UNIVERSITY OF ALBERTA
FACULTY OF MEDICINE & DENTISTRY

ALBERTA TRANSPLANT INSTITUTE

# RESEARCH DAY

# "Registry-based science: Transplantation and beyond"

## Keynote Speaker

Josef Stehlik, MD, MPH

**Allard Lecture Theatre**
**Wednesday, June 7th 2017**
**9:00am - 10:15am**

### ATI Research Day Schedule
### (Katz Atrium and Allard Lecture Theatre)

8:00 - 8:30 Continental Breakfast
8:30 - 9:00 Opening Remarks
9:00 - 10:15 Keynote address
10:15 - 10:30 Coffee
10:30 - 11:15 Posters
11:15 - 12:15 Presentations
12:15 - 1:00 Lunch
1:00 - 2:00 Presentations
2:00 - 2:15 Coffee
2:15 - 3:00 Posters
3:00 Closing Remarks and Announcements

**Register by June 2nd**
Visit the ATI website for more details

**50th Anniversary of Transplantation**

## Keynote Speaker – Dr. Josef Stehlik

## "Registry-based science: Transplantation and beyond"

**Josef Stehlik, MD, MPH** is an Associate Professor of Medicine at the University of Utah School of Medicine. Dr. Stehlik has received his medical degree from Charles University in Prague and Masters of Public Health degree from Harvard School of Public Health in Boston, MA. He completed his training in Internal Medicine and in Cardiovascular Diseases at Allegheny General Hospital, MCP*Hahnemann University and advanced training in Heart Failure and Transplantation at the Cleveland Clinic.

Dr. Stehlik serves as Head of the Advanced Heart Failure section and Medical Director of the Heart Transplant Program at the University of Utah Health Sciences Center and at the George E Wahlen Veterans Affairs Medical Center in Salt Lake City, UT. He also serves as the Director of ISHLT International Registry for Heart and Lung Transplantation. He has been active in clinical work, education and research. He has received extramural funding for research in the areas of heart failure, heart transplantation and mechanical circulatory support. His awards have included funding from the American Heart Association, the Veterans Health Administration through a CSR&D Merit Review Award, the Veterans Affairs Center for Innovation, the International Society for Heart and Lung Transplant and other sources. He is an author of over 100 peer-reviewed publications and a number of book chapters.

# Schedule of Events

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| 8:00 - 8:30 am | Continental Breakfast |
| 8:30 - 9:00 am | Opening Remarks: Dr. Richard Fedorak, Dr. Lori West, Dr. Darren Freed |
| 9:00 - 10:15 am | **Keynote Address and Discussion: Dr. Josef Stehlik**<br>**"Registry-based science – transplantation and beyond"** |
| 10:15 - 10:30 am | Break - Coffee and snacks |
| 10:30 - 11:15 am | **Poster Session**<br>**Themes:** Clinical Outcomes, Infectious Diseases/Complications, and Transplant Diagnostics |
| 11:15 - 12:15 | **Presentations**<br>Themes: Cellular Transplantation, Infectious Diseases/Complications, Policy/Advocacy, Transplant Diagnostics |
| | Boris Gala-Lopez: Beta cell death by cell-free DNA: Correlation with clinical outcomes after islet allotransplantation |
| | Minal Borkar: Prognostic biomarkers and prospective treatment for BK polyomavirus associated nephropathy |
| | Kieran Halloran: Molecular Phenotyping of Lung Transplant Biopsies using Archetype Analysis |
| | Benjamin Adam: Chronic antibody-mediated rejection in nonhuman primate renal allografts: validation of human histological and molecular phenotypes |
| 12:15 - 1:00 pm | Lunch |
| 1:00 - 2:00 pm | **Presentations**<br>**Themes:** Vascular Biology, Clinical Outcomes, Ex Vivo or Organ Regeneration, Immunity/Tolerance |
| | Daniel Kim: Unfolded Protein Pathway Expression Patterns in Human Heart Transplant Patients |
| | Chantal Allan: Let's get physical: Aerobic capacity, muscle strength, and muscle endurance in pediatric heart transplant recipients |
| | Peter Dromparis: Molecular Repair and Injury in Ex Vivo Perfused Swine Lung Transplants |
| | Esmé Dijke: Human CD27+IgM+ B Cell activation is regulated by cis-binding of the inhibitory molecule CD22 to CD22 Ligand (CD22L) |
| 2:00 - 2:45 pm | **Poster Session**<br>Themes: Cellular Transplantation, Ex Vivo or Organ Regeneration, Immunity/Tolerance, Vascular Biology, Other |
| 2:45 - 3:00 pm | Break - Coffee and snacks |
| 3:00 - 3:30 pm | Closing Remarks and Announcements |

# Presentations

**Morning presentations (11:15 – 12:15)**

**Themes: Clinical Outcomes, Infectious Diseases/Complications, and Transplant Diagnostics**

1. Beta cell death by cell-free DNA: Correlation with clinical outcomes after islet allotransplantation: Boris Gala-Lopez
2. Prognostic biomarkers and prospective treatment for BK polyomavirus associated nephropathy: Minal Borkar
3. Molecular Phenotyping of Lung Transplant Biopsies using Archetype Analysis: Kieran Halloran
4. Chronic antibody-mediated rejection in nonhuman primate renal allografts: validation of human histological and molecular phenotypes: Benjamin Adam

**Afternoon presentations (1pm – 2pm)**

**Themes: Cellular Transplantation, Ex Vivo and Organ Regeneration, Immunity/Tolerance, and Other**

1. Unfolded Protein Pathway Expression Patterns in Human Heart Transplant Patients: Daniel Kim
2. Let's get physical: Aerobic capacity, muscle strength, and muscle endurance in pediatric heart transplant recipients: Chantal Allan
3. Molecular Repair and Injury in Ex Vivo Perfused Swine Lung Transplants: Peter Dromparis
4. Human CD27+IgM+ B Cell activation is regulated by cis-binding of the inhibitory molecule CD22 to CD22 Ligand (CD22L): Esmé Dijke

**Beta cell death by cell-free DNA: Correlation with clinical outcomes after islet allotransplantation**

**Boris L. Gala-Lopez**[1,6], Daniel Neiman[2], Tatsuya Kin[1,5,6], Doug O'Gorman[1], Andrew R. Pepper[1,6], Andrew J. Malcolm[1], Sheina Pianzin[2], Peter A. Senior[3,5], Benjamin Glaser[4], Yuval Dor[2], A.M. James Shapiro[1,3,5,6]

1. Department of Surgery and Clinical Islet Transplant Program, University of Alberta, Edmonton, AB , Canada.
2. Department of Developmental Biology and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.
3. Department of Medicine and Clinical Islet Transplant Program, University of Alberta, Edmonton, AB, Canada.
4. Endocrinology and Metabolism Service, Department of Internal Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
5. Alberta Transplant Institute (ATI), Alberta, Canada.
6. Canadian National Transplant Research Program (CNTRP).

Optimizing engraftment and early survival after clinical islet transplantation is critical to long-term function, but there are no reliable, quantifiable measures to assess beta cell death. Circulating cell free DNA (cfDNA) derived from beta cells has been identified as a novel biomarker to detect cell loss, and was recently validated in new-onset type 1 diabetes and in islet transplant patients.

Herein we report beta cell cfDNA measurements after allotransplantation in 37 subjects and the correlation with clinical outcomes.

A distinctive peak of cfDNA was observed 1hr after transplantation in 31/37 (83.8%) of subjects. This signal represents dead beta cells carried over into the recipient after islet isolation, combined with acute cell death post infusion. The presence and magnitude of the 1hr signal did not correlate with transplant outcome. Beta cell cfDNA was also detected 24hrs post-transplant (8/37 subjects, 21.6%). This signal was associated with higher 1-month insulin requirements ($p=0.04$), lower 1-month stimulated C-peptide levels ($p=0.01$) and overall worse 3-month engraftment, by insulin independence (ROC:AUC=0.70, $p=0.03$) and Beta 2 score (ROC:AUC=0.77, $p=0.006$). cfDNA-based estimation of beta cell death 24hrs after islet allotransplantation correlates with clinical outcome and could predict early engraftment.

## Prognostic biomarkers and prospective treatment for BK polyomavirus associated nephropathy

**Minal Borkar**[1], Vikas Srivastava[2], Ryan Cunnington[1], Tejeswin Sharma[1], Steven Greenway[1], Lee Anne Tibbles[1]

1. Department of Medicine, University of Calgary; Calgary, AB, Canada
2. CSIR-Indian Institute of Toxicology Research, Lucknow, U.P. India

Introduction: BK polyomavirus reactivation in kidney causes chronic kidney disease in immuno-compromised renal transplant and non renal transplant recipients. Due to lack of appropriate antiviral therapy against BKV it is important to study the underlying mechanism of pathogenesis causing renal fibrosis and BK polyoma virus associated nephropathy (BKPVAN).

Objective: To investigate BK polyomavirus pathogenesis from epigenetic point of view. This will also help to determine potential anti-viral therapy against BKPVAN.

Methods: Human Proximal Tubular Epithelial Cells (HPTCs) and CCD1105 cell lines were infected with BK virus. Another set of cells were treated with DNMT1 inhibitor RG108. Urine samples were collected from BKV viruria/viremia positive patients. RNA/DNA was isolated to perform Methylation Specific PCR (MSP) and Bisulfite Sequencing (BS) to asses DNA methylation. Expression study was done by using Real-time PCR and western blot assays. Immunoflourscence staining experiment and flow cytometry were performed to demonstrate fibrosis and necroptosis respectively.

Results:The downregulation of E-cadherin (CDH1) and collagen IV (COLIVA1) gene expression was observed in BKV infected cells, whereas, increase in expression of fibrotic marker collagen I suggests that BKV infection induces epithelial to mesenchymal transition (EMT). MSP confirmed silencing of those genes through DNA methylation mechanism by demonstrating hypermethylation of promoters of CDH1 and COLIVA1 genes. Immunoflourescence staining has shown increase in Vimentin and disruption of actin filaments in BKV infected cells confirming EMT. RG108 treatment had shown altered COLIVA expression and decrease in methylation of promoter, as demethylating agent, verified that BKV may uses DNA methylation for inducing EMT and eventually fibrosis.

The protein study confirmed that BKV induced necroptosis by inducing expression of Receptor-interacting serine/threonine-protein kinase 3, phospho-Mixed Lineage Kinase Like pseudokinas and High Mobility Group Protein B1, whereas it can be regulated by DNMT inhibitor indicating that BKV may induce necroptosis epigenetically.

We observed that BKV hypermethylates the RB1 gene promoter to silence it and instigate host cell division for its own replication however, RG108 treatment had demonstrated significant decrease in BKV DNA ($p$-value $<0.037$).

Conclusion: We have investigated BKV pathogenesis from epigenetic point of view and first to report that BKV orchestrates EMT and necroptosis by using DNA methylation mechanism. The hypermethylated genes may serve as biomarkers for prognosis of fibrosis. The use of DNMT inhibitors could prevent progression of fibrosis and block BKV replication, therefore, may use as antiviral therapy. However, further studies exploiting DNA methylation mechanism are much needed to prevent graft loss due to BKPVAN.

## Molecular Phenotyping of Lung Transplant Biopsies using Archetype Analysis

**Kieran Halloran**[1], Michael Parkes[2], Justin Weinkauf[1], Alim Hirji[1], Ali Kapasi[1], Dale Lien[1], Philip Halloran[2]

1. Lung Transplant program, University of Alberta, Edmonton, Canada
2. Alberta Transplant Applied Genomics Center, University of Alberta, Edmonton, Canada

PURPOSE: Accurate diagnosis of T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) in lung transplant recipients is an unmet need, but limited by the reproducibility of the current histology system for TCMR and absence of histologic criteria for ABMR. We previously defined molecular rejection phenotypes in kidney and heart transplant biopsies using kidney-derived rejection-associated transcripts (RATs). We studied whether RATs could be used to characterize transbronchial lung transplant biopsies (TBBs) and to separate those biopsies into those with ABMR-like, TCMR-like and no-rejection related molecular features.

METHODS: We used microarrays to analyze the expression of 453 kidney-derived RATs in 58 TBBs. We then used principal component analysis and archetype analysis with N=3 clusters to group biopsies into those with ABMR-like changes, TCMR-like changes and no-rejection-like changes.

RESULTS: Archetype analysis of TBBs based on expression of kidney-derived RATs was characterized by three clusters: TCMR-like (A1), ABMR-like (A3), and non-rejection (A2) (Figure 1). RATs separated TBB samples in a pattern similar to kidney and heart transplant biopsies: principal component 1 (PC1, x-axis) = rejection (right) vs. no rejection (left), and principal component 2 (PC2, y-axis) = ABMR (up) vs. TCMR (down). Expression of the 453 individual RATs correlated with PC1 and PC2 in a similar fashion to kidney and heart transplant biopsies. The agreement with histologic diagnosis was limited.

CONCLUSION: Kidney-derived RATs, previously shown to separate TCMR, ABMR and non-rejection phenotypes in kidney and heart transplant biopsies, can be used to distinguish rejection-like phenotypes in TBBs. We propose that this approach can now be used to develop a new classification of lung rejection phenotypes to compare with and potentially recalibrate conventional clinical and histologic phenotypes.

## Chronic antibody-mediated rejection in nonhuman primate renal allografts: validation of human histological and molecular phenotypes

**Benjamin Adam**[1], Rex Neal Smith[2], Ivy Rosales[2], Bahman Afzali[1], Masa Matsunami[2], Tetsu Oura[2], Benedict Cosimi[2], Tatsuo Kawai[2], Robert Colvin[2], Michael Mengel[1]

1. University of Alberta,
2. Massachusetts General Hospital and Harvard Medical School

Background: Molecular testing represents a promising adjunct for the diagnosis and classification of antibody-mediated rejection (ABMR). However, several obstacles hinder the translation of molecular diagnostics to routine clinical practice. These include the lack of a true diagnostic 'gold standard' for validation, heterogeneity of existing data associated with different Banff classification iterations, absence of robust validation studies, and lack of consensus on platforms to be used, transcripts to be assessed and criteria for positivity. We aimed to address these challenges by analyzing the expression of ABMR-related transcripts in a nonhuman primate (NHP) animal model free from the confounding variables of inconsistent immunosuppression, rejection treatment, and non-compliance.

Methods: 197 archival renal allograft samples were obtained from 81 NHP treated with tolerance induction protocols at the time of transplantation and then permanently taken off immunosuppression. 102 of the samples came from 29 recipients in which the tolerance protocol failed and chronic ABMR developed. 80 samples from recipients without ABMR and 15 normal native nephrectomies were also included. Expression of 34 genes previously associated with ABMR in humans was measured in each sample using the NanoString® nCounter® platform. The 34 genes included 18 endothelial, 6 NK cell, and 10 inflammation-related transcripts. Gene expression results were correlated with clinical, histological and serological data. Repeated 10-fold cross validation was used to rank individual gene performance and derive a refined gene set for ABMR.

Results: Hierarchical clustering identified endothelial genes as having the strongest association with Banff 2013 diagnoses of ABMR. A cross validation refined subset of three endothelial genes (VWF, DARC, CAV1) was found to optimally discriminate ABMR from non-ABMR samples (AUC=0.92). This 3-gene set correlated with classical features of ABMR, including glomerulitis, peritubular capillaritis, transplant glomerulopathy, C4d deposition, and donor specific antibodies ($r=0.39$-$0.63$, $p<0.001$). Principal component analysis confirmed the association between 3-gene set expression and ABMR and highlighted the ambiguity of v-lesions and ptc-lesions between ABMR and T-cell mediated rejection (TCMR). In sequential protocol biopsies, elevated 3-gene set expression corresponded with the development of immunopathologic evidence of rejection and often preceded it. 69% of the recipients that developed ABMR demonstrated concurrent TCMR.

Conclusion: These data provide NHP animal model validation of recent updates and discussions around the Banff classification, including the assessment of molecular markers for diagnosing ABMR, the non-specificity of v-lesions and ptc-lesions, and the growing recognition of mixed ABMR/TCMR as the potential natural history of renal allograft rejection.

**Unfolded Protein Pathway Expression Patterns in Human Heart Transplant Patients**

**Daniel H Kim**[1,4], Jody Groenendyk[2], Lindsey Carter[1], Philip F Halloran[3,4], Marek Michalak[2]

1. Division of Cardiology, University of Alberta,
2. Department of Biochemistry, University of Alberta,
3. Division of Nephrology, University of Alberta,
4. Alberta Transplant Institute

Introduction: Cardiac Allograft Vasculopathy (CAV) and late myocardial dysfunction remain a significant challenge in heart transplantation (HTx). Pathological and imaging studies have suggested that myocardial fibrosis is associated with CAV and worse long term outcomes in HTx. The lack of understanding of mechanisms underlying fibrosis presents a basic challenge for any therapeutic approach. Endoplasmic Reticulum (ER) stress and the Unfolded Protein Response (UPR) have been implicated in the development of cardiac fibrosis and may be triggered by events such as ischemia-reperfusion injury, acute and chronic rejection. Our group has also shown that in a murine heart failure model, blocking this UPR pathway by tauroursodeoxycholic acid (TUDCA), prevents fibrosis and improves survival. The aim of this pilot study was 1.To confirm the expression of the UPR pathway in the transplanted heart and 2. To compare UPR expression between rejecting and non-rejecting hearts.

Methods: Using RNA isolated from transplanted human heart endomyocardial biopsy samples, cDNA was generated and quantitative PCR (qPCR) was used to quantify markers of ER stress, the UPR, inflammation and fibrosis, specifically spXBP1, BiP, CHOP, PERK, ATF4, ERN1, ATF6, INFγ, IL-2, PTGS2, TNFα, TGFβ1, Collagen 1A, Fibronectin, and Periostin. Briefly, 500 ng of RNA was used to generate cDNA using the BioRad iScript Reverse Transcription Supermix. The cDNA generated was diluted 1:10 with ddH2O and 2 µl was used for qPCR. Quantification was performed by online monitoring for identification of the exact time-point at which the logarithmic linear phase could be distinguished from the background (crossing point) by setting a threshold. The threshold was expressed as a cycle number (Ct) and was normalized to mRNA abundance of housekeeping genes, including GAPDH.

Results: A total of six samples were obtained; rejection (n=3) and no-rejection (n=3). As expected, UPR signaling pathways were present in the human heart transplant samples. Although a gross trend towards differences in UPR between rejection and no-rejection samples was noted, specifically when comparing spXBP1, TGFβ1, ATF4 and Periostin, these were not statistically significant (See Figure ).

Conclusion: Initial data from our pilot study showed that UPR signaling pathways were present in transplanted human hearts. Preliminary analysis showed no differences between rejection and no-rejection samples. There appeared to be a slight increase in ER stress in rejection samples but larger sample numbers are needed to adequately assess this question. Future investigations may assess the therapeutic potential of suppressing the UPR pathway to prevent fibrosis in the cardiac allograft.

## Let's get physical: Aerobic capacity, muscle strength, and muscle endurance in pediatric heart transplant recipients

**Chantal Allan**[1,2,3], Simon Urschel[1,2,3,4], Ingrid Larsen[1,2,3], Sunita Mathur[2,5], Lori West[1,2,3,4,6]

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Introduction: Pediatric heart transplant recipients (HTx) often appear to have lower physical fitness than healthy children (HC). The purpose of this study was to assess the aerobic capacity, muscle strength, and muscle endurance of transplant recipients, and investigate clinical and lifestyle factors that may affect their fitness. The impact of fitness on quality of life was assessed to show the relevance of fitness level in daily functioning.

Methods: In this cohort, there were HTx (n=5) and HC (n=7). The HC group consisted of patient siblings when possible to control for lifestyle factors and genetics. To complete matching of the control group, non-related HC were selected based on age, height, and sex. Age-specific questionnaires assessed quality of life (PedsQL 4.0) and physical activity (PAQ). Anthropometry and body composition (BodPod, triceps skinfold) were measured. Aerobic capacity was assessed by six-minute walk test (6MWT). Muscle strength was measured by hand-held dynamometry of deltoid, abdominal, and quadriceps muscle groups. Muscle endurance was measured by push-ups, curl-ups, and timed wall sit. Clinical data regarding transplant course, medications, physical therapy, underlying diagnosis, and onset of disease was collected from patient charts. HTx measures were compared to HC by Mann-Whitney rank sum test. Spearman correlation was used to assess relationships among clinical

Results: The pediatric heart transplant group was age-matched (HTx=9.3±4.7 y; HC=12.2±2.9 y, p=0.10) and sex-matched (HTx=2F, 3M; HC=2F, 5M) with healthy controls, and 7.1±5.5 y post-transplant. Despite comparable levels of physical activity (p=0.20), quality of life (p=0.40), and fat-free mass (p=0.07), wall sit time (HTx=28±15 s, HC=94±63 s) and absolute 6MWT distance (HTx=540±49 m, HC=681±98 m) were lower in HTx as compared to controls (p<;0.05). HTx were receiving tacrolimus and mycophenolate mofetil, but not prednisone at the time of the study. The length of pre- and post-transplant physical therapy varied among HTx, ranging from 0-12 weeks pre-transplant, and 0-52 weeks post-transplant. In the entire cohort of HTx and HC (n=12), there were strong, positive correlations among PedsQL 4.0 score, and abdominal strength (r=0.81, p=0.01), number of push-ups (r=0.82, p=0.01), and number of curl-ups (r=0.82, p=0.01).

Conclusion: Lower body muscle endurance and aerobic capacity were impaired in HTx versus HC. Since physical activity, quality of life, and fat-free mass were matched in the study sample, muscle endurance and aerobic capacity were likely impaired by other factor(s). Ongoing recruitment will allow further investigation the effects of clinical and lifestyle factors on fitness outcomes.

**Molecular Repair and Injury in Ex Vivo Perfused Swine Lung Transplants**

**Zofia Czarnecka**[1], Peter Dromparis[1], Siegfried Wagner[1], Nader Aboelnaz[2], Sayed Himmat[2], Jessica Luc[2], Darren Freed[2], Jayan Nagendran[2], Michael Mengel[1], Benjamin Adam[1]

1. Department of Laboratory Medicine and Pathology
2. Division of Cardiac Surgery

Introduction: Due to acute lung injury (ALI), only 20% of donated lungs are suitable for transplant. Evidence suggests ex vivo lung perfusion (EVLP) may improve lung quality over the current gold-standard, cold static preservation (CSP), but the molecular mechanisms driving this have not been thoroughly investigated. We aim to define and validate a set of molecular markers that quantify ALI to monitor lung quality before, during and after EVLP.

Methods: Swine lung biopsies were collected immediately prior to explant (in vivo; IV) (N=13) and from lungs exposed to EVLP (N=41) or CSP (N=20) treatments at 0 hours (T0) and 12 hours (T12). RNA expression of 53 literature-derived ALI-associated genes were quantified with the NanoString nCounter system and normalized to 5 housekeeping genes. Histological analysis was performed on H&E slides. Functional parameters were documented during EVLP. Correlational analysis was performed using Spearman's rank correlation coefficient. Data were analyzed with nSolver and R. Significance was considered at $p<0.05$.

Results: Heat map analysis demonstrated gene expression that differed between EVLP T12 and all other groups. Volcano plot analysis comparing EVLP T12 and T0 identified 26 'repair' genes and 6 'injury' genes significantly increased and decreased, respectively. The geometric means of the 26 'repair' genes were significantly increased in EVLP-T12 but not CSP-T12 group compared to respective T0 controls. 'Repair' gene expression correlated with the PaO2:FiO2 ratio ($r=0.587$; $p<0.001$), lung compliance (0.578; $p<0.001$) and perivascular neutrophils/HPF ($r=0.283$; $p=0.022$) but inversely correlated with interstitial edema ($r=-0.373$; $p=0.002$) and interstitial inflammation ($r=-0.279$; $p=0.023$). 'Injury' gene expression correlated with pulmonary artery pressure ($r=0.606$; $p<0.001$), pulmonary vascular resistance ($r=0.485$; $p=0.004$), and interstitial neutrophil count ($r=0.259$; $p=0.037$), but inversely correlated with PaO2:FiO2 ratio ($r=-0.618$; $p<0.001$), and lung compliance ($r=-0.711$; $p<0.001$). Principal component analysis confirmed positive association of 'repair' and inverse association of 'injury' gene expression with EVLP treatment exposure.

Conclusion: EVLP alters the molecular phenotype of lung tissue, suggestive of an active repair process, potentially contributing to the favorable clinical outcomes with this modality. We identify repair and injury gene sets for ALI quantification that may be used for molecular monitoring of tissue repair during EVLP, a potential tool for tailoring ex vivo protocols in human transplant lungs.

**Human CD27+IgM+ B Cell Activation is Regulated by Cis-binding of the Inhibitory Molecule CD22 to CD22 Ligand (CD22L)**

**Esmé Dijke**[1,2,3,4], Kim Derkatz[1,3], Jean Pearcey[1,3], Fred Wong[1,3], Bruce Motyka[1,3,4], Lori West[1,3,4,5,6]

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5. Dept. of Surgery, University of Alberta, Edmonton, AB,
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BACKGROUND: ABO-incompatible (ABOi) heart transplantation (HTx) can be performed safely in infants and results in B cell tolerance to donor ABO antigens by mechanisms that remain unclear. We showed that the inhibitory molecule CD22 is more highly expressed on splenic CD27+IgM+ B cells, which contain the vast majority of ABO-specific antibody secreting cell precursors, compared to other splenic B cell subsets. CD22 expression was highest on infant CD27+IgM+ B cells and decreased with age. Binding of CD22 to sialic acids (CD22L) on the same cell (cis-binding) or different cells (trans-binding) regulates B cell activation and may lead to B cell tolerance. Here, we studied whether CD22-CD22L cis-binding affects activation of CD27+IgM+ and CD27-IgM+ B cells from pediatric and adult spleen.

METHODS: CD27+IgM+ and CD27-IgM+ B cells were isolated via fluorescence-activated cell sorting from pediatric (n=3; age range: 0-2.5 yr) and adult (n=3; age range: 21-46 yr) spleen obtained from organ donors. To “break” CD22-CD22L cis-binding, sorted B cells were treated with neuraminidase (NEU) to cleave surface sialic acids. Activation of B cells was defined by analyzing intracellular phosphorylation of PLCÎ³2 (pY759) after stimulation with anti-IgM antibodies using “phospho-flow” cytometry.

RESULTS: Kinetics of pY759 signaling were similar between untreated CD27+IgM+ and CD27-IgM+ B cells as well as pediatric and adult samples. At peak response (1-3 min after stimulation), the median fluorescence intensity (MFI) of pY759 in pediatric CD27+IgM+ B cells was comparable to that in pediatric CD27-IgM+ B cells (350-614 vs. 259-565), whereas in adult cells the MFI was higher in CD27+IgM+ B cells than in CD27-IgM+ B cells (540-912 vs. 248-328). NEU treatment had a strong effect on kinetics and magnitude of pY759 signaling in both pediatric and adult CD27+IgM+ B cells: the MFI of pY759 increased to 506-2023 and 1086-2051, respectively, at peak response (4-8 min after stimulation). In contrast, treatment had minimal effect on pY759 signaling in CD27-IgM+ B cells.

CONCLUSION: NEU treatment strongly increased the magnitude of pY759 signaling in stimulated CD27+IgM+ B cells, but not CD27-IgM+ B cells, suggesting that CD22-CD22L cis-binding may play a role in control of activation of CD27+IgM+ B cells. This finding may contribute to unraveling mechanisms of B cell tolerance in ABOi HTx in children.

## Posters

### AM Posters (10:30am – 11:15am):

### Micronutrient Monitoring and Bone Health in Adults with Cystic Fibrosis Undergoing Lung Transplantation

**Grace Hubert**[1], Connie Prosser[2], Dale Lien[3], Justin Weinkauf[3], Neil Brown[4], Marianne Goodvin[1], Kathy Jackson[1], Joan Tabak[5], Josette Salgado[5], Abeer Alzaben[6], Diana R Mager[6]

1. Lung Transplant, University of Alberta Hospital, Alberta Health Services, Edmonton, AB, Canada
2. Laboratory Medicine, University of Alberta Hospital, Alberta Health Services
3. Lung Transplant, Division of Pulmonary Medicine, Department of Medicine, University of Alberta
4. Adult Cystic Fibrosis, Division of Pulmonary Medicine, Department of Medicine, University of Alberta
5. Adult Cystic Fibrosis, University of Alberta Hospital, Alberta Health Services
6. Department of Agricultural, Food and Nutritional Science, University of Alberta

Purpose: Low bone mineral density (BMD) is experienced frequently by patients with cystic fibrosis (CF), especially as the disease becomes more severe and the need for lung transplant (LTx) becomes apparent. The purpose of this study was to describe mincronutrient status and BMD in adults with CF pre- and pos-LTx

Methods: Twelve paitents with CF (29 +/- 8 years) were recruited from the CF clinic at the University of ALberta Lung Transplant Program. Vitamins A, D, E and K status and parathyroid hormone were measured pre- and at 3, 6 and 12 months post-LTx. BMD was measure pre-LTx and at 9 and 18 months post-LTx.

Results: No significant differences pre- and post-LTx were observed at the bone sites measured (P>0.05). BMD T-scores (<-2) was present in lumbar-spine, femoral neck, hip, and femoral-trochela in 33%, 14%, 17% and 25% of individuals pre-LTx, and 58%, 33% 58% and 33% post-LTx, respectively. Greater than 50% of individuals had sub-optimal vitamin K levels (PIVKA-II values >3ng/ml) pre- and post-LTx. 33% of pre-LTx patients and 42 % post LTx had low vitamin D levels (<80 nmol/L).

Conclusion: Adults with CF pre- and post-LTx were shown to have sub-optimal vitamin K status and reduced BMD. Although vitamin D status was better than vitamin K status, it too required attention. This undersocres the need for specialized medical teams to address ongoing micronutrient deficiencies in this population. Registered dietitians are uniquely positioned to help optimize bone health by developing effective nutrition care plans and exploring barriers and facilitators to achieving nutrition goals.

**Think outside the 'three': A case of allo-sensitization from an alternate route**

**Deanna Manna**[1], Luis Hidalgo[1,3], Anne Halpin[1,2,3,4], Patricia Campbell[1,3,5]

1.Department of Laboratory Medicine, University of Alberta Hospital
2 Canadian National Transplant Research Program
3 Alberta Transplant Institute
4 Department of Pediatrics, University of Alberta
5 Department of Nephrology, University of Alberta

When we think about HLA sensitization risk for transplant patients we tend to focus on the three main routes: pregnancy, transfusion, and previous transplant. However, there are other means of non-self HLA (allo)-immunization to consider.

We present a case of a 27 year old male renal transplant candidate listed in March 2014. At the time of listing his cPRA was 29%. His serum initially showed non-specific bead reactivity on single antigen beads but did not suggest significant sensitization. In May 2015, the patient underwent a ligament replacement procedure for a knee injury during which he received a tendon and patella allograft from a deceased donor; the renal transplant program was not informed of this procedure at the time. In August 2015, a dramatic increase in HLA sensitization was noticed as part of our routine testing algorithm resulting in a cPRA increase to 100%, initially seen in the class II HLA antibody results then progressing to both class I and II soon after. The patient's antibody levels and specificities have continued to increase over time due to continued exposure to the graft's non-self HLA.

Being homozygous for any HLA antigen is a disadvantage as it increases the likelihood of being exposed to non-self HLA in any immunizing event. This patient's HLA typing makes him more vulnerable to HLA class I and class II antibody development as he is homozygous for HLA A*02, HLA DR*15, and HLA DQB1*05.

In order to increase the chance of finding a compatible donor, this recipient has been listed in our national Canadian Blood Services Highly Sensitized Patient (HSP) registry but as of yet, no suitable donor has been offered.

This occurrence has been investigated at our centre, through the Histocompatibility Laboratory and the Comprehensive Tissue Centre. This event has triggered process improvement initiatives to look at the informed consent policy for procedures that may jeopardize a transplant candidate's likelihood of finding a suitable donor. Allograft sensitization is a growing concern for all transplant programs and should be investigated and discussed at the time of listing. For patients on a transplant waitlist, this type of surgery may seem unconnected to their transplant journey. However, our case clearly demonstrates that tendon replacement can lead to extensive HLA sensitization and perhaps additional patient education is required on the routes of sensitization and the risks involved with exposure to non-self HLA antigens.

**Early Islet Engraftment Measured by BETA-2 Score Predicts Islet Transplant Outcomes**

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Introduction: Optimizing islet engraftment with the aim of ensuring adequate primary graft function is a potential target in improving islet transplant outcomes. Unfortunately, measuring islet engraftment is difficult and so little is known about it in the clinical setting.
Objective: The aim of this study was to characterize islet engraftment post-CIT and to determine whether early engraftment predicts long term graft function.

Methods: We calculated the BETA-2 score, a novel and validated composite score that incorporates several indices of islet function, on a weekly basis for the first 6 months post-CIT. Fifteen patients transplanted with allogenic islets between 2009 and 2014 were selected on the basis of having optimal graft function (insulin independent for at least 1 year after single islet infusion; group 1CIT, n=8), or suboptimal graft function (requiring a second islet transplant within 3-6 months of initial transplant before achieving insulin independence; group 2CIT, n=7).
Results: In both groups, engraftment was apparent at one week post initial CIT and appeared to continue over the next few weeks with BETA-2 score increasing and reaching a plateau by four to six weeks. Group 1CIT subjects showed better early engraftment with significantly higher BETA-2 score at 1 week compared to group 2CIT subjects (BETA-2 score 15 + 3 vs. 9 + 2, P=0.001). This difference was maintained until 16 weeks when most group 2CIT subjects had received their second CIT (BETA-2 score 25 + 4 vs. 17+ 6, P=0.07). Long term graft function, as assessed by the BETA-2 score at 2 years, was higher in group 1CIT compared to group 2CIT (22 + 4 vs. 14 + 7, P=0.02). Linear regression showed that the BETA-2 score at one week predicted graft function at 24 months ($R2= 0.496$, $P < 0.05$), whereas fasting blood glucose and fasting C-peptide at one week post-CIT were not associated with graft function at two years.
Conclusion: This study demonstrates that islet engraftment takes place rapidly over the first week post-CIT and is almost complete by 1 month. Furthermore, measurement of islet engraftment using the BETA-2 score, as early as 1 week post CIT, may be useful in predicting long term transplant outcome and may be an endpoint of interest in optimizing CIT.

**Common Iliac Artery Stenosis: An Often Overlooked Cause of Renal Graft Dysfunction**

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Introduction:Transplant renal artery stenosis is a well recognized complication post kidney transplantation and a potentially reversible cause for hypertension, allograft dysfunction and recurrent heart failure. This case series describes successfully treated upstream native common iliac artery stenosis resulting in improved allograft function and blood pressure control, including one patient with a recovery off dialysis.

Methods: Six cases were retrospectively reviewed over a 15 year period from our University of Alberta Renal Transplant program.

Results: Half of the cases were deceased donor grafts; average presentation from transplantation was 7 years. Culprit stenotic lesions were proximal to the transplanted renal artery, located at the common iliacs or aortic bifurcation. One patient who was dialysis dependent for over 3 weeks, recovered to baseline eGFR of 57 ml/min. The remaining had mean improvements in eGFR of 32.7 ml/min. Systolic blood pressure was reduced by a mean 16.8 mmHg and diastolic 5.4 mmHg, allowing reduction of antihypertensives in four cases. No patients developed contrast induced nephropathy despite significant renal impairment at the time of angiography.

Conclusions: As we expand the criteria for kidney transplant recipients, the prevalence of vascular disease is likely to increase and consequently the incidence of upstream vascular stenosis will become a more frequently encountered issue. As a potentially reversible cause of progressive allograft dysfunction, concerns regarding contrast exposure should not delay investigation for vascular insufficiency especially in those with significant risk factors.

**Recurrence of cytomegalovirus (CMV) infection after secondary antiviral prophylaxis post-lung transplantation.**

**Harjot Malhi**[1], G. Sanjaya Chandrarathne[1], Cristina Hernandez Garcia[2], Dima Kabbani[2], Justin Weinkauf[1], Ali Kapasi[1], Kieran Halloran[1], Dale Lien[1], Jutta Preiksaitis[2], Carlos Cervera[2], Alim Hirji[1]

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Background: Cytomegalovirus (CMV) infection commonly occurs in lung transplant (LTx) recipients, and prior to prophylaxis regimens, was associated with a high rate of morbidity and mortality. Patients now receive universal CMV prophylaxis post-transplant, however a third of patients still go on to develop a CMV infection. In our institution, the use of post-CMV infection antiviral prophylaxis is not protocolized, and decided upon by the treating physician. This study reviews the role of secondary antiviral prophylaxis in reducing the occurrence of a subsequent CMV infection in LTx recipients once treatment of the initial infection has been completed.
Methods: Retrospective data analysis of LTx recipients performed between September 2005 and December 2013 in the University of Alberta Lung Transplant Program. CMV-related data was collected to two years of follow up post-transplant. Duration of secondary prophylaxis was calculated as number of days a LTx recipient received prophylaxis following treatment of first CMV infection. Primary outcome was recurrence of CMV infection. Chi-square analysis and logistic regression were utilized for categorical outcomes.

Results: 301 LTxs were performed during the study period with 93 (31%) recipients being treated for first CMV infection. Of those treated, 49/93 (53%) recipients received secondary prophylaxis, of which 31/49 (63%) recipients developed a second CMV infection and 18/49 (37%) recipients did not (p=0.132). In 25/93 (28%) recipients receiving secondary prophylaxis of at least 21 days, there was no difference in incidence of a second CMV infection [15 (60%) had a second CMV infection and 10 (40%) did not, p=0.599]. Among those developing a second CMV infection, 44/54 (85%) had a CMV status associated with higher risk of CMV infection [23 donor IgG +/recipient IgG+ (D+/ R+) and 21 D+/R- patients].

Conclusion: The use of secondary antiviral CMV prophylaxis for a minimum of 3 weeks did not alter the incidence of a second CMV infection in LTx recipients.

**LOLRB1 Polymorphisms Influence Post-Transplat CMV Susceptibility and the Functional Interaction with UL18 and Classical MHC-1**

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NK cells play an important role in the response to HCMV, and HCMV encodes a human MHC-I homolog UL18 to evade NK cell responses. From the study of the two cohorts of transplant patients in Canada and Switzerland, respectively, we found a significant association of HCMV replication in kidney transplantation patients with minor alleles of LILRB1 gene in the regulatory regions that are correlated with lower surface expression of LILRB1 on NK cells. The SNPs in the regulatory regions of the distal lymphoid-specific promoter of LILRB1 gene are in strong linkage disequilibrium with two of four non-synonymous SNPs located in the first two immunoglobulin domains. The NK cell lines expressing associated variants of LILRB1 (denoted PTTI) are functionally inhibited by UL18 as well as classical MHC-I molecules to a greater extent than the more common variant (denoted LAIS). Consistently, we observed stronger binding of the LILRB1 PTTI variant with both UL18 and classical MHC-I molecules than LAIS variant, and three of the four residues greatly (P45, T70, T119) contribute to the stronger interaction. The threonine at position 119 is required for the glycosylation of a nearby glutamine which facilitates stronger interaction of the minor variant with classical MHC-I and UL18. The results suggest alleles of LILRB1 gene that allow superior immune evasion by HCMV are restricted by the mutations that regulate LILRB1 expression selectively on NK cells.
Acknowledgments: The work was funded by grants from CIHR MOP 123257 and the Li Ka Shing Institute of Virology and trainee support from AIHS, NSERC and the China Scholarship Council.

**Influence of donor characteristics on the rate of CMV transmission in CMV mismatch transplant recipients.**

**Cristina Hernandez Garcia**[1], Dima Kabbani[1], Karen Doucette[1], Juan G. Abraldes[2], Sonia Fuentes[1], Jutta Preiksaitis[1], Carlos Cerver[1]

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Introduction: Cytomegalovirus (CMV) is the most important opportunistic pathogen after solid organ transplantation. CMV seronegative recipients of an organ from a seropositive donor (D+/R-) are at the highest risk of CMV infection and antiviral prophylaxis is recommended, although CMV transmission is not universal. Our aim is to investigate donor characteristics that may influence the rate of CMV transmission to seronegative recipients.

Methods: This is a retrospective cohort study at University of Alberta Hospital. We included CMV seronegative adult transplant recipients who received an allograft from a CMV seropositive donor from January 2000 to December 2013. All recipients received valganciclovir prophylaxis for 3-12 months depending on organ type. CMV transmission was defined as either seroconversion or DNAemia.

Results: We included 418 CMV mismatch recipients from 348 donors. Living-donor transplantation accounted for 77 (18%) transplants, 70 were kidney. The mean number of recipients per deceased donor was 1.3. The prevalence of seroconversion was 71.5% at 2-years post-transplant. It was highest in lung (83.6%) and lowest in heart transplant (64%).
After prophylaxis, CMV infection monitoring was done in 410 patients; 140 (42%) of non-lung and 50 (68%) lung transplant patients, respectively, had a positive CMV DNAemia by 1 year posttransplant. The median time to first positive CMV DNAemia detection was 153 days (range 127-192) and 219 days (range 175-246), respectively.
CMV transmission at 2-years occurred in 63.6% of all patients; 88% of lung recipients. Examining donor characteristics:
a) Donor/recipient sex mismatch occurred in 167 transplants: CMV transmission rate was 64.4% and 53.3% (p=0.197) for male-donor to female-recipient and female-donor to male-recipient, respectively.
b) The donr's age and a donor-recipient age difference more than 10 years were not significantly associated with CMV transmission.
c) Living donor: There were 156 kidney transplants and 44.9% of them were living donors. The rates of transmission were higher in deceased donors (66.3% vs. 3.1%, p<.001).
We identified 59 donors who donated to at least two recipients; 28 transmitted CMV to all recipients, 8 did not transmit CMV to any recipient and 23 transmitted CMV inconsistently: 12/14 lungs, 6/13 livers, 3/5 heart, 4/17 kidneys and 1/6 SPK had CMV transmission at 2 years (p=0.002).

Conclusion: We found different rates of CMV transmission by donor and organ type. Further research is warranted to investigate the mechanisms of, factors influencing and role of the allograft in CMV transmission.

**CD3 T-cell Infiltrates at Diagnosis Predicts Overall Survival in Solid Organ Transplant Recipients with Post-Transplant Lymphoproliferative Disorders (PTLD)**

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6. Department of Hematology, University of Alberta, Edmonton, Canada,Introduction

The host's immune status is central to both the pathogenesis and treatment of Post-Transplant Lymphoproliferative Disorders. (PTLD) We hypothesized that CD3 positive T-cell infiltrate density in the tumor microenvironment, a surrogate of the potency of the host versus tumor immune response, may be prognostic of overall survival in PTLD.

Methods: A database consisting of 131 biopsy confirmed PTLD cases occurring in pediatric and adult solid organ transplant recipients after the year 2000 in Alberta were analyzed for clinical prognostic variables and overall survival. CD3 infiltrate was determined by a blinded pathologist (JZ) using an integer scoring system (0 - 3) to quantify CD3-positive cells in archived, formalin-fixed paraffin embedded tissue stained by immunohistochemistry. Tissue was available on 85 patients. Survival analysis was done by Kaplan-Meier analysis or Cox regression, with between group differences tested by a Pearson's chi-square test, with $p < 0.05$ being taken as significant.

Results: Histology subtypes included early (n = 7), polymorphic (n = 17), monomorphic, (n = 100) Hodgkin, (n = 8) and unavailable. (n = 2) A less dense CD3 T-cell infiltrate, defined as a CD3 score of 0-1, had a statistically significant adverse effect by univariate Cox regression with respect to overall survival. (HR 3.021, $p = 0.001$) A CD3 score of 0-1 was positively associated with a monomorphic histology ($p < 0.001$), but was not statistically associated with lymphocyte count, early PTLD, EBV status or bone marrow involvement. Multivariate Cox regression with respect to overall survival, including monomorphic histology, IPI 3-5, lymphocyte count < 1.0 and CD3 score of 0-1 again showed a statistically significant adverse effect of a low CD3 score. (HR 3.328, $p = 0.013$)

Conclusions: A less dense CD3 T-cell infiltrate in the tumor microenvironment at diagnosis is an adverse marker with respect to overall survival in PTLD by both univariate and multivariate Cox regression, versus clinical prognostic markers. This is reflective of the critical role that the host immune response, which can be altered in this setting by changes in exogenous immunosuppression, may play in treatment response. In the future, validated histologic measures of immune status such as the CD3 score may be integrated into existing models, such as the IPI, to provide additional prognostic power in PTLD.

**Impact of Donor and Recipient CMV Serology on Long Term Survival of Thoracic Transplant Patients**

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Background: Pre-transplant cytomegalovirus (CMV) serostatus has been associated with thoracic patient survival. Improvements in CMV molecular diagnostics and prevention strategies have significantly impacted morbidity and mortality. We retrospectively analyzed the relationship between pre-transplant donor/recipient CMV serostatus and long-term mortality in a large cohort of heart and lung transplant recipients transplanted at the University of Alberta Hospital over a 30 year period.

Methods: Adult (Age >17y) heart (n=603) and lung (n=652) recipients transplanted between July 1985-December 2015 were analyzed. Variables included age, sex, pre-transplant donor (D)/recipient (R) serostatus [D-/R-, D-/R+, D+/R+, D+/R-], CMV infection within 2 years of transplant and transplant eras divided by changes in CMV prevention strategies: Era 1 (Pre-oral ganciclovir, July 1985-April 1998), Era 2 (Oral ganciclovir, May 1998-December 2004), Era 3 (Oral valganciclovir, January 2005-December 2015). Data on each patient was collected from time of first transplant until December 31, 2016. Kaplan-Meier survival curves at 10 years were generated and curves were compared using log-rank test. Cox regression was used to identify risk factors associated with 10 year mortality, and CMV infection was treated as a time-dependent variable in the Cox model.

Results: No difference in 10 year survival was observed in hearts as compared by CMV serostatus (p=0.17) or Era (p=0.16). In lungs, a trend of worse 10 year survival was observed in high risk recipients compared to D-/R- (p=0.036) and survival improved in subsequent transplant eras (p<0.001). Multivariate Cox regression revealed that age at transplant, transplant era and lung transplantation but not CMV serostatus or infection were significant predictors of 10 year mortality. Age at transplant was associated with a 1.5% (0.7% - 2.4%) increased risk of death per year. Transplantation in the oral ganciclovir era was associated with a 26% (6.7% - 41.4%) reduced risk of death compared to the pre-oral ganciclovir era. Similarly, the valganciclovir era was associated with a 45.5% (30.6% - 57.2%) reduced risk of death compared to the pre-oral ganciclovir era. Lung transplants were associated with a 63.9% (34.8% - 99.1%) increased risk of death compared to hearts.

Conclusions: Our data suggests that the effect of CMV in heart and lung transplantation was confounded by an era effect, with no impact observed in the current era of valganciclovir prophylaxis. Further studies are warranted to prove the decreased impact of CMV in long-term mortality of thoracic transplant recipients.

**Monomorphic Post Transplant Lymphoproliferative Disorder (Diffuse Large B Cell Lymphoma subtype) successfully treated without chemotherapy after Solid Organ Transplant**

**Marta Rojas Vasquez**[1,4], Sunil Desai[1,4,5], Anthea Peters[2,4,5], Raymondi Lai[3,4,5], Simon Urschel[1,4,5], Jason Yap[1,4,5], Catherine Morgan[1,4,5], Jutta Preiksaitis[2,4,5]

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Background: Post-transplant lymphoproliferative disease (PTLD) represents a spectrum of clinically and morphologically heterogeneous lymphoid proliferations. Histologies observed in the monomorphic subtype are similar to those observed in Non Hodgkin Lymphoma. The majority of the PTLD are of B-cell origin, with diffuse large B-cell lymphoma (DLBCL) being the most common. Rituximab as monotherapy to treat PTLD after organ transplant has been efficacious in adult patients, but data are lacking in pediatric patients. Similarly, low-intensity chemotherapy has been effective in EBV-positive, CD20-positive B-lineage PTLD. A study by Children's Oncology Group with rituximab plus cyclophosphamide and prednisone in children with PTLD post solid organ transplantation in parallel with immune suppression reduction demonstrated a 67% event-free survival.The aim of the study is to determine if Pediatric monomorphic PTLD DLBCL subtype can be treated without chemotherapy.

Methods: A single institution retrospective study (November 1998 to April 2017) of pediatric patients after solid organ transplant who developed monomorphic PTLD (DLBCL subtype).

Results: Fourteen out of forty-three patients (32%) presented with monomorphic PTLD (DLBCL subtype). Types of organ transplantation included 7 heart, 4 liver, 2 kidney and 1 combined liver-small bowel transplant. Eight patients were male. Average age at transplant was 3.74 years (range 0.03-16.02) and at PTLD presentation was 8.05 (range 1.13-21.98) years. Four patients were previously treated for PTLD, non DLBCL type. All patients had PTLD B cell subtype, 10 patients were EBER positive on histology, 3 negative and 1 unknown. Thirteen out of fourteen were CD 20 positive. Two patients presented with stage II, seven with stage III and five with stage IV diseases. Four patients (29%) were treated with reduction of immunosuppression (RIS)+/- antiviral +/- intravenous immunoglobulin G (IVIG), 8 patients (57%) received Rituximab + RIS +/- antiviral +/- IVIG, 2 patients (14%) Rituximab and chemotherapy concomitantly. One patient treated with Rituximab + RIS, relapsed after being in remission for 3 months, required chemotherapy treatment. Nine/fourteen (64%) patients remained alive at 4.82 (range 0.01-19.05) years follow-up. Five patients died as a result of other causes than PTLD. One patient presented with Classical Hodgkin Lymphoma PTLD 48 months later.

Conclusion: This cohort shows that it is possible to omit chemotherapy in pediatric monomorphic PTLD (DLBCL type). Prospective data is required to further assess this novel treatment approach.

## PM Posters (2:15 – 3:00 pm):

### 3-D Modelling and Printing for Facial Allotransplantation Donor Reconstruction

**Alexander Hopkins**, Adrian Mendez[1], Heather Loga [2], Hadi Seikaly[1], David Cote[1]

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Donor reconstruction following organ transplantation is considered an ethical obligation and has become the standard of care. Donor reconstruction following procurement in facial allotransplantation is of particular significance with current methods of reconstruction using a plaster based moulding technique. Three dimensional (3-D) rendering and printing technology has been proposed as a possible reconstructive modality. The aim of this study is to evaluate 3-D printing technology for donor reconstruction after facial allotransplantation in a cadaveric model.

Using an embalmed cadaver, two progressive facial defects were created. The first involved only a soft tissue defect while the second involved both soft tissue and bony resection. Photos prior to and following facial resection were obtained using a 3-D rendering camera (Canfield-Canon) at two separate angles. Soft tissue and bony reconstruction was done using computer software (VECTRA). The completed 3-D reconstruction was printed using an Objet260 Connex® PolyJet 3D printer and a silicone based medium (Tango ™). The mask was inlayed onto the donor site and evaluated for reconstructive accuracy and cosmetic outcome.

Total time to acquire pre-reconstructive photos was approximately 10 minutes including camera set up. Post-reconstructive images were taken in approximately 10 minutes with no tissue preparation required. The 3-D reconstructed mask required approximately 10 hours the recreate the soft tissue defect t and 30 hours for the bony defect. We found the aesthetic result of the masks to be accurate.

We propose that 3-D modeling and printing is a simple, accurate, and easily implementable way to reconstitute donor tissue following facial allotransplantation.

**Public Solicitation & the Canadian Media: Two cases, two different stories [working title]**

**Alessandro Marcon**[1], Maeghan Toews[1,3], Timothy Caulfield[1,2]

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The public solicitation of organs has become intensified in the modern digital era where social media, the popular press, and various crowdfunding/crowdsourcing platforms can be leveraged to generate considerable attention for particular causes and initiatives. In Canada, when two high profile cases of public solicitation occurred in the latter half of 2015, it created a large amount of media discourse including the ethical, legal, and issues concerning organ donation in this manner. The two cases involved 1) Eugene Melynk, billionaire CEO and owner of the hockey franchise The Ottawa Senators, and 2) Binh and Phuoc Wagner, adopted 4-year-old twins born in Vietnam and living in Kingston, Ontario. Not only did both cases receive significant media attention individually, but many articles were also published comparing the two respective public appeals for organs. It is the goal of this study to investigate how popular Canadian media covered these stories in similar and diverse manners, shedding light on which social, ethical or legal issues were highlighted and the manner in which those issues were discussed. In order to build a dataset of articles, our research team will use the FACTIVA database and search for articles in popular Canadian digital and print media using relevant search terms over a relevant time frame. From there, we will perform content analysis using inductive and deductive methods, which will include the construction of a coding frame and subsequent coding of all articles. We expect to see the two cases covered in diverse manners with a more favorable tone used for the twins, and a more critical one used for Melynk. In addition, it's possible that certain ethical, legal, or social issues are brought forth when discussing one case but not the other. Faced with encountering either the presence or absence of discussions taking place concerning the need for regulation or increased public policy attention surrounding public solicitation, we will be able to begin shedding light on the public's understanding and values concerning public solicitation in Canada. This study will have relevance for public policy makers, organ donation organizations, and also academics working in areas of public health, specifically public health communications.

**Development of an Immune-Chromatographic Assay for Point-of-Care Detection of Donor-Specific Antibodies (DSA) After Organ Transplantation**

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Antibody-mediated rejection (AMR) is the leading cause of late allograft failure, especially after kidney transplantation. AMR is caused by donor-specific antibodies (DSA) developed in the recipient directed against donor HLA molecules. The timing, and clinical impact of DSA are highly variable. Treatment of AMR is expensive and of limited success, especially once a patient presents with advanced AMR which can progress asymptomatically for a long time once DSA starts acting on the allograft.

Currently, post-transplantation patients must undergo regular blood screening for DSA. Since screening is expensive, it is only done in a sub-set of high risk patients, 2-3 times a year, and rarely more than 2 years post transplantation. As such, a significant number of patients develop DSA undetected, progress to AMR and advanced DSA-mediated allograft damage. There is a critical need for more frequent, reliable DSA screening in organ recipients.

We are designing a point-of-care approach for detection of DSA in transplant recipients. We are developing a hand-held chromatographic assay on pocket-sized slides that patients could use to test for DSA (cost per slide <10$). Similarly to diabetic patients, transplant recipients could monitor their DSA status at home, and only see their physician for follow-up appointments or when the assay detects DSA. This would instigate earlier DSA detection, allowing earlier intervention and increased likelihood of graft survival.

We have developed a prototype lateral flow immune-chromatographic assay. Human MHC Class I antigens were isolated from the immortalized, T-lymphoblast derived cell line MOLT-4 and mounted on slides. The slides are made of tantalum (anodizable color-generating metal) allowing us to coat them with immobilized MHC antigens. Anti-human HLA class I serum (pooled from sensitized renal allograft recipients, i.e. = a pool of a broad variety of DSA) was pipetted onto linker spots on the slides. Afterwards, Peroxidase-AffinPure Goat Anti-Human IgG and chromogenic substrate were pipetted onto the slide and color change from blue to yellow (positive for HLA) was monitored.

We are presently modifying the slides to improve MHC I antigen binding and increase specificity of IgG binding for more precise colorimetric read-outs. Once completed, a validation of the slides will be performed by screening patient serum. If successful, this project has the potential to significantly improve and personalize post-transplant monitoring for DSA and improve early detection of AMR.

**Negative Pressure Ventilation (NPV): A Novel Modality for Ex Vivo Lung Perfusion?**

**Nader S. Aboelnazar**[1], Sayed Himmat[1], Sanaz Hatami[1], Mohamad S. Burhani[1], Christopher W. White[1,2], Peter Dromparis[3], Michael Mengel[3,4], Darren H. Freed[1 2,4,5], Jayan Nagendran[1,2,4]

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Background: Normothermic Ex Vivo Lung Perfusion (EVLP) has increased the rate of donor organ utilization, and increased volumes of lung transplantation at centers that have adopted the technology. Current ventilation methodology for EVLP uses Positive Pressure Ventilation (PPV) with all clinically available devices. As ideal mimicry of native lung physiology would apply a negative pressure to the pleural surface of the lung (Negative Pressure Ventilation, NPV), we developed a novel ventilation system that replicates in vivo lung ventilation. We hypothesize that NPV would be superior to PPV during EVLP.

Methods: A fully automated NPV EVLP platform was developed and compared to conventional (PPV) EVLP. Pig and human lungs were perfused for 12 hours and physiologic parameters, cytokine profile, bullae and edema formation were analyzed. A total of 32 pig lungs were perfused, divided equally into 4 groups based on ventilation strategy and perfusate composition: acellular (STEEN solution ™) and cellular (packed Red Blood Cells + STEEN solution ™). Preliminary unutilized human lungs compared NPV-Cellular (N=3) and PPV-Cellular (N=3).

Results: Using a Student t test and repeated ANOVA (mean±SE), pig and human lungs showed stable trends in lung oxygenation (>400 mmHg) and physiological parameters. Cytokine analysis of pig and human lungs showed significantly lower TNFα, IL-6, and IL-8 production with an NPV strategy regardless of perfusate ($p < 0.05$). Moreover, there was a 42% reduction incidence of bullae with a NPV vs PPV strategy ($p = 0.02$). Porcine lung edema demonstrated lower weight gain with NPV, irrespective of perfusate (NPV-Cellular: 20.1 ± 4.1% vs PPV-Cellular: 39.0 ± 6.6%, $p < 0.01$; NPV-Acellular: 40.4 ± 5.3% vs PPV-Acellular: 88.1 ± 11.0%, p <; 0.01). However, an acellular perfusate had greater edema formation, irrespective of ventilation strategy ($p = 0.01$). Interestingly, in human lungs a weight loss ("drying"• ) was observed (-8.0 ± 2.1% and 39.4 ± 5.6%, $p < 0.01$).

Conclusion: Negative pressure ventilation (NPV) has the potential to be more beneficial compared to the conventional positive pressure ventilation (PPV) with significantly less inflammation, bullae, and edema formation during extended EVLP for both perfusate groups. The value of a NPV strategy may lead to further improvements to currently available clinical EVLP platforms. Further studies are warranted on the value of NPV-EVLP in pre-clinical transplant models and clinical trials.

**Normothermic Ex Vivo Kidney Perfusion and DAMPs**

**Yilun Wu**[1], Alison Muller[1], Nader Aboelnazar[2], Sanaz Hatami[2], Sayed Himmat[2], Mohamad Burhani[2], Xiao Qi[2], Nobutoshi Matsumura[4], Jody Levasseur[4], Darren Freed[1,2,3,5,6]

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Organ transplantation is the definitive therapy for chronic, end-stage organ failures such as end-stage kidney failure. The transplant process invokes an ischemia reperfusion phenomenon that is accompanied by sterile inflammation. Sterile inflammation can lead to kidney injury and transplant rejection. One important factor in sterile inflammation is the interaction of damage-associated molecular patterns (DAMPs) and toll-like receptor 4 (TLR-4). Drug therapy to modulate TLR-4 activation can be difficult, due to ethical concerns in treating the donor and systemic side-effects in the recipient. However, in Normothermic Ex Vivo Kidney Perfusion (NEVKP), a novel organ preservation technique, the kidney is preserved in an isolated system outside of both donor and recipient bodies. NEVKP provides a unique opportunity to treat or prevent the ischemia reperfusion injury and sterile inflammation with TLR-4 inhibitors. Although NEVKP is an attractive platform for preserving organs, the device itself may induce inflammation and TLR-4 activation as a consequence of the artificial environment. Though NEVKP's clinical implementation is in its infancy, it has been shown to provide superior preservation compared to the traditional cold preservation techniques. We have established a large animal model of NEVKP to investigate the effects of TLR-4 inhibitors such as statin drugs and/or TAK-242 ex vivo. To date, we have successfully perfused 6 kidneys in the NEVKP system. Preliminary data show an increase in perfusate concentrations of cytokines downstream of TLR-4 (IL-1b and IL-18) from hour 5 to hour 11 of NEVKP. If statin drugs and/or TAK-242 can be used to reduce sterile inflammation in NEVKP caused by DAMPs, it may translate to increased graft function and the ability to treat dysfunctional organs ex vivo.

## Adiponectin gene therapy on ex vivo lung perfusion to improve lung transplantation outcome

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Introduction: A growing body of evidence highlights normothermic ex-vivo lung perfusion as a valuable platform for the delivery of therapeutic agents to donor lungs while evaluating them prior to transplantation. Progression of in-vivo gene therapy faces anchoring heralds from fatalities in early trials. Adiponectin, a bioactive peptide, has significant anti-inflammatory and cytoprotective properties to the pulmonary vascular endothelium and airway epithelium. In addition, low levels of plasma adiponectin increase the susceptibility of the lung to inflammatory related injury.

Objectives: We propose to optimize the efficacy and assess the functional as well as immunologic benefits of ex-vivo adenoviral adiponectin (Ad-ApN) gene delivery in conferring protection against primary graft dysfunction and development of chronic allograft dysfunction.

Methods: Procured lungs from nine Yorkshire female domestic pigs (35-45kg) are randomly assigned to 3 treatment groups, where lungs are perfused on EVLP for 12 hours: 1) Ad-mCherry: with adenovirus-mCherry in the perfusate, 2) Ad-ApN Gene Therapy: adenovirus-adiponectin group, and 3) EVLP-Control. Baseline physiologic functional and cytokine profile will be reported from EVLP-control. Ad-mCherry, facilitates the visualization of viral distribution using florescence overlay pictures and histologic evidence. In addition, levels of expressed adiponectin over 12 hours, will be measured in Ad-ApN perfused lungs using immunoassays and tissue western blots.
Results (to date): Thus far, perfusion of control lungs (N=6) showed stable physiologic parameters over 12 hours. Lung dynamic compliance (Cdyn) remained stable from 16.4 Â± 2.6 ml/cm H2O (T1) to 25.6 Â± 2.5 ml/cm H2O (T11). There was a trend towards improved pulmonary vascular resistance (PVR) from 609 ± 78 dyn-s/cm5 to 423 ± 53 dyn-s/cm5, (p = 0.055). Weight gain was 60% on average. Moreover, analysis of pro-inflammatory cytokines in perfusate samples taken 2 hourly, showed a significant increase in TNFα, IL-6 and IL-8 on average. Ad-mCherry perfused lungs showed increased florescence in all lobes compared to controls, on pictures taken at T12.

Conclusions: Our results so far showed feasibility of intravascular delivery of gene therapy using EVLP. We hope to show evidence of reduced inflammatory markers and improved function of adiponectin transduced lungs. Moreover, measure an increase in adiponectin expression in the perfusate over time from respective lungs. Finally, using transplant models to demonstrate pre-clinical safety and efficacy of ex-vivo Ad-ApN gene therapy.

**Endoplasmic reticulum stress in Ex vivo heart perfusion: a comparison in working versus non-working modes**

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Background: Ex vivo heart perfusion (EVHP) provides the opportunity to preserve the donated heart in a semi-physiologic beating status, to monitor function and potentially improve the quality of marginal/declined hearts. Currently, the clinically available EVHP apparatus supports the heart in non-working mode (NWM). EVHP in working mode (WM) however, may be better in terms of function assessment and preservation. Endoplasmic reticulum (ER) is an organelle of particular importance for cardiac function. In response to different stresses and pathophysiologic stimuli, the unfolded protein responses (UPR) can be activated, which if severe/prolonged, can lead to functional decline/cardiac failure. The aim of the present study was to investigate the ER stress responses in the EVHP setting and to compare it in the working vs non-working modes.

Methods: 19 female domestic breed pigs (37-47 kg) were included. The procured hearts were immediately mounted on a custom EVHP apparatus and perfused for 12 hours. In NWM group (n=6), perfusion continued in non-working mode with no left atrial flow, whereas in WM group the heart perfused with a left atrial pressure of 6 mmHg from the first hour until the end of perfusion (n=9). Cardiac function parameters were compared between two groups. At the end of perfusion, left ventricle (LV) tissue specimens were collected and analyzed for ER stress markers using protein immunoblotting method. The results were compared between two groups and with baseline EVHP samples (n=4).

Results: In both groups, cardiac function declined overtime but the cardiac function parameters were better preserved in WM group (e.g. cardiac index, $P<0.01$). Compared to baseline, hearts in both groups showed upregulated levels of sensors/branches of ER stress responses including downstream effectors of activated PKR-like ER kinase (PERK) ($p<0.01$), inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6), and their products including protein disulfide isomerase (PDI) ($p<0.05$). No significant difference was observed in ER stress markers levels between WM and NWM groups ($p>0.05$).

Conclusion: There was upregulation of ER stress markers in ex vivo perfused hearts over 12 hours of perfusion. The enhanced UPR was not associated with ventricular load and perfusion mode during the perfusion. To optimise the EVHP protocols for the aim of better cardiac function preservation and recovery, further studies are warranted to clarify the causes of ER stress during EVHP and its specific consequences such as myocardial energy metabolism disarrangements.

## Pro-Fibrotic Marker Expression in Primary Human Mesenchymal Cells Reseeded in Decellularized Human Cardiac tissue

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Endogenous mesenchymal cells contribute to fibrosis in the heart, leading to congestive heart failure by impairing its function as a result of pathological extracellular matrix (ECM) protein deposition. It has been established that both atrial fibroblasts (AFs) and bone marrow-derived mesenchymal progenitor cells (BM-MPCs) contribute to ECM protein deposition by adopting a pro-fibrotic phenotype. Previous research in other labs, including ours, has determined that AFs and BM-MPCs become pro-fibrotic in response to a stiff ECM environment. Patients with severely impaired heart function who are unable to receive a donor heart may be implanted with a left ventricular assist devices (LVADs). Although LVAD unloading has been shown to allow for structural and functional remodeling, there is an increase in both interstitial and total fibrosis. Our lab is interested in understanding endogenous AF and BM-MPC differentiation into pro-fibrotic phenotypes and we have recently optimized a technique that allows us to better understand the molecular mechanisms involved. Human ventricular tissue from an un-utilized donor heart and an explanted heart equipped with an LVAD were sliced and subject to a multi-step washing process. After decellularization, tissue slices were co-incubated with primary human atrial fibroblasts (hAFs) or human BM-MPCs (hBM-MPCs) with plates of hAFs and hBM-MPCs cultured on standard plastic culture dishes as a control. HAFs and hMPCs were incubated separately on sections of decellularized human LVAD or un-utilized donor heart extracellular matrix (ECM) over a week. Recellularized samples were fixed for scanning electron microscopy (SEM) to visualize the decellularized and recellularized tissue, or homogenized followed by RNA isolation. qRT-PCR of pro-fibrotic markers indicating that expression of Col1a2, MYH9, and MYH10 were decreased compared to plastic control, whereas Col1a1expression was significantly increased compared to plastic control. There were also increased expressions of Col1a1 and MYH9 in hAFs seeded in explanted tissue compared to control. When comparing between healthy and LVAD ECM, hAFs exhibited different responses in their unique environments indicating that ECM alone can influence a pro-fibrotic phenotype. In addition to LVAD patients, this has implications in stem cell therapies targeting tissue regeneration in pro-fibrotic environments, where additional measures may need to be implemented in order to prevent the ECM environment from influencing unfavourable pro-fibrotic differentiation of mesenchymal-derived progenitor cells.

## A-Antigen-Specific Tolerance in a Mouse Model of ABO-incompatible Heart Transplantation

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Introduction: In contrast to adults, ABO-incompatible heart transplantation (ABOi HTx) can safely be performed in infants due to absent/low levels of anti-A/B antibodies. Tolerance develops to donor blood group A/B-antigen(s) after ABOi HTx by mechanisms not well-defined. Using a novel human blood group A-antigen transgenic (A-Tg; C57BL/6, B6) mouse model, we showed A-antigen specific tolerance following HTx into young (4 wk old) wild-type B6 (WT) mice. Here, we explored tolerance induction by exposure to A-antigen in a form other than a transplant. A-Tg erythrocytes (RBC) express A-antigen at high levels. We hypothesized that treatment of infant WT mice (≤3 wks old) with A-Tg RBC would induce A-specific tolerance, allowing subsequent ABOi HTx.

Methods: To induce tolerance, WT mice were injected ip at age 7, 14, 21 days with: 1) intact A-Tg RBC (n=12); 2) A-Tg RBC membranes (n=4); 3) human A RBC membranes (hA-RBCs, n=6); or 4) left untreated (n=9). As adults (7 wks), all mice were injected ip (weekly x4) with hA-RBC in an attempt to elicit anti-A antibody (ie, to break tolerance). Serum anti-A and third-party (non-A anti-human RBC) antibodies were assessed by hemagglutination/ELISA.

Results: A-sensitization stimulated the production of high levels of anti-A antibodies in untreated mice (median titre 1:1024) and mice neonatally-treated with hA-RBCs (1:2048) or A-Tg RBC membranes (1:256). In contrast, anti-A production was not stimulated in mice treated as neonates with intact A-Tg RBC (median titre ≤1:2); third-party antibody production was high in all groups (1:64).

Conclusion: The inability to elicit anti-A in A-sensitized adult mice neonatally-treated with intact A-Tg RBCs, together with production of abundant third-party antibodies, indicates development of robust A-antigen-specific tolerance. Our findings suggest that the form of A-antigen (intact cells vs membrane), which may relate to antigen persistence, is critical to B cell tolerance in this setting. Intentional induction of tolerance to A/B-antigen(s) in infancy may allow later ABOi HTx.

**An In Vitro Model Of Human Antibody-Mediated Rejection**

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INTRODUCTION: Antibody-mediated rejection (AbMR) remains a leading barrier to long term transplant survival. The immune mechanisms of AbMR are incompletely understood but recent evidence suggests that monocytes and NK cells are potential effectors against HLA antibody-targeted endothelium. We have developed an in vitro model of human AbMR that incorporates the immune cellular components currently known.

METHODS: Human umbilical vein endothelial cells (HUVECs) served as endothelial targets and were pretreated with IFN-g for 48 hours to maximize HLA expression. HLA antibodies were derived from sera pooled from highly sensitized patients identified by the UAH HLA laboratory. Primary NK cells and monocytes were isolated by immunomagnetic depletion from healthy controls and NK cells were added to HLA antibody-coated HUVECs. Wells containing monocytes had the monocytes added to tissue culture inserts that allow the passage of soluble mediators but not cells. RNA was extracted for global gene expression analyses after eight hours of culture. Analyses focused on three representative sets of transcripts: pro-inflammatory chemokines, IFN-g response, and endothelial activation.

RESULTS: NK cell/ HLA antibody-mediated attack led to greatly increased chemokine expression, particularly those that promote additional NK cell and monocyte recruitment, many reflecting IFN-g effects on endothelial cells and monocytes. Adhesion molecules key to recruitment and leukocyte engagement and indicative of endothelial activation were also increased, some of these being required for optimal NK cell activation (eg. ICAM1). The presence of monocytes in the system (despite being separated from NK-HUVECs) increased all three sets of transcripts beyond levels observed with NK cells alone, particularly endothelial activation.

CONCLUSIONS: Our results suggest that we have a working model of human AbMR that incorporates two effector cell types, HLA antibodies, and HUVEC (endothelial) targets. This model argues for a key effector role by NK cells in AbMR, whose function is greatly enhanced by soluble mediators from monocytes in close proximity.

**Fc-receptor-activated NK cells in ABMR are highly similar to CD3-activated effector T cells in TCMR**

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BACKGROUND: NK cell Fc-receptor (CD16a) and T cell TCR/CD3 activate shared signalling pathways. NK cell CD16a activation in antibody-mediated rejection (ABMR) and T cell CD3 activation in T cell mediated rejection (TCMR) are the key initiators of their respective rejection processes in the allograft. We hypothesized that shared effector lymphocyte activation events would be commonly observed in both ABMR and TCMR.

METHODS: To map shared CD16a/CD3-inducible molecules in vitro, primary human NK and CD8 T cells were cultured +/- CD16a or CD3 stimulation respectively. We measured global gene expression changes using gene expression arrays, and assayed production of 28 soluble proteins in supernatants using protein arrays. Transcripts selective to both T and NK cells were identified by examining expression in a primary human cell panel. In vivo expression of shared transcripts in rejection was examined in 703 clinically-indicated kidney transplant biopsies collected from 579 patients at six kidney transplant centres.

RESULTS: Four hours stimulation induced 231 shared activation transcripts in both T and NK cells (>2x vs unstim. FDR<0.05). The transcripts most increased in both cell types included effector cytokines IFNG, TNF, GM-CSF; pro-inflammatory chemokines CCL3, CCL4, XCL1; and membrane proteins that support lymphocyte effector function such as 4-1BB and CD160. Both cell types produced a limited 5/28 proteins after stimulation (IFNG, TNF, GM-CSF, CCL3, CCL4) and did not produce 23/28 (XCL1 not assayed). Whereas many transcripts were expressed in other cell types, IFNG, XCL1, 4-1BB, and CD160 were selective for activated T and NK cells. All four were increased in and highly associated with both ABMR and TCMR. Differential transcript signal strength in vitro between T and NK cells were reflected in vivo between TCMR and ABMR: CD160 had 11-fold higher expression in stimulated NK cells vs stimulated T cells in vitro and was more strongly associated with ABMR than with TCMR. IFNG showed the opposite pattern.

CONCLUSIONS: NK CD16a and T cell CD3 stimulation induce highly shared transcript expression and effector cytokine production. T/NK-selective gene expression in biopsies indicates that T and NK cell activation events are shared in ABMR and TCMR. Effector lymphocyte activation events in rejection are thus surprisingly conserved in TCMR and ABMR despite differences in triggering mechanisms and location. Selectivity of CD160 for NK cells and ABMR makes it a candidate marker for specifically assessing effects of treatment on NK cell activation in ABMR.

**Expansion of thymic regulatory T cells (tTregs) with Good Manufacturing Practice (GMP)-compatible tetrameric antibody complexes**

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BACKGROUND: Treg-based cellular therapy to suppress graft-directed immunity could reduce the need for life-long immunosuppressive drugs. Challenges include isolation of pure Tregs and expansion to clinically relevant numbers while maintaining stable function. We previously showed that abundant CD25+ thymocytes can be isolated from discarded human thymuses, routinely removed during pediatric cardiac surgery, and expanded to highly suppressive and stable Tregs using artificial antigen-presenting cells (APCs). For transition into GMP setting, we studied whether tTregs can be expanded using GMP-compatible non-APC-based tetrameric antibody complexes.

METHODS: Thymocytes were isolated by mechanical dissociation from thymuses obtained during pediatric cardiac surgery. CD25+ tTregs were isolated by magnetic-bead cell separation and expanded in the presence of IL-2 and rapamycin with anti-CD3/CD28 antibody complexes, anti-CD3/CD28/CD2 antibody complexes or artificial anti-CD3-loaded APCs. Treg expansion was assessed by analyzing expansion rate, viability, FOXP3 expression and suppressive capacity of the proliferation of anti-CD3/28-stimulated T cells.

RESULTS: tTregs cultured with anti-CD3/CD28/CD2 antibody complexes had a higher expansion rate than those cultured without anti-CD2 (mean±SEM fold expansion at day 14: 46±31 vs. 29±19, respectively). Although their expansion rate was lower compared to tTregs cultured with APCs at day 11, it was comparable on day 14. Viability at day 14 was >80% for all culture conditions. The vast majority of tTregs cultured with antibody complexes maintained high FOXP3 expression (mean±SEM % FOXP3+ cells: anti-CD3/CD28: 83±5% and anti-CD3/CD28/CD2: 85±4%), comparable to APC-cultured tTregs. tTregs cultured with antibody complexes potently suppressed proliferation of anti-CD3/CD28-stimulated T cells (>70% inhibition at a Treg:responder cell ratio of 1:2.5).

CONCLUSION: Thymic Tregs can be expanded with tetrameric antibody complexes and maintain FOXP3 expression and potent suppressive capacity. By using non-APC-based tetrameric antibody complexes, we will be able to transition into a GMP setting for further optimization of an expansion protocol for therapeutic tTregs.

## CD4 Cells And Co-Engagement With Forigen Protein Are Required In Antibody Response To Blood Group A-Antigen

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Introduction: ABO-incompatible heart transplantation (ABOi HTx) is safe during infancy and allows increased donor access. B cell tolerance develops to donor A/B-antigen(s) after ABOi HTx by mechanisms not well defined. We developed A-transgenic (A-Tg) mice constitutively expressing human A-antigen on vascular endothelium and erythrocytes (RBC) to study B cell immunity and tolerance. Here we studied A-incompatibility in the context of syngeneic, allogeneic and xenogeneic stimulation.

Methods: Part I: Adult wild-type (WT) C57BL/6 (B6; H-2b), BALB/c (BALB; H-2d), or C3H/He mice (C3H; H-2k) received i.p. injection weekly x3 of B6 or BALB A-Tg RBC membrane (100 µl of 10% vol/vol); or human RBC membranes (100 µl of 10% vol/vol) from blood group A or O; or A-incompatible heart allografts (Table). Serum anti-A antibody was measured by hemagglutination and ELISA (IgG and IgM); graft survival was assessed by palpation. Part II: a) to assess requirement for ‘linkage' of the non-self carbohydrate A-antigen with foreign protein to stimulate anti-A, human O-RBC were co-injected in B6 WT mice with syngeneic A-Tg cells; b) to assess T cell dependence of anti-A response, CD4+ T cells were depleted (mAb GK1.5) from B6 WT mice before human A RBC injection.

Results: Part I: Exposure to incompatible A-antigen in the context of allogeneic stimulation (MHC-mismatched A-Tg blood or heart graft) or xenogeneic stimulation (human A-RBC) induced abundant anti-A production (Table), whereas A-Tg syngeneic (MHC-matched) cells did not. Part II: a) a mixture of syngeneic A-Tg and xenogeneic human O-RBC did not induce anti-A; b) after CD4+ T cell depletion, xenogeneic cells failed to elicit anti-A.

Conclusion: Exposure to non-self A-antigen in syngeneic cells is insufficient to induce anti-A production; stimulation of antibody depends not only on exposure to the foreign carbohydrate but co-engagement with allogeneic or xenogeneic protein, and requires CD4 T cell help. These results are consistent with our previous data showing a requirement for chemical/physical linkage of non-self A-antigen and foreign protein.

**CD4 and CD8 T Cells: Respectively Reprogrammed and Recapitulated in the Neonate to Induce Transplant Tolerance**

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Introduction: Non-conditioned neonatal mice develop acute graft-versus-host disease (aGVHD) when injected with adult allogeneic spleen and bone marrow cells (allo-SC/BMC). To prevent aGVHD and induce transplant tolerance with allo-SC/BMC we reprogrammed donor/host CD4 T cells by co-stimulation blockade to preserve regulatory CD4 T cells while recapitulating depleted donor/host CD8 T cells.

Methods: C3H (H-2k) neonatal mice were injected iv with total or depleted B6 (H-2b) GFP+ SC/BMC. CD8 T and CD49b NK cells were depleted from donor inocula using StemCell Technologies EasySep® kits. In vivo neonatal host CD8 T cells were depleted (Mab 53-6.7) and donor/host CD4 T cells reprogrammed (CD154 Mab MR1). Trafficking of injected cells was monitored by whole body/organ imaging; donor cell interactions/fates in lymphoid organs was determined by high resolution microscopy. Tolerance induction was assessed by transplanting treated mice as adults with donor-type hearts.

Results: Neonatal mice injected with GFP+ allo-SC/BMC developed aGVHD, with diarrhea, reduced growth and early death. GFP+ cells proliferated and spread throughout injected mice indicating systemic inflammation. Depletion of donor/host CD8 T cells together with co-stimulation blockade of donor/host CD4 T cells (CD154) resulted in GFP signal being reduced and restricted to lymphoid organs (day 6). High resolution microscopy showed donor T and B cells positioned in PALS and follicular regions, respectively, of host spleen; lack of Ki67 immunostaining indicated many donor cells were not proliferating. Donor DC were detected in host thymus (day 6) suggesting a possible role in central tolerance. Donor-type B6 hearts transplanted into neonatally-treated adult C3H mice (n=5) (CD8 depleted/CD4 co-stimulation blockade) all continued to beat at 100 days post-transplant, 3 grafts with maximal strength and 2 grafts with diminished strength. In untreated control mice (n=3) B6 hearts stopped beating by day 10. H&E staining of a strongly beating B6 heart graft at 100 days showed undamaged cardiomyocytes with little if any cellular infiltrate and absence of macrophages and T cells by immunostaining.

Conclusion: Reprograming CD4 T cells while depleting/recapitulating CD8 T cells in allo-SC/BMC treated neonates leads to 100% survival at 100 days of cardiac allograft transplanted as adults; low beat scores in 2/5 B6 grafts suggests further optimization is required. These findings provide insight into robust tolerance induction in neonates.

**A Minimum Conditioning Protocol towards Transplantation Tolerance in NOD Mice by Mixed Hematopoietic Chimerism**

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Stable mixed hematopoietic chimerism is a robust method for inducing donor specific tolerance. However, its clinical application is dampened by the toxicity of current recipient conditioning regimens. We previously showed an irradiation-free mixed chimerism protocol in diabetes prone NOD mice is achievable with antibodies to T cells and CD40L together with busulfan and high dose rapamycin. We sought to generate a more clinically feasible chimerism protocol and tested the hypothesis that more efficient recipient T cell depletion would eliminate the need for anti-CD40L (known to cause thromboembolism in humans) and rapamycin.

We preconditioned NOD mice with donor specific transfusion from fully mismatched mice (d -10), cyclophosphamide (CYP) (d -8), antibodies against CD90 and/or CD4 + CD8 (d -6, -1, 4, 9, 14), busulfan (d -1) and donor bone marrow transplant (d 0). Flow cytometry was used to detect chimerism.

Mixed chimerism was induced in 33/45 NOD mice. Stable chimerism with multilineage donor cells was maintained in 21/33 recipients. Loss of chimerism could be predicted by a lower early level of chimerism at d 4, 9 or 14. The inclusion of Î±CD90 mAb, busulfan and CYP was critical for chimerism induction. With anti-CD90 17/23 mice became stable chimeras. 5/5 chimeric mice accepted skin from bone marrow donors but rejected skin from MHC-matched and minor antigen mismatched donors. The loss of anti-donor Vβ11+ T cells and anti-recipient Vβ17+ T cells in stable chimeric mice indicated the establishment of chimerism involves clonal deletion. Conclusion: A protocol causing rapid and robust recipient T cell depletion generated chimerism without the need for anti-CD40L or rapamycin in tolerance induction resistant NOD mice.

**Characterization of vascular endothelial repair of Apelin-deficient hearts in Chronic Allograft Vasculopathy model in Mice**

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INTRODUCTION: Heart transplantation is a lifesaving treatment for end-stage cardiac patients. Long-term cardiac allograft survival is limited by chronic allograft vasculopathy (CAV), hence, CAV is a leading cause of mortality beyond the 1st year of transplantation. Immune injury targets the endothelial cells (ECs) lining the coronary arteries and microvasculature. The maladaptive repair response results in obliterative arterial intimal expansion, and microvessel injury. In turn, a decreased blood supply contributes to graft failure. Apelin is an EC-specific protein, coded on the X chromosome that participates in vascular repair from myocardial infarction and kidney glomerular microvasculature injury. We hypothesize that Apelin-dependent pro-angiogenic signals are exploited for repair of CAV injury.

METHODS: CAV was induced via transplantation of male mouse hearts into female recipients to elicit a HY-minor histocompatibility antigen-directed, cell-mediated allo-immune response against the male donor hearts. Apelin-/y (knockout; KO) or Apelin+/y (wild type; WT) hearts were transplanted into WT female recipients. The heart grafts were harvested two or six weeks after transplantation. We characterized intima area, and endothelial loss in medium to large-sized arteries, inflammatory cellular infiltration, and microvasculature density, using Immunohistochemistry (IHC).

RESULTS: Apelin-/y donor hearts show an increase in circumference area of endothelial loss ($1.4 \pm .1$ vs $0.4 \pm .1\%$; $1.9 \pm 0.2$ vs $0.5 \pm 0.1\%$; $P<0.05$), and intima expansion in conduit arteries at 2 and 6 weeks respectively. Further, Apelin-/y donor hearts have decreased microvessel density at 2 and 6 weeks. An enhanced inflammatory cellular infiltration is observed in Apelin-/y donor hearts compared to controls.

CONCLUSIONS: Loss of Apelin exacerbates CAV, loss of microvessel density, and is associated with an enhanced inflammatory infiltrate evident at an early (2 week) timepoint after transplantation.

**Reduced naive regulatory T-cells and B-10 cells in thymectomized children predispose for allergic disorders after heart transplantation**

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Introduction: Pediatric heart transplant (HTx) recipients are at higher risk for allergic and autoimmune disorders than non-transplanted children. We found young age at HTx and thymectomy (TE) to be associated factors. We hypothesized that TE and immunosuppression at an early age affect the development of T and B-cell subsets, especially regulatory T-cells (Tregs), which are key in maintaining peripheral tolerance. We investigated the impact of TE and lymphocyte-depleting induction on lymphocyte subtype proportions, and asthma and allergies in HTx patients.

Methods: Flow cytometry phenotyping was used to determine proportions of lymphocyte subsets in peripheral blood samples from patients transplanted before age 18, detecting expression of specific surface markers. Clinical data were collected in standardized questionnaires on allergic and autoimmune disorders including asthma and eczema. Medical charts were reviewed for confirmation.

Results: Proportion of CD45RA+CD27+ naïve Treg cells within the Treg population (CD4+CD25+CD127low) was lower in thymectomized (n=8) than non-thymectomized (n=4) patients ($p<0.05$). Of thymectomized patients, 71% developed asthma or eczema post-transplant, compared to 41% of all HTx children and 15% of the general pediatric population. A markedly reduced proportion of total Tregs in patients with asthma and eczema did not reach statistical significance. Memory CD4+ cell proportions were higher in thymectomized patients. CD5+CD1d+ “B10” populations were higher in thymectomized patients. Memory B-cell proportions were not different between groups.

Conclusions: Lower percentages of naive Tregs following TE may contribute to the increased risk of asthma and eczema in HTx children. Other lymphocyte subsets such as “B10” populations likely further contribute to increased risk. Confirmation of this phenotype pattern correlated to allergic disorders in a larger sample is needed in order to risk stratify patients and consider individualized treatment modification.

## Age-Related Differences In The Regulatory Capacity of CD5+CD1D+ B-Cells In The Context Of Heart Graft Acceptance

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Introduction: Young children show better graft survival and reduced need for immunosuppression following heart transplantation (HTx). The CD5+CD1d+ subpopulation of B-cells contains IL-10-producing “B10 cells” found to have immune regulatory capacity in mice. We found this subtype to be up to ten times more prevalent in infants than older children, and hypothesized that they contribute to the more tolerogenic environment. We aimed to determine if IL-10 in humans originates mainly from CD5+CD1d+ B-cells, and if this cell type impacts B- and T-cell proliferation. Age-related differences in the functionality of these cells were also explored.

Materials and Methods: Splenocytes were sorted by flow cytometry (FACS) to separate CD5+CD1d+ from all other B-cells and obtain 2 populations, which were cultured with stimuli reflecting T-dependent (TD; IgM+CD40L) or T-independent (TI; CpG) activation. IL-10 secretion was measured by ELISA. To assess effects on proliferation, CellTrace™-marked splenocytes were stimulated with Staphylococcal enterotoxin B, IgM+CD40L, CpG, or CD3+CD28 in absence of CD5+CD1d+ B-cells, natural proportion (CONTROL), and with addition of 2 and 5 times their natural proportions.

Results: CD5+CD1d+ B-cells produced IL-10 following TD and TI activation. However, the remaining phenotypes produced higher amounts of IL-10 with TI-activation than the CD5+CD1d+ subset. In adult samples, IL-10 secretion appeared to be reduced. Compared to the CONTROL, B-cell proliferation after TD activation was 27% higher in the CD5+CD1d+ depleted cultures ($p = 0.081$). The suppressive effects were strongest on non-class-switched memory (CD27+IgM+) B-cells ($p = 0.059$). No effects of addition or removal of the CD5+CD1d+ B-cell on proliferation of T-cells or TI-activated B-cells were observed. As with IL-10 secretion, CD5+CD1d+ B-cell effects on proliferation appeared diminished in adult samples.

Conclusions: CD5+CD1d+ B-cells encompass some, but not all, IL-10-producing B-cells. The presence of these cells in increasing proportions decreases the proliferation of TD-activated B-cells, especially the non-class-switched memory cells, indicating that their high proportion in early childhood contributes to better graft acceptance. Further analysis is required to confirm age-related difference in IL-10 secretion and suppression of proliferation.

## Notification of the VADs to the HLA laboratory: Now we are talking

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Introduction: The implantation of a ventricular assist device (VAD) has been shown to be a risk factor for development of HLA antibodies. HLA antibodies are known to be associated with poor post-transplant outcomes and increase the difficulty of finding a compatible donor. Although the exact role of VADs in the development of HLA antibodies is not yet clearly understood, it is important that patients with VADs are identified to the HLA lab so that relevant samples are tested after this sensitizing event. This study examined whether or not a sample was submitted for testing pre- and post-VAD and if so, whether the HLA laboratory had been notified of the implant.

Methods: Adult and pediatric patients who received a VAD between 2005 and 2013 were included. Data were collected from the HLA laboratory's information system and the VAD database. HLA antibody testing that was performed was reviewed and the samples were categorized as pre- or post- the first implant.

Results: Patients were identified from the VAD database (n=140) with samples received from 79 adult and 42 pediatric patients. There were 19 VAD recipients for whom no samples was sent to the HLA laboratory. For adult patients, samples for HLA antibody analysis were sent both pre- and post-implantation in 66% (n=52); 21% (n=17) had only pre-VAD samples and 13% (n=10) had only post-VAD samples. For children, samples were sent both pre- and post-implantation in 83% (n=35), with one patient having only pre-VAD samples and 15% (n= 6) only post-VAD samples. The majority of patients from whom no samples were analyzed had been implanted with short-term VADs and were unlikely to have proceeded to transplant; 2 patients with no testing had long-term VADs.Some samples (n=42) from both adults and children were sent without identifying the VAD implant. Furthermore, some patients in both groups proceeded to transplant without the laboratory's knowledge of the VAD implantation.

Conclusion: The majority of pediatric and adult VAD patients in this local cohort underwent both pre- and post-VAD testing for HLA antibodies. However, there were a number of patients not identified in the HLA lab as having received a VAD. Communication with the HLA lab is essential to establish which samples are most important for determining potential treatment options for these critically ill patients, including the risks of heart transplantation in sensitized patients. Missing VAD implant dates have been entered into the HLA laboratory information system. As a result of this study, the HLA laboratory has updated the 'Activation Form' utilized by the transplant teams to include specific questions related to VAD implants.

## Sensitization across the age spectrum post-VAD implantation: Maybe memory matters

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Introduction: Ventricular assist device (VAD) implantation is a risk factor for development of Human Leukocyte Antigen (HLA) antibodies (Ab). These Ab increase transplant (Tx) waitlist times and are associated with poor transplant outcomes. Few data exist regarding impact of age on HLA Ab development. This study aimed to compare class I and II Ab production in adult vs pediatric patients with VADs.

Methods: Long-term VADs at our centre between 2005-13 were evaluted. Patients were included who were within one year post-VAD implantation, pre-heart transplant, with their first long-term device, and for whom both pre- and post-VAD HLA Ab results were available. N=40 adults and 27 pediatric patients were eligible for analysis. Class I and II panel reactive antibodies (PRA) were measured by either FlowPRA® or cPRA; cPRA was assessed by single antigen beads and was available only in patients with positive HLA screens. cPRA values were calculated using the Canadian cPRA Calculator by entering all known HLA antibody specificities. Class I and II PRA values pre-VAD were compared to the first sample post-VAD. Sensitization was defined as PRA ≥ 10%.

Results: Patient demographics including age, sex, weight, cardiac disease, and sample collection days pre- and post-VAD were captured. Median time to first HLA sample was 13 days (IQR 7-38) post-VAD. N=13 patients were sensitized to class I pre-VAD (7 adults, 6 peds), increasing to 24 patients (15 adults, 9 peds) post-VAD. Three patients sensitized pre-VAD had PRA <10% post-VAD. In both pediatric and adult patients, class I but NOT class II FlowPRA values were increased post-VAD compared to pre-VAD. The overall cPRA values (class I and II) were increased post-VAD only in adult patients (p=0.02).

Conclusion: HLA Ab detected early post-VAD implantation are predominantly against class I antigens. Increased class I Ab may be driven by platelet transfusion at the time of VAD implantation. Adults, but not children, had significantly increased cPRA following implantation. It is unclear whether Ab developing early post-implant will persist over time and whether the difference between the two groups represents an amnestic response to previous sensitization. Future studies will examine long-term Ab responses in these patients and age and sex will be evaluated as risk factors for the development of post-VAD HLA antibodies.

## Proof of Principle: Bead-Based ABO Antibody Assessment

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Introduction: For clinical management of ABO-incompatible (ABOi) organ transplantation, serum ABO-antibodies are traditionally assessed by hemagglutination. However, hemagglutination cannot readily differentiate antibody isotype nor distinguish between antibodies to ABO-subtype antigens, which are expressed differently on organs vs erythrocytes. We explored Luminex as a potential platform for characterization of IgG and IgM antibodies to ABO subtype antigens.

Methods: Phase I: Human blood group antigen subtype A-II was synthesized and conjugated to bovine serum albumin (BSA). BSA-A-II conjugates were coupled to Luminex beads. Coupling was assessed using phycoerythrin (PE)-labeled mouse monoclonal IgG and IgM anti-human ABO-A. Increasing BSA-A-II antigen concentrations were tested to optimize mean fluorescence intensity (MFI) output. A Luminex-200 cytometer was used for bead fluorescence detection.
Phase II: Antigens ABO A-III and A-IV were similarly tested (alone and multiplexed) using additional Luminex beads.
Phase III: Dilutions of PE-labeled anti-human IgG secondary antibody were tested against dilutions of anti-ABO-A plasma (blood group B donor) in a checkerboard titration. Blood group A donor plasma was included as a negative control. BSA-A-II coupled beads were used for this phase of testing.

Results: Bead coupling was optimal at 5ug of BSA-A-II antigen. The coupling of BSA-A-II, -III and -IV was successful as demonstrated by a linear increase in MFI with increasing concentrations of primary antibody. MFI values approaching 20,000 and plateau of MFI values were achieved, indicating bead fluorescence saturation. Beads were successfully labeled with monoclonal ABO-antibody when multiplexed. Based on the patient serum results, the assay was able to detect IgG ABO antibody with increasing sensitivity depending on the dilution of patient plasma and secondary antibody. As expected, there is high background in undiluted patient serum that decreases with dilution.

Conclusion: Histocompatibility laboratories are uniquely situated to assess ABO antibodies by Luminex as expertise and instrumentation already exist. This novel assay has the potential to be adopted by clinical histocompatibility laboratories for rapid, specific, and sensitive assessment of IgM/IgG subtype-specific ABO antibodies in ABOi transplantation. We will continue to optimize the assay, include additional controls, and assess additional ABO subtypes. We are also testing flow-based bead platforms. The ABO antibody results from these platforms will be compared to the existing micro-array technique already shown to provide additional specificity in transplantation.

## Refinement of an Old Faithful: a Standardized Flow Cytometry Approach to Measure Donor-Recipient Alloreactivity (a Pilot Study)

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Introduction: Donor-specific antibody (DSA) and antibody (Ab)-mediated rejection reduce long-term survival, accelerate graft vasculopathy and limit options for re-transplantation. Sensitizing events such as homograft use in surgical procedures, assist device implantation, and transfusions may lead to sensitization and development of HLA-Ab. Thymectomy is routinely performed during pediatric heart surgery; our overarching goal is to study the impact of thymectomy and its role in development of post-transplant (de novo) DSA. In order to study donor-recipient alloreactivity, we evaluated the mixed lymphocyte reaction (MLR) for T cell proliferation combined with a standardized flow cytometry lymphocyte phenotyping panel (Duraclone IM, Beckman Coulter) widely used in the Canadian National Transplant Research Program and the ONE Study, providing standardized data across sites.

Methods: Pre- and post-transplant de novo DSA data from our clinical laboratory were analysed (n=117; data not shown). MLR and flow phenotyping were performed using adult and pediatric control peripheral blood mononuclear cells and irradiated pooled HLA-mismatched third-party splenocytes. Cell proliferation was visualized using CellTrace Violet dye combined with the flow panel (Duraclone Immunophenotyping panel) or BrdU incorporation ELISA (n=12). The flow cytometer output was controlled for daily variation using pre-defined median channel colours and an additional calibration bead set.

Results: The BrdU assay demonstrated proliferation in response to non-self HLA but lacked the ability to identify which cell populations proliferated. Proliferation dye was readily detected within the standardized panel. Unstimulated cells did not proliferate whereas mitogen stimulated cells showed strong proliferation. Phenotypic changes comparing pre- to post-MLR analysis included decreased %T cells (although %CD4 and %CD8 remained consistent), increased %B cells, increased %NK cells, decreased % NKT cells and monocyte disappearance. These are % values and not absolute counts of cells. Small sample size (0.2Ã—106 cells) was sufficient for phenotyping thus enabling use with pediatric samples.

Conclusion: Our preliminary results show that using a highly standardized flow-panel in combination with CellTrace proliferation dye, provides a feasible way to detect cell specific proliferation in an MLR assay with small amounts of blood; the standardized assay may allow detection of alloimmune responses in individual patients in a reproducible manner from patient to patient and centre to centre, and provide an opportunity to standardize clinical investigation of transplant immune responses.

**HLA Experssion in Flow Crossmatch: Cell Source and Pronase Matter**

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Introduction: Flow cytometry crossmatch (FCXM) is an important tool in pre-transplant assessment of immunologic risk. Variation in HLA antigen density (AgD) may affect FCXM interpretation. The objectives of this study are to measure HLA AgD on lymphocytes and compare the differences in AgD between cell source, donor type, and pronase treatment.

Methods: AgD was measured with monoclonal antibodies (clones: W6/32, WR18 (pan-Class II), and HLA-DQ1 (binds DQB1 except DQ2)). Lymphocytes were isolated by immunomagnetic depletion from living donors (LD-PBL, n=39), deceased donors (DD-PBL, n=7), and spleen (DD-spleen, n=20). Cells were pronase treated. AgD was assessed with and without pronase (n=3). HLA-DQ AgD analyses were limited to DQ2-negative cells. AgD was plotted against different cell sources using box-whisker plot. One-way ANOVA with Tukey Post Hoc analyses were performed to compare AgD between groups.

Results: Class I AgD detected on T-cells from LD-PBL and DD-PBL were comparable with a p-value of 0.915; DD-spleen T-cells showed the lowest AgD that was statistically insignificant when comparing to other cell sources ($p = 0.072$ & $p = 0.561$). There were no significant differences across the three cell sources between class I AgD on B-cells. In contrast, class II AgD was lower on spleen-isolated B-cells vs LD-PBL ($p=0.000$) and DD-PBL B-cells ($p=0.007$). DQ AgD was lower on both DD-PBL ($p=0.009$) and DD-spleen B-cells ($p=0.000$) as compared to LD-PBL. Pronase treatment enabled increased detection of class I AgD on T-cells but not B-cells. Cell isolation technique, immunomagnetic depletion vs ficoll density gradient, did not appear to affect AgD.

Conclusion: Although T or B cell class I AgD did not differ significantly between cell sources, B cell class II was substantially decreased, particularly in DD-spleen while HLA-DQ expression was lower on all deceased donor cells. Our results suggest that FCXM results may be affected when assessing deceased donor FCXM in the context of class II DSA. Pronase treatment primarily affected/increased the detection of class I on T-cells with minimal impact on B cell AgD, but this was observed in a limited number of samples. We are continuing to collect data and will assess the impact of class/loci specific HLA antibody and the relationship to AgD.

## Chronic antibody-mediated rejection in nonhuman primate renal allografts: validation of human histological and molecular phenotypes

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Background: Molecular testing represents a promising adjunct for the diagnosis and classification of antibody-mediated rejection (ABMR). However, several obstacles hinder the translation of molecular diagnostics to routine clinical practice. These include the lack of a true diagnostic 'gold standard' for validation, heterogeneity of existing data associated with different Banff classification iterations, absence of robust validation studies, and lack of consensus on platforms to be used, transcripts to be assessed and criteria for positivity. We aimed to address these challenges by analyzing the expression of ABMR-related transcripts in a nonhuman primate (NHP) animal model free from the confounding variables of inconsistent immunosuppression, rejection treatment, and non-compliance.

Methods: 197 archival renal allograft samples were obtained from 81 NHP treated with tolerance induction protocols at the time of transplantation and then permanently taken off immunosuppression. 102 of the samples came from 29 recipients in which the tolerance protocol failed and chronic ABMR developed. 80 samples from recipients without ABMR and 15 normal native nephrectomies were also included. Expression of 34 genes previously associated with ABMR in humans was measured in each sample using the NanoString® nCounter® platform. The 34 genes included 18 endothelial, 6 NK cell, and 10 inflammation-related transcripts. Gene expression results were correlated with clinical, histological and serological data. Repeated 10-fold cross validation was used to rank individual gene performance and derive a refined gene set for ABMR.

Results: Hierarchical clustering identified endothelial genes as having the strongest association with Banff 2013 diagnoses of ABMR. A cross validation refined subset of three endothelial genes (VWF, DARC, CAV1) was found to optimally discriminate ABMR from non-ABMR samples (AUC=0.92). This 3-gene set correlated with classical features of ABMR, including glomerulitis, peritubular capillaritis, transplant glomerulopathy, C4d deposition, and donor specific antibodies ($r=0.39$-$0.63$, $p<0.001$). Principal component analysis confirmed the association between 3-gene set expression and ABMR and highlighted the ambiguity of v-lesions and ptc-lesions between ABMR and T-cell mediated rejection (TCMR). In sequential protocol biopsies, elevated 3-gene set expression corresponded with the development of immunopathologic evidence of rejection and often preceded it. 69% of the recipients that developed ABMR demonstrated concurrent TCMR.

Conclusion: These data provide NHP animal model validation of recent updates and discussions around the Banff classification, including the assessment of molecular markers for diagnosing ABMR, the non-specificity of v-lesions and ptc-lesions, and the growing recognition of mixed ABMR/TCMR as the potential natural history of renal allograft rejection.

**Development of an Immune-Chromatographic Assay for Point-of-Care Detection of Donor-Specific Antibodies (DSA) After Organ Transplantation**

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Antibody-mediated rejection (AMR) is the leading cause of late allograft failure, especially after kidney transplantation. AMR is caused by donor-specific antibodies (DSA) developed in the recipient directed against donor HLA molecules. The timing, and clinical impact of DSA are highly variable. Treatment of AMR is expensive and of limited success, especially once a patient presents with advanced AMR which can progress asymptomatically for a long time once DSA starts acting on the allograft.

Currently, post-transplantation patients must undergo regular blood screening for DSA. Since screening is expensive, it is only done in a sub-set of high risk patients, 2-3 times a year, and rarely more than 2 years post transplantation. As such, a significant number of patients develop DSA undetected, progress to AMR and advanced DSA-mediated allograft damage. There is a critical need for more frequent, reliable DSA screening in organ recipients.

We are designing a point-of-care approach for detection of DSA in transplant recipients. We are developing a hand-held chromatographic assay on pocket-sized slides that patients could use to test for DSA (cost per slide <10$). Similarly to diabetic patients, transplant recipients could monitor their DSA status at home, and only see their physician for follow-up appointments or when the assay detects DSA. This would instigate earlier DSA detection, allowing earlier intervention and increased likelihood of graft survival.

We have developed a prototype lateral flow immune-chromatographic assay. Human MHC Class I antigens were isolated from the immortalized, T-lymphoblast derived cell line MOLT-4 and mounted on slides. The slides are made of tantalum (anodizable color-generating metal) allowing us to coat them with immobilized MHC antigens. Anti-human HLA class I serum (pooled from sensitized renal allograft recipients, i.e. = a pool of a broad variety of DSA) was pipetted onto linker spots on the slides. Afterwards, Peroxidase-AffinPure Goat Anti-Human IgG and chromogenic substrate were pipetted onto the slide and color change from blue to yellow (positive for HLA) was monitored.

We are presently modifying the slides to improve MHC I antigen binding and increase specificity of IgG binding for more precise colorimetric read-outs. Once completed, a validation of the slides will be performed by screening patient serum. If successful, this project has the potential to significantly improve and personalize post-transplant monitoring for DSA and improve early detection of AMR.

**Human derived pancreatic endoderm cells reversed diabetes in mice post-transplantation**

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Aims of Study: Beta cell replacement therapy is an effective means to restore glucose homeostasis in a subset of individuals with type 1 diabetes. The scarcity of "healthy" human donor pancreata further restricts the broad application of this therapy. "Beta-like" cells derived from human embryonic cells (hESC), have been developed in vitro. However, translation to clinical investigation, especially immune isolating approaches, has been limited by host immune response to foreign material. Herein, we examine the efficacy hESC derived pancreatic endoderm (PE) cells to differentiate and reverse diabetes post-transplantation into the prevascularized subcutaneous "device-less" (DL) site in mice.

Methods: Subsequent to a month catheter implantation period, chemically induced diabetic immune deficient mice were transplanted with 10-20µL (0.5-1.0 x 107 cells) of PE cells. Recipient mice were randomly distributed into three transplant groups: 1) epididymal fat pad (FP), 2) subcutaneous alone (SC), or 3) DL. Post-transplant function was assessed through twice-weekly non-fasting blood glucose measurements, human C-peptide secretion and intraperitoneal glucose tolerance testing (IPGTT).

Results: Transplanted PE cells were able to reverse diabetes in 33% (2/6) of the recipients in both FP and SC groups, with a mean time to euglycemia of 110.5 ± 10.5 and 116.0 ± 19.0 days, respectively. In contrast, in the DL group, 100% of the mice (22/22) transplanted with PE cells became euglycemic within 99.8 ± 3.8 days ($p<0.01$, $p<0.01$ respectively, log-rank)(Figure 1A). Glucose homeostasis was abolished upon DL graft excision (Figure 1B). Furthermore, only PE-DL transplanted mice demonstrated glucose responsive human C-peptide secretion ($p<0.01$, paired two-tail t-test)(Figure 1C). In response to IPGTT, PE-DL mice demonstrated glucose clearance profiles similar to non-diabetic controls. Retrieved grafts stained positive for insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin.

Conclusions: This advancement mitigates chronic foreign body response evoked by biomaterials, utilizes a device- and growth factor-free approach, facilitates in vivo differentiation of PE cells into functional, glucose-responsive insulin-producing cells, and restores glycemic control in a reliable and effective manner.

## MicroRNA-301a Utilizes Dicer to Attenuate Pro-Fibrotic Phenotypes in Human Mesenchymal Cells

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Bone marrow-derived mesenchymal progenitor cells (MPCs) and atrial fibroblasts (AFs) are cell types that contribute to cardiac fibrosis. Fibrosis is a result from the pro-inflammatory molecule; transforming growth factor β (TGF- β). TGF- β plays a crucial role in the transition of MPCs to a pro-fibrotic phenotype causing a pathological accumulation of collagens within the heart, leading to heart failure. It has been observed that cellular phenotypic changes and differentiation results in a change in the micro-RNA profile as a result of Dicer, the molecule responsible for activating miRNAs. These molecules are critical in determining what proteins a cell is expressing which directly influences its phenotype. To determine if Dicer is involved with TGF- β induced change, human mesenchymal progenitor cells were cultured and treated with TGF- β, after which protein isolation, western blotting techniques were performed. The separation of proteins using western blot test trended towards TGF- β treated cells expressing elevated levels of Dicer. This indicates that TGF- β could be stimulating Dicer to cause the phenotypic transition of MPCs to a fibrotic phenotype. Additionally, previous studies have shown that MPCs display a myofibroblast phenotype that is linked to the altered expressions of mircoRNA(miR)-301a. miR-301a is affiliated with the regulation of cell proliferation and dichotomous phenotype which contribute to cardiac fibrosis. Regulating Dicer expression to target miR-301a could increase coding of miR-301a. An increase of coding in miR-301a could furthermore inhibit or promote degradation of targeted RNA's, possibly affecting phenotype and therefore regulating myofibroblasts. Techniques such as quantitative real-time PCR we can measure miR-301a levels, as well as we can detect protein levels of miR-301a targeted mRNA to see if it's happening at both transcriptional and translational level. Understanding these molecular mechanisms could lead to treatment of fibrosis, not just in the heart, but in organs throughout the body, as currently, there is no intervention for fibrosis.