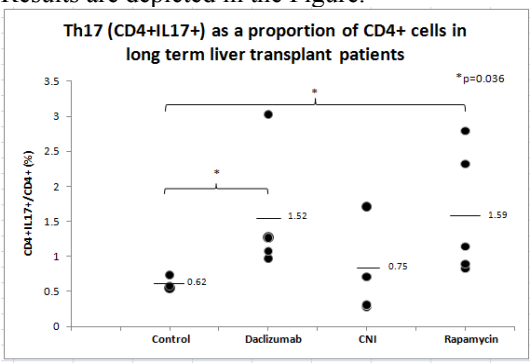
Background: Anti-CD25 monoclonal antibodies (mAb) have been shown to reduce the incidence of acute rejection in solid organ transplantation (Tx). We have used anti-CD25 mAb or rapamycin for maintenance immunosuppression as a substitute to CNI in pts with chronic kidney disease (CKD).
Purpose: To determine whether there is a difference in Th1, Th2 and Th17 between liver Tx pts on maintenance CNI-, rapamycin- or anti-CD25 mAb-based immunosuppression.
Methods: Four groups were compared. Group 1 (n=5) included liver Tx pts who were converted from CNI to CD25 mAb (daclizumab) for >6 months due to CKD; Group 2 (n=4) included liver Tx pts on CNI; Group 3 (n=5) included liver Tx pts on rapamycin and Group 4 (n=3) included healthy controls. All pts on CNI were on mycophenolic acid (3 on mycophenolate mofetil (MMF) and 1 on mycophenolic sodium). Four pts on rapamycin were on MMF and 1 was not. Four pts on anti-CD25 mAb were on MMF and one was not. Peripheral blood mononuclear cells were isolated on density-gradient, and cultured with PMA, ionomycin and brefeldin for 6 hours. Cell surface staining with anti-human CD4 (FITC) and intracellular staining with anti-human IFN-γ, IL-4 and IL-17A antibodies (all PE) was then performed for flow cytometry analysis of Th1, Th2 and Th17 respectively.
Results: Th17 cells as a proportion of CD4+ T helper cells were higher on daclizumab and rapamycin treated pts vs. healthy controls. No differences were observed between groups for proportion of Th1 or Th2 cells.

Results are depicted in the Figure.



Conclusion: These results suggest that liver Tx pts on anti-CD25 mAb and those on rapamycin have an elevated Th17 population as a proportion of CD4+ T helper cells when compared to healthy controls. This may have implications for risk of infection, malignancy and acute rejection in liver Tx pts.

#508

THE EFFECT OF PREVIOUS BARIATRIC SURGERY ON PHARMACOKINETICS OF? IMMUNOSUPPRESSIVE DRUGS IN RENAL TRANSPLANTATION??

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Introduction: Obesity is a significant co-morbidity in patients with end-stage renal disease (ESRD) with increased risks of mortality after transplantation, delayed graft function and development of post-transplant diabetes. Listing is usually denied to morbid obese patients at most centers, though a defined limit does not exist.

Gastric bypass (GB) has been shown to be effective in the ESRD and renal transplant (RT) populations. Recently, laparoscopic GB performed after RT has demonstrated effective weight loss and improvement in diabetes, hypertension and hyperlipidemia. Pre-transplant, GB may also result in recovery of renal function, even obviating dialysis.

Few studies have described the pharmacokinetics of immunosuppressive medications post-GB and none have reported on newer delayed release formulations.

Case: 62 year old male had ESRD secondary to focal segmental glomerulosclerosis. His medical history included hypertension, diabetes, morbid obesity and Roux-en-Y GB. The pre-transplant BMI was 31.2 kg/m^2.

Prior to transplantation, enteric coated (EC) mycophenolic acid (720 mg BID) was given and the area under the concentration vs. time curve was calculated using the linear trapezoidal method. The mycophenolate C_{max} = 6.5 mg/L, T_{max} = 4 hours and AUC_{0-12h} = 58 mg*h/L. A trial of tacrolimus 8 mg BID had a C_{trough} = 16.7 mcg/L.

Subsequently, he received a living donor kidney transplant from his brother, also post-GB, who was a complete HLA match. There were no peri-operative complications. The serum creatinine at discharge was 136 umol/L. Immunosuppression included prolonged-release tacrolimus, EC-mycophenolic acid and steroids.

Conclusion: Delayed-release formulations have adequate intestinal absorption in a recipient with prior bariatric surgery. In the early post-transplant period, they have been a safe and effective immunosuppressive regimen.

#509

MICROVASCULAR LESIONS IN RENAL BIOPSIES FROM DSA+ PATIENTS ASSOCIATE WITH THE NK CELL BURDEN BUT NOT THE T CELL BURDEN

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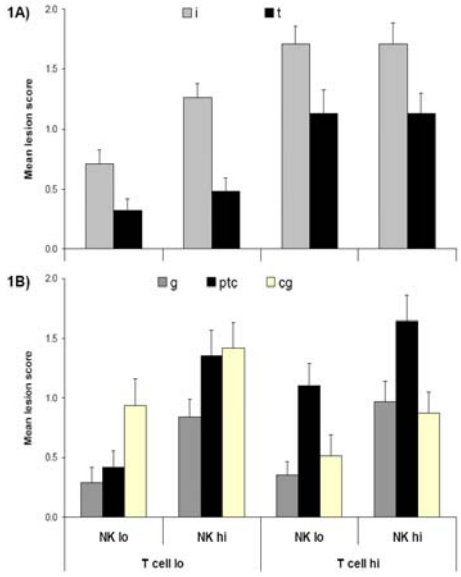
Introduction: The diagnosis of ABMR relies on the presence of donor specific antibody (DSA), C4d deposition, and certain histologic lesions. However, the histopathology of ABMR is subtle, C4d is insensitive, and not all DSA+ patients progress to ABMR. We previously showed that NK cells selectively associate with ABMR but not T cell mediated rejection (TCMR). To assess the T and NK cell burdens in a biopsy, we generated transcript lists exclusive to each cell type and examined their associations with histologic lesions and C4d staining.

Methods: 124 clinically indicated biopsies from DSA+ patients were processed for histopathology and microarray analyses. Transcripts with high expression in purified T cells (5 transcripts) or NK cells (4 transcripts) but not B cells, macrophages, endothelial or renal epithelial cells were selected. T and NK cell burdens were calculated as the geometric mean fold change of a biopsy versus normal kidney calculated across all transcripts in each transcript set. Paraffin sections were graded according to the Banff criteria.

Results: Transcripts exclusive for T cells or NK cells did not include any cytotoxic molecule transcripts commonly associated with rejection. T and NK cell transcript set scores in biopsies from DSA+ patients correlate with one another (Spearman r=0.5283) but skewing towards each cell type was evident (not shown). Biopsies were split into high ($\geq 50^{th}$ percentile transcript set score) or low ($<50^{th}$ percentile) T cell burdens and then each subdivided in high or low NK cell burdens. Interstitial inflammation (i) and tubulitis (t) scores were primarily associated with the T cell burden with NK cells contributing little particularly in the high T cell burden biopsies (Figure 1A). Microvascular inflammation lesions; glomerulitis (g) and peritubular capillaritis (ptc), both showed a strong association with the NK cell burden. The T cell burden contributed little to g lesions, but had a more dramatic effect with ptc (Figure 1B). Transplant glomerulopathy (cg) did not show associations with the T or NK cell burdens with higher cg scores in biopsies with a lower T cell burden. T and NK cell burdens across C4d negative, C4d focal, and C4d diffuse staining biopsies did not differ ($p>0.09$, 1 way ANOVA).

Conclusion: The individual assessment of T cell and NK cell burdens in a biopsy is possible using a small number of transcripts. Interstitial inflammation and tubulitis are primarily driven by the T cell burden in a biopsy in contrast to microvascular inflammation where the NK cell burden is predominant. Transplant glomerulopathy is largely T cell and NK cell inactive reflecting the lack of immune activity in many such biopsies. Neither the T nor NK cell burdens differed across the C4d staining patterns further demonstrating the prevalence of C4d negative

ABMR. Assessment of these two cell types will be critical in deriving a more accurate threshold for the diagnosis of TCMR, ABMR, and Mixed types of rejection.



#513

THE IMPACT OF A PREDICTABLE ORGAN ALLOCATION SCHEME ON MORTALITY AFTER KIDNEY TRANSPLANTATION

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The growth in the kidney transplant waiting list has led to an increased interest in strategies that preserve the benefit of transplantation despite increased waiting times.
One such strategy is a predictable organ allocation scheme which allows for the identification of upcoming transplant candidates and optimization of their peri-operative medical management. One of the potential benefits of the UNOS ECD program is the implementation of a predictable allocation scheme in which organs are allocated by waiting time only.
Using data from the USRDS, we studied n=13,168 recipients of ECD transplantation. We calculated the change in mortality during the first year after transplantation before and after the implementation of the ECD program in the United States using Poisson Regression.
The unadjusted rate of death in the first year after ECD transplantation increased from 8.1 to 10.2 per 100 patient years after the implementation of the ECD program in October 2001. However after adjustment for differences in donor quality (Donor Profile Index(1)) and recipient characteristics, implementation of the ECD program was associated with a decreased relative risk of death in the first year after transplantation (RR=0.73 (0.64, 0.82)).
In stratified analyses by wait-time, the associated benefit of ECD allocation was lost in recipients with more than 3 years of waiting time (RR=0.87 (0.74, 1.02)).
These results suggest that a predictable organ allocation scheme which provides an opportunity to maximize the peri-operative management of wait-listed candidates is associated with improved post-transplant outcomes but this benefit is not maintained after prolonged waiting times > 3 years.
Predictable allocation of deceased donor organs may be an important strategy to maintain the benefits of transplantation under current conditions in which there are prolonged waiting times for DD transplantation. The benefits of this strategy on outcomes after SCD transplantation should be further studied.

1 Rao P et al. A Comprehensive Risk Quantification Score for Deceased Donor Kidneys: The Kidney Donor Risk Index. Transplantation 2009; 88: 231-236

#515

THE SAFETY AND EFFICACY OF AN ADJUVANT-CONTAINING PANDEMIC H1N1 VACCINE IN RENAL TRANSPLANT RECIPIENTS

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Background: Influenza in renal transplant patients is associated with a higher morbidity and mortality compared to the general population as well as graft rejection. While seasonal influenza vaccination is strongly recommended for renal transplant recipients, there are concerns regarding its efficacy as well as its safety. There is a theoretical concern of sensitization and graft rejection after vaccination, either due to non-specific immune activation or induction of cross-reactivity to transplant antigens. The emergence of the pandemic H1N1 strain of influenza (pH1N1) led to a broad vaccination campaign targeting at risk populations. We sought to determine the safety and efficacy of an adjuvant-containing vaccine against pH1N1 administered to Canadian renal transplant recipients.

Methods: We prospectively followed 42 renal transplant recipients that received the pH1N1 vaccine in the fall of 2009. Seroprotection (titer ≥ 1:40) and seroresponse (≥ 4-fold increase in titer) were assessed using the hemaglutination inhibition assay. Vaccine safety was assessed my measurement of serum creatinine, documentation of vaccine side-effects, and change in clinical condition.
Results: There was a low rate of baseline seroprotection to pH1N1 in our transplant cohort (7%) and a low rate of seroconversion after immunization (31%). In recipients < 1 year post-transplantation (n=7), there was a 0% response to immunization. Recipients receiving double immunosuppression had a higher rate of seroprotection compared to recipients receiving triple immunosuppression (80% vs. 24%, p=0.01). Vaccination was safe with no difference in creatinine pre- or post vaccination (133 umol/L vs. 140 umol/L, p=n.s.) and no rejection episodes occurred. No medical attention was necessary for vaccine-related side effects. The most common side effects were sore arm at the injection site (36%) and muscle or joint pain (21%).
Conclusions: An adjuvant-containing vaccine to pH1N1 was safe in a cohort of Canadian renal transplant recipients. Seroprotection after vaccination was low in our cohort. Recent transplant within 12 months and triple immunosuppression were associated with a poor seroresponse.

#519

HMGB-1 And HSP-70 In Mechanically Preserved Kidney Allografts As Markers Of Long Term Function
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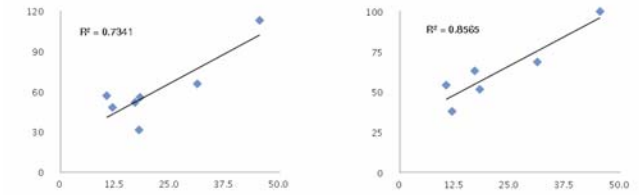
Introduction: Biomarkers measurable during kidney preservation, and which could accurately predict post-transplant function have not been identified. High-mobility group box 1 (HMGB1) is a nuclear protein that is released into the extra-cellular space in the event of non-programmed cell death. Heat-shock protein-70 (HSP-70) is part of a family of stress proteins, released in response to various stresses. We examined these 'danger signals' to investigate their use as surrogate markers for pre-transplant kidney allograft viability, and as predictors of post-transplant kidney function.

Methods: Eight consecutive adult renal allografts were placed on the LifePort pulsatile perfusion system prior to transplantation. Samples of KPS-1 (Belzer)preservation solution were taken from the unit after 10 minutes, 1 hour, 6 hours (if possible) and pre-transplantation. Using commercially available ELISA kits, we detected t he presence of HMGB1 and HSP-70. We compared the concentrations of HMGB and HSP-70 at the above time points to glomerular filtration rate (GFR) using the Modification of Diet in Renal Disease (MDRD) equation, at 6 months and 1 year post-transplantation. The coefficient of determination was calculated to identify the relationship. Pearson's correlation coefficient was used to determine statistical significance.
Results: The concentration of HMGB1 at 1 hour of preservation correlated well with both the 6-month and 1-year MDRD GFR (R^2=0.7341 p=0.008 and 0.8565 p=0.014, respectively). The 6 hour HMGB1 correlated less well with these outcomes (R^2=0.3978 p=0.129 and 0.5418 p=0.095 for 6 month and 1 year GFR, respectively). The concentration of HSP-70 at 1 hour and 6 hours of preservation correlated poorly with both 6-month and 1 year GFR.
Conclusion: In this study, we have shown for the first time, that concentrations of HMGB1 measured after 1 hour of pulsatile preservation can predict kidney allograft function at 1 year. Surprisingly, the relationship is direct, rather than inverse, suggesting that early accumulation of HMGB1 in preservation fluid could represent active secretion by healthy cells, while later accumulation could be the result of a combination of processes including cell death.



#522

ALLORECOGNITION OF DENDRITIC CELLS PREVENTS THEIR REGENERATION AND DIMINISH CD4 HOMEOSTATIC PERIPHERAL EXPANSION DURING GRAFT-VERSUS-HOST-DISEASE.
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Allogeneic bone marrow transplant (BMT) is an effective treatment for numerous types of haematological malignancies. However, graft-versus-host disease (GVHD) is a serious complication and its adverse effect on T cell regeneration greatly exaggerates the immunodeficiency normally associated with BMT. To understand how anti-

host reactivity constrains T cell regeneration, we used the mouse model B6→B6D2F1 to measure the effect of GVHD on CD4 homeostatic peripheral expansion (HPE). Our lab demonstrated that IL-7 signalling in DCs constrains CD4 HPE during lymphopenia. Since IL-7 levels are also elevated during GVHD, we hypothesized that this mechanism might also contribute to diminish T cell regeneration in this setting. To regenerate a peripheral niche permissive for CD4 HPE, we transplanted B6D2F1 with bone marrow from IL-7R□-/- mice and GVHD was induced with 1x106 B6 T cells. CD4 HPE was measured at day +28 by transferring CFSE labelled anti-HY transgenic CD4+ T cells. As predicted, TCR transgenic CD4 T cells transferred into non GVHD host underwent robust HPE. In contrast, GVHD animals failed to support CD4 expansion, suggesting that IL-7 signaling in DCs is not the factor limiting CD4 HPE during GVHD. Loss of CD4 HPE in GVHD host was associated with a severe depletion of all DC subsets by day +35 post-BMT. Surprisingly, BM progenitors obtained from GVHD and non GVHD hosts produced similar number of DCs when cultured in vitro. Importantly, using an in vivo cytotoxic assay, we showed that B6 DCs obtained from B6→B6D2F1 chimeras are specifically recognized and eliminated by alloreactive T cells when transferred into GVHD host, suggesting that allogeneic peptides derived from DBA mice are presented by B6 DCs. In conclusion, our data demonstrates that alloreactive T cells can sustain immunosuppression by eliminating and preventing DC regeneration after allogeneic bone marrow transplantation.

#523

OPTIMIZATION OF FLOW CYTOMETRIC CROSSMATCH ASSAY TO EXPEDITE PRE-TRANSPLANT IMMUNOLOGIC RISK ASSESSMENT.

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Aim: A pre-transplant flow cytometric crossmatch (FCXM) is performed to assess whether potential allograft recipients have donor directed HLA antibodies. The standard three color FCXM protocol was described over 20 years ago and is currently used by most HLA labs in Canada and United States. The original protocol is time intensive and can contribute significantly to transplant delays and increased graft ischemia time. Therefore, the goal of this study was to develop an expeditious FCXM procedure without compromising sensitivity of the assay.
Methods: Initially, pooled positive sera was used to investigate the effects of varying serum incubation time (3-30 min), anti-IgG-FITC incubation time (5-30 min), and wash time (5 and 1 min) on FCXM . The effects of cell concentration and serum volume were also assessed. Based on those results, an optimized FCXM protocol was developed and then compared to the standard assay. Briefly, FCXM studies were performed with 20 well characterized patient sera and five volunteer donor cells predicted to result in a positive (n=15) or negative (n=5) crossmatch. Median channel fluorescence (MCF) shifts of ≥70 and ≥105 channels beyond the negative control were considered as positive crossmatches for T and B cells respectively.
Results: Decreasing wash steps from five minutes to one minute had no impact on MCF shifts or FCXM interpretation. MCF shift values plateued after 15 minutes of serum incubation with cells, and although there was a marginal increase in MCF shift at 30 minutes, differences were not significant. Importantly, there were no appreciable differences in MCF shifts at any anti-IgG-FITC incubation time points ranging from 5-30 minutes. As expected, MCF shifts increased slightly when cell concentrations were decreased and/or serum volumes were increased. The optimized FCXM protocol based on the above results included: a) decreased serum incubation time (from 30 to 15 minutes), b) decreased anti-IgG-FITC incubation time (from 30 to 10 minutes) and c) decreased wash times (from 5 minutes to 1 minute per wash). Comparison between the standard and the optimized protocols in 20 FCXM studies demonstrated 100% concordance in FCXM results with no significant differences in MCF shifts between the two methods.
Conclusion: An optimized FCXM protocol has been developed which reduces crossmatch assay time >50%. Implementation of this protocol will expedite pre-transplant testing.

#524

TIMING OF RABBIT ANTI-THYMOCYTE GLOBULIN INDUCTION THERAPY: IS THERE AN IMPACT ON KIDNEY TRANSPLANT OUTCOMES?

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Background: Rabbit anti-thymocyte globulin (rATG) is a commonly used induction agent in kidney transplant recipients (KTR). Literature regarding timing of first dose of rATG administration and effect on outcomes is limited.
Objectives: To compare graft and patient outcomes in KTR receiving the first dose of rATG post-operatively vs.

prior to cross-clamp removal over a median 2.5 years of follow-up.

Methods: Incident KTR from 1 Jan 2002 to 31 Dec 2009 receiving rATG induction were included. Multi-organ recipients, cases of hyperacute rejection, primary non-function, and patients in desensitization protocols were excluded. Standard baseline recipient, donor and transplant characteristics were assessed. Indication for induction, timing of first dose, and characteristics of treatment (*e.g.* total weight-adjusted dose) were also collected. Outcomes included: (1) CKD-EPI eGFR at 1-year post-transplant; (2) change in eGFR at 12 vs. 1 month; (3) delayed graft function (DGF); (4) biopsy-proven acute rejection (BPAR); and (5) total graft failure (including death). The impact of timing of induction on the various outcomes was adjusted for potential confounders and assessed using linear, logistic and Cox proportional hazards regression models.

Results: Of the 487 KTR included, 349 (71.7%) received rATG post-op (Post) and 138 (28.3%) prior to cross-clamp removal (Pre/Intra). Post patients were slightly older but other baseline characteristics did not differ. The most common indication for rATG induction was a high-risk recipient in the Post (37.5%) and Pre/Intra groups (58.7%). Of patients surviving with graft function at 12 months (n = 424) there was no significant difference in mean CKD-EPI eGFR (69.1 vs 73.2 mL/min) and delta eGFR (1.6 vs 1.2 mL/min) for Post vs. Pre/Intra groups at 1-year. Incidence of DGF was 34.9% vs. 24.6% with an adjusted OR 1.67 (95% CI, 0.95-2.93, $P = 0.07$) for Post vs. Pre/Intra. Time to first BPAR (HR 0.67 [95% CI, 0.37 – 1.21, $P = 0.18$]) and total graft failure (HR 1.07 [95% CI, 0.59-1.95, $P = 0.82$]) did not significantly differ between Post vs. Pre/Intra. Results remained unchanged when patients with DGF were excluded.

Conclusions: Post-operative administration of rATG has no appreciable negative impact on renal function at 1-year post-transplant or on the risks of DGF, BPAR and total graft failure. These results offer support for more flexible timing of rATG induction therapy in KTR.

#525

DIALYSIS PATIENT ATTITUDES TOWARDS DECEASED DONOR KIDNEY ALLOCATION: A QUALITATIVE STUDY

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We conducted a qualitative study of patients currently on dialysis and awaiting kidney transplantation in order to understand their attitudes and concerns toward the current provincial transplant association kidney allocation policy. Two groups (individuals age 54 years and under and those 55 years and older) participated in professionally moderated focus groups. Aspects of the current allocation system were explained to participants including blood type matching, priority for highly sensitized patients and pediatric patients and allocation of kidneys based on donor and recipient age matching. 11 participants expressed a broad range of opinions on the allocation system, though there was general concensus that the system seemed reasonable and fair. There were few major concerns with the allocation of organs from younger donors to younger recipients and older organs to older recipients. Common themes in participant responses included: 1. lack of knowledge regarding organ allocation criteria and wait-list management 2.need for better patient education 3. concern regarding the lack of transparency and possible favoritism in organ allocation 4.involvement of patient representatives or groups in future allocation decisions. The results of the focus groups have spurred major policy change within the provincial organ allocation system, including greater transparency about the organ allocation criteria to the public and the establishment of an allocation review panel that reports to the executive board of the health region and ensures that allocation rules are being followed.

#526

POST-TRANSPLANT VENOUS THROMBOSIS AND EFFECT ON GRAFT SURVIVAL

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Introduction: Venous Thromboembolic events (VTE) are major causes of postoperative morbidity and mortality. The objective of this study is to establish the prevalence of VTE in liver and kidney transplant recipients and to assess its impact on graft survival.

Methodology: Using our prospectively collected transplant database, patients diagnosed with VTE from 1985 till 2010 were identified. After excluding recipients of combined grafts and late VTE development (90 days post-transplantation), 2228 recipients were included. Prevalence of postoperative VTE, graft and patient survival were determined. Other prognostic factors were also collected (age, sex, cold ischemia time, donor risk index, and extended criteria donors).
Results: The prevalence of VTE in Kidney and Liver recipients was 1.634 % and 2.292 % respectively.
Of the 1530 kidney transplant patients, 25 developed VTE and of those 3 had PE. Mean graft survival in patients without VTE was 20.5 years while for those with VTE was 12.3 years. Hazard ratio (HR) obtained using Cox regression models was 1.5 (95%CI: 0.5-3.9) but such an effect was not statistically associated (p = 0. 447). Adjusting for confounders did not alter this finding.
Of 698 liver transplant patients, 16 had VTE and of those 4 had PE. Mean graft survival in patients without VTE was 11.9 years while for those with VTE was 4 years. HR associated with VTE was 1.3 (95%CI: 0.5-3.3) but such an effect was not statistically associated (p =0.551). Adjusting for confounders did not alter this finding.
Conclusion: VTE increases the risk of graft failure by 30%. However, in our study these effects were not statistically significant due to under power in detecting the effect of small VTE prevalence. If the impact of VTE and PE are proven in further larger scale studies there could be important clinical implications in the prevention of VTE in transplant recipients.

#527

POOR SEROPROTECTION AFTER H1N1 VACCINATION IN KIDNEY TRANSPLANT RECIPIENTS

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Although influenza vaccination is widely recommended for people that are immunosuppressed, the same immune dysfunction that can increase the risk and consequences of influenza infection might also compromise vaccine responses and effectiveness. These distinctions are particularly important in settings of vaccine shortages or in response to a new pandemic, where selective and prioritized allocation of vaccine is necessary. We surveyed all adult kidney transplant recipients seen in our outpatient clinics between October 2009 and January 2010 to determine if they had received the recommended vaccinations for both seasonal (H3N2) influenza and H1N1 influenza. Patients who received H1N1 were tested for a serological response to H1N1.
Serological response was determined using the hemagglutination inhibition assay. Titers were based on duplicate results and a titer of ≥40 was considered to be seroprotective. Overall 16/58 (28%) patients had seroprotective titres against H1N1. Surprisingly, seroprotected patients did not have higher GFR, but more frequently received MPA (Table). No patient developed a confirmed H1N1 infection in follow up. No immunized patient experienced acute rejection within 6 months post transplant, and one patient report prolonged fatigue after immunization. We conclude that vaccination provides limited serological protection against H1N1. Studies are needed to determine the clinical effectiveness of H1N1 vaccination and/ or to identify strategies that may lead to a higher serological response.

	Seroprotected N = 15	Not Seroprotected N = 39	
Age at Vaccine (mean ± std)	54±15	54±15	0.62
% male	12 (80%)	22 (56%)	0.11
Race Caucasian Other	 8(53%) 7(47%)	 14(36%) 25(64%)	0.24
Years since transplantation at time of vaccination ≤ 1 year 1-5yr 6-10yr >10 yr	 6 (40%) 3 (20%) 1 (7%) 5 (33%)	 14 (36%) 14 (36%) 7 (18%) 4 (10%)	0.15
eGFR at vaccination mean (std) median (q1, q3)	 42.8 (14.1) 40 (31, 52)	 56.4 (22.7) 54 (42, 74)	0.03
MPA Azathioprine Neither	7(46.7) 6(40.0) 2(13.3)	34(87.2) 3(7.7) 2(5.1)	0.003
Cyclosporine Tacrolimus Sirolimus Neither	5(33.3) 8(53.3) 1(6.7) 1(6.7)	9(23.1) 29(74.4) 1(2.5) 0	0.08
Maintenance Prednisone Yes	8 (53%)	24 (62%)	0.58
Diabetes	5 (33%)	12 (32%)	0.90
Immunized Before Transplantation	1/15(7%)	3/39(8%)	0.9

#528

BERLIN HEART EXCOR USE IN PEDIATRIC RESTRICTIVE CARDIOMYOPATHY: A CASE REPORT

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The use of ventricular assist devices (VAD) for pediatric end stage heart failure is increasing. The most common indication worldwide is dilated cardiomyopathy as a bridge to cardiac transplantation. There is limited experience in the use of VADs for restrictive cardiomyopathy. We report a case of biventricular support in a 3 year old child with restrictive cardiomyopathy.

The patient presented prenatally with a pericardial effusion which persisted after birth requiring a pericardial window. By 2 years of age she showed typical findings of restrictive cardiomyopathy and was listed for transplantation. The patient had failure to thrive and heart failure which were managed medically. At three years of age, she developed acute renal injury on maximal heart failure treatment. The decision was made to support her with biventricular assist devices (Berlin Heart EXCOR®) while awaiting transplantation. Unique bi-atrial cannulation was performed using a 6 mm inflow/outflow cannula with a 6/9 mm connector which allowed the cannulas to be connected to 25 ml RVAD and 30 ml LVAD pumps. The patient was supported for 369 days during which three pump changes were required for fibrin and clot formation. The patient underwent successful ABO compatible orthotopic cardiac transplantation after 658 days on the transplant waiting list. She was discharged home 28 days post transplantation and continues to do well.

This case illustrates successful long-term use of Berlin Heart EXCOR®biventricular support in a pediatric patient with restrictive cardiomyopathy as a bridge to transplantation. It represents the largest duration of support currently reported for this pediatric indication and illustrates a novel method of cannulation which facilitated successful bi-atrial cannulation. We believe patients with restrictive cardiomyopathy with evidence of extra-cardiac end-organ dysfunction should be considered for VAD support.

#529

A NOVEL APPROACH TO OLDER ORGAN DONORS: SCREENING GUIDELINES

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Background: Guidelines for cancer screening of the elderly donor must be evidence-based, but systematic review of such data is sparse. Based on a review of our 20-year experience, we propose a new series of guidelines concerning screening for the three most common malignancies: breast, lung and colon.
Methods: Donor and recipient data was collected on heart (n=245), kidney (n=1084), liver (n=706) and pancreas (n=163) transplants performed between 1990 and 2010 at our institution (n=2189). Transmission of donor malignancy was suspected in 13 recipients, 6 of whom received organs from donors aged between 60-70, and 4 cases over 70. The most common diagnosis was undifferentiated adenocarcinoma (n=6). The donor age distribution was as following: 1115 donors younger than 40, 517 between 40 and 49, 404 aged between 50 and 59, 276 aged between 60 and 69, 83 between 70 and 79 and 3 older than 80. We performed a chi square test with a Yates correction comparing the incidence of cancer in the younger population when compared to the older one. We have conducted a literature review of population statistics for breast, colon and lung and calculated the predicted incidence of cancer in each age group.
Results: The incidence of donor malignancy in the group 70 and older is 4.7% whereas it is 0.18% in the donor population younger than 40 ($p<0.00001$). Women have one chance in 9 of being diagnosed with breast cancer. According to NCI statistics the relative risk (RR) increase to 3.75 in the 70-79 age group (38%), would result in an expected 14 cases in our donor population. The lifetime risk of developing colorectal cancer is about 1 in 20, and the RR in the 70-79 age group when compared to the general population is 2.03 (11.5%), accounting for 8 expected cases. One in 11 men and one in 16 women are expected to develop lung cancer during their lifetime, the RR being 3.96 in the 70-79 population, with an expected 24 cases in our donors. Therefore, we observe that the incidence of cancer transmission was considerably lower than the expected rate of occult cancer in the 70-79 year old donor population.
Conclusion: Cancer transmission to transplant recipients has been relatively rare. However, the potential for cancer transmission, based on changing donor demographics, will only increase in the future. In order to balance demand with safety of the organ supply, we propose standardized screening for all donors above the age of 70 for breast, colon and lung cancer. This screening program needs to be

examined prospectively and longitudinally for efficacy and cost effectiveness.

#530

THE EFFECT OF CAVAL ANASTOMOTIC TECHNIQUES ON RENAL FUNCTION POST LIVER TRANSPLANTATION

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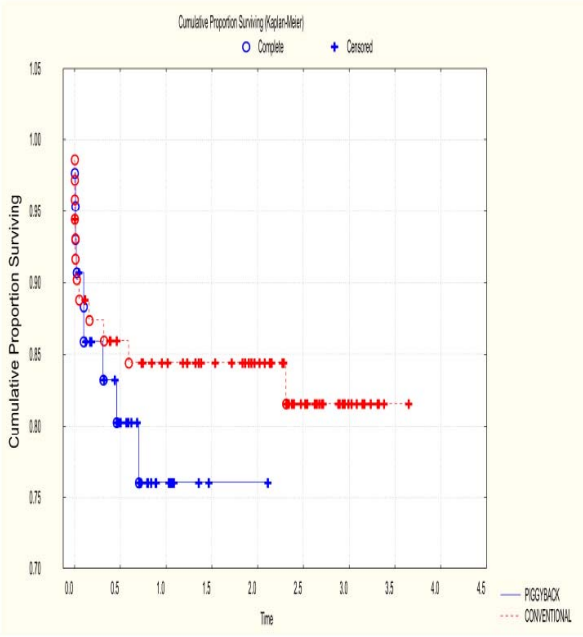
Introduction: Renal impairment is not uncommon following liver transplantation and has major effects on graft and patient survival. Our aim is to determine the impact of caval anastomoses employed during liver transplantation on post-operative renal function. This involves comparing the traditional caval replacement without veno-venous bypass to the piggyback technique.

Methods: Retrospective review of cadaveric liver transplants performed between January 2007 and June 2010. Cases involving other methods of caval anastomosis, sever pre-operative renal impairment, and combined transplants were excluded. A total of 117 patients were analyzed, 43 in the piggyback group vs 72 in the caval replacement group. Prospectively collected data regarding patients demographics, cause of liver cirrhosis, MELD score and donor risk index were analyzed retrospectively. Renal function status was determined by estimating MDRD values pre-operatively and on post-operative days 1, 7 and 30. We also included in our analysis patient and graft survival, blood loss, ischemia times, operative time, hospital stay and biliary and vascular complications. Univariate comparisons of variables between anastomosis groups were done using the Mann Whitney U test. Multivariate Cox regression analysis was used to determine factors related to graft survival.

Results: Univariate analysis identified higher MDRD at 30 days post-operatively, shorter hospital stay and lower cold ischemia time in the piggyback group (p= 0.033, 0.045, 0.021 respectively). However greater blood loss was encountered in the piggyback group (p= 0.064). There was no significant difference in graft survival between the anastomosis groups (Figure 1). Multivariate analysis showed a direct association between graft failure and longer warm ischemia (p=0.043) as well as vascular thrombosis (p=0.003).

Conclusion: Piggyback anastomosis offers less impact on kidney function with higher post-operative MDRD and is associated with shorter hospital stay compared to standard caval replacement. This represents less morbidity and probably better graft outcome on long term follow up.



#531

ACUTE CELLULAR REJECTION HAS A NEGATIVE IMPACT ON 10-YEAR GRAFT SURVIVAL IN KIDNEY TRANSPLANT RECIPIENTS OF EXPANDED CRITERIA DONORS

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Background: Acute cellular rejection (ACR) impacts kidney graft survival depending on the time of onset, severity, and treatment response. The purpose of this study is to determine the impact of ACR on graft survival in kidney transplant recipients of expanded criteria donors (ECD).
Methods: We analyzed 386 recipients with actual 10-year graft survival data of deceased kidney transplants from 1990 to 2000. Kaplan-Meyer survival curves were generated for patients with and without ACR, adjusted for recipient age, donor age, gender, ACR frequency, cold time ischemia and UNOS ECD criteria definition.
Results: Median graft survival of all kidney transplants was 10.6 years (95% CI: 9.9 to 11.3). Median graft survival in patients with ACR was 9.4 years (95% CI: 8.1 to 10.7) and 11.3 years (95% CI: 10.7 to 11.8) in those without ACR. Compared to patients without ACR, the hazard ratio (HR) for graft loss secondary to ACR was 1.12 (95% CI 0.86 to 1.45, p=0.08). When adjusted for age and gender of recipients and age of donors, the ACR effect became significantly associated with graft loss (HR 2; 95% CI 1.5 to 2.7; p=0.016). The ACR effect is increased by younger recipient age (HR 0.98; CI 0.97 to 0.99; p=0.02). The impact of ACR on graft survival was not changed by the UNOS ECD criteria definition or by the other variables.
Conclusion: The risk of graft loss with ACR becomes twice as high when adjusting for age and gender of recipients and age of donors. Older recipient seems to have some protection against this effect. This analysis was based on an actual 10-year survival and revealed no effect of cold ischemia time or UNOS ECD criteria on graft survival.

#532

ALPHA-FETOPROTEIN GRADIENT BEFORE LIVER TRANSPLANTATION AS A PREDICTOR OF POST TRANSPLANT TUMOR RECURRENCE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Introduction: Progression of Alphafetoprotein (AFP) before Liver Transplantation (LT) was recently found to predict post transplant tumor recurrence (TR) in patients with Hepatocellular Carcinoma (HCC). The aim of our study was to examine such predictive value of AFP in patients with or without pre transplant interventional therapy for control of HCC.
Methods: We reviewed 144 patients transplanted for HCC at the University of Alberta between 1992 and 2009. Group 1 patients received therapeutic interventions on the waiting list while group 2 didn't. The AFP gradient (AFP-G) was calculated by dividing the difference between the last 2 AFP readings before LT by the time interval and it was expressed as ng/ml/month.
Results and Discussion: The median follow up was 4.96 years. Recurrence free survival (RFS) for all patients was 86.8%. 19 patients (13.2%) developed TR by the time of data collection, 11 of 71 patients in group 1 (15.5%) and 8 of 73 patients in group 2 (11%). There were no statistically significant differences between the two groups regarding total tumor volume, highest AFP values, and AFP-G. Maximum tumor diameter was significantly larger in group 1 (mean 3.49, SD ±2.05cm) compared to group 2 (mean 2.86, SD ±1.6cm), (P=0.041). Receiver operating characteristic (ROC) analyses for the AFP-G showed no significant correlation between pre transplant AFP-G and post transplant TR in all 144 patients, nor in group 1 patients. Significant correlation was found in group 2 (area under the curve [AUC] 0.965 & p <0.001). At 60ng/ml/m, AFP-G could predict TR with sensitivity 83.3%, specificity 94.7%, positive predictive value 71.4%, and negative predictive value 97.3%. RFS was 28.6% in 7 patients with AFP-G >60ng/ml/m versus 97.3% in 37 patients with AFP-G <60ng/ml/m (P<0.001). No one with AFP-G <10ng/ml/m developed TR.
Conclusion: AFP gradient before liver transplantation correlates with post transplant tumor recurrence in patients with no pre transplant therapeutic interventions for HCC. We couldn't establish a clear correlation in those who had interventions on the wait list. Patients with AFP-G <10ng/ml/m are highly unlikely to develop post transplant

tumor recurrence. Our data argue for AFP-G of 60ng/ml/m in patients with no interventions; recurrence remained infrequent below this value despite broader patient inclusion.

#533

TOTAL TUMOR VOLUME AND ALPHAFETOPROTEIN >400 ARE INDEPENDENT PREDICTORS OF POST TRANSPLANT TUMOR RECURRENCE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Introduction: Improving prediction of tumor recurrence (TR) post liver transplantation (LT) for hepatocellular carcinoma (HCC) is crucial for better organ allocation. We have previously shown that total tumor volume (TTV) can predict post transplant TR. Assuming that HCC lesions are more or less spherical, we calculated TTVs as such using maximum tumor diameters. In reality, most HCC lesions are ellipsoid or spheroid and their actual tumor volumes (ATVs) are considerably smaller. It was questioned whether non transplant candidates due to large TTV could still be considered for LT based on the smaller ATV?. Our aim was to study the ATV and its value among other predictors of TR.
Methods: We reviewed 144 patients transplanted for HCC between 1992 and 2009. ATVs were calculated using online volume calculators and tumor diameters on explant pathology.
Results: The median follow up was 4.96 years. Recurrence free survival (RFS) was 86.8% for all patients. Receiver operating characteristic (ROC) analyses for ATV showed c-statistic of 0.713 (area under the curve). ATV of 75cc could predict TR with sensitivity (SN) 35.3%, specificity (SP) 96.6%, positive predictive value (PPV) 60%, and negative predictive value (NPV) 91.2%. At the 115cc cutoff for the original TTV calculations, SN was 29.4%, SP 94.8%, PPV 45.5%, and NPV 91%. Four patients with TTV >115cc had ATV <75cc and none of them developed TR. RFS was 40% in 10 patients with ATV >75cc versus 91.2% in 125 patients with ATV <75cc (P<0.001). A univariate proportional hazard regression model was used to examine potential risk factors for association with TR and revealed 4 factors at a statistical level of P <0.05 including: vascular invasion (hazard ratio [HR] =4.6; confidence interval [CI] =1.6-12.9), TTV >75cc (HR =8.4; CI =3.1-22.8), AFP gradient (HR =1; CI =1.0-1.002), and AFP >400ng/ml (HR =5.1; CI =1.9-13.9). None of the following was significantly associated with TR: Milan Criteria, maximum tumor diameter, number of tumors, tumor location, and pre transplant HCC treatment. The 4 significant factors were assessed by multivariate analysis with Milan Criteria enforced into the model. ATV >75cc (P =0.002; HR =11.4; CI =2.4-54.9) and AFP >400ng/ml (P =0.009; HR =6.2; CI =1.6-24) were found to be independent risk factors.
Conclusion: Actual tumor volume (ATV) >75cc and AFP >400ng/ml are independent predictors of post transplant HCC recurrence. Calculation of the actual tumor volume based on the true form of the tumor is easy, fair, may increase patient inclusion and it improves the predictive value of the total tumor volume for post transplant tumor recurrence.

ALLIED HEALTH

#397

CATCH YOUR BREATH: A GUIDE TO LUNG TRANSPLANT FOR FAMILIES AND SUPPORT PEOPLE

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Purpose: Lung Transplant (LTx) is a life altering event for both patients and their support people (SP). SP perform an important caregiving role by providing daily care, emotional support, and attending to organizational issues often at the expense of their own needs. A previous study identified a lack of resources available specific to the needs of LTx SP. This abstract describes the development of a guidebook for LTx SP to enhance caregiver education.
Methods: A multidisciplinary approach was adopted to address the biospychosocial needs of the SP. A collaborative team from the transplant program edited the guidebook and previous SP were asked to review it and provide feedback. Comments and suggestions from SP were incorporated into the guidebook and reviewed by a multi-disciplinary panel. Readability and comprehensiveness were evaluated using the LTx center standard patient education assessment tool.
Results: A 55-page booklet was produced. The guidebook encompasses the full trajectory of LTx. A wide range of topics is addressed including stress reduction, health promotion, family issues, including young children, relocation, information management, bereavement, and grief. SP identified “intimacy” as an important issue to include. Feedback was obtained from 6 past SP who

reviewed the guidebook (4 spouses, 2 parents (of the same recipient); 4 female, 2 male; 2 deceased recipients; 3 survivors). Overall reaction was very positive and provided valuable comments and editorial changes. There was unanimous agreement regarding the readability and understanding of the guidebook. LTx program team members identified the guidebook as a means of enhancing communication with SP.

Conclusion: In keeping with patient centered care principles, the guidebook addresses the expressed needs of the SP in managing the challenges associated with their role in the LTx process. The guidebook has been in use for the past 12 months. The guidebook was described by SP as a valuable and realistic tool for other families and SP, especially when used in conjunction with other education resources. Comments and suggestions made by SP reflect their sense of pride, achievement, and empowerment in the role.

#409

EVIDENCE FOR EXCELLENT ADHERENCE WITH IMMUNOSUPPRESSIVE MEDICATIONS IN LUNG TRANSPLANT RECIPIENTS UTILIZING A CENTRALIZED DISPENSING STRATEGY

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Purpose: Non-adherence (NA) to immunosuppression (IMS) regimens following organ transplantation is associated with poor allograft and patient outcomes. The goal of this study was to determine the prevalence of NA in a lung transplant population where consequences of NA may be severe.

Methods and Materials: This cross-sectional retrospective cohort study evaluated prescribed dosing and central IMS prescription records of all living lung transplant recipients followed in our outpatient clinic between September 2008 and August 2010. Medication Possession Ratio (MPR), as defined as the number of days of medication supplied to the patient over the two-year study period, was used as a surrogate marker for adherence based on methods described in a large renal transplant population (Pinsky, AJT 2009). A gap in IMS prescription fills was defined as a >30 day lapse between expected depletion of supply and next medication refill.

Results: The charts of seventy-five single (n=44) or double (n=31) lung transplant recipients with mean±SD age 55±13 years, who were 6±4 years post-transplant, were reviewed. Mean MPR for IMS use was 95±8% (range 65-100%). Only 6 patients had MPR < 90% with only 2 collecting < 70% of the prescribed medication. Twenty patients (27%) had at least one gap in filling prescriptions during the two-year follow-up.

Conclusions: Lung transplant recipients at our centre demonstrated excellent adherence as measured by MPR > 90%. This finding is reassuring in view of results reported for a large group of recipients early post renal transplant where MPR indicated overall low compliance. A number of explanations could account for this difference including the use of a centralized dispensing strategy, and possible variability in adherence between organ recipients. Determination of the variables contributing to NA within and between organ recipients will be helpful in developing educational and other strategies to optimize adherence.

#418

A COMPARISON OF RECENT INTERNATIONAL STRATEGIES TO INCREASE ORGAN DONATION

Campbell, Michael (University Health Network, Toronto, ON, CAN); Wright, Linda (University Health Network, Toronto, CAN)

The goal of organ transplantation is to save the most lives possible within acceptable medical, ethical and legal parameters. A major limitation is the absolute scarcity of available organs in many countries, which has led to the introduction of a variety of initiatives to boost organ donation (OD) rates. Some aspects of the highly successful Spanish model have been utilized elsewhere with varying results. As OD reflects local beliefs and customs, not all initiatives are appropriate in every country. Recent changes to the Israeli and British systems consider non-medical criteria in their allocation algorithms, hoping that peoples' inclination to help family members may motivate them to donate their organs after death. However, these systems differ in their application of the ethical principles of equity and impartiality to OD. The Israeli system allots priority points to those who have agreed to donate after death or whose close relatives have donated or agreed to donate. It preferences non-directed donation rather than directed donation from both the living and the dead. Israel departs from most other countries in its policies towards living donors (LDs) i.e., giving priority points only to previous LDs who did not direct their donation to a named recipient. The UK system prohibits conditional donation from the dead but allows requested direction of organs after death to family members and long-standing friends. This presentation will discuss some of the relevant ethical issues in the Spanish, Israeli, UK and Canadian allocation models.

#419

ALLOCATION ISSUES IN ALTRUISTIC KIDNEY AND LIVER DONATION

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Altruistic or "Good Samaritan" organ donors are a source of much needed organs at a time of great scarcity. Altruistic donors (ADs) are living persons who give an organ anonymously to a transplant centre which allocates the organ to a suitable recipient. The recipient and AD are unknown to each other. ADs' donations are unsolicited; they are willing to assume the risks of the donation procedure and are motivated by a desire to help others. The practice is justified by good medical outcomes for recipients, psychological benefits for donors, and the belief that altruism and beneficence are legitimate motivations for organ donation. AD donation promotes equity and increases utility in organ allocation by providing organs to those who lack suitable living donors (LDs).

Traditionally, an organ from an LD has been directed to a recipient with whom the donor has a close personal relationship. As ADs' organs are allocated to recipients unknown to the donor, they are often allocated according to a different model i.e., that for deceased donation (DD). One source of ethical tension in donation from ADs arises from the merging of elements of LD and DD models, which differ in regard to equity and impartiality, harms and benefits to donors, and donor motivation. This presentation will explore the ethical dimensions of these differences and compare donation from ADs with kidney chains and exchanges and public appeals for organs.

#423

ORGAN AND TISSUE DONATION: WHAT HEALTH PROFESSIONALS NEED TO KNOW

Bonin, Mark (Capital District Health Authority, Halifax, NS, CAN)

Background: Many missed organ and tissue donor referrals had been identified by Provincial chart audits conducted in Nova Scotia. In 2006, The Legacy of Life: Nova Scotia Organ and Tissue Donation Program defined one of its strategic directions as providing education to healthcare professionals regarding the need, value and process of organ and tissue donation.

Goal: To make donation information accessible to healthcare professionals by developing a binder to be placed in each Intensive Care Unit (ICU) and Emergency Department (ED) containing the basic information needed to make a donor referral.

Method: A working group was formed to develop the resource binder, comprised of staff from the Critical Care Organ Donation Program, Regional Tissue Bank and the Legacy of Life Provincial Donation Program. Topics included regulations and standards, roles and functions, identifying and referring organ and/or tissue donors, approaching families, family support, monitoring and evaluation, resources and glossary. Pilot binders were introduced in the ICUs and EDs, and healthcare professionals were given three months to add comments and make suggestions.

Result: As a result of the feedback received, information was clarified and simplified. The referral contact numbers and referral process were laminated and placed at the front of the binder. In Spring 2009, the completed binders "Organ and Tissue Donation: What Healthcare Professionals Need to Know" were placed in all ICUs and EDs. Donor referrals increased significantly over the following year, at least partially attributed to the new resource. In 2010, the binder was added to the Legacy of Life website.

The development and dissemination of the binder are presented. Lessons learned and future plans are presented. The standards contained in the binder have provided provincial healthcare professionals with valuable information for clinical practice, and have provided a helpful framework for introducing the new Accreditation standards for organ and tissue donation.

#425

SURFING FOR HEALTH: THE DEVELOPMENT OF A TRANSPLANT WEBSITE FOR TEENS

Korus, Moira (Sick Kids, Toronto, Toronto, ON, CAN)

Objectives: 1) Discuss the importance of interactive tools to promote the development of self management skills and education in the adolescent transplant population; 2) Review the current literature on internet tools for adolescents; 3) Summarize the steps that are required to build an interactive self-management website for adolescents; 4) Describe potential outcomes to evaluate the impact of website interventions.

Description: Organ replacement is now standard therapy for patients with end stage organ failure with improving graft and patient survival. However chronic illness, such as the need for an organ transplant during adolescence can negatively impact all aspects of their lives including physical, cognitive, emotional, social and vocational functioning Non-adherence in adolescents with chronic illness is a significant problem There are disturbing rates of rejection in adolescents and young adult transplant recipients, believed to be attributable to non-adherence.

Furthermore, significant graft loss after transfer from pediatric to adult centers has been observed.
A needs assessment in our renal transplant population pointed to the need for more innovative ways to educate teenagers about their condition. Young people wanted to know everything possible about their treatment and outcomes, but they wanted it gradually and they wanted to make some choices as to how they could receive the information. They felt an interactive website would meet these needs. Self Management interventions typically include information based materials as well as cognitive and/or behavioral strategies designed to increase the participant's knowledge, self-efficacy and use of self-management behaviors. Studies have shown that comprehensive interactive interventions that augment information and medical treatments with self-management lead to better health care outcomes. The Internet is the main health information source for adolescents and young adults and thus e-health technologies offer teenagers an innovative and attractive means of acquiring skills in health management.
This presentation will describe the current literature in the development of self-management skills in youth with chronic health conditions, the research evidence that interactive Internet interventions are effective in improving certain health outcomes in youth and the process of developing and evaluating an interactive Internet program to improve self-management and health outcomes in youth who have undergone transplantation.

#444

THE BEANSTALK PROGRAM: DEVELOPMENTALLY FOCUSED CARE FOR THE YOUNG TRANSPLANT CHILD DURING PROLONGED HOSPITALIZATION

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Purpose: Solid organ transplantation (SOT) is standard therapy for patients with end stage disease; however young children undergoing SOT are at increased risk for developmental delay, particularly those with prolonged hospitalizations. The Beanstalk Program (BP) provides an optimal environment, ongoing education and facilitates positive experiences to enhance early childhood development for children <3 years old who require hospitalization >3 weeks. This study investigates parental experiences and perception of the developmentally focused care during their child's hospitalization.
Methods: Following IRB approval, parents whose children were part of the BP between 2003-2008 were recruited to participate in a mixed method study. Participants completed the *Measures of the Process of Care (MPOC-20),* with additional questions regarding the BP, and were invited to participate in a follow-up qualitative semi-structured interview. *MPOC-20* scores are reported descriptively. Interviews were transcribed and analysed until saturation of themes was achieved.
Results: Twenty parents (of children hospitalized between 3 weeks-15 months) completed the *MPOC-20*. Scores rate the extent of healthcare provider's behaviour as perceived by the family ranging from 1 (worst) to 7 (best). Highest scores included Respectful and Supportive Care (6.33) and Beanstalk Programming (6.34); lowest scores were for Providing General Information (5.65). Interview data generated several key themes: a) Parents describe striving for "normalcy" with the importance of routines, creating safe spaces for their child and accommodating the family/child's needs; b) Shifting the focus to their child's development in the midst of complex medical issues helped parents reframe their perspective and prepare for the journey home; c) Receiving appropriate developmental information and skills helped with empowerment and confidence as a parent.
Conclusion: These results emphasize the importance of enhancing child development for young SOT patients. Parental perceptions of experiences during prolonged hospitalizations attest to the importance of developmentally focused care. The BP has positively impacted the culture of care for this population.

#446

LUNG TRANSPLANT PATIENTS' ASSESSMENTS OF QUALITY OF CARE

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Purpose: Health care providers are interested in assessing quality of care. Patient assessments of health care provide important information about how well health care providers meet the needs of their patients. The present study examine whether patients assessments of health care varied by transplant status, education, age and gender using Consumer Assessment of Health Plans Study (CAHPS®).

Methods: Consecutive pre- and post-lung transplant patients attending the lung transplant outpatient clinic in a tertiary institution completed the CAHPS®. CAHPS® focuses on physician-patient communication and care management. This survey contains 38 items including one global rating about the patient's personal doctor on a 0 to 10 rating scale. The questions are grouped into three composites: access (getting care quickly, getting needed care), provider (communication skills, shared decision making, knowledge of medical history), and provider's office staff (follow-up on test results, courtesy/respect, and helpfulness). Data were analyzed using linear regression models. The dependent variables were CAHPS® global rating (best to worst doctor) and two composites created assessing doctors' and nurses' communication skills and shared decision making. The independent variables were patients' transplant status (pre- and post-transplant) and gender. The covariates included age, education and self-rated health.
Results: Two hundred and thirteen patients (105 pre- and 108 post-transplant) mean age 53 of whom 50% were female completed CAHPS® from July 2005 through April 2007. Females pre-transplant patients with poor self-health rating and low education level tended to report lower scores when assessing doctors' and nurses' communication skills and shared decision making.
Conclusion: This study suggests that a sub-group of patients perceived difficulties in communicating with their doctors and nurses. In order to maximize quality of care the observed disparities should be addressed.

#452

TAILORING IMMUNOSUPPRESSION TO PREVENT NODAT: DOES IT WORK?
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It remains unknown if tailoring immunosuppressants will reduce the incidence of new onset diabetes after transplantation (NODAT) in non-diabetic renal transplant patients. In 2005, we changed our protocol so that patients at high pre-transplant risk for NODAT (Hep C positive, obese, age>60, non-Caucasian race) were given CSA instead of TAC. Primary objectives of this study were to compare the incidence of NODAT one year post-transplant between tacrolimus (TAC) and cyclosporine (CSA) in non-diabetic renal transplant patients and incidence of NODAT before and after 2005; compare effects of NODAT on time-to-graft-survival and mortality in two time periods; and to determine whether switching from TAC to CSA resulted in discontinuation of hypoglycemics or insulin.
A retrospective chart review of adult non-diabetic patients admitted to the renal transplant program between January 2003 and December 2007 was conducted. Post-transplant data was collected at the time of transplant then 1, 3, 6 and 12 months after.
Out of 188 patients receiving TAC, 21 (11.2%) developed NODAT, compared to 2/16 (12.5%) receiving CSA (P=0.70). Nine patients out of 72 (12.5%) in period 1 (January 2003 - December 2004), developed NODAT compared to 14/132 (10.6%) in period 2 (January 2005 - December 2007) (P=0.68). Mortality rate was observed to be 8.1 times more prevalent for people who developed NODAT (95% CI: 29.4 – 2.25) than those who did not. Post-hoc analysis determined that age, ethnicity and BMI were all associated with NODAT. Although we did not see a statistically significant reduction in NODAT with the selective use of CSA, the 16 patients who received CSA were at high risk for NODAT and may have developed NODAT had they been given TAC. A prospective trial of TAC vs CSA in patients at high risk for NODAT is needed.

#456

EVALUATION OF AN ADVANCED PRACTICE NURSE COLLABORATIVE CHRONIC KIDNEY CARE MODEL FOR RENAL TRANSPLANT PATIENTS
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Purpose: The purpose of this study was to evaluate an Advanced Practice Nurse (APN) collaborative chronic kidney care model on the achievement of clinical target outcomes and care processes for renal transplant chronic kidney disease (CKD) patients.
Design/Setting: This was a non-randomized, controlled design guided by the Chronic Care Model (CCM). A propensity-score matched analysis was used to account for confounding variables and selection bias in the non-randomized sample. The study took place in an ambulatory care post kidney transplant clinic at an academic Health Science Centre.
Intervention: The intervention consisted of a multifaceted APN led inter-professional renal transplant CKD model of care based on the CCM disease self-management elements and CKD clinical practice guidelines. The CCM included patient focused strategies for disease self-management, APN/Physician collaborative practice,

shared decision making, and medication adherence interventions. A set of medical directives, based on CKD practice standards were used by the APN to guide the medical management and processes of care for patients with renal transplant CKD.
Methods: A prospective group of adult renal transplant stage three or greater CKD patients, who received 12 months of care under the new renal transplant APN led CKD model were propensity-score matched to a retrospective control group who received 12 months of care under the traditional transplant nephrologist led model. The propensity-score matching included the covariates of glomerular filtration rate, age, sex, kidney donor type, and diagnosis of diabetes. The proportion of patients in each group achieving a target score of 78%, based on K/DOQI clinical targets and participation in discussions about end-stage renal disease (ESRD) options, was compared after 12 months of care. The outcomes were systolic and diastolic blood pressure, lipids, hemoglobin, phosphate, calcium, parathyroid hormone levels, and acid base balance. The process measures included the implementation of K/DOQI treatment standards for the comorbidities associated with CKD.
Results: The propensity-score matching of 61 treatment patients to 119 retrospective controls, resulted in 40 matched pairs with an equivalent balance of measured covariates between the two groups. The target score of 78% was achieved by 68% of treatment patients as compared to 10% of the control patients. In addition, 88% of treatment patients participated in ESRD options discussions as compared to 13% of control patients. When compared to controls, implementation of recommended standards of care occurred in a significantly higher proportion of treatment patients (ASA 50% vs 23%, ESA 65% vs 33%, ACE-I/ARB 53% vs 13%, Statins 80% vs 45%, Calcium 73% vs 25%, Calcitrol 65% vs 18%).
Conclusions: The APN/physician collaborative chronic kidney disease model of care, guided by the chronic care framework, as an alternate model, may provide a feasible approach to optimizing the clinical outcomes and implementation of standards of care for the complex health care needs of renal transplant CKD patients. While there has been limited research on this patient population, this study points the way for future expansion of the an APN/Physician inter-professional collaborativepractice environment. Further research is required to determine the effectiveness of this model, among a broader group of renal transplant CKD patient populations and settings.

#459

DOES THE PRESENCE OF A DISTRICT RESOURCE NURSE INCREASE REFERRALS FOR ORGAN AND TISSUE DONATION?

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Motivation: Since 2002 the district health authorities in an Atlantic province have been engaged in efforts to improve organ and tissue donation processes to help to close the gap between the need for, and supply of organs and tissues for transplantation. In 2007, a fill time district resource nurse position was implemented in one district. The position is managed by the district and accountable to the Department of Health. The goal of the position is to provide education, develop policy, and promote the processes to thereby increase referrals to the Tissue Bank and Critical Care Donation program in this province.
Problem Statement: Despite having 25% of the population of the province within its boundaries, and having a 15-bed intensive care unit, there has been relatively few referrals, nineteen for organ and tissue donation between 2003 and 2006.
Approach: The District Resource Nurse position was implemented in 2007, initially as a 0.5FTE, but increased to a full time position once the donation potential was understood. The position is a partnership funded by the Dept of Health and managed by the DHA that seeks to enhance the processes, policies, procedures and education around organ and tissue donation. The district nurse engaged both the public and health professionals from across the district in education sessions. A local steering committee made up of people interested in donation was developed. Additionally, medical chart reviews were conducted in 2008 to identify missed potential donors. The audit results were communicated broadly to key decision-makers in the district.
Results: Organ and Tissue donor referrals increased by 289% between 2007 and 2009.
Conclusion: The presence of a full time district nurse positively influenced both referrals and actual donors.

#485

WORLD TRANSPLANT GAMES: INCENTIVE TO IMPROVE PHYSICAL FITNESS AND HABITUAL ACTIVITY IN PAEDIATRIC SOLID ORGAN TRANSPLANT RECIPIENTS

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Toronto, CAN; Drabble, Alison (Hospital for Sick Children, Toronto, CAN); Dipchand, Anne (Hospital for Sick Children, Toronto, CAN);

Purpose: Solid organ transplantation (SOT) is an accepted treatment option for children. However, recipients have varying degrees of exercise intolerance, reduced physical fitness and decreased habitual activity. This study examined the impact of training for and participating in the 2009 World Transplant Games (WTG) on measures of fitness and self-reported habitual physical activity.
Methods: SOT patients from a single centre were enrolled and divided into 2 groups: participants in the WTG (intervention group) and non-participant controls. Baseline physical fitness was assessed in both groups using components of the FITNESSGRAM® 4 months pre-WTG and re-assessed in the intervention group 4 months post-WTG. Habitual activity of both groups was assessed using the Habitual Activity Estimation Scale (HAES) 4 months pre-WTG, immediately before and 4 months post-WTG. The intervention group took part in event-specific training and received home training programs prior to the WTG. Controls received no intervention.
Results: There were 19 children in the intervention group (12 heart, 4 lung, 2 kidney, 1 liver; mean age at transplant (Tx) 9.6±6.5y; mean age at testing 14.5±2.7y) and 14 controls (9 heart, 4 liver, 1 multi-organ; mean age at Tx 8.0±5.3y; mean age at testing 13.2±2.6y). There was no difference in physical fitness or habitual activity between the groups at initial assessment. The intervention group demonstrated an increase in habitual weekday ("very active" hours: $p<0.002$; "active" hours: $p<0.02$) and weekend ("very active" hours: $p=0.01$; "active" hours: $p<0.04$) activity over the training period, while control habitual activity did not. The intervention group demonstrated an improvement in select components of the FITNESSGRAM® (cardiorespiratory fitness, muscular endurance and strength) after the WTG. At initial assessment 42.8% of the control group had ≥ 1 fitness component in the Healthy Fitness Zone (HFZ) compared to 36.8% of the intervention group. Following the WTG 56.2% of the intervention group had ≥ 1 component in the HFZ. All children in the intervention group improved in ≥ 1 measure of health related fitness and 69% improved in ≥ 2 of the 3 measures. The increase in habitual activity levels over the training period was not sustained beyond 4 months post WTG.
Conclusion: Motivational tools, such as the WTG, can positively influence habitual activity and health-related physical fitness of paediatric SOT recipients in the short term. Further study is needed to determine the optimal intervention strategies to assist with long-term maintenance of lifestyle changes.

#496

PHYSICAL ACTIVITY BEHAVIOURS AMONG TRANSPLANT RECIPIENTS

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Physical activity can influence the health and well-being of transplant recipients. Current physical activity recommendations for healthy adults state that individuals should engage in moderate-intensity aerobic physical activity for a minimum of 30 minutes, five days per week, or vigorous-intensity aerobic physical activity for 20 minutes or longer, three days per week (Haskell, Lee, Pate, Powell, Blair, Franklin, et al., 2007). This study sought to describe the physical activity behaviours of transplant recipients and related mental health outcomes. One hundred and thirty eight (58.1% male) transplant recipients volunteered for this study and completed scientifically-supported questionnaires. The participants ranged in age from 20 to 76 years (M = 47.63, SD = 12.85). Participants reported a variety of transplantations, including kidney (37.4%), liver (25.2%), heart (16.0%), lung (10.4%), bone marrow (5.5%), and either multiple organs or other organ not specified (5.5%). The average length of time participants waited for an organ was 10 months (M = 10.57, SD = 15.65). The length of time since the most recent transplant averaged at 90 months (M = 89.36, SD = 81.08 months). In spite of the heterogeneity of the sample transplant characteristics, these factors were not associated with physical activity levels. Transplant recipients participated in moderate amounts of health enhancing physical activity (M = 152.17, SD = 132.72 minutes per week). Participants engaged in an average of 78 minutes per week (M = 78.04, SD = 86.17 minutes) of leisure activities. In addition, participants reported engaging in screen time activities (computer and television use) ranging from 0 to 13 hours per day (M = 4.73, SD = 2.58). These findings suggest that a focus on facilitating adherence to the guidelines for physical activity is warranted

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#506

BODY IMAGE AND EATING ATTITUDES AND BEHAVIORS AMONG ADOLESCENT HEART AND LUNG TRANSPLANT RECIPIENTS

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Background: Adolescents with a chronic illness are at increased risk for body image and eating disorders. Life after transplant is akin to living with a chronic illness with rigid medication and regimen requirements, adverse medication effects and morbidities. We sought to ascertain rates and characteristics of eating attitudes and behaviors amongst thoracic transplant recipients.
Methods: Adolescent (11-18 years) heart and lung transplant recipients (>3 months post-transplant) completed standardized questionnaires that assessed eating attitudes including body dissatisfaction, drive for thinness, and weight preoccupation, and eating disordered behaviors. Detailed demographic, body mass index (BMI), medical history and medications were obtained through chart review.
Results: 25 heart and 3 lung transplant recipients completed the questionnaires (54% female; median time post-transplant 1.6 yrs, median age at survey 14.5 yrs). 80% were in the normal BMI range for weight-for-age, 7% overweight and 10% underweight. Median change in z score from transplant to survey was +0.67 (range -1.3 to +4.5); 37% perceived current weight as too high or low. 82% were dissatisfied with current weight (38% wanted to lose and 44% wanted to gain weight), however, few reported engaging in disordered behaviors such as taking less medication, diet pills or purging to control weight. Caloric reduction, skipping meals, and exercise to control weight were reported in the normal range.
Conclusions: Despite high body dissatisfaction, low rates of disordered behaviors were observed in this young cohort of adolescent thoracic transplant recipients. Weight dissatisfaction was high but bidirectional. Future assessment should include insidious activities such as medication non-adherence, as well as traditional weight control behaviors such as binge eating or assiduous exercise. Further research will explore the impact of body dissatisfaction and eating behaviors and outcomes in longer-term transplant survivors, and should focus on older adolescent cohorts who may have increased risk for body image disorders after transplant.