UNIVERSITY OF ALBERTA
FACULTY OF MEDICINE & DENTISTRY

ALBERTA TRANSPLANT INSTITUTE

RESEARCH DAY

Thursday, May 31st

ECHA L1-490

8:00am - 3:00pm

William H. Lakey Lecture in Transplantation

"Building an Organ in 2018"

Dr. Doris Taylor

Director, Regenerative Medicine Research
Director, Center for Cell and Organ Biotechnology
Texas Heart Institute

ATI Research Day Schedule

8:00 - 8:30 Continental Breakfast
8:30 - 9:00 Opening Remarks
9:00 - 10:15 Plenary Session
10:15 - 11:00 Coffee and Poster Session
11:00 - 12:00 Oral Presentations
12:00 - 12:45 Lunch
12:45 - 1:30 Poster Session
1:30 - 2:30 Oral Presentations
2:30 - 3:00 Announcements and Closing Remarks

Plenary session presented in partnership with the Department of Surgery, Division of Urology

Register by May 18th

Visit the ATI website for more details

Schedule of Events		Room
8:00 - 8:30 am	**Continental Breakfast**	L1 - 420
8:30 - 9:00 am	**Opening Remarks: Dr. Richard Fedorak, Dr. Darren Freed, Dr. Lori West**	L1 - 490
9:00 - 10:15 am	**Introduction - William H. Lakey Lecture in Transplantation: Dr. Ronald Moore** **Plenary Address and Discussion: Dr. Doris Taylor** **"Building an Organ in 2018"**	L1 - 490
10:15 - 11:00 am	**Poster Session and Refreshments**	Hallways
11:00 - 12:00 pm	**Presentations**	L1 - 490
	Sanaz Hatami - The Functional Decline of Ex Vivo Perfused Heart Is Not Due To Cell Death	
	Ahmed Abdelbasit - Lung Transplantation from Hepatitis C Viremic Donors to Uninfected Recipients	
	John Staples - The role of microsurgical technique in hepatic artery reconstruction in pediatric liver transplantation	
	Esmé Dijke - Low HLA antigen expression on expanded regulatory T cells (Tregs) isolated from human discarded thymus: potential for "off-the-shelf" tolerogenic therapy?	
12:00 - 12:45 pm	**Lunch**	L1 - 420
12:45 - 1:30 pm	**Poster Session**	Hallways
1:30 - 2:30 pm	**Presentations**	L1 - 490
	Max Buchko - Common hospital ingredient perfusate equivalent to standard Krebs-Henseleit buffer with serum albumin derived perfusate in negative pressure ventilation ex vivo lung perfusion	
	Alison Müller - Increased Expression of Pro-Fibrotic Markers in Human Progenitor Cells Caused by Recellularizing Fibrotic Human Extracellular Matrix is Attenuated by Transfection with miR-301a	
	Tiffany Kim - Lymphocyte Proportions Post Thymectomy are Associated with Allergic Disorders in Heart Transplanted Children	
	Sayed Himmat - Adenoviral Adiponectin Gene Therapy to Improve outcomes on Ex vivo Lung Perfusion	
2:30 - 3:00 pm	**Closing Remarks and Announcements**	L1 - 490

Thank you to our Judges

Dr. Darren Freed
Dr. James Shapiro
Dr. Patricia Campbell
Dr. Shokrolla Elahi
Dr. Simon Urschel
Dr. Lori West
Dr. David Williams
Dr. Ron Moore
Dr. Carlos Cervera
Dr. Lee Anne Tibbles
Dr. Doris Taylor
Dr. Jelena Holovati
Dr. Kieran Halloran

Alberta Transplant Institute Research Day 2018

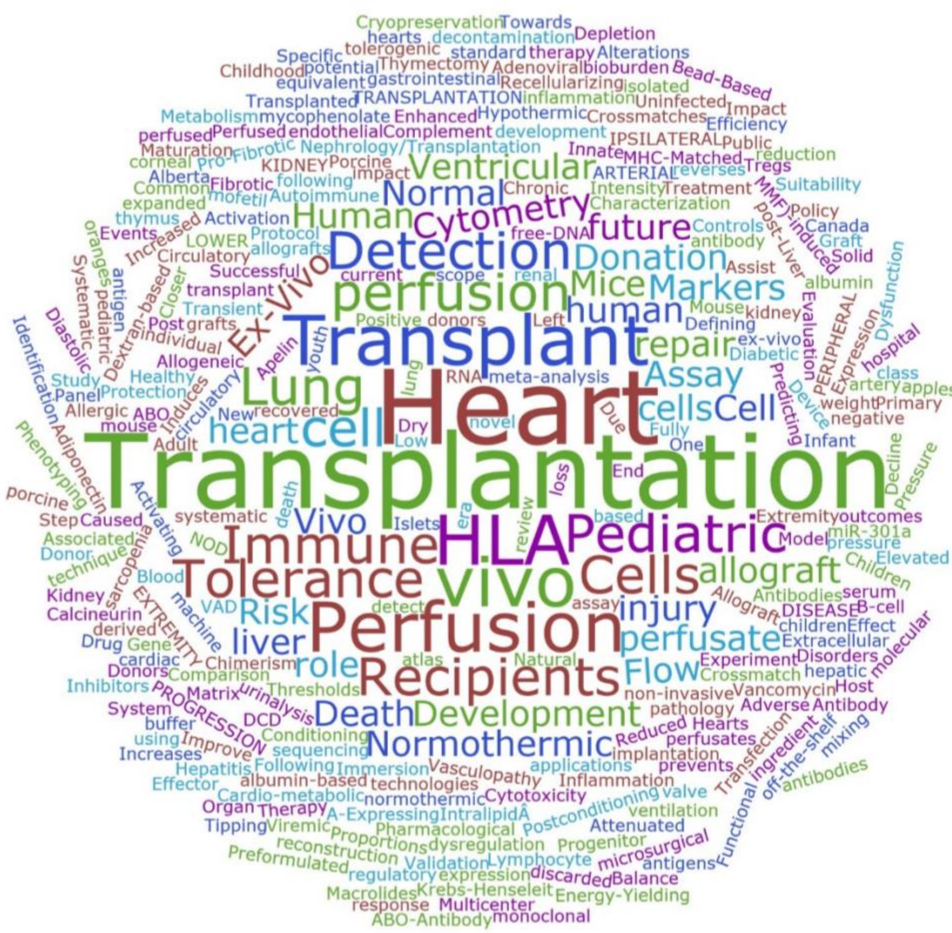
Dear Colleagues,

Welcome to the second annual ATI Research Day! Today, we are showcasing some of the excellent research in donation and transplantation being carried out by ATI members and ATI trainees. This year, the William H. Lakey Lecture in Transplantation is being presented as the plenary address, in partnership with the Department of Surgery, Division of Urology. We are honoured to have Dr. Doris Taylor, of the Texas Heart Institute, giving the plenary address “Building an Organ in 2018”. We hope you enjoy the day!

Lori West
Director, ATI

Darren Freed
Director of Research, ATI

ATI Research Day Committee: Darren Freed, Nowell Fine, Carlos Cervera, Luis Hidalgo, Jason Acker, Karen Hunter, and Ingrid Larsen



The William H. Lakey Lecture in Transplantation

Presented jointly with the Department of Surgery, Division of Urology

Doris A. Taylor, Ph.D., FAHA, FACC

Doris A. Taylor, Ph.D., FAHA, FACC, has worked in the field of cardiovascular regenerative medicine since its inception and is widely acknowledged as a major thought-leader in the field. Her mechanistic insights and effective approaches to cardiac repair and replacement are well established and include a number of firsts.

She currently serves as director of the Department of Regenerative Medicine Research and the Center for Cell and Organ Biotechnology at Texas Heart Institute, in Houston, Texas. She also heads the Cardiovascular Cell Therapy Research Network (CCTRN) Biorepository, the cell and cytokine profiling core lab that serves multiple NIH, National Heart Lung and Blood Institute (NHLBI) networks, medical centers, and research foundations in the U.S. and Canada. She has published extensively, authoring or co-authoring more than 140 scientific publications and co-editing two textbooks.

Dr. Taylor earned a B.S. in biology and physical sciences from Mississippi University for Women in 1977 and a Ph.D. in pharmacology from the University of Texas Southwestern Medical Center in Dallas, Texas in 1987. She holds many honors, including appointments as a Fellow of the American Heart Association and the American College of Cardiology, among others. She has been recognized by the AHA for Top 10 Research Advances. She was awarded an honorary Doctorate Science degree by her alma mater, Mississippi University for Women, in 2015 and the national Distinguished Alumnus Award presented by the American Association of State Colleges and Universities (AASCU) in 2016. She received the international Madrid Award for Excellence in Basic and Preclinical Regenerative Research in June 2017.

She frequently appears as an expert on stem cell therapy and cardiac repair in the media as well as in the scientific arena. Her work has been recognized and featured by 60 Minutes, CNN, The New York Times, The Wall Street Journal, BBC Horizon, BBC News Health, ABC News, NBC News, CBS News, Associated Press, National Public Radio, NOVA Science Now, Science Channel STEM CELL UNIVERSE with Stephen Hawking, Huffington Post (Canada), and numerous other worldwide media outlets.

Morning Presentation Abstracts

The Functional Decline of Ex Vivo Perfused Heart Is Not Due To Cell Death

Sanaz Hatami[2], Xiao Qi[2], Alois Haromy[4], Martin Ondrus[2], Alexandra Kinnear[2], Sayed Himmat[2], Christopher W. White[2], Max Buchko[2], Allan Wu[3], Jayan Nagendran[1,2], Darren H. Freed[1,2,3]

1. Alberta Transplant Institute
2. Faculty of Medicine and Dentistry, Department of Surgery, Division of Cardiac Surgery
3. Faculty of Medicine and Dentistry, Department of Physiology
4. Faculty of Medicine and Dentistry, Department of Medicine

Background: Ex vivo heart perfusion (EVHP) provides the opportunity to preserve/monitor/treat the donated heart in a semi-physiologic status. We have observed a decline in cardiac function over time during EVHP, regardless of perfusion mode [working mode (WM) versus non-working mode (NWM)]. Cell death and metabolic alterations may contribute to this phenomenon, limiting the safe perfusion period and the potential of EVHP to expand the donor pool.

Objectives: Our aim was to determine the rate of apoptosis and alterations in energy metabolism in ex vivo perfused hearts.

Methods: 17 female domestic breed pigs (37-47 kg) were included. The procured hearts were perfused on a custom EVHP apparatus (12 hours, NWM n=6 and WM n=7) with insulin and glucose infusions for metabolic support. Cardiac function was assessed during perfusion. Apoptotic cells were detected using Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay. Cell death effectors [cleaved caspase-3, apoptosis-inducing factor (AIF), C/EBP homologous protein (CHOP)] and enzymatic activity of pyruvate kinase (PK), and expression of different PK isoforms were analyzed using immunoassay techniques. The results were compared with baseline values (n=4, in vivo). After the last functional assessment, sodium pyruvate was added to the perfusate solution to achieve a concentration of 5 mmol/L and post-intervention changes in functional parameters were recorded.

Results: Cardiac function declined over time but function was better preserved in WM (e.g. cardiac index change, p=0.025). The cleaved caspase-3 was higher in NWM compared to in vivo (p=0.009), but AIF and CHOP were not significantly higher after 12 hours of EVHP. At the end of EVHP, although significantly higher than in vivo, only a negligible percentage of apoptotic cells were present (in vivo 0.13 % ± 0.01, WM 0.54 % ± 0.05, NWM 0.88 % ± 0.15, p<0.001). The activity of pyruvate kinase was lower in the ex vivo perfused hearts compared to in vivo (p=0.001). Total pyruvate kinase M1 (PKM1) protein expression was significantly lower in WM hearts compared to in vivo (p=0.001). Total PKM2 protein expression was significantly higher in ex vivo perfused hearts compare to in vivo (WM p<0.001, NWM p=0.015). Addition of pyruvate was associated with a significant recovery of cardiac index in both WM and NWM hearts (p=0.01).

Conclusions: Hearts preserved ex vivo experience a significant decline in myocardial function over time. Metabolic alterations happening during EVHP may contribute to this phenomenon, and represent an avenue to improve donor heart preservation.

Lung Transplantation from Hepatitis C Viremic Donors to Uninfected Recipients

Ahmed Abdelbasit[1], Alim Hirji[1,2], Kieran Halloran[1,2], Justin Weinkauf[1,2], Ali Kapasi[1,2], Dale Lien[1,2], Jayan Nagendran[3], Karen Doucette[1,4],

1. Department of Medicine, University of Alberta.
2. Division of Pulmonary Medicine, University of Alberta.
3. Department of Surgery, Division of Cardiac Surgery, University of Alberta.
4. Division of Infectious Disease, University of Alberta.

Importance: Intentional transplantation of organs from hepatitis C virus (HCV) viremic donors to HCV uninfected recipients and subsequent treatment with direct acting antiviral (DAA) therapy has been recently shown to be feasible in kidney transplantation, but has not been systematically performed in lung transplant recipients. Lung transplant recipients receive more aggressive immunosuppression regimens and often cannot tolerate oral therapy immediately post-transplant, and efficacy of DAAs in this cohort is unclear.

Objective: To determine the safety and efficacy of lung transplant from HCV viremic donors to uninfected recipients treated post-transplant with DAA therapy.

Design: This is a case series of five HCV uninfected recipients who received lungs from HCV viremic donors.

Setting: This study was conducted at a single lung transplant center at the University of Alberta, Canada.

Participants: The five patients included were selected as the HCV viremic donor was otherwise an appropriate match. All were deteriorating rapidly with only a small window remaining for transplantation.

Main Outcome: The primary outcome of interest was sustained virologic response 12 weeks (SVR12) following completion of HCV DAA therapy.

Results: All five recipients were documented to have donor-derived HCV and all achieved SVR12 following 12 weeks of DAA therapy.

Conclusion: Transplantation of lungs from HCV viremic donors to HCV uninfected recipients with post-transplant DAA therapy can be safely done and has the potential to expand the donor pool.

The role of microsurgical technique in hepatic artery reconstruction in pediatric liver transplantation

John Staples[2], Peter Kwan[2], James Shapiro[1], Norman Kneteman[1], David Bigam[1], Adil Ladak[2]

1. Transplantation Surgery, Department of Surgery, University of Alberta Hospital, Edmonton, Alberta, Canada.
2. Division of Plastic Surgery, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Failure of hepatic artery (HA) reconstruction in pediatric liver transplantation can lead to biliary complications, graft necrosis, and failure. Microsurgical reconstruction of the hepatic artery is being employed by many centers to minimize these complications. The purpose of this study is to report the outcomes of a single centre experience in pediatric liver transplantation with the institution of microsurgical HA reconstruction.

Methods: A retrospective review of pediatric orthotopic liver transplantations in Edmonton between 1990 to present was performed. Patients were divided into two groups: cohort 1 underwent HA reconstruction by the primary transplant surgeon; cohort 2 had reconstruction of the HA performed by a plastic surgeon using microsurgical technique. Primary outcomes included hepatic artery thrombosis (HAT), hepatic artery stenosis

(HAS), anastomotic bleeding, and arterial ischemic time. Secondary outcomes included length of PICU stay, length of hospital stay, graft and overall survival.

Results: A total of 223 pediatric patients underwent liver transplantation (cohort 1 = 180; cohort 2 = 43). While operative times (498 vs. 414 min, p=0.001) and arterial ischemic times (94 vs. 43 min, p=0.000) were higher in cohort 2, the use of microsurgical technique resulted in significantly lower vascular complications (HAT 2.3% vs. 12.8%, p=0.047) and a trend towards improved graft survival (14% vs. 24%, p=0.157). PICU and hospital stay were similar between the 2 groups.

Conclusions: Microsurgical HA reconstruction in pediatric orthotopic liver transplantation has resulted in a decrease in vascular complications and improved graft survival, supporting use of this technique for pediatric liver transplantations.

Low HLA antigen expression on expanded regulatory T cells (Tregs) isolated from human discarded thymus: potential for "off-the-shelf" tolerogenic therapy?

Esme Dijke [1, 2, 3, 4], Tess Ellis [2, 3], Jean Pearcey [2, 3], Ingrid Larsen [2, 3], Ivan Rebeyka [3, 5], David Ross [3, 5], Darren Freed [3, 4, 5], Mohammed Alaklabi [3, 5], Megan Levings [4, 6], Lori West [1, 2, 3, 4, 5, 7],

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6. Dept. of Surgery, University of British Columbia, Vancouver, BC,
7. Dept. of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB.

Aim: Tregs modulate immune responses and are a promising therapeutic tool in organ and cell transplantation. We showed that abundant CD25+FOXP3+ Tregs can be isolated and expanded from discarded human thymuses, demonstrating potential of thymic Tregs as an ‘off-the-shelf’ tolerogenic therapy. Immunogenicity of allogenic Tregs is a possible obstacle, thus we studied HLA class I/II expression on thymic Tregs.

Methods: Thymocytes were obtained from infant thymuses (n=3) by mechanical dissociation; FOXP3+CD25+ Tregs were isolated by magnetic-bead-based cell separation. Tregs were also isolated from infant and adult peripheral blood mononuclear cells (PBMCs; n=3 for each group). After expansion with artificial antigen-presenting cells loaded with anti-CD3/IL-2/rapamycin, Treg phenotype/function were confirmed. HLA-ABC and HLA-DR expression was assessed by flow cytometry.

Results: Before expansion, HLA class I expression was similar between thymic, infant blood and adult blood Tregs (Median Fluorescence Intensity (MFI) range: 3673-6059 vs. 3841-3830 vs. 6355-7952, respectively). In contrast, class II expression differed: few thymic Tregs or infant blood Tregs expressed HLA-DR (<3%), whereas 11-28% of adult blood Tregs were HLA-DR+. After expansion, HLA-ABC expression strongly increased on both infant and adult blood Tregs (6299-34745 and 14490-20799, respectively), but only moderately on thymic Tregs (7440-8365). Interestingly, HLA-DR+ thymic Treg quantity remained low (2-13%), in contrast to infant (17-56%) and adult (81-86%) blood Tregs.

Conclusion: Lower HLA class I expression levels and fewer HLA-DR+ cells in expanded thymic Tregs compared to blood Tregs suggests that thymic Tregs may be less immunogenic. These findings provide further evidence that discarded human thymus is potentially an excellent source of Tregs for ‘off-the-shelf’ tolerogenic therapy.

Afternoon Presentation Abstracts

Common hospital ingredient perfusate equivalent to standard Krebs-Henseleit buffer with serum albumin derived perfusate in negative pressure ventilation ex vivo lung perfusion

Max Buchko[1,2], Sayed Himmat[1], Catherine Stewart[1], Nader Aboelnazar[1], Sanaz Hatami[1], Darren Freed[1,2,3,4], Jayan Nagendran[1,2,3,4]

1. Division of Cardiac Surgery, Department of Surgery, University of Alberta, Edmonton, AB, Canada
2. Mazankowski Alberta Heart Institute, Edmonton, AB, Canada
3. Alberta Transplant Institute, Edmonton, AB, Canada
4. Canadian National Transplant Research Program, Edmonton, AB, Canada

INTRODUCTION: Normothermic ex-vivo lung perfusion (EVLP) using negative pressure ventilation (NPV) and red blood cell-based perfusate solutions have been shown to decrease edema formation and pro-inflammatory cytokine production compared to positive pressure ventilation (PPV). We sought to develop a common hospital ingredient derived perfusate (CHIP) with equivalent functional and inflammatory characteristics to the standard Krebs-Henseleit buffer with 8% serum albumin derived perfusate (KHB-Alb) in order to improve access and reduce costs of ex vivo organ perfusion.

METHODS: Porcine lungs were perfused using NPV-EVLP for 12 hours in a normothermic state, and were allocated to two groups: KHB-Alb (n=8) vs CHIP (n=4). Physiologic parameters, cytokine profiles, and edema formation were compared between treatment groups.

RESULTS: Perfused lungs in both groups demonstrated equivalent oxygenation (partial pressure of arterial oxygen/ fraction of inspired oxygen ratio >350 mmHg) and physiologic parameters. There was equivalent generation of tumor necrosis factor-Î±, irrespective of perfusate solution used, when comparing CHIP vs KHB-Alb. Pig lungs developed equivalent edema formation between groups (CHIP: 15.7 ± 5.8%, STEEN 19.5 ± 4.4%, p>0.05).

CONCLUSION: A perfusate derived of common hospital ingredients provides equivalent results to standard Krebs-Henseleit buffer with 8% serum albumin based perfusate in NPV-EVLP.

Increased Expression of Pro-Fibrotic Markers in Human Progenitor Cells Caused by Recellularizing Fibrotic Human Extracellular Matrix is Attenuated by Transfection with miR-301a

Alison L Müller[1,4,5], Xiuhua Wang[1,4,5], Darren H. Freed[1,2,3,4,5]

1. Department of Physiology
2. Department of Surgery
3. Department of Biomedical Engineering
4. Mazankowski Alberta Heart Institute
5. University of Alberta

Decellularized matrix is a material that is increasingly being used in tissue engineering to optimize cell and tissue repair therapies. The heart is an important target for tissue repair as it lacks effective endogenous healing ability after suffering injury. This technique provides an opportunity to utilize native cardiac ECM to better understand the endogenous response of pro-fibrotic cells to healthy and damaged ECM environments. Previous research has alluded to over-expression of a pro-proliferative microRNA, miR-301a, inhibiting pro-fibrotic differentiation of endogenous human bone-marrow derived mesenchymal progenitor cells (hMPCs).

These experiments investigate the reaction of hMPCs in decellularized unutilized human donor cardiac LV tissue and explanted human cardiac LV tissue from a patient who had been equipped with a left ventricular assist device.

Decellularization was performed on 100-200 µm thick slices of tissue using a variety of reagents over the course of three days to ensure removal of cellular debris. ELISA analysis of pro-inflammatory markers (TNFα ± and IL-6) was performed to evaluate the status of the decellularized tissue prior to seeding with hMPCs. The effect of these matrices on hMPC pro-fibrotic differentiation was compared to regular culture methodology using cells cultured on traditional plastic plates. In order to evaluate the effect of these ECMs on pro-fibrotic differentiation, q-RT-PCR and ELISA was performed on a variety of pro-fibrotic markers including α-smooth muscle actin, collagen1, cellular fibronectin, and myosin heavy chain-10 and -11. These are important indicators of a myofibroblast-like phenotype that contributes to pathological fibrosis in heart disease. In order to evaluate the efficacy of mir-301a on attenuating pro-fibrotic differentiation, miR-301a transfected hMPCs were also seeded on healthy or explanted human LV tissue. There were increases of pro-fibrotic markers in cells seeded in the fibrotic explant LV which was attenuated by miR-301a transfection. These results highlight the importance of a physiologically relevant disease model as the effectiveness of a single microRNA in attenuating the affects of diseased myocardial ECM. Increased expression of miR-301a could be applied to cell therapy techniques when looking to repair damage in fibrotic areas in order to prevent the influence of this pathological environment from impairing reparative processes.

Lymphocyte Proportions Post Thymectomy are Associated with Allergic Disorders in Heart Transplanted Children

Tiffany Kim[2], Lavinia Ionescu[1,2,3] , Nicholas Avdimiretz[4], Ingrid Larsen[1,2,3], Faye Murdoch[2,3], Susan Gilmour[2,3], Bruce Motyka[1,2,3], Lori West[1,2,3], Simon Urschel[1,2,3]

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2. Faculty of Medicine and Dentistry, Department of Pediatrics, University of Alberta
3. Stollery Children's Hospital
4. The Hospital for Sick Children

Allergic disorders are more common in pediatric heart transplant (HTx) recipients than non-transplanted children, with young age at HTx and thymectomy being risk factors. We hypothesized that thymectomy and immunosuppression at early ages affect development of T and B cells, especially regulatory T cells (Tregs), which help maintain peripheral tolerance. We investigated impact of thymectomy and lymphocyte-depleting induction on lymphocyte subsets, and allergic disorders after HTx. Flow cytometry phenotyping was used to determine subset proportions in peripheral blood. Clinical data were collected in standardized questionnaires and from medical charts. An age-matched, immunosuppressed but non-thymectomized liver transplant (LTx) comparison group was included. 69% of thymectomized patients experienced new/worsening asthma and eczema post-transplant, compared to 33% of non-thymectomized patients. CD45RA+CD27+ naive Treg proportions within CD4+CD25+CD127low populations was lower in thymectomized patients although not significantly. Patients with asthma and eczema had significantly lower naive Treg proportions than patients without (p=0.038), and showed increased memory CD4+ populations with age. Memory CD4+ and CD5+CD1d+"B10"populations were higher in thymectomized patients although not significantly. 71% of thymectomized patients were EBV carriers compared to 29% of non-thymectomized patients. Comparison to LTx recipients is pending due to small sample size. Lower naive Treg proportions are found in children with allergic disorders after HTx, likely resulting from thymectomy. Persisting EBV infection was more common after thymectomy and may impact T-cell maturation. Memory and “B10” populations may contribute. Larger sample sizes may help quantify independent risk of these factors and allow us to stratify immunosuppression type and intensity.

Adenoviral Adiponectin Gene Therapy to Improve outcomes on Ex vivo Lung Perfusion

Sayed Himmat[1,3], Nobutushi Matsumara[2,6], Shingo Takahara[2,6], Mohamad Burhani[1,3], Nader Aboalnazar[1,3], Sanaz Hatami[1,3], Max Buchko[1,3], Catherine Stewart[1,3], Xiao Qi[1,3], Yulin Wu[1,3], Jody Levasseur[2,6], Amy Barr[2,6], Xiuhua Wang[1,3],Jason Dyck[2,6], Darren Freed[1,3,4,5], Jayan Nagendran[1,3,4,5],

1. Division of Cardiac Surgery, University of Alberta
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3. Mazankowski Alberta Heart Institute
4. Alberta Transplant Institute
5. Canadian National Transplant Research Program (CNTRP)
6. Cardiovascular Research Centre (CVRC)

Background:Ex vivo Lung Perfusion (EVLP) is an ideal platform to deliver organ-specific therapies to donated lungs. We investigate delivering adiponectin gene therapy to lung grafts on EVLP. Adiponectin is a hormone mainly secreted by adipocytes, has anti-inflammatory and cytoprotective benefits to pulmonary vasculature and airway epithelium.

Objectives: We investigate the safety and efficacy of delivering adenoviral-adiponectin gene therapy to donor lungs on EVLP. Analyze the potential benefits of the expressed protein in lung function and inflammatory response.

Methods: Procured lungs from six domestic pigs (35-45kg) were randomly assigned to three groups, where lungs were perfused on EVLP for 12 hours. Vehicle (PBS+10% glycerol) (N=3), Ad-mCherry (N=1) or Ad-ApN (1x1010 PFU) (N=2) was delivered trans-bronchially to segmental bronchi using flexible video bronchoscopy at hour 1 (T1) perfusion. Physiologic parameters and oxygenation were evaluated every 2 hours. Overlay fluorescence pictures were taken for Ad-mCherry lungs at T12. In addition, real time PCR was done to measure mRNA expression levels of adiponectin gene in central and peripheral lung biopsies taken at T12.

Perfusion of virus transduced lungs showed stable physiology, average oxygenation index (ratio of arterial partial pressure of oxygen to inspired oxygen fraction) of 562±14.3 at T12. Lung dynamic compliance (Cdyn) at T12 was significantly higher in Ad-ApN group (35.4±1.1) compared to Vehicle perfusion (24.1±2.5) (p=0.01). Interestingly, average lung edema (% weight gain) at the end of perfusion was 22.9±4.8 for Ad-ApN lungs compared to 88.5±22.1 for Vehicle perfused lungs. Significant fluorescence was more prominent along the bronchial tree with interstitial diffusion in all lobes of Ad-mCherry lungs. Furthermore, absolute adiponectin gene mRNA levels from central and peripheral biopsies averaged 37.5 ng/µl and 7.52 ng/µl respectively.

Conclusions: Our results show feasible adenoviral adiponectin transduction on EVLP. Gene therapy lungs reported lesser edema and significant improvement in compliance compared to non-treated grafts. Analysis of inflammatory markers will demonstrate adiponectin's anti-inflammatory effect. Further research will determine adiponectin's role in improving outcomes in lung donation and transplantation.

Poster Abstracts

Validation of cardiac valve allograft decontamination and bioburden reduction

Christina Crossie[1], Jelena L. Holovati[1], Graeme Dowling[2]

1. Department of Laboratory Medicine and Pathology, University of Alberta
2. Comprehensive Tissue Centre, Alberta Health Services

Bacterial contamination of recovered tissue allografts poses a serious threat to transplant recipients. Cardiac valve (CV) allografts are typically disinfected in an antibiotic cocktail at 4°C; however, antibiotic availability and recent changes to Canadian health standards have resulted in a requirement for a further validation of disinfection practices at the AHS Comprehensive Tissue Centre. Disinfection solution, composed of vancomycin (50 µg/mL), tobramycin (80 µg/mL), and cefoxitin (240 µg/mL) in RPMI-1640 and was inoculated with 105 CFU/mL of five challenge organisms considered medically significant in tissue banking at 4°C and 21°C. Following 24 hours of incubation, the remaining CFUs were counted and log reduction was calculated. To further evaluate the appropriateness of a disinfection incubation range of 24 hours ± 2 hours, four CVs were bisected and inoculated with 106 CFU of challenge organisms before being placed in the disinfection solution. At each time point one half of the allograft was removed and washed in Lactated Ringer's solution before recovering the remaining bacteria in sterile saline by sonication and mechanical shaking. The final recovery solution was filtered and tested for bacterial growth. The initial results demonstrate that 4°C incubation reduced the bacterial growth between log 1.00 and log 2.00 whereas incubation at 21°C reduced the bioburden by log 4.00. The study established that incubation of CVs in the updated disinfection solution for 24 hours ± 2 hours at 21°C was sufficient to reduce the bioburden above the minimum required standard of 4.00 logs.

The Alterations in Energy-Yielding Metabolism During Ex Vivo Heart Perfusion

Sanaz Hatami[2], Xiao Qi[2], Sayed Himmat[2], Christopher W. White[2], Martin Ondrus[2], Alexandra Kinnear[2], Max Buchko[2], Jayan Nagendran[1,2], Darren H. Freed[1,2,3]

1. Alberta Transplant Institute
2. Faculty of Medicine and Dentistry, Department of Surgery, Division of Cardiac Surgery
3. Faculty of Medicine and Dentistry, Department of Physiology

Background: Ex vivo heart perfusion (EVHP) is a novel method for preservation of the donated heart in a semi-physiologic status. It also provides the opportunity to evaluate the function /metabolism of the donated heart during preservation time, and to potentially improve the dysfunctional hearts. Considering the high energy demands of the heart, an efficient metabolic support for the ex vivo perfused heart is critical for the preservation of its function and viability. Metabolic alterations/inefficiency can negatively affect cardiac function, and limit the safe perfusion period and the potential of EVHP to expand the donor pool.

Objectives: Our aim was to determine the changes happening in energy metabolism/energy substrates during ex vivo perfusion of the hearts in two different perfusion modes, working mode (WM) and non-working mode.

Methods: 17 female domestic breed pigs (37-47 kg) were included. The procured hearts were perfused on a custom EVHP apparatus (12 hours, NWM n=6 and WM n=7) with insulin and glucose infusions for metabolic support. Cardiac systolic and diastolic function was assessed during perfusion. The rates/over-time changes in myocardial oxidative metabolism, glucose utilization, the perfusate concentrations of lactate, free fatty acids

(FFA) and triglycerides (TG) were evaluated during EVHP. The enzymatic activity of pyruvate kinase was also assessed using immunoassay techniques. The results were compared with baseline values [in vivo perfusate, and the left ventricular tissue control, (n=4)].

Results: Cardiac systolic and diastolic function declined over time but function was better preserved in WM (e.g. cardiac index change, p=0.02). The glucose utilization increased during EVHP in both groups, compared to baseline values (WM p=0.04, NWM p=0.04) however, the activity of the pyruvate kinase (PK) was significantly lower in the ex vivo perfused hearts compared to in vivo (p<0.01).Venous and arteriovenous lactate did not change over time and were not different between WM and NWM. FFA concentrations declined considerably during EVHP in both groups (WM p<0.01, NWM p=0.03) but were not different between WM and NWM. Perfusate TG concentrations did not change over time in WM (p= 0.662) but slightly increased in NWM during EVHP (p=0.047).

Conclusions: The alterations in oxidative energy metabolism and energy substrates utilization during EVHP may contribute to the functional decline of the ex vivo perfused heart. For the aim of an optimal cardioprotective metabolic support for the heart in the setting of EVHP, more studies are warranted to characterize these changes and their underlying reasons, and how to address them.

Identification of a molecular repair response in ex vivo perfused porcine hearts

Nicole Herbers[1], Sanaz Hatami[2], Jayan Nagendran[2], Michael Mengel[1], Darren Freed[2], Benjamin Adam[1]

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

Background: Cardiac transplantation is a life-saving intervention for advanced heart failure but is limited by a shortage of suitable donor organs. Ex vivo heart perfusion (EVHP) represents a promising alternative for organ preservation and repair. We aimed to assess the feasibility of using gene expression to monitor cardiac tissue injury and repair during EVHP.

Material and Methods: Heart samples were obtained from 24 pigs either in vivo (IV, n=5) or after 12 hours of ex vivo heart (EVHP, n=15) or combined heart and liver (H+L, n=4) perfusion. Functional parameters were recorded during EVHP. Histology was assessed for features of cardiac injury. NanoString was used to measure 68 genes related to cardiac injury and repair. Molecular data were assessed for differential expression and correlated with function and histology.

Results: 43 genes were significantly up-regulated and 8 genes were significantly down-regulated in EVHP vs. IV (FDR<0.05) (Figure 1). As an aggregate ‘repair’ gene set, the up-regulated genes exhibited higher expression in EVHP vs. IV (p<0.001), EVHP vs. H+L (p=0.002), and H+L vs. IV (p=0.02). As an aggregate ‘injury’ gene set, the down-regulated genes showed lower expression in EVHP vs. IV (p<0.001), EVHP vs. H+L (p=0.004), and H+L vs. IV (p=0.02). No statistically-significant correlation was observed between gene set expression and function or histology.

Conclusions: These data suggest that EVHP induces a molecular repair response that is independent from functional and histological parameters. This response appears to be abrogated in high-demand H+L perfusion. This represents a novel approach for measuring donor heart quality during EVHP.

The Impact of Childhood Solid Organ Transplantation on B-cell Maturation

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BACKGROUND: The developing immune system of childhood promotes graft acceptance and longevity but bears a higher risk for infections and post-transplant (Tx) lymphoproliferative disorders. This may be due to lack of memory B-cells or higher proportions of immune modulatory B-cells (CD24hCD38h transitional (TrB) and CD5+CD1dh (Breg)). We assessed the role of age, Tx and organ type on B-cell maturation around Tx.

METHODS: 115 children listed for Tx were enrolled in the Canadian National Transplant Research Program (CNTRP) POSITIVE multicenter collaborative study. Samples were collected at listing, 3 and 12 months post-Tx. Isolated PBMC were analyzed by flow-cytometry.

RESULTS: Pre-Tx infants (0-2y) had higher B-cell proportions than children (2-10y) and teenagers (10-17y; $p=0.01$), but fewer CD27+ memory B-cells of IgM+ ($p<0.05$) and switched IgM- ($p<0.01$) phenotype. Memory B-cells increased with age throughout childhood ($p<0.05$), unaffected by Tx or immunosuppression compared to age-matched pre-Tx children. TrB were highest in infants ($p<0.05$) and declined to adult values thereafter ($p<0.05$). Pre-Tx, liver recipients had more B-cells, TrB and CD21+ B-cells ($p<0.05$), while post-Tx all organ groups were similar. Breg proportion was not associated with age, Tx stage or organ type.

CONCLUSIONS: While Tx and immunosuppression significantly alter T-cell proportions in childhood, B-cells show similar maturation profiles with age as in a non-manipulated immune system. Lack of memory and increased proportions of immature B-cells likely contribute to the better graft acceptance in younger children. The role of the individual immune profile and organ-related differences on the clinical course are being prospectively assessed in long-term follow-up.

The Effect of Public Policy on Heart Donation and Heart Transplantation Efficiency in Alberta, Canada.

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Aim: To evaluate an Alberta Government health policy change designed to promote organ and tissue donation, using the introduction of a public organ and tissue donation registry in Alberta in 2013-2014. We assessed the impact of the policy change on heart donation and heart transplantation (HTx) rates in Alberta.

Methods: A retrospective analysis from January 1 2007 and December 31 2016 (10 years) was conducted using administrative and health care data sets. Analysis was performed using interrupted time series techniques with simple linear regression. The pre-policy time period of 2007-2013 was used to reconstruct the counterfactual of the effect of the policy change observed from 2014-2016. Heart organ donation offers by

source of origin, HTx recipient listings, and completed HTx were determined as quarterly aggregate rates. The efficiency of heart donation and HTx completion, relative to active heart transplant listings was assessed using production frontier analysis.

Results: From January 2007 to December 2016, a total of 451 unique individuals were listed for HTx, while 2661 donor heart offers were made from all sources. Of these offers, 439 (16.5%) were derived from donors within Alberta. HTx occurred following 306 offers, indicating an overall conversion ratio to HTx of only 11.5% of offers. Simultaneously, 68 listed patients (15.4%) died in Alberta while awaiting HTx. A time-related 41% increase in donor heart offers, from 59.2 to 83.7 per quarter (p<0.01) was observed, but the population-adjusted donation rate within Alberta was unchanged. There was no increase in heart donations, HTx recipient listings, or completed HTx demonstrated to have occurred as a direct consequence of the policy change. There was evidence of variable efficiency of donor heart offer conversion to completed HTx during the entire time frame using production frontier analysis.

Conclusions: While overall heart donation rates have increased over time, this appears to be a result of increased externally derived donors. As yet, no improvement in heart donation rates and HTx rates in Alberta can be demonstrated to have occurred following the policy change of 2013-14. More attention to promoting deceased organ donation, and increasing the efficiency of conversion of heart offers to heart transplantation is required in Alberta.

Elevated Left Ventricular End Diastolic Pressure Increases Risk Of Primary Graft Dysfunction In Lung Transplant Recipients

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Ischemia-reperfusion injury contributes significantly to primary graft dysfunction (PGD), a major cause of morbidity and mortality after lung transplant. However, elevated left ventricular end diastolic pressure (LVEDP) may exacerbate hydrostatic capillary leak and therefore PGD. We assessed the relationship between LVEDP and PGD development with comparison to two other measures of LV pressure: mean pulmonary capillary wedge pressure (mPCWP) and echocardiographic E/E ratio. We hypothesized that elevated LVEDP would increase the risk of PGD.

We reviewed the records of 330 adult double lung transplant recipients at UAH between 2004 and 2016. The primary outcome was Grade 3 PGD (lung edema and PaO2/FiO2 < 200 mmHg) 48 or 72 hours post-transplant. We used logistic regression to assess the relationship between LVEDP > 15 mmHg and grade 3 PGD, adjusting for known PGD risk factors. We assessed mPCWP > 15 and E/E ratio > 8 similarly in separate models.

Mean LVEDP in patients who developed Grade 3 PGD was 17 ± 7 mmHg and 12 ± 5 mmHg in those who did not (p<0.0001). LVEDP >15 mmHg was associated with an adjusted odds ratio (OR) of 4.0 (p<0.0001) for the development of Grade 3 PGD. mPCWP >15mmHg showed similar findings (adjusted OR 4.3, p=0.0005), while E/E ratio >8 showed no association. Agreement between LVEDP and PCWP was fair (K=0.39).

Elevated LVEDP is associated with severe PGD, as is elevated mPCWP. These measurements appear to be complementary and can each be used as markers of prospective PGD risk during candidate evaluation.

A Reduced Intensity Conditioning Protocol Induces Transient Chimerism and Transplant Tolerance to Fully Allogeneic Islets in Autoimmune Diabetic NOD Mice

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Hematopoietic chimerism is a robust method for generating donor specific tolerance with the potential to allow islet transplant tolerance. However, its clinical application is prevented by the toxicity of current recipient conditioning regimens. We recently developed a T cell depletion based chimerism protocol in pre-diabetic, non-obese diabetic (NOD) mice. As generating chimerism in diabetic NOD mice is even more challenging compared to pre-diabetic ones, we sought to test if we could induce chimerism and transplant tolerance to allogeneic islets in spontaneously diabetic NOD recipients.

We preconditioned spontaneously diabetic NOD mice with donor specific transfusion from fully mismatched FVB mice (d-10), cyclophosphamide (d-8), antibodies against CD4/8/90 (d-6, -1, 4, 9, 14), and busulfan (d-1). Donor islets and/or bone marrow transplantation (BMT) were done at d0. Blood glucose levels of recipients were assessed weekly. Flow cytometry was used to detect chimerism.

We induced transient mixed chimerism in 6/8 diabetic NOD mice. Although chimerism in the diabetic recipient was less stable compared to pre-diabetic NOD mice, with lower chimerism levels at the early time points (d4/9/14/28) post-BMT, islet recipients (3/3) with high-level chimerism at d28 were able to maintain normal blood glucose even after donor bone marrow was rejected (2/2). Recipient T cells in diabetic NOD mice were depleted as efficiently as in pre-diabetic NOD mice but rebounded quickly, starting at d14, which might be associated with the instability of chimerism.

Conclusion: A T cell depletion based chimerism protocol induces chimerism in diabetic NOD mice and promotes tolerance to fully allogeneic islets.

Defining Positive Thresholds in Flow Cytometry Crossmatches: Not so Normal

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Background/Aim:Histocompatibility laboratories use flow cytometry crossmatch (FCXM) to assess donor-recipient compatibility. Positive thresholds must be determined, as this is a laboratory-developed test. Our aim was to evaluate methods to establish FCXM thresholds using different lot numbers of this assay's key reagents: FITC-labelled anti-human IgG (FITC-IgG) and negative control serum (NS).

Material/Methods: Sera (n=24) from healthy/non-transfused donors were screened for human leukocyte antigen (HLA) antibodies. Using these sera, T- and B-cell FCXMs were performed using a previously-validated method (n=24 cells). Data normality was measured: Shapiro-Wilk test, normal Q-Q and histogram plots (SPSS Statistics 24). Outliers were excluded by mean±3SD and Tukey's methods. Expected positive FCXMs were performed using current/new lot numbers of FITC-IgG and NS; multiple positive thresholds were evaluated.

Results: Negative FCXM results are not normally distributed (Figure 1). Although NS were screened for HLA antibodies, many FCXM showed non-specific reactivity. Tukey's method excluded more outliers than applying

mean±3SD, but may exclude valid results (Table 1). Based on known/expected positive results, a threshold of mean+3SD appears best for peripheral blood T-cells (Table 1). B-cell thresholds for HLA class II antibodies and threshold analysis of spleen and frozen spleen cells is still ongoing.

Conclusions: Evaluating data for normality is important to select valid methods for determining thresholds and removing outliers; these statistics may be misleading if data are skewed. Careful evaluation of individual lots is important as differences in normal samples may yield specific challenges in threshold studies. A more thorough screening of NS may also be required.

Characterization of the role of Apelin in injury repair of the Chronic Allograft Vasculopathy in Mouse heart allografts

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Heart transplantation is the mainstay treatment for end-stage cardiac patients. Unfortunately, chronic allograft vasculopathy (CAV) limits long-term graft survival, causing mortality beyond the 1st year of transplantation. CAV develops as a maladaptive repair response to the injured endothelial cells (ECs) lining coronary arteries and microvasculature, developing obliterative arterial intimal expansion, micro-vessel injury, decreased blood supply and graft failure.

Apelin (APJ receptor-ligand), a novel peptide (77 amino acids) encoded on the X chromosome, participates in vascular repair from myocardial infarction and kidney glomerular microvascular injury. We hypothesize that Pro-reparative cues (e.g. Apelin) attenuate graft arterial endothelial injury, and subsequent maladaptive repair. Minor antigen-mismatched heart allografts were used to evaluate CAV.

CAV was induced via transplanting Apelin-/y (knockout) or Apelin+/y (wild type) hearts into females to elicit a HY-minor histocompatibility antigen-directed, cell-mediated allo-immune response against the male donor hearts. Heart grafts were harvested two or six weeks after transplantation. We characterized intima area, endothelial loss in medium to large-sized arteries, inflammatory cellular infiltration, microvascular density and proliferation.

We observed Increased Apelin expression 2 weeks following heart transplantation. Apelin-/y hearts showed increased circumference area of endothelial loss (1.337 ± 0.08253 vs 0.3564 ± 0.03327; 1.906 ± 0.1767 vs 0.5721 ± 0.05901; $P<0.0001$), intima expansion in conduit arteries (6 weeks), decreased microvessel density (2 and 6 weeks), decreased proliferation and enhanced inflammatory cellular infiltration when compared to controls.

We concluded that Apelin is induced following heart transplantation; loss of Apelin exacerbates endothelial damage, microvascular rarefaction, an inflammatory cellular infiltrate (2 weeks) and CAV.

Nephrology/Transplantation Immersion - A Successful Natural Experiment

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The story described here began nine years ago in January 2009 when Medical Resident 1 began working on Nephrology/Transplantation research tasks with the last author when she was a first-year undergraduate student. It is a "natural experiment" because the scenario was not conceived of as an experiment until the present first author undergraduate student began doing similar Nephrology/Transplantation research tasks with the last author in January 2018 and decided to see if the work could be structured so she would learn Nephrology/Transplantation while doing it despite having no medical training. This "jumping into the deep end of the pool" immersion approach is quite different from the usual from-basics-to-specifics progressive acquisition of knowledge approach to medical education. A necessary component is that the student must "suspend belief" that she has truly mastered the subject until she is in medical school and learns the surrounding context of related subjects necessary to completely understand nephrology. However, if this sort of nephrology immersion education can succeed safely and comfortably, such students preloaded with Nephrology knowledge before medical school are a solution to the looming recruitment problems in Nephrology/Transplantation, because such students are very likely to choose a career in Nephrology/Transplantation as the path of least resistance in medical school. Medical Resident 1 has chosen a career in Nephrology/Transplantation. Immersion is an effective education technique in many other areas of human endeavour. It will probably also work here and have many advantages for the first author as she pursues a medical career.

ABO Tolerance Following Treatment of Infant or Adult Mice with MHC-Matched A-Expressing Blood Cells

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Purpose: ABO-incompatible heart transplantation (ABOi-HTx) is safe during infancy and allows increased donor access. Post-ABOi-HTx, B-cell tolerance develops to donor A/B-antigen(s) by mechanisms not fully defined. We developed A-transgenic mice (A-Tg) expressing A-antigen on vascular endothelium and erythrocytes, and demonstrated A-antigen-specific tolerance induced by HTx into MHC-identical juvenile wild-type (WT) mice. Herein, we explored A-Tg blood cells (BC) to induce tolerance in infant and adult WT mice. Methods: WT BALB/c mice were injected ip (weekly×3) with A-Tg BALB/c BC (±40Gy-irradiated), at age 7-days (neonates) or 5-months. Two weeks after treatment, all mice were injected ip (weekly×5) with human A-erythrocytes ('A-sensitized') to elicit anti-A antibody production. Serum anti-A and 3rd-party (non-A anti-human) antibodies were assessed by hemagglutination assay using A-Tg or human O erythrocytes.

Results: In response to A-sensitization, high anti-A antibody levels were produced in untreated mice (median 1:256, n=11). In contrast, anti-A remained undetectable (≤1:2) in A-sensitized mice treated as neonates with irradiated (n=5) or non-irradiated A-Tg BC (n=6). Treatment of adult mice with A-Tg BC resulted in reduced anti-A antibody in response to A-sensitization compared to untreated mice. Adult mice with undetectable natural anti-A prior to A-sensitization produced less anti-A following A-sensitization (≤1:2 to 1:4, n=5) vs mice with pre-existing natural anti-A (1:16 to 1:64, n=5). Third-party antibody responses were high for all groups (≥1:128).

Conclusions: Our results suggest that a radiation-resistant component of A-Tg BC can induce robust A-antigen-specific tolerance in WT mice. Our findings further suggest that tolerance is not limited to neonates but can be induced in adults. Intentional tolerance induction to A/B-antigen(s) may allow safe ABOi-HTx.

Inflammation and Innate Immune Activation During Ex-Vivo Heart Perfusion

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Purpose: The generation of circulating mediators of inflammation during EVHP has not been previously investigated. We hypothesized that inflammatory mediators will be activated during EVHP and that the inflammatory response would play a role in declining of donor heart function.

Methods: The procured porcine hearts were perfused ex vivo in a beating state for 12 hours (normothermic, whole blood-based perfusate, no steroids) on a custom EVHP apparatus. Group 1 hearts (n=9) were perfused in a working mode (WM, left atrial pressure=6 mmHg, heart rate=100 beats/minutes) for the entire EVHP interval. Group 2 hearts (n=6) were briefly transitioned into a working mode at hours T1, T5, and T11 for inflammatory and functional assessment, but were otherwise perfused in a non-working mode (NWM, left atrial pressure=0 mmHg). In vivo hearts (n=4) without perfusion were treated as baseline control. Cardiac functional parameters were compared between two perfusion modes groups. The pro-inflammatory cytokine levels in the perfusate and myocardial tissue were measured by ELISA and western blot and compared between two groups.

Results: Myocardial function declined over time but the function parameters were better preserved in WM (T11 cardiac index (mL/minute/gram): WM=6.9±1.0 vs NWM=2.0±1.2, p=0.02; LV stroke work (mm Hg·mL): WM=1012.5±245.7 vs NWM=303.4±121.6, p=0.03). The perfusate concentration of pro-inflammatory cytokines TNF-alpha, IL-6, IL-8, IL-1alpha, IL-1beta, IL-18 increased significantly during the 12-hour perfusion interval ($p<0.05$), but were not significantly different between WM and NWM groups (Figure 1A). The IL-6 and TNF-α increased in the left ventricular tissue after perfusion compared to baseline myocardial samples ($p<0.05$), with no significant difference between WM and NWM.Conclusion: A significant pro-inflammatory cytokine response is generated during prolonged EVHP in the perfusate and left ventricular tissue, independent of perfusion mode. The inflammatory responses may play role in declining of myocardial function. Further studies are warranted to elucidate the stimulants, consequences of inflammatory responses, and methods to mitigate these effects.

HLA antibody development following Ventricular Assist Device (VAD) implantation in the current era

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Introduction: Anti-HLA antibodies (HLA-Ab) are recognized as an important contributor to transplant outcomes. There are a number of events that may lead to the development of HLA-Abs, including VAD insertion. The true incidence of HLA-Ab development post-VAD insertion remains unclear. We sought to prospectively

characterize HLA-Ab development post-VAD insertion, in the current era using a standardized detection method and efforts to minimize HLA exposure from blood products.

Methods: Adult and pediatric patients were prospectively recruited prior to the insertion of a durable VAD starting in 2016. HLA-Ab testing was sent pre and post-implant as per standard protocol. Low reactivity, non-specific patterns HLA-Ab patterns, not representative of true HLA epitope were interpreted as negative. Sensitization with HLA-Ab was defined as a panel reactive antibody level >10%.

Preliminary Results: To date, 21 patients (3 peds) have been prospectively recruited (81.5% male; 85.7% non-congenital heart disease). Only 3.6% of patients had previous cardiac surgery and 39.3% required short-term mechanical support prior to VAD insertion. Two patients (9.5%) had a PRA >10% prior to VAD insertion. Following VAD therapy, 23.8% (n=5) of the patients developed new HLA-Abs, all within the first 3 months post-implant; both patients with pre-VAD sensitization had an increase in PRA to >80%.
Discussion: In this contemporary cohort of patients, preliminary results show that only one-quarter developed new HLA-Abs. This finding is much lower than previous reports in the literature, which have been mostly been retrospective, lacked uniformity of HLA-Ab methods and did not employ efforts to decrease HLA exposure.

Cardio-metabolic dysregulation and sarcopenia in children and youth post-Liver transplantation.

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Introduction: Sarcopenia and cardio-metabolic dysregulation are known co-morbid conditions that adversely impact post-liver transplant (LTx) patient outcomes in adults. We have recently shown that approximately 40% of children post-LTx have sarcopenia, but no data is available regarding the associations between cardio-metabolic dysregulation. We hypothesized that cardio-metabolic dysregulation would be associated with sarcopenia in youth post-LTx. Methods: A chart review was conducted in youth (ages 5-18 years; 33F; 30 M) who underwent LTx at the Stollery Children's Hospital (1995-2015). Sarcopenia was determined at annual Dual Energy-X-ray absorptiometry (DXA) scans using age-gender data to determine skeletal muscle mass (SMM-z). Anthropometric (weight, weight-z, height, height-z, BMI, BMI-z), demographic (age, liver disease), liver biochemistries (AST, ALT, total bilirubin, GGT, albumin, PTT, INR), fasting metabolic panel (TG, insulin, total-cholesterol, HDL-and-LDL cholesterol, HOMA-IR), blood pressure (systolic-diastolic absolute and z scores) and medications were collected at LTx assessment, LTx, and DXA were collected from the medical chart. Results: Mean (± SD) age, weight-z, height-z, BMI-z , and SMM-z at DXA scan was 9.8 ± 3.5 years (3.5-17.9), 0.20 ± 1.03, -0.015 ± 1.2, 0.35 ± 1.06, -0.91 ± 0.90 respectively. Systolic/diastolic BP was within healthy references ranges for age-gender in 83.1% and 93.5% children, respectively. Decreased insulin and HDL-cholesterol were found in 86.5% and 45.7% of patients. With the exception of serum TG, abnormal findings for metabolic parameters were independent of weight-z, BMI-z, systolic/diastolic BP (absolute/z-scores), and presence of sarcopenia. Conclusions: With the exception of serum TG, sarcopenia in youth post-LTx was not associated with cardio-metabolic dysregulation.

A New Pediatric Normal: An Evaluation of Pediatric T Cell Markers in Healthy Controls

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Background: Study of pediatric patients involves challenges including limited normal controls and small sample volumes. Standardized flow cytometry (FC) lymphocyte immunophenotyping panels are commercially available and normal adult ranges are published; no comparable pediatric control data exist. Our aim is to establish reference data for pediatric studies using standardized FC panels. Our group collaborated with the clinical laboratory to obtain normal pediatric samples with the secondary aim of evaluating these panels for clinical implementation.

Material and Methods: Blood from healthy children (n=10) aged 66 days-16 years was included; samples were tested in five, 10-colour T-/B-cell panels. Acquisition was performed by Navios cytometer. Programmed cell death protein-1 (PD-1), a measure of T-cell exhaustion, and γδ T-cells and associated markers Vd1 and Vd2 were analysed due to lack of reference data and potential interest in transplantation.
Results: Preliminary analyses show increasing PD-1+ CD4 T cells with age (R2=0.701); this association is weaker for CD8 T cells. CD3+ T cell number, including γδ T cells, decreases with age, while the % of each population of cells remains constant. There is a trend indicating decreasing ratio of Vd1 to Vd2 γδ T cells with age.

Conclusions: Duraclone is a rapid, standardized immune-phenotyping method using small blood volumes. These results begin establishing useful pediatric reference data and suggest these panels show promise for standardized research/clinical applications. The use of fresh whole blood differs from published pediatric studies utilizing frozen cells and ensures populations remain unaltered by isolation or thawing. We continue to explore age-related trends.

Comparison of Normothermic and Hypothermic Ex-Vivo Extremity Perfusion (EVEP)

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Introduction: The potential of extremity transplantation is critically limited by the 4-6 hours of preservation time currently offered by standard cold static storage, Ex-Vivo Extremity Perfusion (EVEP) is a novel technology striving to safely extend the preservation time of donated Vascularized Composite Allografts (VCA) allowing for greater applicability of upper limb transplants. As the concept of EVEP is limited to few centers of expertise in the world, there is yet to be optimization of EVEP protocols to determine ideal conditions for safe extended EVEP preservation times. Specifically, there remains controversy whether VCAs should be perfused at normothermia with a blood based perfusate or at hypothermia with an acellular perfusate during EVEP. We hypothesize that normothermic EVEP will allow for improved VCA preservation during extended EVEP protocols.

Methods: Porcine forelimbs are perfused for 12 hours on a novel mobile ex-vivo perfusion system that was developed at the University of Alberta with a solution consisting of Common Hospital Ingredients Perfusate

(CHIP) and 25% bovine serum albumin. The EVEP protocols were stratified to limbs being perfused in either a hypothermia (8-10 C) group with acellular CHIP or a normothermia (38 C) group perfused with CHIP and autologous packed red blood cells. Muscle, nerve, and blood vessel biopsies for histological analysis are taken in-vivo, at that start of perfusion, and at the end of perfusion from both groups. Parameters to be compared include: compartment pressure, muscle response to electrical nerve stimulation, edema formation, myoglobin and CK-MM levels, and perfusate cytokine profiles.

Results/Discussion: Interim results of cellular ex-vivo perfusions run at normothermia and acellular ex-vivo perfusions run at hypothermia will be presented.

Tipping the "Immune Balance" Towards Tolerance by Specific and Systematic Depletion of Donor and Host Effector Cells

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Purpose: To induce robust heart transplant tolerance in neonates with fully allogeneic spleen and bone marrow cells (SC/BMC) it is crucial to define the complex interplay amongst donor and host cells. To that end, we specifically and systematically depleted donor and host effector cells, studying the effects of each depletion on immune outcome.

Methods: C3H (H-2k) neonates were injected iv with total or depleted GFP+ SC/BMC from B6 (H-2b) adults. Donor effector cell types were depleted from inocula before injection using positive selection kits. Host cell types were depleted in vivo with monoclonal antibodies.

Results: Neonates injected with GFP+ allo-SC/BMC developed acute graft-vs-host disease with diarrhea, reduced growth and early death. GFP+ cells trafficked to secondary lymphoid organs, proliferated and spread throughout injected mice indicating systemic inflammation. Donor cell proliferation in neonatal hosts was prevented when the tolerizing inoculum was depleted of CD8 T cells but not when CD4 T cells were depleted. Loss of GFP signal suggested the neonatal host immune system became dominant and cleared injected cells. Combined depletion of CD8 T cells from both inoculum and host allowed donor cells to become dominant again with strong GFP+ signal in spleen, lymph node, lung and kidney (but not in other organs as with total allo-SC/BMC inocula). B6 hearts transplanted after combined depletion (n=4) underwent delayed rejection, only one heart beating 100 days post-transplant.

Conclusion: Some neonatal host effector cell types are dominant over other adult effector types necessitating their removal/inactivation for tolerance induction.

Epigenetic inhibitors as potential anti-viral treatment against BK polyoma virus associated nephropathy: an elucidation of mechanism

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Introduction: BK polyomavirus reactivation in immuno-compromised renal transplant patients may cause rapid graft loss within six months of transplant due to serious complication i.e. BK polyoma virus associated nephropathy (BKPVAN). Due to lack of an appropriate antiviral therapy against BKV it is important to study the underlying mechanism of pathogenesis causing BKPVAN.

Objective: To investigate BKV pathogenesis and to determine the potential anti-viral therapy against BKPVAN.

Methods: Human Proximal Tubular Epithelial Cells (HPTCs) and CCD1105 cell lines were infected with BKV. In order to elucidate the epigenetic mechanism another set of cells were treated with DNA methyl transferase enzyme 1 inhibitor RG108 and Histone acetyl transferase inhibitor CPTH2. Urine samples were collected from BKV viruria/viremia positive patients. RNA/DNA was isolated to perform Methylation Specific PCR (MSP) to assess DNA methylation. Further, Real-time PCR, RNA sequencing, western blot and Immunofluorscence staining were performed.

Results: The downregulation of epithelial cell marker 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 mesenchymal transition (EMT). MSP confirmed silencing of those genes through a DNA methylation mechanism by demonstrating hypermethylation of the promoters of CDH1 and COLIVA1 genes in patient's samples. Imunofluorescence staining has shown an increase in Vimentin and disruption of actin filaments in BKV infected cells confirming EMT. RG108 treatment, a demethylating agent, has shown altered COLIVA expression and a decrease in methylation of the promoter, demonstrating that BKV uses DNA methylation for inducing EMT and eventually fibrosis.
RNA sequencing data has revealed that GCN5 (HAT family) expression is increased in BKV infected cells which is required for viral pathogenesis and during replication whereas HATi treatment has shown significant decrease in VP1 expression (the marker of BKV infection) conferring that histone modification also plays an important role in BKV pathogenesis.
Conclusion: Investigating BKV pathogenesis from an epigenetic point of view revealed that BKV orchestrates EMT and pathogenesis by using a DNA methylation and histone modification mechanisms. The use of DNMTi and HATi could reverse or prevent progression of disease and block BKV replication, therefore, these epigenetic inhibitors may be potentially useful as an antiviral therapy for BKPVAN.

Detection of HLA class II antigens using monoclonal antibodies: mixing apples with oranges?

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BACKGROUND: The flow cytometry crossmatch (FCXM) is a widely used method to assess pre-transplant immunologic risk. One variable that may affect FCXM reactivity is variability in lymphocyte HLA surface expression. Assessing HLA density on donor cells in FCXM could be a useful tool to guide FCXM interpretation, particularly HLA class II due to the inherent high background reactivity of B cells. We defined the specificity of two commercially available monoclonal antibodies specific for all HLA class II.

METHODS: Mouse anti-human HLA class II antibodies, clones WR18 and Tu39, were tested by Luminex single antigen beads (SAB; One Lambda). PE-labeled goat anti-mouse IgG antibody was used as a detection antibody for both clones. Patterns of reactivity across antigens of different HLA loci was compared.

RESULTS: Whereas WR18 bound to all HLA-DR antigens, Tu39 showed lower reactivity to DR7, DR12, and specific alleles of DR9 (DRB1*09:01), DR14 (DRB1*14:01 and 14:54) and DR52 (DRB3*01:01). This reactivity aligned with a lack of reactivity to a specific epitope 60Y on these HLA antigens. Although reactivity varied, WR18 detected all HLA-DQ antigens. In contrast, Tu39 only bound to DQ2 and DQ4. HLA-DP was detected by both clones with similar reactivity.

CONCLUSION: Differences in specificity and reactivity were observed between WR18 and Tu39. These findings indicate that Tu39 lacks reactivities required to be considered a true pan-HLA class II-reactive monoclonal antibody. This limited reactivity would affect assessment of HLA expression in FCXM in cases where the donor HLA types include antigens identified as weakly reactive to Tu39.

One Step Closer to a Bead-Based ABO-Antibody Detection Assay

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INTRODUCTION: Accurate, clinically-relevant characterization of ABO-antibodies (ABO-Ab) is critical to assess their impact in ABO-incompatible transplantation. The current ABO-Ab detection method using erythrocyte agglutination is limited by lack of specificity, difficulty in ABO-Ab isotype differentiation, and poor inter-laboratory reproducibility. We developed a slide micro-array method for ABO-Ab analysis to address these limitations. Our aim is to create a similar bead-based assay.

METHODS: ABO-A subtype antigens (I,II,III,IV,V,VI) were coupled to Luminex beads and quantified using monoclonal ABO-Ab. Bovine serum albumin (BSA) and alpha-Gal antigen were coupled as negative/positive control beads, respectively. Optimal plasma and anti-human IgG and IgM labelling-antibody dilutions were determined. IgG and IgM isotype antibodies with specificities for ABO-A-subtypes were measured (n=40 healthy plasma donors). Differences between IgGvsIgM ABO-A-Ab were evaluated (Wilcoxon signed-rank test). Positive thresholds for each ABO-A-subtype bead were determined (median+2SD of the ABO-A samples, n=11).

RESULTS:ABO-A-Ab were successfully measured. There was no statistically significant difference between IgG and IgM ABO-Ab levels (paired data) although variation in ABO-Ab levels between ABO-A-subtypes was detected. Calculated positive thresholds accurately interpreted "positive" ABO-A-Ab in ABO-O samples for the IgG-assay and most beads in the IgM assay. Reactivity to alpha-Gal bead was present but inconsistent.

CONCLUSION:This bead-based method successfully measures ABO-Ab with specificity for ABO-A subtype and shows promise for implementation into the clinical laboratory. The specificity of these solid-phase assays enables accurate assessment of IgG and IgM ABO-Abs in ABO-incompatible transplantation and better donor-recipient selection and transplant monitoring. Further optimization of this assay, positive control selection, and ABO-B and ABO-H panel development is underway.

Immune Phenotyping in a Pediatric Multicenter Transplant Study: Suitability of a Preformulated Dry Antibody Panel System

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BACKGROUND: Flow-cytometric immune-phenotyping is influenced by cryopreservation and inter-laboratory variability limiting comparability in multicenter studies. The DuraClone IM system (DC) provides optimized pre-mixed dry antibody panel tubes for use with small amounts of blood (1mL/6 panels) and a standardized reading protocol. We assessed DC within a multicenter study (CNTRP POSITIVE) with long-distance sample shipping.

METHODS: 38 children awaiting transplant (Tx) were enrolled for parallel immune-phenotyping with DC and validated, optimized in-house panels (ST). Samples were collected before, 3 and 12 months post-Tx. Quality assurance measures and congruence of phenotypes were assessed using Bland-Altman comparisons, linear regression and group comparisons.

RESULTS: Both assays showed excellent viability (mean 94.8%) and lymphocyte recovery when processed <30h. Recovery was lower and more dependent on time-to-processing in immunosuppressed post-Tx samples. Comparing ST and DC, mean difference <5% and range of deviation (2SD)<15% were found for T-cells: CD4+ (-3.8%), CD8+ (+4.1%) and regulatory CD4+ T-cells (-2.5%), with variation 2SD<20% for CD19 B-cells (+1.7%) and CD56 NK-cells (+4.1%), and highly significant correlation between methods ($p<0.001$). Variation of 2SD<25% was found for CD27+IgM- switched memory B-cells (-2.1%) and CD24hCD38h transitional B-cells (+3.4%). DC systematically under-detected CD27-CD28- 'exhausted' T-cells and plasmablasts (-35%).

CONCLUSIONS: DC is reliable for standardized analysis of many immune phenotypes after long-distance shipping when processed within 30h. Some phenotypes are under-detected and plasmablasts appear to decline, possibly due to red cell lysis or EDTA preservation. Within these limitations, DC is attractive for pediatric studies due to small amounts of blood required and highly standardized processing and analysis.**Calcineurin Inhibitors, Macrolides, and the Risk of Adverse Drug Events in Kidney Transplant Recipients**

Rachel H. Jeong[1], Robert R. Quinn[2], Krista L. Lentine[3], Anita Lloyd[1], Pietro Ravani[2], Brenda Hemmelgarn[2], Amit X. Garg[4], Branko Braam[1], Kevin Wen[1], Anita Wong[1], Sita Gourishankar[1], Ngan N. Lam[1]

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Background: Calcineurin inhibitors (CNI; cyclosporine, tacrolimus) are critical for kidney transplant immunosuppression, but have multiple potential drug interactions. Macrolide antibiotics are often used for atypical infections. Clarithromycin and erythromycin inhibit CNI metabolism and increase the risk of CNI nephrotoxicity, while azithromycin does not.

Methods: We conducted a retrospective study using linked databases in Alberta, Canada to study treatments and outcomes in kidney transplant recipients (2008-2015). We identified recipients on continuous CNI therapy who were co-prescribed clarithromycin/erythromycin or azithromycin, and compared outcomes according to macrolide use. The primary outcome was a composite of all-cause hospitalization, acute kidney injury (creatinine increase μmol/L or 1.5-times baseline), or death within 30 days of the macrolide prescription. Results: Of the 293 recipients who were co-prescribed a CNI and a macrolide, 38% (n=112) were prescribed clarithromycin or erythromycin while 62% (n=181) were prescribed azithromycin. Clarithromycin/erythromycin users were less likely to have outpatient serum creatinine monitoring post-prescription compared to azithromycin users (56% vs. 69%, p=0.03). There was no significant difference in the primary outcome between the two groups (17% vs. 11%, p=0.11); however, the risk of all-cause hospitalization was higher in the clarithromycin/erythromycin group (10% vs. 3%, p=0.02).

Conclusion: Clarithromycin and erythromycin were frequently prescribed in kidney transplant recipients on CNIs. Compared to azithromycin, clarithromycin and erythromycin users were less likely to have post-prescription monitoring of kidney function and were at higher risk of hospitalization. This highlights the need to improve safe prescribing practices in kidney transplant recipients.

Cryopreservation of corneal endothelial cells for future transplant applications

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The cornea is the clear covering at the front of the eye that refracts light and focuses vision. The single layer of cells making up the endothelium is responsible for maintaining optical transparency. Corneal endothelial cells (CECs) do not regenerate in vivo; loss of CECs leads to impaired vision and ultimately blindness that can only be treated with a corneal transplant. Corneal blindness afflicts over 10 million people globally and current treatment involves replacement of the entire cornea or the defective endothelial layer. Because of the shortage in quality donor corneas, recent efforts have focused on the development of protocols for the expansion of CECs for cell injection therapy or for seeding onto natural or synthetic scaffolds for transplantation. These approaches require a readily accessible source of a sufficient number of viable and functional CECs.

Cryopreservation is a technology that enables the long-term storage of cells and tissues for research and clinical use. While many cells have been successfully cryopreserved, others have proven to be difficult or requiring optimization. Previously, using human umbilical vein endothelial cells (HUVECs), we systematically identified the variables that can be manipulated to recover cells with high membrane integrity and functionality after cryopreservation. We found that cooling HUVECs at 1 °C/min in the presence of 5% dimethyl sulfoxide (DMSO) plus 6% hydroxyethyl starch (HES) to −35 °C, and then plunging into liquid nitrogen for storage, followed by rapid warming at 37 °C yielded cells of the highest membrane integrity (94.0 ± 0.9% when normalized against unfrozen control) and with tube forming ability similar to that of fresh cells (Sultani et al., Sci. Rep., 2016;6:34393). We then investigated whether the cryopreservation protocol we have optimized for HUVECs will be applicable to CECs. Due to their similarity to human corneas, we first isolated CECs from pig corneas and applied the protocol optimized for HUVECs in the presence of DMSO, with or without HES. Next, we isolated and expanded human CECs and applied the best protocol verified using porcine CECs. We obtained porcine and human CECs with normalized membrane integrities of 91.1 ± 2.0% and 92.8 ± 0.3%, respectively, having the ability to form monolayers that express the tight junction protein ZO-1, cytoskeletal protein F-actin, and ion transporter protein Na+/K+ ATPase (Marquez-Curtis et al., Cryobiology, 2017;77:1-13). The availability of cryopreserved CECs will be valuable in the preparation of tissue-engineered endothelial grafts and for cell-based injection therapies as alternatives to full donor corneal transplant.

Predicting the future scope and impact of human cell atlas technologies (individual cell RNA sequencing) on renal pathology and urinalysis

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Next-generation DNA- and RNA-sequencing technologies have been decreasing in cost and increasing in computing power at an impressive rate (Shapiro, Biezuner, & Linnarsson, 2013). This technology will allow us to distinguish between morphologically identical (but genetically and functionally distinct) cells (Regev et al., 2017), as well as examine the genetic components in ubiquitous kidney disorders, such as IgA nephropathy and Chronic Kidney Disease (CKD). Single-cell technology is now able to do individual cell RNA sequencing in quantities of up to a million cells at a time, which opens the door for whole-organism sequencing using the single-cell approach; this is the target of the Human Cell Atlas (HCA) initiative. We plan to describe the future scope and impact of this new technology on kidney biopsy interpretation and urinalysis. Villani et al. Science. 2017 356(6335) used single-cell RNA sequencing to gain a previously inaccessible view into human blood dendritic cells. The number of blood dendritic and monocytic cell types jumped from the traditional 6 to 12 in their study, which in itself is meaningful for kidney medicine due to the highly analogous nature of the white blood cell immune system and the kidney (both having 26 cell types traditionally). We predict that kidney cells will experience a similar increase from the 26 cell types currently classified to 52. The discovery and detection of new renal cell types will drastically affect the future of kidney pathology over the next decade, and it seems that this process has already begun, at least conceptually. https://youtu.be/oS3SzQ49A9A

Normothermic ex-vivo machine perfusion for liver grafts recovered from donors after circulatory death: a systematic review and meta-analysis

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Background: In response to a shortage of transplantable organs, donation after circulatory death (DCD) liver grafts are being used increasingly. Lower patient and graft survival, and higher rates of cholangiopathy have been reported in these grafts. This has led to growing research interest in normothermic machine perfusion (NMP) as an alternative preservation strategy. This study aims to systematically review the current literature comparing NMP to SCS for DCD liver grafts and complete a meta-analysis of published large animal and human studies.

Methods: Searches were conducted in Ovid MEDLINE, OVID EMBASE, EBSCO CINAHL, WOS, SCOPUS, Proquest Dissertations and Theses, PROSPERO by a librarian June, 2017 and updated in July, 2017. All full text, porcine and human trials comparing NMP to SCS for the preservation of DCD livers were included. Liver enzymes and histological evaluation were used to assess hepatocellular injury and hepatic function marked by bile production.
Results: A total of 9 porcine articles comparing SCS to NMP for DCD grafts were included. There was a significant reduction in AST; mean difference -2291 U/L, CI= (-3019, -1563); p=<0.00001 and ALT; mean difference -175 U/L, CI= (-266, -85); p=0.0001, for NMP relative to SCS. Total bile production was also significantly higher, mean difference =174 ml, CI= (155, 193) p<0.0001.
Although the strength of this meta-analysis is limited by significant heterogeneity between studies, NMP demonstrates less hepatocellular injury and improved preservation of hepatic function relative to SCS for DCD liver grafts.

The full text article has been accepted for publication to HPB Surgery.

Development of a novel non-invasive assay based on cell free-DNA to detect allograft injury after heart transplantation

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Immune-mediated injury (rejection) of the transplanted organ is a serious problem that can lead to allograft dysfunction and patient death. At present, monitoring for rejection after heart transplantation (HT) requires an endomyocardial biopsy (EMB) - an expensive and invasive procedure with significant limitations. Non-invasive tests are safer and allow for more frequent monitoring but currently lack adequate sensitivity and specificity. Dying cells release fragments of DNA into the circulation and, after HT, levels of donor-derived cell-free DNA (dd-cfDNA), quantified based on SNP differences between donor and recipient, have been associated with rejection. We hypothesize that the quantification of dd-cfDNA based on ventricle-specific methylation patterns will be superior to SNP-based approaches for the detection of allograft injury. We have bioinformatically identified five candidate differentially methylated regions (DMRs) unique to human ventricles using publicly-available data from both the Roadmap Epigenomics and Blueprint Epigenome projects, as well as the software package Metilene. Specific parameters, including an average length of 120 bp and a minimum of four methylation sites, were used to ensure that the candidate DMRs would be clinically applicable. Thus, using Illumina sequencing these DMRs will be validated for their tissue specificity and detection in cfDNA. Levels of

the validated ventricle-specific DMRs will then be measured in plasma collected from patients after HT and correlated with levels of dd-cfDNA measured using an established SNP-based cfDNA assay and rejection grade as diagnosed by EMB. A non-invasive blood test allowing close monitoring of the donated heart for damage will be an important advance in HT.

Kidney transplantation and progression of peripheral arterial disease in the ipsilateral lower extremity

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Objectives: We aimed to investigate whether receiving a kidney transplant allograft graft increases the risk of ipsilateral lower limb amputation in patients with pre-existing peripheral arterial disease (PAD).

Methods: We conducted a retrospective review of adult patients with pre-existing PAD who received a kidney transplant at the University of Alberta from 2005 to 2014, using data abstracted from the kidney transplant patient database (OTTR). Exclusion criteria included patients who did not have pre-existing PAD and pediatric patients. The primary outcome was laterality of lower extremity amputation in relation to transplant graft. Baseline demographics and time elapsed from transplant to amputation were also collected. Data were analyzed using a combination of descriptive and comparative statistics.
Results: A preliminary dataset containing 766 patients who underwent transplantation between 2005 and 2014 was analyzed. 77 patients were identified to have pre-operative PAD. Patients were predominantly male (55:22 M:F) with a mean age at transplant of 56 (31-77). 60/77 (78%) patients were diabetic, 42/77 (55%) patients had known coronary artery disease and 14/77 (18%) patients had a history of stroke. 40/77 (52%) patients had a post-operative amputation. There were significantly more amputations ipsilateral to the transplant graft than contralateral amputations (27/40 vs. 13/40 p=0.0385). There was no significant difference in baseline comorbidities between groups.
Conclusions:The preliminary data suggests that patients experience a higher rate of lower limb amputation ipsilateral to their transplant allograft. This finding has important implications for patient selection, pre-operative optimization, and post-operative risk modification.

Vancomycin prevents and reverses mycophenolate mofetil (MMF)-induced weight loss and gastrointestinal inflammation in the mouse

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MMF is widely prescribed after transplantation but usage is frequently complicated by gastrointestinal side effects including diarrhea, abdominal pain, intestinal inflammation and weight loss, often reducing patient compliance and necessitating dosage reduction or discontinuation. The mechanism underlying MMF-induced GI toxicity is not understood but recent work by our lab suggests involvement of the gut microbiome. In our mouse model, MMF resulted in significant weight loss accompanied by intestinal inflammation and microbiome changes. Treatment with broad spectrum antibiotics reversed and prevented MMF-induced weight loss. In this study, we determined which specific antibiotics were most effective in countering MMF induced GI toxicity.

Mice were treated with MMF in the chow (0.563%) and individual antibiotics (ampicillin, metronidazole, neomycin, or vancomycin) in the drinking water. Mice were monitored daily and serial fecal pellets collected. After 9 days, animals were sacrificed, and blood and tissues collected. DNA for 16S rRNA sequencing and metabolites for mass spectrometry were isolated from fecal pellets. Activity of 31 cytokines was measured in colonic tissue.

Vancomycin most effectively prevented MMF-induced weight loss and gut inflammation, and caused broad changes in the fecal metabolome. Neomycin was effective but to a lesser degree. Metronidazole and ampicillin had no effect. Changes in gut microbiome composition and function following antibiotic exposure are under investigation.

Amelioration of the MMF-induced weight loss and intestinal inflammation phenotype with vancomycin suggests that a specific microbe or group of microbes mediates the negative GI effects of MMF. Identification these microbes will lend to a mechanistic understanding of MMF induced toxicity.

Dextran-based and albumin-based perfusates in normothermic ex vivo kidney perfusion

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Kidney transplantation is the definitive treatment for end-stage renal disease. Transplantation is superior to the alternative, dialysis, in survival, quality of life, and cost-savings. However, the gap between kidney donation and demand for donor kidneys is growing, and improved utilization of renal grafts, especially extended criteria donors, is necessary. Normothermic ex vivo kidney perfusion (NEVKP) is a novel method for graft preservation; it maintains metabolism, allows functional assessment, and therapeutic interventions. Currently there are various types of perfusate compositions being used in NEVKP systems, most composed of a combination of physiological saline, red blood cells, and supplemental source of oncotic pressure and nutrients. We examined the differences between using bovine serum albumin and dextran 40 in NEVKP. We found that dextran and albumin produced similar intra-renal pressure during 12 hours of normothermic perfusion. However, dextran produced improved urine production rates and quality, maintained better perfusate biochemistry, and resulted in lower circulating inflammatory markers. Both perfusates groups produced kidney injury molecule-1 (kim-1) immediately after start of NEVKP, but no significant increases were found between each group or between start and end of perfusion. With improved perfusion characteristics and reduced inflammatory profile, we believe a dextran-40 based perfusate is superior to albumin based perfusates in NEVKP.

Development of a Cytotoxicity Flow Cytometry Crossmatch Assay For The Detection of Complement Activating HLA Antibodies

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Background: CDC assays are the gold standard for determining donor-specific HLA antibodies associated with high-risk antibody-mediated transplant rejection. These assays suffer from low sensitivity, subjective scoring, and limited reproducibility. We aimed to develop a next-generation CDC flow cytometry assay benefitting from flow cytometry's enhanced sensitivity and reproducibility and using a fixable viability dye.

Material and Methods: Lymphocytes purified from peripheral blood mononuclear cells (PBMCs) of healthy volunteers were incubated with and without increasing dilutions of serum containing HLA antibodies, followed by low-toxicity rabbit complement. Cell death was defined by the cell percentage stained with viability dye. Cells were paraformaldehyde fixed and analyzed by flow cytometry.

Results: Lymphocytes were killed by heating/cooling and number of dead cells were titrated along with viability dye to determine optimal dye concentration. T- and B-cell labelling antibodies did not affect CDC. Patient sera

characterized for HLA specificities were tested against lymphocytes from HLA-typed volunteers. Cell death was readily detected following incubations with sera lacking rabbit complement (T-20.3%, B-32.0%). EDTA-treated sera had lower background cytotoxicity (T-3.8%, B-16.3%). Figure 1 demonstrates the EDTA-treated sera experiment on Bw4-specific serum against T cells with 1 Bw4 target. The top Bw4 bead reactivity was 17275 mean fluorescence intensity (MFI).

Conclusions: CDC flow cytometry assay was developed measuring T and B-cell cytotoxicity following incubation with sera containing HLA antibodies. Upcoming projects will focus on decreasing non-specific B-cell cytotoxicity before expanding to using larger numbers of patient sera and specifically sera containing

Enhanced Protection of Donation after Circulatory Death (DCD) Hearts by Pharmacological Postconditioning with Intralipid® in a Porcine Model

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Intralipid® (ILE), a clinically used lipid emulsion, reduces ischemia-reperfusion (IR) injury in healthy and infarct-remodeled rat hearts. We tested whether ILE is also cardioprotective in porcine hearts in the context of the donation after circulatory death (DCD) model, where hearts are procured for transplantation after cardiac arrest and thus exposed to IR injury. After termination of ventilator support and cardiac arrest with a 15 min standoff period, hearts of female Yorkshire pigs were procured with a previously optimized tepid normokalemic crystalloid adenosine-lidocaine cardioplegic solution and randomly allocated to ex vivo reperfusion (35Â°C) in the presence or absence of 1% ILE. Hearts were perfused with blood and STEEN solution (1:1), initially in the Langendorff mode. Working mode was established after 60 min for assessment of functional recovery. Left ventricular (LV) biopsies were obtained after 5 min of reperfusion for measurements of reactive oxygen species (ROS) production and cardiac tissue was preserved at the end of perfusion for biochemical analysis. ILE postconditioning increased inotropy (average dP/dtmax 2001 (SD 345) vs 1584 (192) mmHg/s), reduced cell membrane damage as assessed by the leakage of glutathione from the myocardium, reduced protein carbonylation, and decreased myeloperoxidase activity. ILE postconditioning increased ROS signaling at early reperfusion and Akt, STAT3 and GSK3Î² phosphorylation in cardiac tissue, recapitulating all features of ILE-protection previously reported in rodent hearts. Our data show that ILE postconditioning efficaciously elicits protective signaling in large mammal hearts, mimicking conditions closer to patients, and is capable of enhancing protection of DCD hearts.