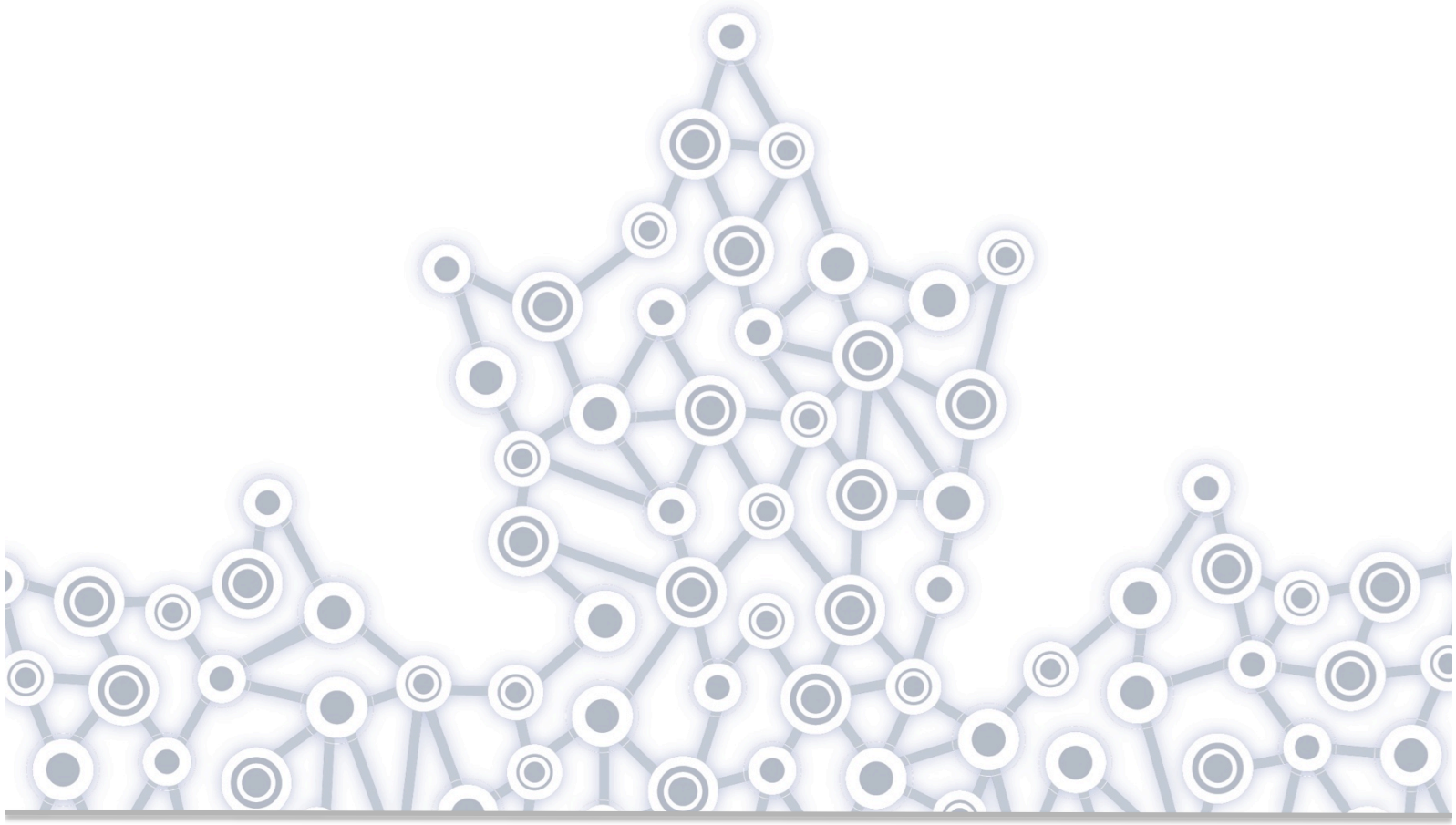
Canadian National TRANSPLANT Research Program | Programme national de recherche en TRANSPLANTATION du Canada

Year 3 Annual Extended Scientific Report

Nov 2015 – Nov 2016



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The Canadian National Transplant Research Program Central Report

Director: Dr. Lori West, University of Alberta
Co-Director: Dr. Marie-Josée Hébert, Université de Montréal

The CNTRP: uniting the community

In January 2012, the Canadian Institutes for Health Research presented the transplant research community with a challenge: bring solid organ transplant (SOT), cellular transplant and donation/critical care researchers together into a national program that would transform the field of donation and transplantation and improve the health of Canadians. The research community responded to this challenge by building a national program that is not only innovative and unique in Canada, but also not achieved elsewhere in the world.

The Canadian National Transplant Research Program (CNTRP) is a nationally funded research network that now unites over more than researchers, collaborators, trainees and patients at 30 universities and institutions across Canada, representing the major health researchers from basic biomedical, clinical, health services/policy and population health arenas. The CNTRP weaves the solid organ transplant, cellular transplant and donation/critical care communities into a highly integrated national coalition. The Program is led by Dr. Lori West, CNTRP Director at the University of Alberta, and Dr. Marie-Josée Hébert, CNTRP Co-Director at the University of Montreal.

The CNTRP develops new knowledge and health care practices to:

- Increase the availability of donors and access to transplants
- Extend the longevity of grafts
- Improve long-term survival and quality of life of transplant patients
- Develop and enhance the pool of talent in the transplant field
- Integrate and coordinate donation and transplantation research nationwide

The CNTRP is a uniquely Canadian initiative, drawing on our strength in scientific collaboration, our history of transplantation research, our publicly funded health care system, and the federal and provincial support and leadership provided to this initiative. The CNTRP framework is innovative and trans-disciplinary, nurturing synergistic and transformative collaborations that did not exist previously and would not be possible without this interactive program structure.

Over the last three years, the major accomplishment of the CNTRP has been to create and nurture interactions and synergies across all levels of the program including:

- uniting teams of basic and clinical scientists in organ and tissue donation, cellular transplantation, and liver, heart, lung, pancreas, and kidney transplantation, as well as health economics, legal and ethics researchers, policy experts and knowledge-users into a dynamic network now focused around seven thematic projects consisting of more than 75 sub-projects, and supported by four comprehensive cores
- bringing investigators **across all career stages** into the program to collaborate on important studies, including clinical trials
- creating new linkages with **industry**, **health charities** and **provincial** and **federal governments** to disseminate new knowledge and to leverage over **$7.7 million** in new research funding over the past year (Table 1).

- linking research across the entire age spectrum of subjects by engaging investigators in **pediatric**, **adolescent** and **adult** transplantation across the country to participate in multicenter studies that share samples and data.
- engaging **patients** and **family members** as co-leads and patient researchers across all levels of the CNTRP structure to integrate their expertise and experience in donation and transplantation.
- expanding our **provincial**, **federal** and **international** partnerships to address important policy issues and clinical gaps.
- working and interacting in both **French** and **English** to unite colleagues across the country and disseminate new knowledge in both languages.

Since 2013 the CNTRP has worked to combine the efforts of researchers across the country to address prevailing problems in donation and transplantation that are obstacles to provision of the best health care for people with (life-threatening diseases) of (end-stage organ failure and hematologic malignancies). The CNTRP is positioning Canada as a world leader in transplantation and donation research and is on track to build an enduring legacy that will transform the field.

Expanding the CNTRP:

Since Year 2 (November 2015), the number of participants in the CNTRP has increased from 358 to 512 individuals. CNTRP by the numbers (compared to Year 2 - November 2015):

- 156 researchers, including 14 Canada Research Chairs[1] (+17)
- 152 collaborators (+40)
- 149 stakeholders (patients, partners, industry, health charities) (+85)
- 32 trainees (+4)
- 15 program/project managers (+7)
- 8 patient researchers or patient co-leads (+8)
- 151 scientific peer-reviewed publications since April 2013

In terms of expanding our funded research and leveraging the success of our program, the CNTRP was successful in helping our researchers secure **over $7.7 million** in new funding and partnerships in Year 3 that are incorporated into and supported by our research structure, including:

- 3 new CIHR Project Grants ($1,888,318 total)
- 6 new CNTRP Research Innovation Grants, funded through partnerships with Astellas Canada, Inc., the Alberta Transplant Institute (Edmonton), the CRCHUM (Montreal) and the UHN Multi-Organ Transplant Program (Toronto) ($150,000 total)
- 3 new industry-partnered clinical trial and research studies ($1,625,000 total) (Qiagen, BD, and Astellas)
- 3 new CIHR Planning Grants ($33,750 total)
- 1 new CIHR SPOR Patient Engagement Grant ($35,000 total)
- 1 new NIH grant ($2,782,901 total)
- 3 new operating grants from Canadian NCEs, including BioCanRx, GlycoNet, and StemCell Network ($575,659 total)

For full list of new projects and funding, see Table 1 and Table 2 below (p. 11-12)

[1] **Canada Research Chairs (CRCs) in the CNTRP**: Antoine Boivin, Tim Caulfield, Jeremy Grimshaw, Lucie Germain, Todd Lowary, Patrick MacDonald, Quim Madrenas, Dan Muruve, Tommy Nilson, Claude Perreault, James Shapiro, Jean-Claude Tardif, Pierre Thibault, Lori West

A major change to the CNTRP structure was creation of a new **Project 7** and **Core 4**, bringing the program to 7 projects and 4 core platforms made up of over **75** interconnected sub-projects or sub-aims.

Project 1: *Ex vivo* organ transplant protection and repair
Project 2: Increasing solid organ and hematopoietic cell donation
Project 3: Understanding, predicting, preventing early graft rejection and GVHD
Project 4: Strategies for immunomodulation and transplant tolerance
Project 5: Predicting and controlling viral complications of transplantation
Project 6: Improving pediatric outcomes in transplantation
Project 7: Improving long-term outcomes and quality of life in transplant recipients
Core 1: Ethical, economic, legal and social (EELS) platform
Core 2: Research infrastructure and registries support platform
Core 3: Training and career development platform
Core 4: Patient researcher partnership platform

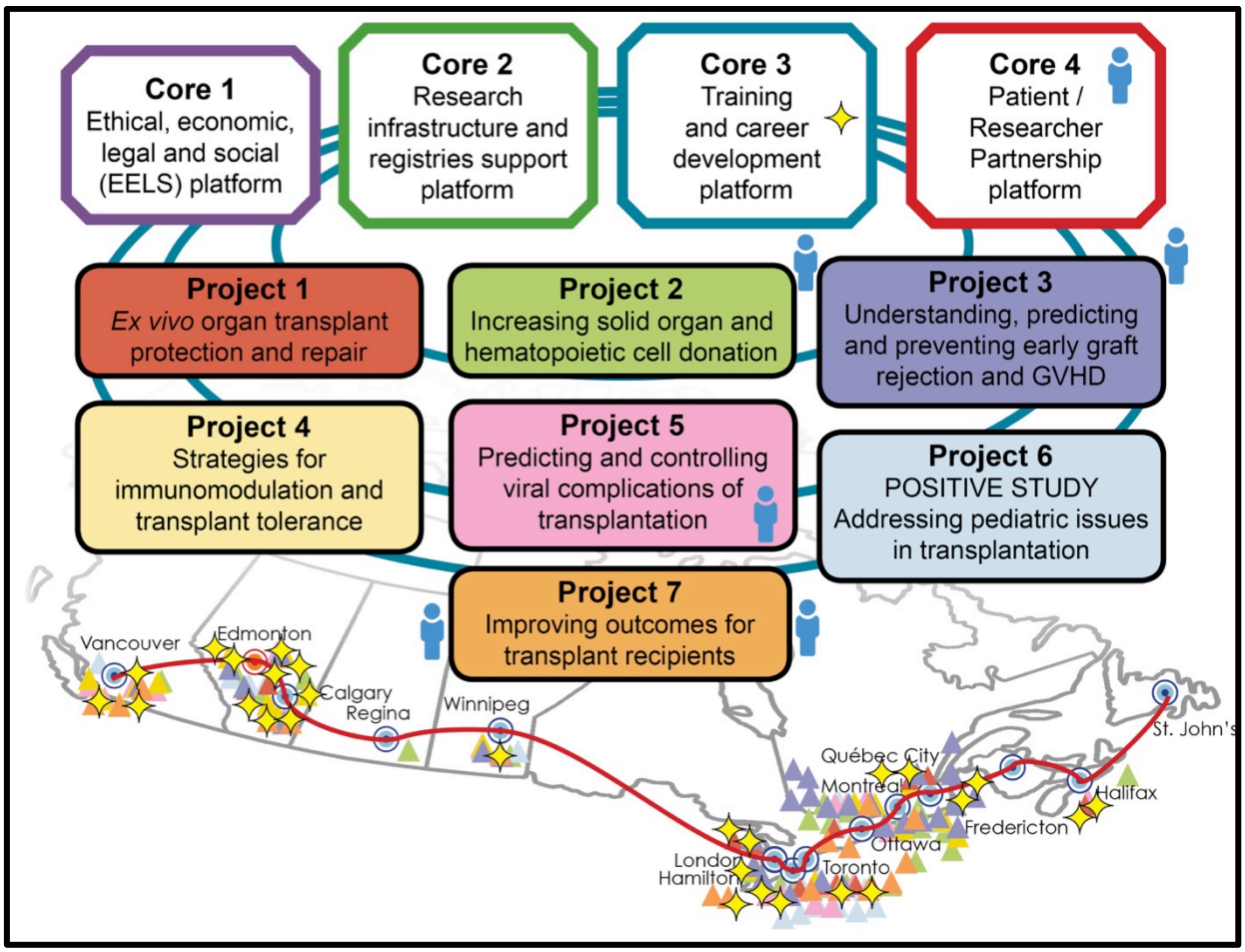


Figure 1. Diagram of the revised structure of the CNTRP (including P7 and C4) and CNTRP researchers and patient partners across Canada.

Introducing our new Core 4: Patient Researcher Partnership Platform

One of the main priorities for the CNTRP is engagement and integration of living donors, families and transplant patients into the research structure. For the members of the CNTRP, patient engagement occurs when patients meaningfully and actively collaborate in the governance, priority setting, and conduct of research, as well as in summarizing, distributing, sharing, and applying resulting knowledge. Involving patients in setting research priorities has been acknowledged as a way to enhance the quality, relevance and transparency of medical research. Until recently, patients and the public were not widely involved in donation or transplantation research. Patient engagement is a priority for the CNTRP and our expanding strategy builds on important consultations attended by patients, caregivers, clinicians and researchers aimed to develop a structure and mechanisms to foster meaningful partnerships and collaboration amongst patients, families and the CNTRP.

The CNTRP **Patient Researcher Partnership Platform (Core 4)** was officially created in April 2015 with new funding from CIHR and Astellas Canada. Composed of a core group of patients and families, together with a core group of researchers (Marie-Chantal Fortin, Greg Knoll, Linda Wright, Samantha Anthony, David Hartell, Vincent Dumez and Antoine Boivin), this platform is responsible for coordinating partnerships and developing a training program for researchers and patients while evaluating the impact of this patient partnership.

Over Year 3, the new Core 4 team carried out the implementation phase of the patient researcher partnership strategy with integration of patients/family members in the following roles across the CNTRP:

- **Patient Core 4 Co-Lead**: A patient working for the CNTRP (2 days a week) and a full member of the CNTRP Executive Committee (Sylvain Bédard)
- **Patient Co-Leads** on 3 CNTRP projects
- **Patient Researchers** who will participate initially in three research projects

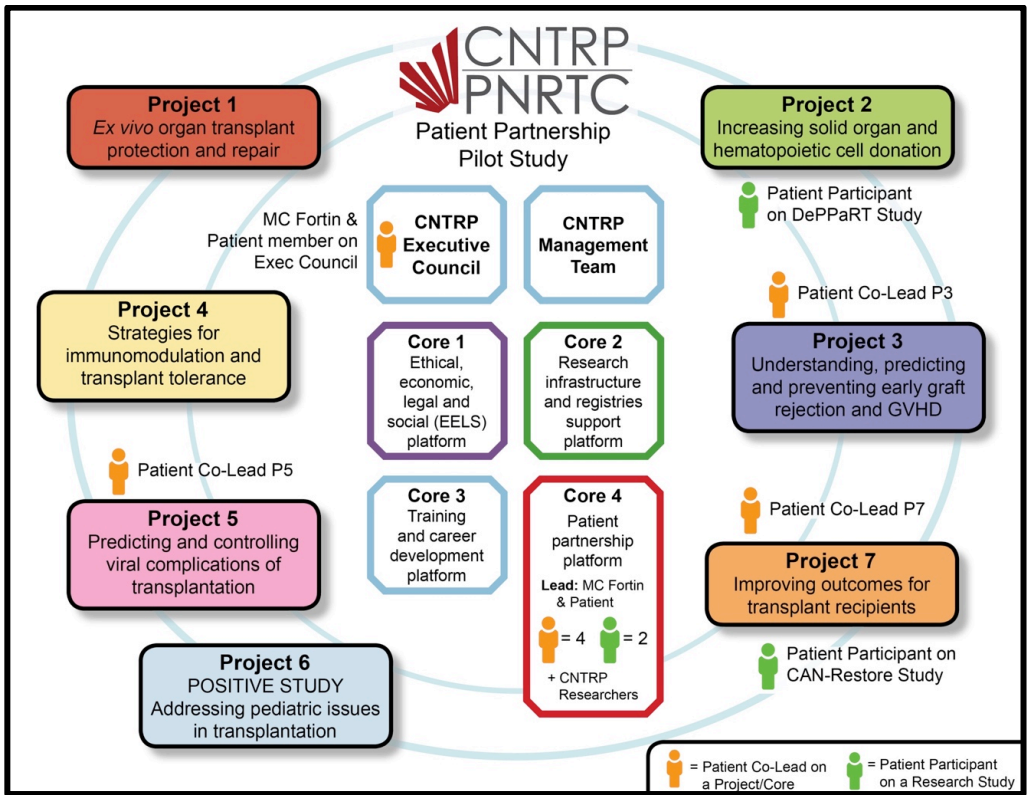


Figure 2. *CNTRP's new patient research partnership strategy implementation*

Introducing our new Project 7: Improving long-term outcomes and quality of life in transplant recipients

Another important addition to the CNTRP structure in Year 3 was the creation of a new project with the main goal to improve long-term outcomes and quality of life in transplant recipients. Project 7 originated from our CAN-Restore study (www.cntrp.ca/exercise) that focuses on exercise and rehabilitation therapy to improve outcomes for transplant recipients. Several other CNTRP researchers and collaborators expressed interest over time to bring independently funded studies and new research ideas into the CNTRP structure that would address long-term outcomes and quality of life. Project 7 is led by Dr. Sunita Mathur, University of Toronto, and is co-led by Dr. Maureen Meade, McMaster University together with Sandra Holdsworth, our Patient Co-Lead. Project 7 now includes **19 investigators** (13 are new to CNTRP), a strong **patient partnership** component, and **$1,950,000** worth of externally funded projects/studies. The aims of Project 7 include:

- ***P7 Aim 1:*** *Evaluation of co-morbidities, disability and quality of life in transplant candidates and recipients*
- ***P7 Aim 2:*** *Effectiveness of interventions in donors, candidates and recipients to mitigate co-morbidities and improve long-term outcomes and quality of life after transplantation*
- ***P7 Aim 3:*** *Improving long-term organ function through innovations in assessments and novel interventions*
- ***P7 Aim 4:*** *To increase knowledge uptake of Project 7 research findings by key stakeholders in transplantation (Knowledge Translation/Knowledge to Action cycle)*

Building excellence through innovative partnerships

Figure 3. Current CNTRP funders, partners, collaborators and supporters

One of CNTRP's key objectives is to integrate and coordinate transplantation research nationwide; much of this is accomplished by developing strong partnerships. Since the launch of the CNTRP, the program has worked closely with our provincial/regional, federal and international partners, including industry, governments, funding agencies and health charities, to strengthen and enhance our national research efforts and integrate with provincial components. New partnership activities are under constant development as we seek to expand the CNTRP effectively.

2016 National Child Health Transplant Team Grant Competition

The **Alberta Transplant Institute (ATI),** the **Transplant Research Foundation (TRF) of BC**, **Astellas Canada, Inc.** and the **BC Children's Hospital Foundation (BCCHF)** partnered with CNTRP to develop and launch the National Child Health Transplant Team Grant Competition aimed to support research to improve outcomes for pediatric transplant patients through cross-Canada, multi-disciplinary collaboration. Initially conceived as the *Addison Pediatric Project* in BC (a $25,000 initial investment), the vision of this new expanded partnership is to support an innovative pediatric transplantation project that unites researchers across Canada to work together toward substantial improvement in outcomes for pediatric transplant patients. In March 2016, the CNTRP brought the TRF, Astellas Canada and the BCCHF into a partnership to increase the initial investment to **$100,000** to fund one team grant (application deadline November 1, 2016) to allow researchers across the country to work together within the structure of the CNTRP (www.cntrp.ca/research) to conduct research not possible at a single site. This national competition, focused exclusively on improving outcomes for pediatric solid organ transplant patients, is a first in Canada.

Emerging Research Leaders Initiative 2016 (ERLI)

The CNTRP was fortunate to receive **$150,000** in direct funding support from the **CIHR Institute of Circulatory and Respiratory Health** to allow the CNTRP to partner on the 2016 Emerging Research Leaders Initiative (ERLI). Partnership on the ELRI program enables the CNTRP to support the successful early career launch of new investigators examining how to increase organ donation and access to heart transplantation in Canada and/or enhance the survival and quality of life of Canadians living with heart transplants. This multi-partnered initiative, led by **Heart and Stroke Foundation of Canada**, includes organizational partners from non-profit, government, industry, and emerging / existing networks; results of the 2016 competition will be announced in January 2017.

CNTRP's 3rd annual Research Innovation Grant Competition

For the 3rd consecutive year, the CNTRP partnered with Astellas Pharma Canada, Inc. to launch the CNTRP Research Innovation Grant Competition to fund peer-reviewed pilot research projects aligning with CNTRP's overall objectives to increase organ and tissue donation in Canada and enhance the survival and quality of life of Canadians who receive transplants with the ultimate goal of improving patient care. In the 2016 competition, the CNTRP expanded the competition by including additional partnered funding from the **Alberta Transplant Institute**, the **Centre hospitalier de l'Université de Montréal**, the **Multi-Organ Transplant Program of the University Health Network** in Toronto, and the **University of Ottawa Heart Institute**, which together is providing **$275,000** in new funding to the research community. Results from this competition will be announced in December 2016 and planning for the next phase of this competition is currently underway.

To support research performed by the CNTRP, we have developed integral partnerships with several international companies who provided funding and equipment, reagents, training, and/or personnel. These include:

- The CNTRP launched a partnership with **QIAGEN** and the **Canadian Liver Foundation** (CLF) to study a new approach to treating cytomegalovirus (CMV) infections in transplant recipients. Through this partnership, we launched a new CNTRP multi-centre clinical trial,

led by Drs. Atul Humar and Deepali Kumar at the University Health Network in Toronto, to use the QuantiFERON®-CMV assay to guide primary prophylaxis treatment for CMV infection in kidney and liver transplant recipients. With a $1.5M investment, this CNTRP interventional clinical trial will determine whether this test can be used to make clinical decisions regarding tailoring the duration of CMV prophylaxis. The study will be performed at several transplant centres across North America and Europe.

- **Beckman Coulter Life Sciences** partnered with the CNTRP on our immune monitoring initiative, part of **Core 2**, to standardize how we organize, analyze and share data across Canada on immune parameters in transplant patients. Beckman Coulter continued to provide three CNTRP research labs with new NAVIOS 10-colour Flow Cytometers, funded access to two clinical NAVIOS machines, provided on-site set-up and training, a substantial discount on the flow panels developed by the ONE Study consortium and the ability to develop our own custom CNTRP panels. Only through this partnership is the CNTRP able to support and provide a comprehensive standardized immune monitoring strategy that is used in our studies and trials across the country.
- **Kiadis Pharma** is partnering with the CNTRP on our CARE Trial, part of **Project 4**, by providing the TH9402 photosensitizer and shipping and installing two Theralux devices in each of our three cell processing centres. The CARE trial opened in 6 centres across Canada and is now actively recruiting patients.
- Professor Peter Friend's group at Oxford University, together with their spin-off company **OrganOx**, have developed a portable normothermic liver perfusion device called the Metra and are partnering with CNTRP **Project 1** to launch the *Ex Vivo* Normothermic Liver Perfusion Marginal Clinical trial in Toronto and Edmonton. OrganOx is providing the CNTRP with considerable in-kind contribution by way of subsidized perfusion circuits and Metra devices to allow these studies to move forward.

Beyond new and existing funding partnerships, the CNTRP is striving to unite the research community and forge important relationships with patient groups, national stakeholders, professional societies, and research programs to increase the impact of the CNTRP.

- To strengthen links between the solid organ transplant (SOT), cellular transplant and donation communities, the CNTRP co-hosted our Annual Scientific Meetings with each of the major professional societies representing these communities including the Canadian Society of Transplantation (SOT) October 2016 in Quebec City, the Canadian Critical Care Trials Group (donation) June 2015 in Montebello and the Canadian Blood and Marrow Transplant Group (cellular transplant) June 2014 in Halifax. By co-hosting our meetings with these professional societies, the CNTRP initiated new conversations and brought together groups of researchers who typically would not attend the same meetings.
- Beyond these three main communities, the CNTRP is also developing new partnerships with CellCAN, the national network representing the five GMP cell processing facilities in Canada, and with GlycoNet, the national glycomics research network, by co-developing training webinars, co-hosting a workshop in May 2016 in Banff, AB, creating new research proposals and exploring joint funding opportunities to link our research communities.
- One of CNTRP's most important partnerships is our relationship with the Canadian Transplant Association (CTA), the patient advocacy group comprised of transplant recipients, athletes and volunteers dedicated to promoting organ donation through public advocacy and events. CNTRP worked closely with the CTA to develop our patient engagement strategy and was involved in the Canadian Transplant Games in August 2016 in Toronto.
- The CNTRP participated in the development of two powerful documentary films on organ donation and transplantation, both directed by Niobe Tompson and produced by Rosie Dransfeld and ID Productions. "Memento Mori" is a full-length documentary that was funded and supported by the National Film Board of Canada. The second, entitled "Vital Bonds", was funded by CBC's The Nature of Things. The two films will be released in November and include participation by CNTRP members Dr. James Shapiro (featured on the movie poster), Dr. Simon Urschel, Dr. Lori West, Dr. Jim Kutsogiannis and other CNTRP members.

Leveraging CNTRP strengths

Over the past year, the CNTRP supported investigators across the three communities to submit and seek new research funding to expand the scope of work included in the network. The following projects and investigators were added to the CNTRP in Year 3:

Table 1 – new CNTRP-leveraged research funding in Year 3

New CNTRP studies bringing new funding into the program in 2016	
A comprehensive model to predict the risk of invasive aspergillosis after hematopoietic stem cell transplantation **Dr. Simon Frédéric Dufresne** - Université de Montréal $25,000 – Astellas Innovation Grant	*Evaluation of a glyconanotechnology tool for personalized ABO-incompatible kidney transplantation* **Dr. Todd Lowary** - University of Alberta $25,000 – Astellas Innovation Grant
A 34-gene signature diagnostic test to determine the individualized likelihood of antibody-mediated rejection and kidney transplant failure using formalin-fixed, paraffin-embedded biopsy samples: a multicenter external validation study **Dr. Banu Sis** - University of Alberta $25,000 – Astellas Innovation Grant	*Characterization of Donor Specific Immune Reactivity in Pediatric Heart Transplant: Impact of Thymectomy* **Dr. Lori West** - University of Alberta $25,000 – ATI Innovation Grant
Rocuronium and Midazolam Metabolomics for the Assessment of Liver Grafts During Subnormothermic Ex Vivo Liver Perfusion **Dr. Markus Selzner** - University Health Network $25,000 – UHN Innovation Grant	*Brain-dead donor management goals, donor biomarkers and the development of delayed graft function in kidney transplant patients* **Drs. Josée Bouchard and Héloise Cardinal** - Université de Montréal $25,000 – CHUM Astellas Innovation Grant
Computed tomography perfusion for neurological death determination: a prospective Canadian multicenter diagnostic test study **Dr. Michaël Chassé** – Université de Montréal $951,803 – CIHR Project Grant	*The molecular heritage of vascular injury: Roles in rejection and renal failure* **Dr. Marie-Josée Hébert** – Université de Montréal $699,520 – CIHR Project Grant
Multi-centered study to test quantiferon-cmv based primary prophylaxis for CMV infection in organ transplant recipients **Dr. Atul Humar** – UHN Toronto $1,500,000 – Qiagen, CLF & CNTRP Partnership	Evaluation of Sarcopenia in Solid Organ Transplantation **Dr. Sunita Mathur** – University of Toronto $236,995 – CIHR Project Grant
Teen Adherence in KidnEy transplant Improving Tracking To Optimize Outcomes (TAKE-IT TOO) **Dr. Beth Foster** – McGill $2,782,901 – NIH RFA-DK-15-017	*ABO GLYCAn MICROARRAY development* **Dr. Lori West** – University of Alberta $75,000 - BD research funding grant
Implementation of a patient-oriented research strategy within the Canadian National Transplant Research Program **Dr. Marie-Chantal Fortin** – Université de Montréal $35,000 – CIHR SPOR Patient Engagement Grant	*Continuous waveform analysis to improve prediction of time to death after withdrawal of life sustaining therapy in critically ill patients* **Drs. Sonny Dhanani & Andrew Seely** – CHEO $168,000 - Physician's Services Incorporated
International Planning Meeting to Develop a cDCD Collaborative **Dr. Sonny Dhanani** – CHEO $10,000 – CIHR Planning Grant	*Immune Responses to Polysaccharides in Transplantation* **Drs. Lori West, Kirk Schultz, Donna Wall, Simon Urschel** – University of Alberta $200,000 - GlycoNet
Turning Garbage into Gold – GMP ramp-up for thymic regulatory T cells from discarded human thymus **Drs. Megan Levings & Lori West -** University of British Columbia and University of Alberta $100,000 – StemCell Network	*The TSS meeting in Victoria* **Drs. Megan Levings & Lori West** - University of British Columbia and University of Alberta $8750– CIHR Planning Grant

Using regulatory T cell (Tregs) from discarded thymuses to prevent GVHD in hematopoietic stem cell transplant patients **Drs. Megan Levings & Lori West -** University of British Columbia and University of Alberta $275,659 – BioCan Rx	

Table 2 – new funding partnerships supported by CNTRP

New partnered funding in 2016 supported by the CNTRP	
Planning meeting to develop ECPR research network (CNTRP supported workshop) **Dr. Sam Shemie and Laura Hornby** – McGill $15,000 – CIHR Planning Grant	*Living Kidney Donation: Improving Safety, Access, and Outcomes (CNTRP supported study)* **Dr. Amit Garg** – Western University $5,336,854 – CIHR Foundation Grant
Data Serving Canadians: Deep Learning and Optimization for the Knowledge Revolution (CNTRP supported program) **Dr. Marie-Josée Hébert** – Université de Montréal $93,562,000 - CFREF	*A randomized controlled effectiveness trial of urine CXCL10 chemokine monitoring post-renal transplant (a CNTRP supported trial)* **Dr. Julie Ho** – University of Manitoba $2,664,284 – CIHR Project Grant

Next Steps:
As CNTRP prepares for its 4th year, the focus will be on integrating patients, families and caregivers fully into the CNTRP structure, exploring additional international partnerships and collaborations, and building the framework with our research community to secure long-term and renewable funding.

Project 1 - *Ex vivo* organ transplant protection and repair

Lead: Dr. A.M. James Shapiro, University of Alberta
Co-Leads: Dr. Markus Selzner, University Health Network and
Dr. Darren Freed, University of Alberta

Original aims/sub-aims and changes

Project 1 represents the national pre-clinical and, safety and efficacy clinical trials of *ex vivo* oxygenated perfusion modalities for organ preservation and repair, with a focus on the elimination of cold ischemic organ preservation, across the following five (5) major transplant organs:

- Heart (Dr. Freed, Edmonton; Dr. Badiwala, Toronto)
- Lung (Dr. Nagendran, Edmonton)
- Liver (Dr. Selzner, Toronto; Dr. Alwayn, Halifax; Dr. Shapiro, Edmonton)
- Kidney (Dr. Luke, London; Dr. Selzner, Toronto)
- Pancreas (Dr. Shapiro, Edmonton; Dr. Paraskevas, Montreal)

Through collaboration, investigators have worked to optimize this new technology by leveraging organ commonalities while respecting physiologic differences that require organ-specific approaches. The team is driven by the desire to increase transplant organ availability and utility by reducing organ injury caused by current transplantation processes and through the possible perfusion of reparative compounds to organs *ex vivo* that will reduce recipient exposure and risk.

Global Aims:

- The use or development of *ex vivo* oxygenated perfusion systems to diagnose, protect and repair organs across all five transplant organ areas.
- Restoration of physiologic organ support promptly after donor removal until recipient receipt.
- Increase utilization and improve outcomes of injured organs for transplantation.
- Elimination of cold ischemic organ preservation.
- Increase the quality of transplantable organs to Canadians with end-organ failure.
- Increase use of “marginal’ and “donation after cardiac death” (DCD) organs for transplant.
- Pre-clinical testing of drugs to protect/repair organs during *ex vivo* perfusion (BMX, F573, AAGP) using animal organs and unused (“discard”) human organs.

Original Sub-Aims (Organ-specific):

Heart

- Establishing preclinical *ex vivo* protocols for cardiac perfusion in a pig transplant model.
- Determine optimal perfusion conditions for recovery of dysfunctional hearts
- Treat dysfunctional hearts with targeted therapeutics during EVHP

Lung

- Completion of clinical trial enrolment (INSPIRE trial; OCS lung TransMedics machine).
- Investigating marginal lungs protected on OCS for clinical transplantation.

Liver

- Optimizing alternative preclinical *ex vivo* of liver perfusates in pig transplant models.
- Establishing a working, survivable transplant model to test injured and repaired *ex vivo* livers.
- Achieve graft modification by inducing resistance against Hepatitis C infection using micro RNA during subnormothermic *ex vivo* liver perfusion (Selzner).
- Investigating novel delivery of hemoxygenase-1 (HO-1) for transfection in mouse models.
- Multi-centre first-in-North-America pilot study of a portable *ex vivo* normothermic oxygenated perfusion system (OrganOx) in clinical liver transplantation.
- Initiation of a further clinical trial with CNTRP oversight, to assess safety and utility of clinical *ex vivo* normothermic perfusion in marginal liver transplantation (Toronto + Edmonton joint study).

Kidney

- Optimize ex vivo renal transplant perfusates for hypo- and normothermic storage to determine optimal renal preservation and reduction and translate research finding in a transplant model (Luke)
- Development of a clinically feasible *ex vivo* normothermic perfusion system tested in a prolonged kidney perfusion and transplant model in pigs, with planned initiation of clinical trials in Toronto and subsequently in other collaborative CNTRP sites across Canada starting in the Autumn 2016.

Pancreas/Islets

- Investigating novel approach(s) to optimize pancreas organs in transit for islet isolation in a first-in-human clinical *ex vivo* trial using a specially designed persufflator machine (Shapiro and Paraskevas)
- Investigate the cytoprotective effects of sHO-1-CPP in a murine model of pancreatic islet transplantation (Alwayn)

Major accomplishments in Year 3

CLINICAL TRIALS

Liver

- Through the CNTRP collaboration, Edmonton and Toronto initiated a first-in-North America testing of an *ex vivo* normothermic liver system provided by OrganOx UK in clinical liver transplantation. The Edmonton trial set out to replicate exactly the previous pilot UK trial with the OrganOx system, and Toronto designed a similar trial but exchanged the preservation loading solution from Gelofusine to Steen, to further investigate whether Steen, an approved perfusion solution in North America, would be a reasonable substitute.
- Dr. Shapiro completed the Edmonton based OrganOx liver pilot clinical trial. 10 livers were perfused in the initial trial, one was discarded due to a technical complication of a twist in the portal vein, and 9 subjects underwent successful liver *ex vivo* perfusion and liver transplantation. Outcomes were compared to concurrent and recent historic control liver transplant data. In the normothermic transplanted livers 30 day graft survival was 100% in both NMP and SCS groups. A manuscript has been reviewed and is pending acceptance in the *American Journal of Transplantation*.
- Dr. Selzner completed a Toronto based pilot clinical trial on normothermic *ex vivo* liver perfusion in human livers, as part of the larger OrganOx clinical trial. 13 transplants were

completed using Normothermic ex vivo perfusion (2 additional donated livers were declined) and all livers had excellent graft function. A manuscript has been published in *Liver Transplantation* 2016.

Islet

- Dr. Shapiro's BMX-010 protocol clinical trial has been completed and is *in press* in *Cell R4*. The addition of a potent metalloporphyrin, BMX-010 across the islet isolation process did not affect human islet yield, post culture survival, or beta cell function.
- Dr. Paraskevas optimized and validated his protocol for back table cannulation of the pancreas to the Giner P3S, and disseminated the protocol to collaborators at the Clinical Islet Transplant Laboratory in Edmonton, the University of Arizona, and St. Vincent's Hospital in Melbourne, Australia.
- The Giner clinical trial completed the goal of 10 pancreases from NDD multi-organ donors using the P3S or static cold storage at the Clinical Islet Transplant Laboratory in Edmonton, using pancreases retrieved in Quebec and couriered to Edmonton. A manuscript is in preparation that summarizes the clinical experience to date in islets transplanted from pancreas organs preserved using the persufflator CNTRP trial system.
- The first clinical islet transplant in Eastern Canada was performed. In addition, 6 human islet isolations were completed at the MUHC Human Islet Transplant Laboratory for comparison with historical data.

Lung

- Dr. Nagendran's Canadian branch of the clinical trial for INSPIRE RCT has had 67 patients consented, and 17 patients successfully randomized into the trial at the U of A.
- Three transplant surgeons at the U of A have been trained for clinical ex vivo lung perfusion on the TransMedics Lung OCS, and two of them have also been trained on the XVIVO clinical ex vivo lung perfusion device which is also been used successfully at the U of A for clinical transplantation.
- Dr. Nagendran's arrangements for the clinical use of ex vivo lung perfusion have progressed. The Lung OCS device and clinical disposable modules were successfully shipped to the U of A. A partnership has been established so that packed red blood cells can be used at distant sites during procurements and preservation of the donor lungs on the Lung OCS. And a partnership with HOPE has resulted in the creation of SOPs for transportation and clinical application of the Lung OCS and the XVIVO devices.
- The Transmedics Lung OCS has been used 18 times at the U of A, with 17 successful transplants using the device on randomized patients, and on 9 sets of marginal lungs resuscitated by the device. In addition, the Lung OCS was used to resuscitate an extremely marginal donor lungs with successful transplantation.

Pre-Clinical

Heart

- Having focused on metabolic support for the *ex vivo* perfused heart this past year, the Freed lab identified a dysregulation of pyruvate drogenase, and found that either a bolus or continuous provision of pyruvate enhances preservation of graft function. They are examining metabolomic markers in perfusate and myocardium, as well as PDH function and ER stress in the myocardium following concomitant heart-liver perfusion.
- Dr. Freed re-established an agreement with HB02 Therapeutics for provision of HBOC-201 and continued the relationship with XVIVO Perfusion for provision of STEEN Solution.

- Dr. Freed obtained three unusable human hearts (2 NDD and 1 DCD) for testing, and demonstrated that the EVHP system is capable of perfusing human hearts, as well as large animal hearts. This is a major step forward that will further facilitate refinement in circuits optimized for future clinical implementation in *ex vivo* cardiac transplantation.
- Dr. Badiwala's lab (Toronto) have established an ex *vivo* heart perfusion system and received animal ethics approval of their porcine donor heart procurement and transplant models.
- A pharmacologic strategy using Somah Solution has been evaluated and compared with current perfusion and preservation solutions with respect to their effect on endothelial activation and function in Dr. Badiwala's lab. An abstract summarizing the results of these investigations has been accepted for oral presentation at the upcoming Canadian Cardiovascular Congress.
- Dr. Badiwala's lab have developed the protocol and ex vivo apparatus to facilitate ex vivo echocardiographic evaluation of cardiac function in collaboration with their institutional cardiac anesthesia echocardiography group.

Liver

- Dr. Alwayn's lab completed experiments to demonstrate cytoprotection of sHO-1-CPP in an *in vitro* model of liver IRI using multiple hepatocyte cell lines under various conditions of ischemia and reperfusion, and confirmed the safety of *in vivo* administration of sHO-1-CPP and have demonstrated penetration in livers when administered intravenously.
- Dr. Alwayn and Dr. Selzner identified a correlation between the level of measured mtDAMPs in the perfusate and post-transplant outcomes of *ex vivo* perfused porcine livers, which will be tested further in the coming year.
- Dr. Alwayn's lab demonstrated that mtDAMPs induce myD88 and NFκB and up-regulate the expression of TLR2, TLR4, and TLR9 in *in vitro* models of hepatic IRI, and hepatocytes co-cultured with mtDAMPs up-regulate the expression of TNFα.
- Initial results from Dr. Alwayn's lab confirmed their ability to reduce hepatic fat content of obese Zucker rats with sub-normothermic oxygenated ex vivo perfusion and additives aimed to increase lipid metabolism for 3-6 hours.
- Dr. Selzner's lab compared the effects of three different vasodilators on pig liver normothermic *ex vivo* liver perfusion and outcomes after transplantation. Compared to prostacyclin treatment, Verapamil and BQ123 administration resulted in lower AST release during NEVKP, and resulted in similar hepatocyte and bile duct injury after transplantation.
- Dr. Selzner's lab compared graft modification by micro RNA administration during cold storage and NEVKP and found that micro RNA application during NEVLP resulted in significantly better RNA uptake.
- Dr. Selzner's lab began a pilot study on the role of rocuronium and midazolam metabolism during NEVLP.
- Dr. Shapiro's lab completed a series of studies to clarify certain issues with normothermic *ex vivo* liver perfusion, and compilation of results is underway. One study demonstrated that livers perfused with a higher hemoglobin concentration performed better, with far less evidence of biochemical and histologic evidence of injury. A second study addressed a notable gap in the literature, and documented the degradation or rise in concentration of surrogate markers of viability on the NMP circuity.

Pancreas/Islets

- To analyze the potential utility of the Oxygen Consumption Rate (OCR) of human islets in culture before transplant, Dr. Shapiro's lab has recently tested 350 human islet isolations,

resulting in 200 islet transplant procedures. Data for OCR determination is being prepared in manuscript form to define the predictive potency of the OCR/DNA and OCR Transplant dose on graft function and clinical outcomes. The Montreal group have also incorporated these OCR measures into their early clinical transplant experience, and are working collaboratively with Edmonton through CNTRP to further foster these efforts.

- Dr. Alwayn's lab completed initial experiments using sHO-1-CPP in pancreatic islet transplantation were performed, however no cytoprotection was observed. Dr. Shapiro's lab carried out a pilot study on HO-1-CPP provided by Dr. Alwayn's lab, and did not observe any clear differences between the groups in the restoration or euglycemia or *in vivo* islet function in this initial pilot experience. This collaborative CNTRP work between Halifax and Edmonton is continuing to further explore the utility of shielding islets using the hemoxygenase pathway, and is now looking at dosing and optimizing islet penetration from periphery to core to further advance this work.
- Dr. Shapiro's study testing a potent antioxidant BMX-001 in a murine DCD syngeneic marginal islet transplant model was completed and is under review at *Islets*. Based on this experience, through the CNTRP the group plans to further test the potency of BMX-001 in heart, liver and kidney *ex vivo* normothermic perfusion systems.
- Dr. Shapiro's Anti-Aging Glycopeptide (AAGP) study was completed and published in the journal *Diabetes*. This study demonstrated that supplementation with AAGP during culture improves islet potency and most remarkably effectively attenuates any long-term Tacrolimus-induced islet toxicity in islets soaked for one hour in AAGP. This has sparked further interest within the CNTRP group to test the potency of AAGP on *ex vivo* circuits in liver, kidney and heart systems. The CNTRP Project 1 group sees strong potential in addition of these protectant molecules to optimize, protect and repair injured organs for transplant.
- Dr. Shapiro's lab demonstrated that culturing a marginal islet mass in the presence of a highly potent pan-caspase inhibitor, F573 resulted in higher diabetes reversal rate and significantly less apoptosis than controls, and importantly found that this protective effect was seen best in islet implantation sites most susceptible to hypoxia (portal vein more effective than the kidney site). These observations also have important translational potential across heart, lung, liver and kidney *ex vivo* normothermic perfusions, especially in the setting of marginal donor organ transplantation.
- Dr. Paraskevas further validated the use of oxygen consumption rate measurement of human islet preparations as an important release criteria test for clinical islet transplantation.

Kidney

- Dr. Luke received approval for the Human REB protocol by the host organization (Lawson research, Western University) and has submitted an application for obtaining human organs to TGLN.
- Dr. Luke's lab determined optimal warm ischemia or DCD condition; established blood based warm perfusion system by using a blood based renal perfusion-reperfusion pump-cassette system; determined optimal perfusion and reperfusion time (8-10h) and optimal temperature (22C); identified several TLRs (TLR2, 4, 6, and 7) which play an important role in initiating early immune responses in IRI as well as during *ex vivo* perfusion-reperfusion processes; anti-inflammatory agent CORM-401 was found to improve kidney injuries and function.
- Dr. Selzner's lab developed a pig model of normothermic *ex vivo* kidney perfusion establishing that prolonged perfusion without graft injury, and that normothermic *ex vivo* perfusion resulted in lower graft injury and improved kidney function after transplantation, as compared to cold storage. Dr. Selzner invited Dr. Luke and team from London to Toronto

to train them further on the Toronto pre-clinical perfusion circuits and to further foster collaborations between CNTRP institutions. This is an important step that will accelerate the opportunity to carry out linked translational CNTRP studies and thereby accelerate clinical implementation in kidney transplantation.

Interactions within the project

As previously noted, investigators have committed to collaborate in order to optimize this new technology by leveraging organ commonalities. Project 1 galvanizes cooperation and synergy between institutes at the University of Alberta, University Health Network (UHN) – Toronto, McGill University, Dalhousie University and London Health Sciences Centre, which would likely not occur otherwise. Building our platform as a bridge between these Institutes draws additional expertise to our team, and has the added advantage of engaging a large cadre of students, fellows and other trainees across all aspects of our CNTRP project; such interactions include:

- Dr. Luke's (London, ON) group is collaborating with Dr. Selzner's group in Toronto, and have actively traveled for on site training.
- Collaborations between Dr. Selzner and Dr. Shapiro and Dr. Alwayn have been, and continue to be very fruitful. Frequent, scheduled interactions between the three labs, as well as numerous informal interactions between PIs and fellows and students resulted in the aims and progress achieved in the liver group. Data from the collaborations within the liver group have advanced understanding of ischemia-reperfusion injury in small and large animal and human ex vivo liver perfusion and transplantation, and has led to new and exciting areas of research.
- Dr. Selzner and Dr. Alwayn are collaborating on a project investigating DAMP release in perfusate and bile during Normothermic *ex vivo* liver perfusion.
- Jayan Nagendran is collaborating extensively with Darren Freed and James Shapiro, who have combined efforts to develop and Ex-Vivo Organ Perfusion Laboratory, where they perfuse pig lungs, hearts, and livers every week. There is synergistic knowledge translation between the organs allowing for rapid development of stable protocols for perfusion of lungs, as well as hearts and livers.
- Dr. Paraskevas' project focusing on delivering oxygen to the pancreas during the retrievals is built on the interaction between the GMP clinical islet isolation laboratories in Montreal and Edmonton. This has required strong collaborative interaction between Transplant Quebec and the Alberta Human Organ Procurement and Exchange (HOPE) Program. Pancreas offers are discussed between the two GMP islet isolation laboratories, especially in terms of careful coordination of the timing of retrievals. Since Edmonton relies on key personnel from the Quebec islet team to retrieve, cannulate, connect to the persufflator, and travel by air to accompany the persufflator machine between Quebec and Edmonton, details must be discussed to minimize cold ischemia times for optimal pancreas viability for islet isolation. In terms of isolation variables, the labs have created datasets from the local and distant retrievals to compare and contrast to determine the relative efficacy of using the P3S persufflator at each centre for improving islet isolation and transplantation outcomes. This is truly a study that spans the country.
- Sharing of the perfusion protocols and determining optimal perfusate conditions for the heart are key components to the establishment of the EVHP system. Dr. Badiwala's group have built upon the seminal work performed by Dr. Freed in this area, and will share their findings with Dr. Freed on establishing an ex vivo functional assessment mechanism using echocardiography. In this manner the investigators are optimizing research on ex vivo heart perfusion by focusing on different aspects of the project.

Interactions across the CNTRP

The interdisciplinary approach to research has helped achieve the team's objectives through synergies between **Project 1** and **Project 3** as follows:

Heart:

- Dr. Freed's lab is trying to establish an interaction with P3 around perfusate analysis.

Liver:

- Dr. Alwayn: Initial contact has been made with Dr. Boillard (Project 3) to discuss future collaborations within the mtDAMPs project. This will likely lead to more substantive collaboration in the next 2 years.
- Samples from the CNTRP OrganOx clinical trials both in Edmonton and Toronto and *ex vivo* animal models continue to be collected systematically, frozen and banked for future potential analysis within Project 3.

Lung:

- Dr. Nagendran met with the collaborators in Project 3 in Montreal at the CR-CHUM under Dr. Hebert to help coordinate the analysis of perfusate samples collected during *ex vivo* lung perfusion. This collaboration is being formalized in the following year.

Pancreas:

- Aside from the collaboration mentioned above, Dr. Paraskevas' work within Project 1 also ties well into their work performed on Project 3: Understanding, predicting and preventing early graft rejection and GVHD. The focus of our work within both projects is to minimize the injury to the islets with the goal of improving islet transplant outcomes. In this way, our laboratory brings together the expertise from members of Project 1 with that of Dr. Eric Boilard at the CRCHUQ, Dr. Pierre Thibault at the Université de Montréal, Dr. Marie-Josée Hébert and Dr. Melanie Dieudé from the CRCHUM from Project 3.

New research & collaborations

As studies have yielded new results or outcomes, directions for further research and collaborations have presented themselves, these include:

- Dr. Freed has established a collaboration with Dr. David Nobes and Dr. Hyun-Joong Chung in the Faculty of Engineering (UofA). They are co-supervising an MSc student who is modeling the flow of perfusate in the EVHP system to determine opportunities for improvement in the materials used in the system with a view to replicating the normal vasculature that the heart interacts with.
- Dr. Alwayn: Collaboration was initiated with Dr. Brunt at Dalhousie University in New Brunswick to assess the role of sHO-1-CPP in conjunction with heme-nanocarriers in IRI in myocardial infarction. These experiments are still in the design phase but are planned for the next 2-3 years.
- Dr. Selzner and Dr. Alwayn plan to extend their collaboration to evaluate the role of DAMPS to predict liver and kidney injury during ex vivo perfusion.
- The Edmonton (Shapiro) and Toronto (Selzner) groups will collaborate on a clinical trial of normothermic *ex vivo* perfusion for marginal liver grafts (OrganOx).
- In February 2016, Dr. Nagendran visited the University of Montreal to give grand surgical rounds and spend 2 days in their labs helping establish their *ex vivo* lung perfusion protocols. This has led to a collaboration between the two centers, and a reciprocal visit of the Montreal group to Edmonton is planned for early fall of 2016. We are aligning EVLP

protocols to allow for uniformity between our centers. The goal is to have a Canadian led investigator initiated clinical trial using a device developed at the University of Alberta that would also be tested at the University of Montreal.

- Dr. Paraskevas reports that the completion of the pilot project of using the P3S persfflator for pancreas preservation prior to islet isolation in conjunction with the CITL has enabled them to build a large enough sample size to compare their P3S islets to those from SCS using the techniques that they have optimized for Project 3. These analyses may lead to additional abstract submissions for presentation and another manuscript for submission. A key member of the Project 1 collaboration has been Dr. Klearchos Papas from the University of Arizona. He has been instrumental at setting up a collaboration between his lab, Dr. Paraskevas' lab, and Giner Inc., the manufacturers of the P3S.
- Dr. Badiwala has developed collaboration with Professor Jean Zu, Chair of the Department of Mechanical Engineering at the University of Toronto, to participate in the development of normal mechanisms for ex vivo cardiac loading and performance evaluation. Dr. Badiwala has also developed collaboration with Dr. Max Meineri, Director of Perioperative Echocardiography at UHN and his group to develop an ex vivo mechanism to perform echocardiographic evaluation of cardiac performance using a TEE probe.

Next Steps (Year 4 plan)

Heart

Dr. Badiwala - Toronto:

- To proceed with *ex vivo* heart perfusion studies comparing the effects of Somah Solution based perfusate to other currently used perfusates.
- To utilize the new *ex vivo* echocardiography apparatus to determine whether this non-invasive method of evaluating cardiac performance correlates to data obtained from pressure volume loop analysis. This will answer the key question of whether cardiac performance can reliably be discerned ex vivo and a non-invasive fashion. Once we have established stability in ex vivo heart perfusion and ability to discern cardiac function using our porcine models, we will then move on to studies using unused human donor hearts.

Lung

Dr. Nagendran - Edmonton:

- Adiponectin is a protective hormone in the circulatory system that has anti-inflammatory ad cytoprotective properties. We aim to develop a research program that will test whether gene delivery of adiponectin to lungs removed from donors confers protection to the lung following transplantation. This organ-specific therapy to improve outcomes in lung transplantation would be of greatest benefit to patients who are diabetic and/or obese, who have significantly lower levels of adiponectin and are at greatest risk of poor short and long term survival.

Liver

Dr. Alwayn - Halifax:

- In year 4 further confirmation of the cytoprotective effects of sHO-1-CPP in transplantation and other models of IRI will be sought.
- The role of mtDAMPs in *ex vivo* perfusion will be further elucidated to establish its role as a biomarker of injury during *ex vivo* perfusion. Interactions with cells of innate and adaptive immunity will be examined to assess potential therapeutic targets.

- The technique of *ex vivo* liver defatting will be optimized for translation to large animal models.

Dr. Selzner – Toronto:

- The next two years will focus on further investigation of biomarkers for graft assessment during normothermic *ex vivo* kidney and liver perfusion, and development of strategies of graft modification during *ex vivo* kidney and liver perfusion.

Dr. Shapiro - Edmonton:

- Begin RCT (including marginal grafts) at 2 sites: Edmonton &Toronto.
- Pig livers and human "discard' livers by *ex vivo* perfusion device optimization. Optimize perfusate in the pig *ex-vivo* model.
- Pre-clinical testing of protective modalities: Anti-oxidants (BMX), caspase inhibitors (F572, IDN-6556), glycol-peptide (AAGP) in pig and human liver (discard organs).

Kidney

Dr. Luke - London:

- Compare DCD-type donor condition vs. non-DCD (NDD) kidneys
- To identify optimal thermal and oxygenation conditions for both hypo- and normothermic storage
- To see the effect of washed/filtered blood based normothermic (36^0C) vs. KPS solution based hypothermic (4^0C) perfusion
- To determine if an anti-inflammatory agent CORM401 added to the cold KPS perfusion solution can minimize kidney injury upon washed blood reperfusion

Pancreas/Islet

Dr. Paraskevas - Montreal:

- Will use the MicroFACS platform of Dr. E. Boilard to analyze the extracellular vesicles produced by our human islet preparations derived from pancreases retrieved using SCS or the P3S. To determine if a difference in the profile of specific injury markers, autoantigens or other biomarkers exists depending on the retrieval method used. In addition, we will continue to build on the number of P3S and SCS cases at the HITL based on donor availability.

Dr. Shapiro - Edmonton:

- Pre-clinical assessment of BMX-001 in islet isolation and transplantation. Studies will be conducted to evaluate whether administration of BMX-001 during organ procurement and islet isolation can improve *in vitro* viability and subsequent engraftment outcomes in a murine, syngeneic, marginal mass model.
- Pre-clinical evaluation of non-caspase-dependent cell death modalities in islet isolation and transplantation. Pharmacological agents capable of inducing non-apoptotic forms of cell death, like ferroptosis, will be evaluated to determine their impact on islet viability and engraftment.
- Expanding the utility of AAGP in islet transplantation. We will seek to determine if the administration of AAGP can protect islets from the instant blood-mediated inflammatory response (IBMIR), when transplanted intrahepatically in a porcine, autograft model.
- Islet isolations of DCD pancreata preserved on the Giner Persufflator (transported from Montreal).

Project 2 - Increasing solid organ and hematopoietic cell donation

Lead: Dr Greg Knoll, Ottawa Hospital Research Institute
Co-Leads: Dr Sam Shemie, McGill University & Linda Wright, University of Toronto

Original aims/sub-aims and changes

The CNTRP provides a unique opportunity to study important issues in donation with immediate clinical impact on increasing, in a low-risk and ethical manner, the number of organs available for transplant. **Project 2** focuses on aspects of organ donation for which Canadian data are lacking, with the expectation that this research will have an immediate impact on patients waiting for transplantation. The research aims of this project address multiple aspects critical to successful organ and cell donation including: public attitudes and opinion, appropriate legal and ethical frameworks, and novel epidemiological and clinical research investigations. Although not specified in each aim, Project 2 encompasses issues relevant to both pediatric and adult donation.

5 Aims of Project 2:

- **Aim 1:** Define national strategies for recruitment of organ and HSC donors
- **Aim 2:** Minimize the risk and understand outcomes for new types of living kidney donors
- **Aim 3a&b:** Monitor the physiology of death following withdrawal of life-sustaining therapies and predict the time of death following withdrawal of life-sustaining therapies (WLST)
- **Aim 3c:** Knowledge translation (KT) intervention to increase donation after cardiac death (DCD) in Canada
- **Aim 4:** Enhance and optimize use of 'increased risk' deceased donors
- **Aim 5:** Improving neurological death diagnosis

Aim1 focuses, to this point, on the issue of family override and on helping to develop the CNTRP patient engagement strategy, in partnership with **Core 1**. **Aim1** also investigates systems of incentives for organ donation and studies ethical issues and disparities in public solicitations for organs and tissue. For the family override study, **Aim1** investigates the legal and ethical issues in this override, specifically the moral weight attached to the wishes of the deceased and of the family; the responsibility of healthcare professionals to respect a registered wish to be an organ donor and the reasons for the override.

The goal of **Aim2** is to minimize the risk and understand outcomes for new types of living kidney donors. Specifically, to 1) determine if it is safe for individuals with risk factors (expanded-criteria donors, e.g. older age, obesity, abnormal glucose) to become living kidney donors, and 2) compare psychological outcomes between standard-criteria donors, paired-exchange donors, and anonymous donors. This is done through continued follow up of living kidney donor participants, conducting medical chart audits for recipients, and maintaining the database for the Living Kidney Donor study.

The goal of **Aim3a&b** is to successfully predict which patients, who are eligible for DCD and undergoing WLST, will die within a time period to enable them to be donors and determine the

time intervals, after cardiac arrest, associated with the disappearance of key cardiocirculatory and neurological measures and to describe the frequency with which there is an unassisted, spontaneous return of these measures. This is achieved by conducting observational studies in 13 Canadian ICUs to determine the natural history of cessation of physiological function, after WLST, in adult and pediatric patients who are eligible for DCD.

Aim3c is working to understand the discrepancy in the utilization of DCD across Canada. Since 2006, DCD has grown in many Canadian provinces while remaining stagnant or not used at all in some regions and the reasons for the wide discrepancy in DCD usage remain unknown. **Aim3c** is identifying stakeholder (clinicians and donor coordinators) beliefs and attitudes to DCD, developing a knowledge translation intervention based upon these beliefs and will then implement the intervention nationally with the ultimate goal to sharply increase numbers of available donors.

The overall objective of **Aim4** is to determine why certain deceased donor organs are not used for transplantation, evaluate the outcomes of organs deemed "increased risk" from either a graft failure or transmissible disease point of view, and to expand the use of these organs in an ethical way. As a group, we:

- Assess the determinants of use or non-use of organs from deceased donors offered to Canadian transplant centres;
- Evaluate the outcomes of organ transplants from deceased donors perceived to be at increased risk for graft failure or transmissible diseases and;
- Define the optimal ethical framework for the use of organs from "marginal" or "increased risk" deceased donors.

Aim5 works to improve the neurological death determination (NDD) process by establishing accurate ancillary test(s) as a reference standard(s) for NDD. This CIHR funded study is led by Dr. Michaël Chassé at the Ottawa Hospital Research Institute and looks to:

1. Investigate the accuracy of currently used ancillary tests;
2. Explore the views of Canadian practitioners involved in NDD the use of ancillary test for NDD, NDD diagnostic practices and their opinion regarding NDD;
3. Understand clinician, families and caregivers' satisfaction/dissatisfaction regarding the NDD process.

Major accomplishments in Year 3

Aim1 completed the literature review on family overrides and is now analyzing this data. The team has also completed a media review on the topic and is presenting the data at upcoming conferences. In collaboration with Trillium Gift of Life Network and BC Transplant, Aim 1 is examining the prevalence of family veto and reviewing decision-making processes of families. Educational initiatives and changes to practice are being examined.
A scoping review of public solicitations was completed and a manuscript is published.
We conducted a comprehensive literature review to explore non-financial incentives that have been proposed or implemented globally to increase organ donation and registration rates. A manuscript has been submitted to a leading transplant journal.
We collaborated with CORE 1 on a national workshop (November 2015) involving patients, caregivers, healthcare professionals and researchers to: (i) identify the future research priorities for the CNTRP in organ and tissue donation and transplantation; and (ii) use the knowledge gained to set the groundwork for involving patients in research.

Aim2 has 820 standard-criteria donors, 150 expanded-criteria donors, 104 paired-exchange donors, and 68 anonymous donors and conducted medical-chart audits for 756 recipients recruited from centres across Canada. The study has completed the first year follow up to collect data measuring the 3-year percent decline in kidney function between standard-criteria and expanded-criteria living kidney donors, the 3-year risk for graft loss among recipients of kidneys from standard-criteria vs. expanded-criteria living kidney donors and the Health-related quality of life (SF-36), the Beck Depression and Anxiety Inventories, and the Rosenberg Self-esteem Scale for these patients. The next two year will concentrate on ensuring excellent data collection and retention of our study participants. This data will help determine whether it is safe and ethical to expand living kidney donation in novel areas with minimal risk (e.g. donors with pre-existing medical conditions, anonymous donors etc).

Aim 3a: Monitor the physiology of death
Major accomplishments in the past year include:

- **Enrolment of 156/500 patients (31%)** over ~12 months of staggered enrolment
  - Average of ~20 patients/month since January 2016
  - **366 screened patients, 166 approached for consent, 156 consented (94% consent rate)**
  - 6 pediatric patients, 89 patients in the Czech Republic
- REB approvals at 11/13 sites, applications started at 1 additional site
- Contracts approved at 11/13 sites; 8/13 sites actively screening & enrolling
- Study procedures training completed in person at 4/11 sites; online training at 6/11 sites; in person training scheduled for 1 additional site in May
- Online study procedures training completed with all 3 sites in Prague
- Finalization of study procedures manuals for unique software setups of all sites
- Design of data query program to integrate with online CRF database
- Case report form refined; new versions approved by all REBs
- Study procedures refined – change to exclusion criteria to expand enrolment; approval at all site REBs
- Presentation of study updates to CCCTG in June 2015 and January 2016
- Held 2 Steering Committee meetings in 2015; next meeting scheduled for April 2016
- **Obtained local funding for neurologic-sub study at London site**
- Established regularly updated website, www.ddepict.com
- **Established contact with patient partner (son was DCD donor in 2009)** – have had 3 calls including 1 training webinar where PI presented study objectives. Plan to hold face to face meeting in late April 2016

Aim 3b: Development of Novel Prediction Tool
Major accomplishments in the past year include:

- **Obtained funding from Physician Services Incorporated for advanced analysis of waveform data to be used in death prediction**
- Completion of 115 (85%) of online CRFs, payment issued for 23 (45% of eligible Canadian patients)
- **103 patients in group 1 (non-donors), 27 in group 2 (DCD eligible but did not donate), 8 in group 3 (DCD donors)**
- Have started up enrolment and screening at 3 sites in Prague, Czech Republic; plan to start at 1 additional site in Liberec in spring 2016

- Refined online waveform viewer; added features to automatically track certain expected patterns of physiology & flag for review
- Submitted application to CIHR for a plan that would investigate the potential for development of a prediction tool using physiologic variability in pediatric patients
- Low rate of protocol deviations and violations (13/140 patients, 10%)

Aim 3c accomplishments:
Achievements of the past year include completing data analysis, hosting a full day intervention development meeting, beginning a collaboration with Canadian Blood Services to host an organ donation consensus meeting to inform our intervention development

Progress to Date:
- Belief statements all written and double checked
- Final data trends are being confirmed
- Team meeting was completed in August 2015 to prioritize intervention components based on the data
- Intervention development is ongoing and will be informed by a consensus meeting to be held in September of 2016

Larger trends have emerged regarding the need to target with an intervention:
1) the identification of a potential organ donor,
2) the referral of a potential donor to the organ procurement organization,
3) general knowledge about DCD,
4) communicating with the family about DCD, and
5) separating the role of the physician from deciding to withdraw life-sustaining therapy and offering DCD

To our knowledge, the information regarding the barriers and enablers to DCD does not currently exist. This data will be used to develop an intervention targeting issues (as indicated in 3) with the intent to increase the use of DCD, without decreasing the number of NDD donors and increase the number of organs available overall.

Aim 4's accomplishments have laid the groundwork to achieve all of the goals of this project within the next year. The research team, including project coordinator, has continued to successfully move the project forward. The team has finalized the list of iTransplant variables to be transferred from the Trillium Gift of Life Network (TGLN) to the Institute for Clinical Evaluative Sciences (ICES); the data sharing agreement has been drafted and is undergoing final review by TGLN and ICES lawyers. After examining the iTransplant variable list and data quality it was determined that this data source was very rich and would provide the data needed to answer this aim's important questions. The ICES data creation plan is being developed to allow analytics to begin shortly after the iTransplant data is transferred. Once the data has been transferred the team has asked ICES to prioritize the analytics for this project ahead of other projects to ensure timely completion of this work.

Regarding sample collection, aim 4 lead Dr. Kim, attended a meeting in September with TGLN (Ronnie Gavsie, Julie Trpkovski, and Clare Payne), Atul Humar, Kathryn Tinckam, and Rulan Parekh. There was agreement to change some of the language in the consent forms to allow for the acquisition of samples for use in future research. The team is in the process of discussing with other OPOs on how to access their samples and will continue to have an open dialogue with BC Transplant to discuss potential collaborations. Given the robustness of the iTransplant data the team thinks they can move forward and answer the research questions without the

sample collection piece to avoid project completion delays. However, they will continue to work with TGLN and other OPOs to solidify sample collection for future CNTRP projects.

Aim 5 accomplishments:

1) Completion of the ancillary testing systematic review (manuscript in preparation)
2) Submission of for funding for two multicenter studies (1) CT-Perfusion for NDD and 2) a multi-method study of beliefs and behaviours of families involved with NDD)
3) Development of a survey of practitioners involved with NDD regarding their diagnostic practices as well as their practices regarding consent (including their views of practitioner/family override)

Interactions within the project/core

Within project 2, **aims 3a and 3b** benefit from access to shared CNTRP resources such as the CNTRP coordinator and online webinar services, which have helped to facilitate many of our site training initiatives. The study team additionally benefits from regular contact with other Project 2 investigators who provide advice on study direction during regular project calls. The CNTRP leadership and core team have been responsive to the DePPaRT team's request and have provided support to help us overcome some of our challenges.

Aim4 will endeavour to increase the potential pool of donors available for transplantation (which will invariably increase the range of donor quality) and thus defining factors that predict transplant outcomes in donor organs at increased risk for graft failure will increase in importance. **Aims3a-c** may increase the availability of DCD organs for transplantation so the influence of prolonged warm ischemia on already vulnerable donor organs (due to advanced age and comorbidity for example) will need to be further clarified via the studies in **Aim4**.

Aim 5 benefits from close collaboration with other project 2 members. Over the next year Aim 5 will develop new collaborations with the new core 4 (patient involvement). P2 Aim 5 will collaborate with the P2 Aim 1 family override study team to combine their surveys into one single survey that we will send to clinicians regarding NDD.

Interactions across the CNTRP

Aim1 lead and supported the creation of new Fast Facts with **Core 1** on the topics of Living Donation and on Public Solicitation (available at www.cntrp.ca/policy_facts).
Aim 1 also worked collaboratively with **Core 1** on the creation and execution of a Patient Engagement Strategy within CNTRP. This initiative included hosting a national survey in the spring of 2015 and leading a national workshop in Toronto in November 2015. This workshop has led to the recruitment of 6 patients integrated into the CNTRP in our pilot implementation phase and the creation of the new CNTRP Core 4 Patient-Researcher Partnership Platform.

Over the past year **Aim 3a&b** have collaborated with researchers facilitating CNTRP's Patient Engagement strategy. Members of the DePPaRT team attended the patient engagement workshop in Toronto in Fall 2015 and were inspired to invite a patient partner to become involved in the DePPaRT project. We continue to build from the extensive resources and support available through CNTRP to advance our patient partnership strategy, and hope that we can provide an example strategy that may help other clinical projects integrate patient partnership. The DePPaRT team has received ongoing support and encouragement in these initiatives from the CNTRP. Additionally, we have collaborated with members of **Project 6** for

the development of our pediatric organ donation focused proposal for the 2016 CIHR Project Scheme opportunity. The DePPaRT project is also integrated within **Core 1** with Jennifer Chandler leading the qualitative study component.

Aim 4 worked closely with Project 5 to begin to develop a streamlined process for donor sample collection. Several meetings were held between the two groups and TGLN to discuss REB and sample collection.

New research & collaborations

Throughout the past year **Aim 3a&b** have continued to expand our international collaborations in the Czech Republic and in the United Kingdom. We now have 3 sites active in the Czech Republic and plan to start up at least 1 additional site. In 2016 we plan to organize a face-to-face meeting with Czech investigators to encourage the development of an international organ donation research network. Our colleagues in the UK were not successful with their local grant application but continue to want to be part of the DePPaRT study and are currently looking into more localized options to pilot the DePPaRT procedures prior to re-applying for additional funding. They also continue to be interested in participating in an international organ donation research network.
Locally, the DePPaRT team has applied for a CIHR Project Scheme opportunity, and DePPaRT lead Sonny Dhanani is involved as a collaborator on several other CNTRP-supported applications (Meade, Chasse) and one CCCTG supported application (Lacroix). Sam Shemie and Laura Hornby are members of a pediatric DCD guideline development committee and a planning committee looking at implications of extracorporeal CPR on organ donation potential. Finally, the DePPaRT team has applied to Canadian Blood Services for a summer student grant to help with a scoping review of prospective research methods for studying potential DCD donors.

Aim2 collaborates with Trillium Gift of Life and with Canadian Blood Services.

Aim3c has started working in collaboration with Canadian Blood Services to host a 2-day workshop on the topic of Potential Organ Donation Identification and System Accountability in Ottawa on 20-21, 2016. This workshop will help inform the intervention development for this aim.

Aim4 continues to meet regularly with the Trillium Gift of Life Network to discuss this project. We also continue to meet with BC Transplant.

Aim 5 started new collaboration with a neuroradiology research group based in Halifax to help with the CT-perfusion study for NDD (including their Canadian neuroradiology and neurology network).

Next Steps (Year 4 plan)

Aim 1

- Publish findings from patient engagement initiative
- Explore potential practice changes to Family Veto including a national survey of critical care physicians, in conjunction with the **Canadian Critical Care Trials Group**, to explore their understanding of the legal and policy implications surrounding family veto

and a provincial study interviewing healthcare professionals, and families who have used family veto
    - Complete and submit a manuscript to an academic journal on the scoping review of the use of social media in organ donation and transplantation.
    - Continue to explore and evaluate the implementation of a partnership between patients and researchers within the CNTRP

Aim 2

- Continue the data collection and retention of our study participants. This data will help determine whether it is safe and ethical to expand living kidney donation in novel areas with minimal risk (e.g. donors with pre-existing medical conditions, anonymous donors etc).

Aim 3a&b

- Starting up screening and enrolment at remaining 4 unopened Canadian sites (2 expected within next 3 months)
- Re-open 2 Canadian sites currently on hold (one due to personnel issues, one due to issues with waveform capture)
- Enrol remaining 360 patients
- Begin preliminary analysis of waveform data
- Co-build a strong patient partnership plan & summarize the process into a working document for other CNTRP projects
- Present preliminary findings at upcoming CCCTG and CNTRP meetings
- Hold a meeting with international collaborators to establish future goals for organ donation research in Canada and internationally

Aim 3c:

- Publish the results from the assessment of the barriers and enablers to the use of DCD
- Continue developing the intervention to increase the use of DCD across Canada
- Plan and begin implementing this intervention (through joint workshop with CBS on Sept 20-21, 2016)

Aim 4:

- Begin and finish analytics for the determinants of use or non-use of organs from deceased donors offered to Ontario transplant centres and the evaluation of the outcomes of organ transplants from deceased donors perceived to be at increased risk for graft failure or transmissible diseases. Complete and submit the manuscripts for these studies.
- Finish literature reviews and survey of transplant professionals in preparation for consensus meeting on the ethical use of deceased organs at increased risk for graft failure.

Aim 5:

- 1 year: Publish systematic review, conduct survey of clinicians, secure funding
- 2 years: Secure funding for the family study and the ancillary test study
- 3-4 years: start the 2 planned multi-center studies.

Project 3 - Understanding, predicting and preventing early graft rejection and GVHD

Lead: Dr. Marie-Josée Hébert, Université de Montréal
Co-Leads: Dr. Dan Muruve, University of Calgary and
Dr. Claude Perrault, Université de Montréal

Original aims/sub-aims and changes

The death and release of inflammatory cytokines by parenchymal cells within solid organ transplants play major roles in modulating the immune response to the graft. In bone marrow transplantation, the programmed death of vascular cells due to immune and metabolic stressors is also central to the development of Graft-vs-Host Disease (GVHD). Although deemed important, the specific molecular mechanisms that regulate «response to injury» (i.e. the inflammatory response to cell death and cell damage) in an alloimmune context remain poorly defined. The goal of Project 3 is to characterize the importance of programmed cell death pathways and inflammasome activation as predictors and regulators of rejection and GVHD. To this end, scientists within Project 3 focus on developing cell and animal models of regulated cell death pathways and inflammasome activation, exploring these models with unbiased proteomics and transcriptomics strategies and validating promising biomarkers of rejection or GVHD in transplant patient samples. Our group also strives to characterize novel targets of pharmacological interventions, within these molecular pathways, for better prevention and/or treatment of rejection/GVHD. This multifaceted approach, developed by closely integrated teams nationwide, is aimed at concomitantly at generating novel mechanistic insights and characterizing novel tools of potential clinical utility in assessing risk of alloimmune activation and transplant dysfunction in patients. Project 3 is led by Dr. Marie-Josée Hébert, senior nephrology clinician-scientist and director of the multi-organ transplant program at Université de Montréal, and co-led by Drs. Claude Perreault, Canada Research Chair in Immunobiology at Université de Montréal, and Dan Muruve, Canada Research Chair in Inflammation and Kidney Disease at the University of Calgary.

Aim 1: Define the participation and potential for modification of clinically relevant signatures of programmed cell death (PCD) and inflammasome activation in solid organ transplant (SOT) and graft-versus-host disease (GVHD) transplant injury

Aim 2: Identify components of the PCD and inflammasome pathways that represent novel, clinically relevant biomarkers and/or pharmacological modifiers to predict and /or prevent graft injury, rejection and GVHD

The overall goal of Project 3 for the reporting period was to identify key components of regulated death pathways in endothelial cells, pancreatic islets and renal epithelial cells that:

- regulate rejection/GVHD
- predict rejection/GVHD
- can serve as pharmacological targets of intervention in treating or preventing rejection/GVHD.

The specific objectives were:

- To characterize the molecular signatures that follow necroptosome and inflammasome activation in renal epithelial cells
- To determine if activation of necroptosis has an impact on renal allograft function and survival.
- To develop NLRP3 modulating drugs using chemoproteomics.
- To initiate development of protocols for using an epoxide-based cysteine protease probe in normal urines, which will then be translated to clinical transplant samples as a new methodology to assess renal caspase activation in transplant patients.
- To perform the proteomic analysis of human islet-derived membrane vesicles (MV) that are produced during ex vivo islet preparation
- To characterize the and RNA and protein markers of the different types of membrane vesicles (MV) produced by apoptotic endothelial cells
- To characterize the immune-modulating functions of the various types of endothelial MV in models of vascular rejection, solid organ rejection and allogeneic hematopoietic cell transplantation (AHCT)
- To characterize protein and RNA components of endothelial MV as biomarkers of rejection or allograft dysfunction in renal and heart transplant patients

There have been only minor changes in the project's design that are discussed in the sub-projects reports. Overall, the global direction has not changed, major goals have been reached within the expected timeline.

Major accomplishments in Year 3

Aim1. With regards to progress on the role of programmed cell death and inflammasome pathways in rejection/GVHD, achievements are:

Endothelial cell focus

1. We have identified and characterized a novel type of membrane vesicle (apoptotic exosome-like vesicles) released by apoptotic endothelial cells as accelerators of vascular rejection and accelerators of GVHD in animal models.
2. We have identified a cryptic fragment of perlecan (LG3) and the 20S proteasome core subunit, as important components of endothelial apoptotic nanovesicles regulating the immunogenic activity of these nanovesicles during rejection/GVHD.
3. We have demonstrated that apoptotic nanovesicles accelerate, at least in part rejection, through the production of autoantibodies that in turn aggravate vascular inflammation and have identified anti-LG3 antibodies as a prototypical autoantibody implicated in this maladaptive auto-immune response.
4. We found that the caspase-like proteasome activity, present within apoptotic nanovesicles, increases allograft vascular inflammation and leukocytic infiltration.
5. We found that apoptotic exosome–like vesicles triggers IL-23-IL-17 autoimmune axis in mice.
6. We found that apoptotic exosome-like vesicles trigger the formation of tertiairy lymphoid organ (TLO) around the graft in murine model of vascular rejection.
7. We found that the caspase-like proteasome activity, present within apoptotic exosome-like vesicles, is essential for the formation of TLO's around the graft murine model of vascular rejection
8. We found that the caspase-like proteasome activity, present within apoptotic exosome-like vesicles, increases allograft vascular inflammation and leukocytic infiltration.

9. We have determined, in a murine model of bone marrow transplantation and GVHD, that very large amounts of anti-LG3 antibodies are produced in recipients early after allogeneic hematopoietic cell transplantation. The production of these antibodies is absolutely dependent on the presence of allogeneic T cells in the graft.
10. We performed RNA sequencing and found that apoptotic exosome-like vesicles contain a vast and complex repertoire of RNAs.
11. Apoptotic exosome-like vesicles transcript repertoire is very different from that found in HUVECs and apoptotic bodies.
12. Vesicle-specific transcripts expressed at more than 150 FPKMs (Fragments Per Kilobase of transcript per Million mapped reads) may represent novel and specific markers of endothelial cell damage.
13. In a model of renal IRI in mice, passive transfer of anti-LG3 IgGs led to enhanced dysfunction and microvascular injury compared to passive transfer with control IgGs.
14. Passive transfer of anti-LG3 antibodies also favored intra-renal microvascular complement activation, microvascular rarefaction and fibrosis post-IRI.
15. Our results suggest that anti-LG3 antibodies are novel aggravating factors for renal IRI. These results provide novel insights into the pathways that modulate the severity of renal injury at the time of transplantation and their impact on long-term outcomes.

Pancreatic islet focus

16. We have completed our initial analyses of EV production by normal human islets in culture, and confirmed the presence of diabetes autoantigens in islet-produced EV by MicroFACS and by immunogold transmission electron microscopy.
17. A more in-depth evaluation of human ICM EV is underway by MicroFACS analyses with a greater number of ICM preparations (n=10) to complete this part of the project with enough robust data to permit high-impact journal submission. Journal submission is planned in the next 3 months.
18. A complete analysis of the effects of inhibiting caspase-activated apoptosis is underway from n=4 ICM preparations to determine if these ICM EV are produced under caspase dependent or independent pathways.
19. We have started the evaluating the effects of the presentation of these diabetes autoantigen-containing EV to PBMC's isolated from the blood of diabetic patients and healthy controls.
20. Experimental project planning is underway to use a beta-cell derived cell line to evaluate if the production of EV can be influenced by the presence of cytokines.
21. Significance: This work will dramatically improve our understanding of how antigen presentation leads to a break in self-tolerance in autoimmune diabetes. In addition, re-appearance or increases in autoantibodies may be related to βcell failure post-transplant. These EV containing β-cell markers may be detected as early indicators of injury for therapeutic intervention, or as an indication of the need for subsequent islet infusions. Circulating, β-cell specific EV may prove useful as a clinical diagnostics tool.

Epithelial cell focus

22. We identified Nlrp3 microparticle formation in epithelial cells composed of Nlrp3, ASC and caspase-8. These non-canonical Nlrp3 microparticles are secreted primarily during apoptosis, but not following canonical inflammasome activation.
23. Nlrp3 microparticle formation in macrophages in contrast occurs during classical inflammasome activation. Consists of Nlrp3, ASC and caspase-1. Inflammasome microparticles are released into the microenvironment and are danger signals to neighboring epithelial cells. Inflammasome particles induce epithelial cell death.
24. We have identified the key components of TEC death observed in the absence of RIPK3 and caspase-8, and the related activation of mitochondrial based intrinsic apoptosis.

25. We have demonstrated for the first time that independent of T and B cells, NK cells have a critical role in mediating long-term transplant kidney injury. Osteopontin produced by TEC plays a crucial role for NK cell activation and NK cell-mediated kidney graft injury after transplantation.

Aim2. With regards to progress on identification of programmed cell death/inflammasome components as predictors/biomarkers and/or modifiers of rejection/GVHD, achievements are:

26. Nlrp3, ASC, AIM2 and caspase-1 are detected in human urine during acute kidney injury.
27. We developed standard operating procedures (SOPs) for the collection, storage and preparation of samples for MRM analysis. Using these SOPs, we successfully detected robust levels of endogenous control proteins (vitronectin, apolipoprotein E, kininogen, Zinc-alpha-2-glycoprotein) in human urine.
28. We have generated heterologous HEK293 cell expression systems to enable multiple reaction monitoring (MRM)-mass spectrometry (MS) method development. This method development system enables the validation of specific MRM-peptides to enable detection of proteins (i.e., confirmation of diagnostic peptides, tryptic digestion conditions, LC retention times, and transition states used in the MRM surveys). We surveyed multiple peptides (up to 6 depending upon protein of interest) for ASC, Aim2, NOD1, NOD2, NLRP1, NLRP3, NLRP6, NLRC4, NLRX1, caspase-1 as well as loading controls GAPDH and tubulin. MRM parameters have been applied to tissue-culture cells (THP-1 monocytes and IECs Caco2 HT29 cells) to detect endogenous proteins as well as with urine samples.
29. We successfully detected AIM2, ASC, NOD1, NOD2 peptides in urine using MRM proteomics. Preliminary results are encouraging for the detection of Nlrp3 and caspase-1 in human urine. However, the MRM assay requires further refinement to detect NLRP proteins as their biophysical characteristics currently suppress effective tryptic digestion. Alternative processing procedures are being assessed.
30. We robustly detect ASC in urine samples as well as samples of macrophages (THP1 cells). The next step will be to obtain absolute quantification of ASC in human urine samples from acute kidney injury. To this end, we have already obtained heavy labelled peptide proteotypic for ASC as well as its unlabelled counterpart. We are currently determining the level of detection and level of quantification of a verified ASC peptide. The preliminary linear range of our heavy peptide is from <100 pg/ml up to 1000 ug/ml.
31. To support the MRM-MS aim, Dr. MacDonald acquired $22,900 in internal support from the Libin Cardiovascular Institute of Alberta (LCIA) for mass spectrometer and LC equipment preventative maintenance and upgrades.
32. We successfully completed a novel synthesis of the inhibitor molecule (called HS190) with Dr. Timothy Haystead's chemical biology laboratory at Duke University. Sufficient material was acquired to demonstrate attenuation of IL-1beta production in ATP-stimulated THP-1 cells. New PhD student recruited (Sept 2015) and is developing skills and is now beginning to advance project.
33. We are Commencing 2nd and 3rd generation chemistry on Nlrp3 inhibiting compound.
34. We identified the active site that is labeled by the cysteine protease probe using mass spectrometry.
35. We identified 8 papain-like cysteine proteases in normal urines using 2D-LC-MS/MS.
36. We developed an initial protocol for cysteine protease ABPP of normal urines that is undergoing further optimization.
37. We have completed the comparative proteomic characterization of different types of endothelial membrane vesicles produced by human and murine apoptotic endothelial cells and identified LG3 and 20S proteasome core as biomarkers of immunogenic endothelial apoptotic nanovesicles.

38. We showed that the circulating levels/activity of LG3 and 20S proteasome increase in two models of vascular injury in mice, supporting the notion that nanovesicle LG3 and 20S proteasome are good candidate biomarkers of vascular injury.
39. We showed that circulating levels of 20S proteasome activity within circulating nanovesicles are detectable in human serum
40. We identified anti-LG3 antibodies, assessed prior to transplantation in kidney transplant recipients as novel predictors of delayed graft function (DGF) .
41. We found that pre-transplant anti-LG3 antibodies are inversely associated with kidney graft function 1 year after transplantation in patients who experience DGF, independent of rejection. While Pre-transplant anti-AT1R and anti-vimentin are not associated with DGF, nor with its functional outcome.
42. In BMT patients, we identified CXCL10 as a diagnostic marker of GVHD; its predictive value was increased in combination with low anti-LG3 antibodies levels.We have started evaluating the effects of the presentation of diabetes autoantigen-containing EV to PBMC's isolated from the blood of diabetic patients and healthy controls.

Interactions within the project/core

Tissue damage has been known for decades as an important regulator of alloimmune activation, but the specific molecular basis of this observation remained elusive. In recent years, major progress in the characterization of the molecular pathways that control tissue injury, cell death and inflammation have opened new research avenues for transplantation. The nationwide, complementary and integrated expertise in programmed cell death and inflammasome activation within CNTRP Project 3 uniquely positions this group to make rapid progress in this booming field of investigation and assume an international leadership position. Hence, sustained interactions and collaboration through, monthly group calls, team integration and using standardized procedures for membrane vesicle purification, proteomic and RNA characterization have been major advantages to help us define the role of programmed cell death and inflammasome pathways in rejection/GVHD and to identify predictors/biomarkers and modifiers of rejection/GVHD.

Interactions across the CNTRP

Project 3 scientists are now uniquely poised to validate candidate biomarkers of allograft dysfunction and GVHD in transplant patient samples. Within Project 3, we have access to renal transplant and bone marrow transplantation biobanks. Project 3 also interacts with other Projects and Cores as follows.

Project 1: Ex vivo *organ transplant protection and repair*

Project 1 investigates pre-clinical models and early clinical pilot studies of *ex vivo* perfusion of heart, kidney, liver, lung and pancreas transplants, taking advantage of commonalities across the organs while also considering the physiologic differences that require organ-specific approaches for optimal effect. Interactions with project 3 allow standardization of new tests for monitoring organ injury and predicting allograft function and risk of rejection. SOP have been discussed by P1 and P3 investigators and implemented to collect perfusate samples from each of the ex vivo studies mentioned above. Sample collection is ongoing. Novel biomarkers of tissue injury such as exosome like vesicles will be examined in perfusate samples by P3 investigators.

Project 2: Increasing solid organ and hematopoietic cell donation
Project 2 defines and tests novel and ethically-acceptable modalities for expanding the donor pool in Canada. The recent characterization of novel predictors of allograft function by Project 3 members raises the possibility of creating algorithms for allocation of organs and donors that take into consideration these new biomarkers. To this end, Andrea Lodi and Yoshua Bengio, mathematicians specialising in mathematical optimisation and artificial intelligence with experience in kidney allocation algorithms have been approached to work in collaboration with the CNTRP and in particular P3 and P2 investigators to develop novel allocation algorithms.

Project 4: Strategies for immunomodulation and transplant tolerance
Project 4 tests several novel modes of immunomodulation in human transplant recipients, including phase I/II trials using extracorporeal photopheresis (ECP) to treat acute renal graft rejection and chronic GVHD. The CNTRP CARE Trial officially opened in December 2015 in Montreal and Hamilton and has enrolled its first patient in Hamilton at the beginning of March. P3 has already received samples from this first enrolled patient. CARE trial patient samples will be stored until examination of the presence of P3 newly characterized biomarkers in these samples.

Project 5: Predicting and controlling viral complications of transplantation
Viral infections are common after transplant and are important contributors to post-transplant morbidity, mortality and graft loss. Due to the close collaboration between Project 3 and Project 5 investigators, the study of the importance of BK virus infection in the generation of unique exosome like vesicles as potential cell-cell communication and virus invasion strategy is now launched. Experimental strategies have been established and P3 SOP sent to P5 investigator.

Project 6: Improving pediatric outcomes in transplantation
The study entitled 'Precision Medicine in Pediatric Transplant Recipients' has been elaborated by P6 investigators in collaboration with Project 3 and this project is under evaluation for external additional founding. Currently there are no sensitive markers of graft rejection or injury that can serve as early warning signals in children after transplant. There is a strong need to develop non-invasive peripheral markers of rejection and dysfunction that are based on children's unique immunologic profile. The CNTRP P6 POSITIVE study has launched recruitment of 300 new (and 500 prevalent) transplant patients from 7 pediatric sites with biological samples and clinical data collection to develop an age-appropriate risk profile for rejection and graft dysfunction. Through Project 3, novel biomarkers of rejection and graft injury have been unravelled. This new collaborative subproject study provides a unique opportunity to evaluate the potential of these novel biomarkers in the pediatric context.

Core 2 Research infrastructure and registries support platform
The CNTRP Core 2 platform has launched a new virtual biorepository tool that links existing and prospective research biobanks with other research sites from across the country. In close collaboration with Project 3 investigators, several P3 research sites received state-of-the-art equipment, enabling a complete sample processing & inventory system for local storage.

Core 4. Patient partnership platform.
In December 2015, several P3 investigators participated in CNTRP Patient Engagement meeting in Toronto bringing patients, families, care givers and researchers together to discuss donation and transplant research and determine how to meaningfully engage patients as

partners across the CNTRP. Project 3 investigators are very enthusiastic about the identification by Core 4 of a patient partner to integrate Project 3. Formations and guidance from Core 4 are underway to assure successful integration of the patient in the group.

New research & collaborations

The following external collaborations have contributed to the progress of Project or will support experiments to come:

- Research on exosome-like vesicles and non-HLA autoantibodies is of broad interest to scientists in two main fields: immunology and oncology. Recent advances by Project 3 scientists form the basis of two new collaborative projects with
    - Dr. Emmanuel Zorn (Columbia University, New-York, USA), a high impact investigator focussing on B cells and antibodies in mechanisms of rejection or acceptance of kidney and heart transplants.
    - Dr Guido Kroemer (Paris, France), a world leader in immuno-oncology.

- Collaboration with Prof. Roslyn Bill (Aston University, Birmingham UK), an expert in protein engineering and biotechnology with links to the Oxford Protein Production Facility at the Diamond Light Source in the UK.
- Partnership with Fisher Technologies/One Lambda for development of clinical grade anti-LG3 testing in transplant patients

Next Steps (Year 4 plan)

Aim1: with regards to the role of programmed cell death and inflammasome pathways in rejection/GVHD we plan to:

- Further the evaluation of necroptosome, apoptosis and inflammasome activation in regulation of vascular, renal and heart rejection and GVHD in murine models.
- Further the characterization of immunogenic pathways triggered by apoptotic exosome-like vesicles and implicated in acceleration of rejection/GVHD
- To assess the immune-modulating function of pancreatic islet-derived microvesicles in models of islet transplantation and in models of type I diabetes in mice
- To evaluate the importance of membrane vesicles on NK cell activation in vitro.
- To evaluate the importance of membrane vesicles on kidney IRI in vivo.
- To decipher the mechanisms responsible for the selective enrichment of specific RNAs in exosome-like vesicles,
- To evaluate how exosome-like derived RNAs impact on other cells

Aim2: With regards to identification of programmed cell death/inflammasome components as predictors/biomarkers of rejection/GVHD we plan to:

- Characterize the proteomic signatures of necroptosis and inflammasome activation in renal epithelial cells
- Characterize membrane vesicles from mouse kidney tubular epithelial cells undergoing death receptor-induced necroptosis by using small particle flow cytometry platform.
- Characterize membrane vesicles from TEC undergoing inhibition of necroptosis.
- Optimize protocols for using an epoxide-based cysteine protease probe in normal urines, which will then be translated to clinical transplant samples as a new methodology to assess renal injury and cell death in transplant patients.

- Complete the characterization of the proteomic signatures of pancreatic islets apoptotic membrane vesicles
- Develop SOPs for the measurement of islet-specific membrane vesicles in human serum/plasma samples from patients pre- & post-pancreas and islet transplantation
- Perform continued biobanking of human islet-conditioned media, MVs and islet cell lysates from human pancreases.
- Further characterize the RNA signature profile of endothelial membrane vesicles and evaluate this signature as putative biomarker/predictor of rejection/GVHD in mice and in serum samples from renal and bone marrow transplant patients
- Further assess the validity and clinical usefulness of exosome-like vesicles, LG3, caspase-like activity and anti-LG3 as predictors of rejection/GVHD in renal, heart and bone marrow transplant patients.
- Continue to refine MRM assay to measure inflammasome activation in human biological specimens. We just demonstrated the ability to recovery most NLRPs family members with ATP-Sepharose affinity resin. Based on these results, we successfully used ATP-Sepharose to enrich endogenous NLRP3 together with its associated adaptor protein ASC. We will further investigate if this enrichment/capture strategy can be applied to enable quantitative assessment of NLRPs by MRM-MS as well as for measuring inflammasome-complex formation and activity status in situ.
- Pursue and initiate collaborative work with Projects 1, 2, 4, 5 and 6 for evaluating above putative biomarkers as predictors of allograft dysfunction or response to treatment as described in collaboration with other projects section.
- Evaluate the potential use of RNAs from exosome-like vesicles as markers of endothelial cell damage
- Characterize urinary cysteine protease ABPP in a highly selected cohort of renal transplant patients.
- Isolate and identify the active cysteine proteases enzymes in renal transplant urines from patients with subclinical and clinical rejection.
- Develop quantitative enzyme assays for the specific active cysteine proteases.

<u>With regards to the characterization of programmed cell death/inflammasome components as targets of pharmacological intervention we plan to</u>*:*

- Continue the characterization of NLRP3-modulating compounds
- Develop multiple expression systems (yeast, bacteria, insect cells) to generate NLRP protein for the mechanistic assessment of small molecule HS190 inhibitor. Perform 2nd and 3rd generation chemistry on HS190 (Nlrp3 inhibitor) and test in vitro
- Analyze the effect of pre- & post-isolation oxygenation on membrane vesicle production in human and murine islets (tying in Project 1 to Project 3), as compared to the normoxic and anoxic states
- Assess the efficacy of proteasome inhibition with bortezomib in preventing the formation of autoantibodies (anti-LG3) in models of ischemia-reperfusion injury and in models of vascular, renal and heart rejection

Project 4 - Translating strategies for immunomodulation and transplantation tolerance

Leads: Dr. Megan Levings, University of British Columbia
Dr. Denis-Claude Roy, Université de Montréal
Co-Leads: Dr. Lori West, University of Alberta and
Dr. Kirk Schultz, University of British Columbia

Original aims/sub-aims and changes

The overall goal of the project is to combine our complementary expertise in transplantation and immunology for translating novel strategies to induce tolerance in the clinical setting. The project includes approaches to target and monitor immunoregulatory cells in patients undergoing hematopoietic stem cell and/or solid organ transplantation and the development of protocols to use regulatory T cells as a cell therapy inducing tolerance. Last year we reported that following executive approval, the original aim 1 was substantially revised and that aim 2 was removed.

This year two new projects have been incorporated in Project 4 (aim 4 and aim 5), which are funded through CNTRP innovation grants in partnership with Astellas and the University of Alberta. The goals of these new projects are to analyse donor specific immune reactivity in paediatric heart transplant and to evaluate a glyconanotechnology tool for personalized ABO-incompatibility kidney transplantation. Therefore the current aims for Project 4 are as follows:

Aim 1: **Conduct a phase I/II clinical trial using extracorporeal photopheresis (ECP) to treat chronic GVHD by promoting immune regulation and design an ECP-based clinical trial to treat acute cellular rejection in patients with kidney transplants**.

Aim 1A. Conduct a phase I/II trial of TH9402 ECP in patients with refractory cGVHD
Aim 1B. Conduct a retrospective study in selected centers and a systematic review of the literature on treatment of acute cellular rejection in renal patients to define criteria, endpoints and sample size to design a clinical trial.
Aim 1C. Monitor changes in cells and biomarkers associated with tolerance in patients undergoing ECP for chronic GVHD or allograft rejection.

Aim 3: Develop methods to use thymically-derived regulatory T cells (Tregs) as a cellular therapy to induce transplantation tolerance

Aim 3A. Optimize expansion of thymus-derived Tregs
Aim 3B. Validate the phenotype & function of thymus-derived Tregs in humanized mice
Aim 3C. Develop and optimize GMP-based protocols to expand thymus-derived Tregs

Aim 4: Characterization of donor specific immune reactivity in paediatric heart transplant.

Aim 4A. Characterize donor specific T cell reactivity in thymectomized pediatric heart transplant recipient compared to third party T cell reactivity.
Aim 4B. Characterize de novo donor-specific HLA antibodies and compare the development of these antibodies to the presence of T cell donor specific reactivity.

Aim 5: Evaluation of a glyconanotechnology tool for personalized ABO-incompatibility kidney transplantation.

Aim 5A. Determine subtype specificity of ABO antibodies in ABOi Kidney Tx recipients.

Aim 5B. Define ABH antigen subtype expression in the kidney.
Aim 5C. Analysis of ABO subtype antibodies with clinical data.

(note: Aim 2 was removed at the end of Year 2 of the CNTRP)

Major accomplishments in Year 3

Aim 1a:

- The most important achievement was the official opening of the CARE trial in November 2015 and the recruitment of the first patient in March 2016. Several steps and hurdles had to be taken to accomplish the opening. First a re-classification of CARE treatment from a medical device to cell therapy was requested to Health Canada prior to the CTA submission. The CTA was submitted upon re-classification in August 2015 and the CARE trial was approved by Health Canada as a cell therapy trial in September 2015. The principal contract between HMR-UBC-ARO and final ethical board approval of modifications requested by Health Canada was obtained in November 2015, allowing the CARE trial to open at the lead center, HMR. Other CARE trial sites have opened after upon ethical board approval and a signed subcontract with HMR. Currently, five of the six clinical sites are open for recruitment: Montreal (November 2015), Hamilton (December 2015), Ottawa (May 2016), Toronto (June 2016) and Winnipeg (July 2016). The opening of the sixth site in Vancouver is pending the qualification of the manufacturing lab, which is expected in August 2016. Other achievements include the finalization of the REDCap electronic CRF, training of study coordinators on REDCap, implementation of the clinical protocol and logistics at each site through documentation and discussion sessions. Furthermore, training sessions on cGVHD symptoms and eligibility were provided by Drs. Walker and Ahmad using the webinar platform of the CNTRP, allowing PIs at site to instruct the local teams for screening patients. Currently, three patients have been enrolled in the CARE trial, and all doing well.

- The equipment at the three manufacturing has been either upgraded or acquired and installed. The laboratory protocol (Batch Record) was harmonized and implemented at all the production sites. Two successful production runs have been performed at the cell therapy center (CETC) at HMR and the cell product was found to be stable during an evaluation period of three months. Furthermore, the capacity of the CETC-HMR cell manufacturing lab in Montreal, serving four clinical sites, has been increased to perform the cell productions for up to 12 patients a year. Trainers at the manufacture centers in Vancouver and Winnipeg were qualified, for which dry- and wet-runs were required, with one complete wet-run performed in the presence of a specialist from the HMR-CETC. Additionally, other technicians were trained on the CARE laboratory protocol in all centers. The MCACTT cell manufacturing lab in Winnipeg has been recently (July 2016) qualified for the cell production on the CARE protocol after two successful runs executed in parallel with the reference center CETC-HMR, in which the cell products met the defined criteria. One such a comparative run has already been successfully executed with the Vancouver cell therapy lab and a second one is scheduled for the beginning of August. On request from Health Canada data on the 3-month stability of the single cell product manufactured in Vancouver has been generated and analysis for both cell products generated in Winnipeg has been initiated.

Aim1b:

- The systematic literature study on treatment of acute cellular rejection in renal transplantation patients has been finalized and a manuscript with the results of these data was submitted to Transplantation, July 2016. The strategy employed and developed by a medical librarian involved a first screening by two independent investigators based on keywords in title and

abstracts. A second screening for adherence to inclusion and exclusion criteria was performed on a set of 589 complete articles. Inclusion criteria were original studies describing treatments and outcomes of acute cellular rejection (acr) in kidney transplant patients, and exclusion criteria were (1) absence of human subjects, (2) no definition for therapeutic response or (3) no response rate according to the Banff 1997 classification system, (4) published in other language than English or French, (5) only pediatric patients, (6) inclusion of ABO-incompatible transplantation and (7) case reports. Ultimately only 5 papers were selected for data extraction.

- The data collection on retrospective studies in two selected Canadian transplantation centers has also been completed. In the last five years a total of 126 acute rejection episodes were recorded in 108 patients. Complete clinical responses were observed in >70% of pure cell-mediated rejections, of either grade 1A, 1B or 2 and higher, while only in 43% of the pure antibody-mediated or mixed rejection episodes a response was obtained. These data were presented at the ATC meeting in June 2016. Additional analysis of biopsies will be performed to assess reversibility of isolated endarteritis alone versus concomitant endarteritis + tubulointerstitial rejection.

- The third objective is to obtain insight into standard practice in treatment of acute cellular rejection across Canada by means of a survey. This survey has been designed by Drs. Tom Blydt-Hansen, Lynne Sénécal and Héloïse Cardinal, tested by 4 independent investigators and put into web-form in English and French with support of CORE2. The survey has been dissemination to the community twice and we are also directly contacting centers to ensure comprehensive collection of high quality data.

Aim 1c:

- Using the SOPs established in years 1 and 2 in our work with the CORE 2 immune monitoring committee, we set up immune monitoring for the CARE trial. Work done to set up the CARE trial included: testing the second generation DuraClone tubes (which are different from the first generation tubes tested in years 1 and 2 in CORE2), optimizing intracellular staining protocols, creating and validating a count tube for determining absolute numbers, writing a detailed SOP, and testing all procedures and cytometer settings at two research site (Vancouver and Montreal) and one clinical site (Hamilton). These sites have successfully performed all assays on two healthy donors. Sites in Winnipeg and Toronto will be set up in the coming months. This work was made possible by the continued collaboration with Beckman-Coulter; application specialists have helped set up standardized cytometer settings and troubleshoot various problems at all three sites.

Aim 3:

- We published our first report on the utility of thymic Tregs in transplantation in the Am J of Transplantation. We are now carrying out experiments to test the potential of expanded thymic Tregs to suppress immune reactivity using neonatal pig islets xenografts. We are also testing protocols to modulate the expression of homing receptors on thymic Tregs so they can be directed towards specific sites of inflammation

- We worked with StemCell technologies to develop GMP-compatible protocols for the isolation and expansion of thymic Tregs and are continuing to test StemCell technology products for Treg expansion. These protocols are being tested and validated in collaboration with Stem Cell Technologies.

- We are pursuing a variety of avenues to secure additional funding to support a clinical trial of thymic Tregs, with a letter of intent accepted by the BioCAN Rx National Centre of Excellence and a proposal funded by the Stem Cell Network to support the development of this technology.

Aim 4:
- This is a new project that has started only January 2016. Building of the REDCap database will start in May and sample selection will follow.

Aim 5:
- Start of this new project within Project 4 in January 2016.
- Testing of the ABO-glycan microarray on plasma samples from kidney patients has been initiated.
- Preliminary results for ABO antibody expression type II and II/IV by immunohistochemistry are obtained.

Interactions within the project/core and across the CNTRP

Project 4 is most closely linked with Core 2 as both are involved in the design and execution of the CARE trial. Essential interactions with Core 2 to develop the CARE trial have included:
- development of the clinical protocol
- logistics of site preparation
- logistics and budget allocation of cell processing and shipment
- ethical applications to local IRBs
- logistics of biosample collection, processing and storage
- development of standardized immune monitoring using the Navios flow cytometer

Core 2 has also supported the web-based survey on standard treatment of acute cellular rejection in kidney patients that has been developed for aim 1b.

Other interactions include:
- with Project 3 to collect urine from subjects in the CARE trial to test for exosomes and the presence of other biomarkers of rejection and/or cell death. Specifically Project 3 has demonstrated that endothelial derived apoptotic nanoparticles can break tolerance to self and lead to the development of anti-LG3 antibodies (Abs). These nanovesicles have been shown to accelerate disease in a GvHD mouse model. As the PDT treatment induces apoptosis, bio-samples from the CARE trial will be used to: 1) evaluate the type of apoptotic vesicles produced by the PDT treated PBMCs from GvHD patients and their proteomic content; 2) monitor the presence of nanovesicles in the plasma by proteasome activity detection and anti-LG3 IgG levels; and 3) analyse urinary LG3 levels.
- with Project 5 to monitor for the changes in anti-viral immunity longitudinally for subjects in the CARE trial. Specifically PBMCs will be sent to project 5 for evaluation of changes in the proportion and/or phenotype of T cells specific for CMV or EBV. See more details below.
- with Project 6 to implement similar standardized immune monitoring to that being used for the CARE trial. Specifically, the same antibody panels, instrumentation and protocols developed by Core 2 and Project 4 will be used to evaluate longitudinal samples from children undergoing solid organ transplantation and enrolled in the POSITIVE study. Some of the resulting data will be sent back to Project 4 for analysis using the automated software that has been developed to analyze data from the CARE trial. In addition to intra-study analysis, this will also be a unique opportunity for inter-study analysis.

New research & collaborations

- We established significant collaborations with investigators in the ONE study as well as Beckman Coulter to develop the immune monitoring platform for the CARE trial (see details in Core 2). We also developed a new collaboration with Dr. Ryan Brinkman at the BC Cancer Agency to develop an automated flow cytometry data analysis platform; the commercial potential of this technology is currently being explored with Beckman Coulter.
- A new research collaboration is being established with project 5 to analyse cell mediated immune response to CMV and EBV on samples from the CARE trial, for those patients who were seropositive or received seropositive donor cells. As cGvHD suppresses immune responses, treating cGvHD might increase the response. Evaluations will be done on PBMCs pre- and post PDT collected at baseline, during and at the end of the treatment. The logistics of analyzing effects on the response to influenza antigen exposure are also being considered. We are also exploring the feasibility of using a novel flow cytometry apparatus (CyTOF) to analyze these samples.
- For Aim 3, a new approach to develop antigen-specific Tregs using Chimeric Antigen Receptors has been developed using peripheral Tregs. This technology will be transferred to the thymic Treg project and tested using in vivo modes. The Chimeric Antigen Receptor technology is being developed with the Centre for Drug Research and Development at UBC, as well as an industry, Tx Cell Inc. We are currently engaging in discussions to develop a collaborative research agreement with TxCell who may wish to work with the CNTRP to conduct a "first in man" trial of CAR-Tregs in transplantation. In addition, data revealed that thymic Tregs have a distinct profile of chemokine receptor expression and a new research aim to study how expression of cell homing receptors is underway. We will also work with Core 1 to study the legal and ethical considerations around using thymic Tregs as a cellular therapy.
- We also brought on two new members to the Project 4, Aim 3 team: Dr. Jamie Piret and Ms Katherine MacDonald. Dr. Piret is a bioengineer and UBC Professor who is specialized in cell culture optimization and process development. Working under the combined mentorship of Piret and Levings, Ms MacDonald is conducting research for her Master's degree in systematic optimization of thymic Treg expansion.

Next Steps (Year 4 plan)

Aim 1a

- Open all the sites of the CARE trial.
- Increase recruitment by increasing the engagement of clinical teams by means of regular teleconference calls and follow-up and re-evaluation of eligibility criteria if necessary.
- Complete data analysis from the first patients of the CARE trial
- Increase collaborations with project 3 based on the new research plan on apoptotic vesicles.
- Explore collaborations with Project 5 to use CyTOF technology as part of the immune monitoring platform for the CARE trial.

Aim 1b

- Complete the data analysis for the retrospective study with a blind analysis of biopsies to assess reversibility of isolated endarteritis alone versus concomitant endarteritis + tubulointerstitial rejection

- Collect and analyse data from the survey to the transplantation community
- Develop the protocol and seek sources of funding for a pilot ECP-Kidney trial, eg CIHR grant

Aim 1c
- Set-up of the clinical flow cytometry lab at Trillium for the immune monitoring of the CARE trial samples.
- Analyse the flow data from the CARE trial samples.
- Work with Ryan Brinkman to create new automated gating pipelines for the second generation DuraClone panels.

Aim 3
- Complete testing of GMP-compatible protocols for isolation and expansion of thymic Tregs.
- Test various protocols for the expansion of antigen-specific thymic Tregs.
- Test the effectiveness of thymic Tregs in suppressing rejection of pig islets xenografts in an in vivo islet transplant model.
- Generate antigen-specific thymic Tregs by transduction with chimeric antigen-receptors and test in in vivo models
- Work with Core 1 to determine the ethical framework under which thymuses could be retrieved for a cell therapy application.
- Secure funding to test GMP-manufacturing of thymic Tregs as well as for the eventual clinical trial.
- Work with TxCell to develop a first-in-man trial of CAR expressing Tregs

Both new aims 4 and 5 are one-year projects and are expected to be completed in year 4.

Aim 4
- Building the REDCap database.
- Sample selection of thymectomized patients and controls.
- Analysis

Aim 5
- Completion of the ABO antibody expression analysis.
- Validate quantification of the Antibodies in Kidney transplantation patients and start analysis of samples.
- Held a workshop to raise interest from clinicians and industry to commercialize the ABO-glycan microarray (ABO-SAMA) device.

Project 5 – Predicting and controlling viral complications of transplantation

Leads: Dr. Atul Humar, University Health Network – Toronto
Co-Leads: Dr. Lee Anne Tibbles, University of Calgary and
Dr. Jean-Sébastien Delisle, University of Montréal

Original aims/sub-aims and changes

Project 5 focuses on viral complications from across the transplantation spectrum. The studies span several critical aspects of virology and encompass themes important to donation, SOT and HSCT. Project 5 started with 3 major sub-projects/aims and over the past three years has added the BK-KIDNI trial, one multicenter study to track incidence of PCP and a new EBV therapeutic subproject and more recently an aspergillus related project.

Aim 1: Our goal is to optimize and standardize utilization practices for high infectious risk organ donors (IRDs) across Canada i.e., those with behavioral risk factors for Human Immunodeficiency Virus (HIV), Hepatitis C virus (HCV) or Hepatitis B Virus (HBV). There is limited data in Canada on increased risk donors (IRDs) (defined as donor with behavioral practices that place them at increased risk for HIV, HBV, and HCV. Epidemiologic data on the incidence and utilization practices for IRDs does not exist for Canada and will help inform strategies and policies for optimal utilization of these donors. Development of Canadian guidelines will allow engagement of utilizers and providers. It is also crucial to understand patient factors that play a role in acceptance or decline of IRD organs and the psychosocial impact use of these organs may have.

Aim 2: Our goal is to assess common infectious problems associated with SOT and HSCT, specifically cytomegalovirus (CMV), BK polyomavirus (BK) and Epstein Barr Virus (EBV).

- For CMV (**Aim 2a**), the goals are to 1) better understand the pathogenesis of CMV reactivation following transplantation, and 2) to develop and validate novel biomarkers that can predict reactivation of CMV.
- For BK (**Aim 2b**), the goals are to 1) determine how BK virus causes aberrant changes in expression of host proinflammatory and profibrotic genes and mediators leading to acute inflammation and interstitial fibrosis, and 2) to determine how BK virus controls host transcriptional regulatory machinery, therefore altering the expression of both inflammatory and fibrosis inducing genes. The BK:KIDNI Trial (BK Virus: Kinase Inhibition to Decrease Nephropathy Intervention Trial), the largest ongoing trial in the treatment of BKV viremia, was brought under **Aim 2b**. This CIHR funded multicentre trial is comparing the standard of care (reduction of immunosuppression) with the intervention (change of immunosuppression to a combination of sirolimus and leflunomide).
- For EBV (**Aim 2c**), we are working closely with the Pediatric Study in Project 6 to study pathogenesis and outcomes related to EBV viremia and P6 Aim 2c is leading the adult cohort validation of the larger pediatric study.
- The new EBV therapy study (**Aim 2d**) was added in January 2015 to use cell therapy to prevent or treat opportunistic viral reactivation or disease following transplantation by generating autologous EBV-specific T-cell lines *in vitro* from prospective EBV seronegative

organ recipients who are at high risk of developing EBV-related disease following their transplant.

Aim 3a: the goal is to discover novel viral agents in transplant patients who develop specific idiopathic disease syndromes using existing metagenomics-based pathogen discovery programs in Canada.
Aim 3b: the goal is to develop a viral immune response monitoring strategies and techniques to evaluate immune competence against infectious agents in patients enrolled in tolerance induction trials in **Project 4.**

We have recently added a new **Aim 4** to study an increased incidence of late onset PCP identified in centres across Canada. To date, we performed a cross Canada survey to better understand the scope of the problem, and based on these results, we are doing a a multi-center case control study looking at risk factors for development of late-onset PCP using funds identified within Project 5. Recently an aspergillus risk-factor project was funded and added to Project 5.

Major accomplishments in Year 3

Aim 1

- The Canadian survey of transplant professionals related to utilization of high risk donors was published in Transplantation as follows:
  A Survey of Increased Infectious Risk Donor Utilization in Canadian Transplant Programs. Transplantation. 2016 Feb;100(2):461-4
- A standardized informed consent for use by transplant programs was distributed through various Organ Procurement Organizations (OPO), and the Canadian Society of Transplantation.
- The Ontario OPO (Trillium Gift of Life Network -TGLN) developed a tool-kit for distribution to all Ontario transplant programs based on the CNTRP consensus guidelines. Currently we are working on a system to capture the impact of these toolkits across Ontario and other provinces.
- A review of approximately 200 high risk donors was completed. This provided novel Canadian data on utilization practices related to high risk donors as well as areas for practice improvement. This was performed in collaboration with TGLN. The abstract was accepted at the American Transplant Congress meeting (2016) and the manuscript is in progress. The second part of the utilization survey [patient perspectives on utilization of high risk donors] has commenced enrolment.

Aim 2: Common viral infections post-transplant
Aim 2a: CMV

- In Year 3, further basic and clinical work was performed assessing novel factors related to the pathogenesis of CMV reactivation. This includes a very in-depth assessment of the complexity of the host miRNA response to CMV. This work has resulted in three Am J Transplant publications including a very recent one assessing the complexity of the host miRNA response to CMV [CITATION].
  Complexity of Host Micro-RNA Response to Cytomegalovirus Reactivation After Organ Transplantation. Am J Transplant. 2016 Feb;16(2):650-60.
- This work lead to follow-up study in a very large cohort of transplant patients assessment miRNAs and their role in viral reactivation from latency (oral presentation ATC 2016)
- An in-depth cytometric profiling of host response to CMV was partially completed. This utilized novel flow-cytometry combined with mass spectroscopy (CyTOF) to simultaneously

assess host immune profiles in patients with CMV reactivation. This work was accepted as an oral presentation at the American Transplant Congress 2016 and the post-doctoral fellow received a young investigator travel award for this work.

- Two additional studies related to host immune response and prediction of infection were presented (as oral presentations) at the ATC. The first assessed CD8 T-cell response to CMV and their utility in guiding prophylaxis. The second include a novel assessment of both innate and adaptive immune responses to predict a range of viral (CMV) and other opportunistic infections.
- A publication related to analysis of factors resulting in CMV relapse, including early phase viral kinetics has been accepted for publication [In press Transplantation]
- In Year-3, negotiations and funding for the multicenter Quantiferon-CMV trial were finalized. The final funding amount that was agreed to is $1,173,750 US (equivalent to ~ 1.6M CAN). Health Canada approval was also obtained. So far, 11 centers have agreed to participate in this trial (Canada, US, and Spain) and enrolment at the lead site (UHN) will start shortly.

Aim 2b

- Patient samples are currently being analyzed (isolated DNA, looking at haplo-methylation*)* to further study the pathogenesis of BK reactivation. A new protocol is being designed to study in vivo analysis of tissue (**Aim 2b**)
- The multicenter CIHR funded BK trial remains in the recruitment phase and data analysis will follow (**Aim 2b**)

Aim 2c: EBV

- Further validation work has been performed related to the role of EBV viral heterogeneity in the pathogenesis of PTLD. We have done preliminary genotyping studies on selective major EBV latent and lytic genes where we have examined viral strains among patients with primary EBV infection after transplantation versus healthy subjects. (**Aim 2c**). Working closely with P6 Aim 2c in order to study EBV subtypes and subsequent EBV complications as part of the adult cohort validation leading the pediatric study

Aim 2c.(2): New EBV project

- The project assessing *ex-vivo* development of virus-specific T-cells has been pursued within project 5. This received competitive funding through the CNTRP grant competition ($25K). (**Aim 2d**)

Aim 3: New frontiers in virology
Aim 3a: Novel viruses

- A novel retroviral retroviral pol gene has been detected in bile and stool samples from a patient with severe recurrent primary sclerosing cholangitis following liver transplantation. However, prevalence studies were subsequently unrevealing (*manuscript in preparation*). (**Aim 3a**)
- In order to optimize detection of relevant viruses associated with colitis, cholangitis and lymphoma (target disorders for **Aim 3a**) capture assays using conserved herpes and retrovirus gene sequences are being employed to enrich viral sequence detection from next generation sequencing libraries.
- Sample collection has commenced for assessing BAL and non-EBV lymphoma (target disorders for **Aim 3a**) for novel viral agents and NGS libraries have been made for analyses.

Aim 3b: assessing host antiviral response in immune tolerance trials

- Although no kidney transplant tolerance trial was ultimately performed the work done as part of this aim has had many novel off-shoots. We have optimized and validated methods for assessing viral host immune response to CMV, EBV and influenza antigens resulting in two publications during year 2 assessing influenza responses (published in *J Infect Dis* and *Plos Pathogens*)
- More recently (in year 3) we successfully developed an influenza microarray with over 100 antigens coated onto an array to assess a broad spectrum of anti-influenza antibodies including against both conserved and non-conserved epitopes. This work was accepted as an oral presentation at the 2016 American Transplant Congress.
- In addition, we successfully utilized CyTOF (combined flow cytometry / mass spectroscopy) to perform an in-depth cytometric profiling of CD8 T-cell response to CMV. This work was also accepted as an oral presentation at the 2016 American Transplant Congress.
- We also assessed heterologous immune response to influenza vaccine, something that has not been previously described in organ transplant recipients. This work has been accepted for publication in *Am J Transplant*.

New Aim 4: Fungal infections

- **Aim 4a:** Several centres have agreed to participate on the Pneumocystis jirovecii pneumonia (PCP) in solid organ transplantation project. We are continuing to identify and contact sites for participation in the hopes of maximizing our data collection.
- Progress to date is as follows
    - 11 sites have agreed to participate (including our site)
    - 9 sites have ethics approval
    - 9 sites have fully executed contracts
    - 3 sites have started entering data into the web based data collection tool

- **Aim 4b** is a newly funded (25K) project added to P5. In order to investigate the risk-prediction model of invasive aspergillosis after HSC transplantation, we are focusing on environmental factors (seasonality), spore exposure, presence of anti-Aspergillosis antibody in patients at time of transplant, and genetic factors (Toll-like Factor 4).

Interactions within the project

The different sub-projects within Aim 1 are all complementary. For example knowledge gained from the consensus conference helped inform the developed of the cross country survey, which subsequently helped inform the knowledge translation plan. Now the results of the survey are helping inform the development of a patient focused survey on the use of increased risk donors.

Aim 2a, b, c, address different viruses but explore complementary aspects of pathogenesis. For example, work in aim3 to develop immune diagnostics for viral infection (i.e. using the CyTOF platform) helped further refine the pathogenesis work being undertaken in aim 2.

The PCP aim has utilized existing infrastructure within CNTRP to rapidly recruit sites (9 sites so far with ethics approval and fully executed contracts).

Interactions across the CNTRP

- The **Aim 1** (utilization of IRDs) is closely linked to **Project 2** that is looking to assess the determinants of use or non-use of organs from deceased donors offered to Canadian transplant centres and evaluate the outcomes of organ transplants from deceased donors perceived to be at increased risk for graft failure or transmissible diseases. The standardized inform consent for IRDs has been developed in close collaboration with **Core1**. This IRD project unites the donation and transplantation communities and received very positive feedback from the ICU research community during our 2nd Annual CNTRP meeting.
- The BK:KIDNI continues collaborating with **Core2** to develop clinical trial management support through the Montreal Health & Innovation Coordinating Centre (**MHICC**).
- **P5 Aim2c** (EBV) closely aligns with Project 6 to establish the pediatric and adult cohort for the study.
- The collaboration with **P1** and **P5** involving the ex-vivo utilization of antiviral miRNA to prevent HCV reinfection of livers is ongoing.
- Aim 2d is a pre-clinical cell therapy project with close ties with project 4 (protocol/expertise sharing for cell manufacturing, cellular assessments, etc)
- **Project 5** is now providing samples to **Project 3** to investigate apoptotic nanovesicles and whether there is a specific proteomics signature that could be used for diagnostic tests for BK virus.

New research & collaborations

As noted above P5 has added a number of new studies and investigators including the EBV CT study, the multi-center PCP study and most recently the aspergillus risk factors after HSCT study. P5 therefore continues to try to incorporate HSCT as much as possible in its mandate.
P5 has ongoing strong collaborations with **Health Canada** (HC). This includes funding of the IRD consensus conference by HC and funding for establishment of a Canadian disease transmission advisory committee.
The recent finalization of negotiations with Qiagen regarding the multicenter CMV CMI study is a major milestone for P5 and brings significant funding into the CNTRP envelope.

Next Steps

Aim 1: To continue develop the framework for prospective data collection among OPOs (in conjunction with **Project 2**). In the meantime, a retrospective evaluation of donors from TGLN will be completed and the aim will be to publish these results in the next year. The patient portion of the increased risk donor survey will be performed this year with the aim to have an abstract for presentation within the next year. The psychosocial sub-study has been abandoned but will have the opportunity to be resurrected if Dr. Susan Abbey comes forward with a proposal.

Aim2: We will continue with what has so far been highly successful analysis of factors that relate to the pathogenesis of BK, CMV and EBV reactivation post-transplant including assessment of potential biomarkers. We will continue to develop the two new projects that have been added to this aim (virus-specific CTL studies and the BK randomized trial). The multicenter Qiagen trial will begin its recruitment phase across the different centers.

Aim 3 Identification and validation of novel viruses post-transplant will continue and samples have recently been transferred (Bronchoalveolar lavage samples and PTLD samples) and will

be assessed using metagenomics techniques. We will participate on the CARE Trial (**Project 4)** regarding assessment of viral response in patients undergoing tolerance related treatments.

Aim 4: We will continue to study Pneumocystis jirovecii pneumonia (PCP) in solid organ transplantation (Aim 4a). Research on the risk-prediction model of invasive aspergillosis after HSC transplantation will continue (Aim 4b).

Project 6 - Pediatric Outcomes in Transplant:

Personalising Immunosuppression to Improve Efficacy

Leads: Dr. Seema Mital, Hospital for Sick Children – Toronto
Co-Leads: Dr. Bethany Foster, McGill University and
Dr. Upton Allen, Hospital for Sick Children – Toronto

Original aims/sub-aims and changes

Aim 1: Develop age-appropriate calcineurin inhibitor (CNI) dosing for pediatric SOT patients

- Create a personalized physiologically-based CNI dosing algorithm in SOT
- Validate pediatric sensitive immunosuppression monitoring tools and their therapeutic targets
- Validate immunologic assays for assessing age-specific immune responses.

Aim 2: Develop risk prediction tools based on viral-host interactions that predispose young SOT & HSCT patients to EBV/PTLD

- Gain knowledge of EBV/PTLD host susceptibility factors (age and immune maturation) at the time of transplant.
- Develop an EBV genotype panel to be used as clinical tool for detecting high risk subtypes in patients with EBV

Aim 3: Develop health care systems strategies to enhance medication adherence in adolescents and young adults

- Characterize differences between Canadian solid organ transplant programs in potentially modifiable meso- and macro-level systems factors.
- Identify potentially modifiable meso- and macro-level factors that are determinants of adherence, adjusting for potential confounders.
- Compare the educational attainment and employment outcomes of kidney, liver, and heart transplant recipients with those of the general Canadian population in the same age group.

Major accomplishments in Year 3

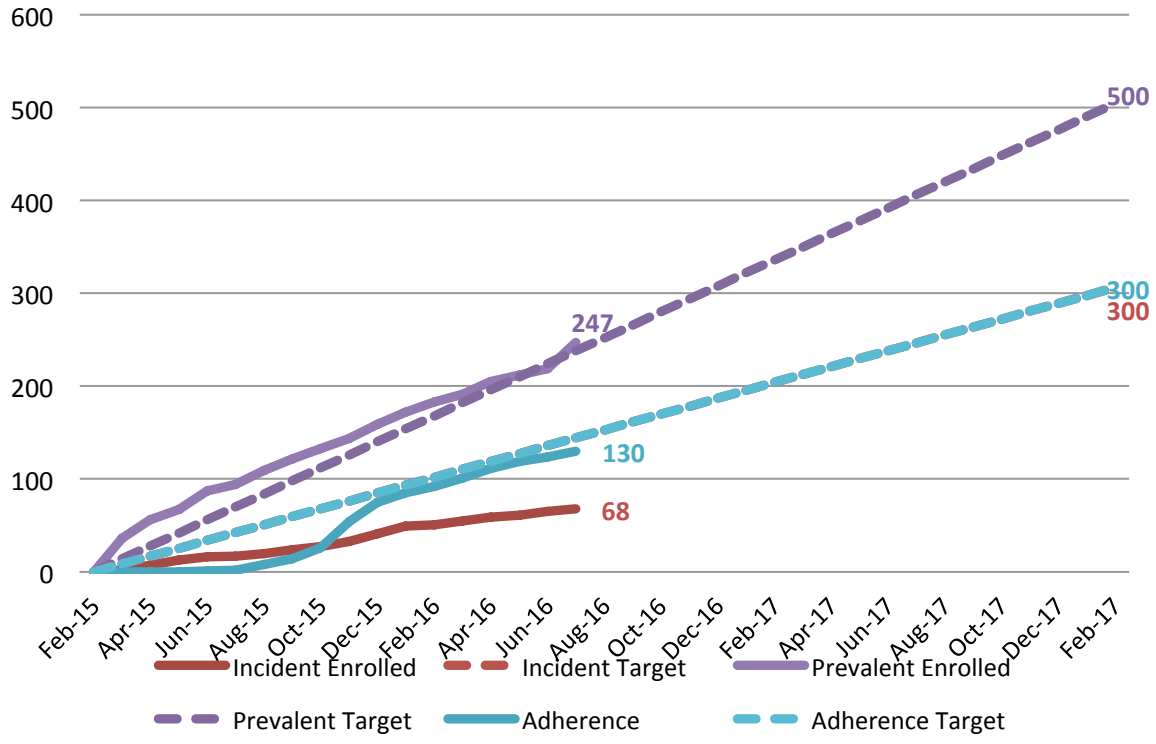
Study Operations & Launch:
Project 6 has obtained research ethics and legal approvals at all 14 centres (7 pediatric and 7 adult) involved in the project. All pediatric sites also have REB approval for biorepositories for the long term storage and use of blood (DNA, biomarkers) & medical and outcome related data. Recruitment has been launched at all 14 centres as of July 2016. The approval status at participating centres and recruitment launch is detailed in Table 1. Our major accomplishment is the effort of 27 research coordinators across Canada towards recruitment. Our 1-year

recruitment is on target for both prevalent and POSITIVE-Adherence subjects despite delayed study launch albeit is behind target for incident patients (Figure 1).

Table 1: Approvals and Recruitment Launch Status

Site	REB Approval	Service Agreement Approval	Institutional Approval	Recruitment Launch
SickKids	Jun 2014	N/A	N/A	Mar 2015
Montreal Children's	Mar 2015	Jun 2015	Y	Aug 2015
Stollery Children's	Jun 2015	Jun 2015	Y	Jan 2016
CHU Sainte Justines	Jun 2015	Sept 2015	Y	Nov 2015
Alberta Children's	Nov 2015	Oct 2015	Y	Jun 2016
Winnipeg Children's	Mar 2016	Nov 2015	Y	Jun 2016
BC Children's	Mar 2016	Nov 2015	Y	July 2016
Toronto General	Y	Y	Y	Nov 2015
Vancouver General	Y	Y	Y	Sept 2015
St.Paul Hospital	Y	Y	Y	Sept 2015
Royal Victoria	Y	Y	Y	Dec 2015
CHUM	Y	Y	Y	Jun 2015
Foothills	Y	Y	Y	Jun 2016
Ottawa Hospital	Y	Y	Y	Nov 2015

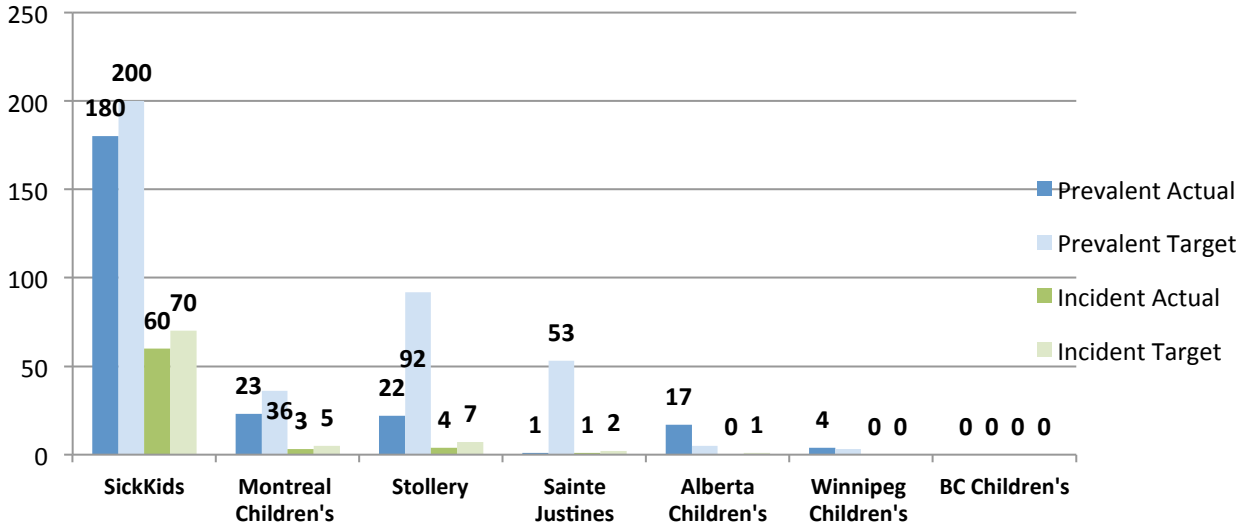
Figure 1: POSITIVE Study Actual Enrollment vs Target



Aim 1: We have successfully recruited 247 of targeted 500 prevalent (101% of 1-year target) & 68 of targeted 300 incident (50% of 1-year target) transplant recipients since study launch in March 2015 (Figure 2). We have also recruited 31 living transplant organ donors through the POSITIVE Study. 87% (n=152) of prevalent participants have completed all CRFs up to 1 year post transplant and 78% (n=21) of all incident participants have completed CRFs up to 3 months post transplant. We analyzed data from patients recruited to date to ascertain incidence

of rejection, infections, PTLD, and other outcomes during 1-year post-transplant follow-up (Figure 3). We have initiated genotyping for several hundred transplant recipients and donors through funding for a P6 ancillary study from Astellas and the SickKids Transplant and Regenerative Medicine Centre for the project *Pharmacogenomic-guided immunosuppression in pediatric transplant recipients* (*Mital*). We will be investigating the genetics of drug-drug interactions among transplant recipients and donors (where available). As of March 2016, we have received approval and have obtained retrospectively collected DNA from deceased donors from the Toronto HLA lab and are currently still awaiting a discussion with TGLN to discuss prospective sampling of transplant donor DNA to be used for aim 1. Extending this analysis beyond Toronto to other centres across Canada will require involvement from other OPOs and HLA labs. Genotyping from this project will provide important information regarding the pharmacogenomics of tacrolimus which will be analyzed as part of Aim 1.

Figure 2: POSITIVE Study Aim 1 recruitment by site



**Targets to date are adjusted for date enrollment started (Mar 2015 – Jun 2016)*

Figure 3: POSITIVE-Adherence recruitment by site

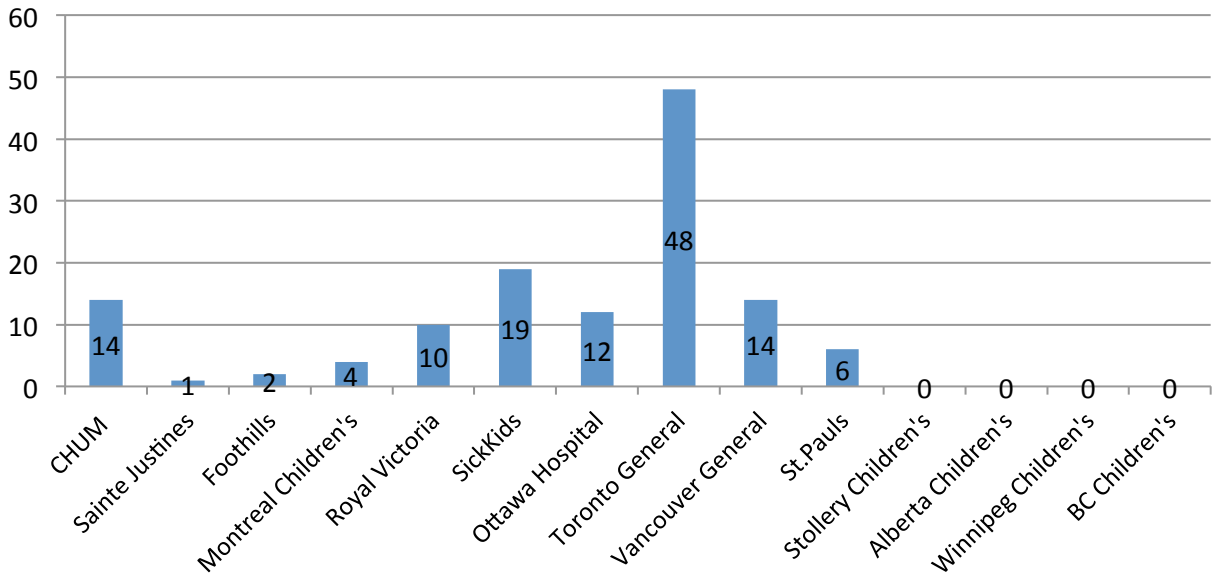
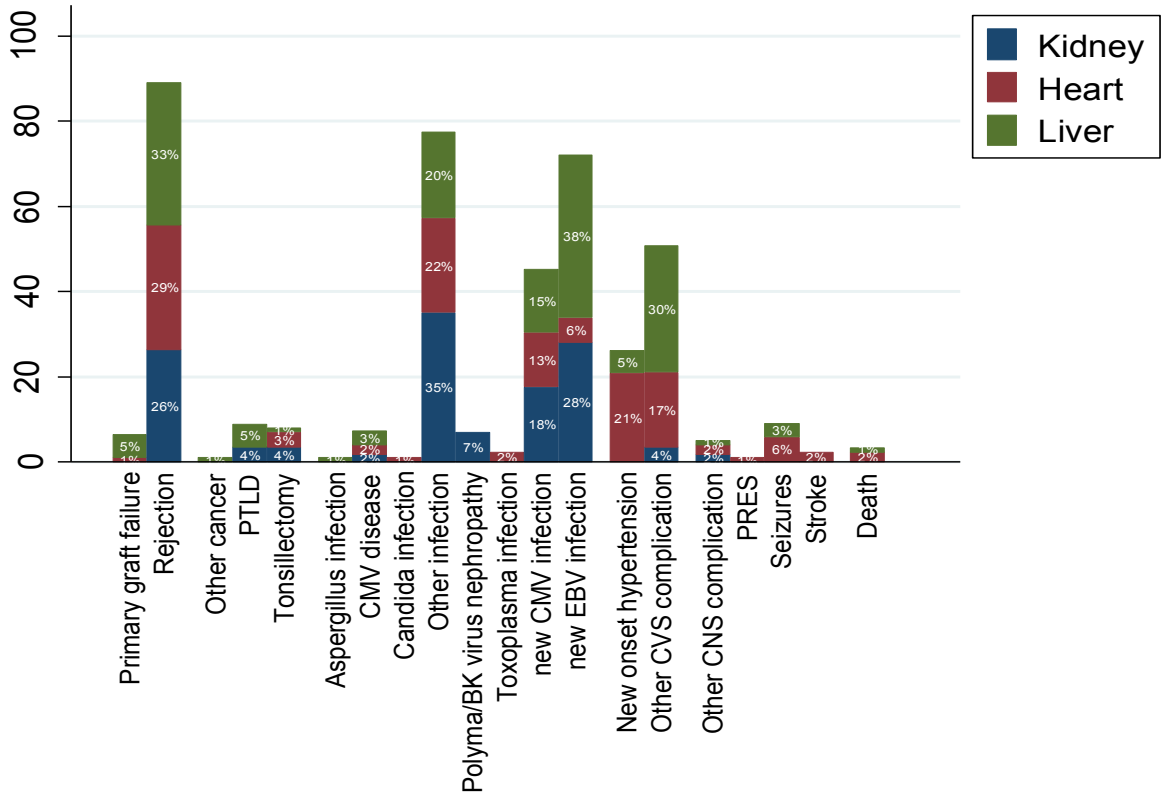


Figure 4: 1 year post transplant outcomes in prevalent patients by organ type



Sub-Aim 1c: The Urschel Lab has begun processing samples collected for Aim 1c as of June 2015. Within this past year, the lab has also hired a new lab technician, Lavinia Ionescu, for the project. To date the lab has processed samples from 28 individual participants out of a target of 90 on their immunophenotyping platform. In collaboration with Core 2, the Urschel Lab has undergone training on the Navios platform and begun running the additional immunoassay panels with Duraclone tubes with the goal of analyzing 30 subjects at 3 time-points (pre-transplant, 3 & 12 months post). Recently published data from previously collected local samples confirms the relevance of immune phenotype and maturation on heart transplanted patients and supports the performed analyses within this aim. Some challenges arise around limited quantities of blood being drawn, especially in the younger patients. A stepwise strategy on the use of the available cells has been developed which allows phenotyping and activation assays for all patients. Cell counts were not sufficient in all subjects to perform 7 day cell cultures. Continued effort was put into standardized immune monitoring across the CNTRP projects in collaboration with Core 2.

Aim 2: In the past year, we expanded recruitment for aim 2 beyond solid organ transplants to include hematopoietic stem cell transplant recipients. The first site to launch HSCT recruitment is SickKids Hospital in Toronto, plans are in place to expand these efforts to other pediatric BMT centres across Canada which include Vancouver, Calgary, Winnipeg and Montreal (Winnipeg and Vancouver have confirmed interest). Adults with primary EBV have been recruited through Project 5 which we plan to utilize for validation of findings from Project 6. We are currently in the process of expanding blood sampling guidelines for the protocol through P5 and reviewing MTA to allow sample transfer between institutions. Currently, 10 SOT participants with EBV in the first year post-transplant and 5 HSCT recipients have been recruited. Specimens and data at acute and convalescent phases of infection have been collected on 8 of these participants.

Aim 3: Since recruitment launch in June 2015 across 11 sites (3 pediatric centres outstanding), 130 participants have been enrolled which is on target for year 1 and represents ~1/3 of the total target enrolment of 300-350 patients. We have completed nurse questionnaires and transplant director questionnaires at 6 sites.

Interactions within the project/core

Project 6 is unique through the establishment of a research network across 14 centres in Canada, the largest of its kind, which includes both pediatric and adult centres and spans solid organ and hematopoietic stem cell transplants. This is a major strength of our network that is allowing us to study and potentially improve outcomes throughout the lifetime of a transplanted patient. A Steering Committee for the POSITIVE Study including POSITIVE-Adherence meets via teleconference monthly to discuss project progress and direction, and more recently to analyze collected data to plan scientific abstracts and manuscripts. All research coordinators for Project 6 also meet on a monthly basis to discuss study operations and recruitment efforts. Project 6 is a standing topic on all monthly National Pediatric BMT conference calls to discuss BMT involvement in Aim 2. A Project 6 in-person meeting is held annually in conjunction with the CST annual meeting to engage members and review the projects progress. Three such in-person meetings have been held for the larger group since the project was funded. A number of P6 investigators serve as liaisons on other CNTRP project calls and likewise we have invited other Projects to provide liaisons to join the P6 calls. Agenda items and minutes are circulated to the members before and after each call.

Interactions across the CNTRP

We are participating in the standardized immune cassette development led by **Core 2** by providing samples from POSITIVE patients for immune phenotyping using the standardized platform. Simon (P4 project liaison) is on the working committee. Immunoassay SOPs are being sent to **Project 4** so as to align sample processing and storage procedures. P6 provides a novel component to this collaboration as Duraclone tubes have never been tested in a pediatric population. Having samples run on the Navios platform will produce additional panels which extend beyond what we had originally proposed. Target number of pediatric samples to be run on this platform are 30 patients at baseline, 3 & 12 months post transplant. A challenge to date has been working within the 26 hour collection to processing time frame which is problematic for samples collected in the east coast. Given this we have had to restrict sample collection to children who are having blood draws in the afternoons only. We requested labs in Toronto and Montreal that have this platform to run these assays but they are not in a position to do so at this time. We have discussed this with Dr. West and are awaiting a resolution to this issue. Currently 2 subjects have undergone Duraclone-based immunophenotyping using the Navios platform.

We are collecting plasma from incident patients at the 3 study points for the purpose of biomarker studies. These samples will be used for proteomic profiling and this effort is being supported by Ashley's Angels Foundation for their 2016 fundraising goals. We are also collaborating with Project 3 to use a portion of the baseline and 1-3 month follow-up samples to validate the findings of inflammasome markers associated with graft injury in a pediatric cohort as well as to analyze the interaction between inflammasome activation and immune maturity and viral infections. As an early goal, we plan to analyze two biomarkers of rejection and graft injury (anti-LG3 & small vesicle proteasomes) identified by P3 as a sub-aim of this proposal.

EBV genotypes associated with EBV disease identified in **Aim 2** of our project will be validated in adults with EBV through **Project 5**. Adult patients with EBV viremia and/or PTLD have been banked through UHN, these samples will be shared and used for validation. We have had an in person meeting between the leads of the P5 & P6 and their respective managers in November 2015 to discuss sample acquisition, exchange and protocol amendments to accommodate for interactions between the projects. In addition, in order to support **P5** in their study of outcomes of SOT from high risk ID donors, we have included data on development of HIV and hepatitis B/C in pediatric SOT recipients during 1 year follow-up in our CRFs. This data will be shared with **P5**.

We are collaborating with **Core 1** in two projects. The first is to analyze cost-effectiveness of implementing healthcare system changes that can enhance medication adherence after transplant. In a conference call with Scott Klarenbach (November 6th, 2014) from **Core 1**, it was determined that no additional data collection is needed at this time beyond the planned data collection for **Aim 3**. It is anticipated that analysis of **Aim 3** data will identify which healthcare system strategies are associated with improved medication adherence. These strategies will then be costed through **Core 1**. The potential impact of improved adherence on clinical outcomes of reduced rejection and improved graft longevity will be modeled to calculate impact on cost savings. This will provide us with cost-benefit analysis of healthcare system strategies for improving medication adherence. The second project is led by Sonny Dhanani which aims to identify predictors of death in pediatric patients that can guide DCD organ donations. Mital is a key collaborator in this project to leverage data collected by P6 to analyze donor data at DCD with outcomes after pediatric SOTs. This project has been submitted for CIHR funding.

Finally, we are working closely with **Project 2 & 5** in an effort to standardize the processes for access to data and samples for research from deceased donors from Trillium Gift of Life for CNTRP projects. The processes implemented in Ontario will be used to involve other OPOs in other provinces and provide a standardized national framework for these efforts. Project leads met with TGLN on September 11, 2015 to discuss access to donor samples and data; however TGLN has not approved the request yet.

New research & collaborations

Since the launch of the CNTRP, we have leveraged the POSITIVE study to support 9 ancillary studies, 4 of which have been funded, 3 are under review and 2 were unfunded. We have highlighted six new research collaborations made this year and provided progress updates on the 3 that were established in years 1-2 below:

New research collaborations from this year:

1) *Teen Adherence in KidnEy transplant Improving Tracking To Optimize Outcomes* (*Take-It TOO) (PI, Bethany Foster; funded by NIH)*: Dr. Foster submitted a grant application to the NIH in Nov. 2015 for a multi-centre study aimed at adapting the successful TAKE-IT medication adherence intervention for use in clinical practice. This project will use the principles of user-centered design in a 3-stage, sequential study to achieve 3 novel and important goals: to adapt and optimize the TAKE-IT intervention for use in the 'real world' clinical setting, to design and test a portable, multi-dose electronic pillbox and companion adherence-tracking website, and to pilot the adapted intervention in preparation for a definitive cluster randomized trial (CRT). Although this project was not a direct result of the CNTRP, collaborations strengthened through the CNTRP helped make the new study possible. This project will include 4 Canadian pediatric CNTRP

centres and 3 American centers, and will be brought into the CNTRP as a new initiative. Data management will be undertaken by the MHICC.

2) *Canadian Patient-oriented Immune balance in Transplantation and Autoimmunity to improve Lives (PI, Kirk Schultz; submitted for SPOR, not funded):* We proposed inclusion of plasma samples from 300 pediatric patients enrolled in the POSITIVE study to analyze biomarkers of immune activation after transplant.

3) *Vital sign waveform analysis to predict time to death in pediatric organ donors and transplant outcomes in recipients (PI, Sonny Dhanani; submitted to CIHR, under review):* This pediatric focused project proposes to collect donation after cardiac death (DCD) data currently missing such as hemodynamic profiles of pediatric donors prior to and during withdrawal of life sustaining therapy. This study if funded will enroll patients from four pediatric centres in Ontario and Quebec with plans to then expand the study across Canada. As P6 is the largest multi-organ network of pediatric transplant centres in Canada, the P6 framework will be utilized by the study to help analyze data at the time of death in DCD donors with recipient outcomes.

4) *Pediatric Biomarkers of Rejection and Graft Failure (PI, Seema Mital):* This is a new research proposal that was submitted to the Ashley's Angels Foundation which was selected as the top proposal for the foundation to fundraise around. Currently there are no sensitive markers of graft rejection or injury that can serve as early warning signals in children after transplant. This project will perform proteomic profiling of paired samples which are already being collected through the POSITIVE Study. We will also assess the correlation of 2 newly identified biomarkers (anti-LG3, small vesicles proteasome) from P3 as biomarkers of rejection and graft injury. Pending final approval, fund raising efforts will begin before 2016 year end for an expected release of funds in early 2017.

5) *Differences in Immune activation by sex and age (PI Beth Foster):* Foster has also recently received internal funding (Research Institute of the MUHC, New Research Directions competition) to support a pilot study to examine differences in immune activation by sex and age in kidney transplant recipients. This work has developed from observations arising from studies of USRDS and SRTR databases showing poorer graft survival among female than male kidney and heart transplant recipients under 40 years of age. Dr. Foster is currently pursuing more detailed analyses of the SRTR database (in collaboration with Dr. Heloise Cardinal) to examine the interaction between sex and age on graft survival. It is expected that the pilot studies will lead to a larger study that will be part of the CNTRP.

6) *A Radical New Strategy for Preventing Post-transplant Lymphoproliferative Disorder: Pre-treating Live Donors (PI Upton Allen, submitted to SickKids Research Institute, pending decision*): Allen and colleagues at UHN are currently exploring the feasibility of a pilot study that targets living kidney donor, whereby if they are EBV-seropositive they would receive an antiviral aimed at reducing the risk of transmitting EBV to seronegative recipients.

Progress on collaborations from Y1-2:

1) *Allergy and Immunity* (*Avitzur)* – The POSITIVE study has incorporated family questionnaires at baseline and follow-up to assess incidence and type to assess incidence of allergic/immune conditions post transplant. This multi-centre effort will

enable development of a future study exploring immune mechanism and predictors of allergy and immunity post transplant. Thus far we have collected 51 questionnaires on allergy and autoimmunity from our incident cohort.

2) *Pharmacogenomic-guided immunosuppression in pediatric transplant recipients* (PI, *Mital; funded Astellas and SickKids Transplant and Regenerative Medicine Centre*): **Aim 1** received funding through Astellas Pharma Canada Inc to collaborate with the iGeneTrain consortium, an international transplant consortium, to genotype POSITIVE study patients using a transplant-specific array (iGeneTrain). Genotyping of approximately 650 participants of which approximately 165 are recipient matched donors will begin by the end of April 2016 with the goal of presenting data at upcoming CNTRP Annual Meeting if analysis is completed in time.

3) *Extreme Phenotypes of PTLD* (PI, *Allen; funded by Enduring Hearts Inc, USA*): This ancillary project will look at whole exome sequencing of Th1/Th2 / Th17 and Treg cyotokines in extreme phenotypes of PTLD. This study will synergize directly with **P6&5** as it shares a subset of the same study population and therefore mechanisms are being put in place to collaborate on recruitment efforts.

Next Steps (Year 4 plan)

Our primary focus will be to ensure that each P6 centre begins recruiting optimally at planned targets as described earlier. A major goal of year 4 is to analyze and submit scientific abstracts and papers from data accumulated to date through the POSITIVE study. The following analyses have been highlighted by the steering committee:

- Factors influencing post-transplant EBV/CMV seroconversion
- Clinical and genetic predictors of infection
- Clinical and genetic predictors of rejection
- Influence of donor clinical and genetic characteristics on recipient outcomes (including genetic data)
- EBV genotype diversity in pediatric versus adult transplant recipients
- Practice variation in healthcare systems
- Influence of socio-economic status on outcomes after pediatric transplant
- Factors influencing POSITIVE study participation
- Methods and Aims paper

The following abstracts will be developed in this coming project year with the goal of presentation at the CST and CNTRP annual meeting in 2016 and/or manuscript submission.

1. Recipient-donor CYP3A5 interaction on tacrolimus availability. This project was presented at CST 2014 but is pending additional donor genotype data for resubmission as a manuscript
2. Pharmacogenomic-guided immunosuppression in pediatric transplant recipients. We will analyze association of pharmacogenotype with tacrolimus levels and drug-drug interactions.
3. Influence of age and organ type on variability in tacrolimus levels after pediatric transplant and incidence of rejection
4. Pharmacogenetic trial of tacrolimus dosing after pediatric transplantation. This pilot clinical trial has closed recruitment and results are expected to be published in the next 6 months. Results from this trial will provide information for study aim 1.

Sustainability: The project leads have engaged in one-on-one discussion with principal investigators of all participating sites to discuss short and long term goals with regards to the sustainability of the project. In this coming year, since all participating centres have obtained approvals, we will work on showing the involvement of each centre by their recruitment contributions towards meeting set targets. We will also increase the productivity of the network through the development of collaborative abstracts and papers from P6 as described above. A number of newly proposed studies have leveraged the network we've created to support these projects, and we will continue to offer support for other studies and are uniquely positioned to do so being the only multi-centre, multi-organ pediatric network in existence in Canada.

Regarding long term sustainability, we envision positioning this network as a trials network (similar to oncology group) where new practices are introduced and monitored on an ongoing basis in a standardized manner. This is an important approach when dealing with small populations. This will enable us to perform follow-on studies based on the results of the current POSITIVE study. This could include (i) developing and implementing age-appropriate, genotype guided immunosuppression to reduce rejection and other post-transplant complications, (ii) developing an immunephenotyping panel that can inform immune maturation and potential for immune tolerance, (iii) developing a point of care test for EBV genotyping of high-risk EBV strains that predispose to EBV disease/PTLD, and (iv) implementing healthcare system modifications that can enhance medication adherence and outcomes.

Core 1 - Ethical, economic, legal and social (EELS) platform

Leads: Prof. Timothy Caulfield, University of Alberta
Co-Leads: Prof. Jennifer Chandler, University of Ottawa and
Dr. Scott Klarenbach, University of Alberta

Original aims/sub-aims and changes

The overall objectives of Core 1 are to:

- Identify emerging EELS issues, including:
  - The relevance of existing legal frameworks and ethical norms;
  - Public perceptions/representations in relation to several issues central to public support of, or fears about donation;
  - The development and implementation of novel incentives and procurement initiatives
- Provide support on EELS issues for the science and policy work of all the relevant CNTRP Projects;
- Lead and facilitate policy translation and public engagement; and
- Create unique interdisciplinary EELS opportunities for training of highly qualified personnel and emerging scholars.

Specific objectives of Core 1 in relation to each of its Research Platforms:

Platform 1: Legal Frameworks and Ethical Norms

- Map relevant legal norms as they relate to concepts such as consent
- Explore the potential impact/benefit and legality of new incentives policies

- Examine the attitudes of families toward the decision for DCD
- Explore the relevance and evolution of ethical concerns such as commodification and exploitation
- Respond to legal/ethical needs of all CNTRP projects

Platform 2: Research Ethics Challenges

- Engage in mixed methods research (surveys, interviews, consensus workshops, and legal scholarship) exploring issues to do with consent, the right to withdraw, the control and management of tissue/health information and governance, and research ethics challenges associated with next generation genome sequencing technologies
- Will provide guidance based on research results to CNTRP projects on a range of practical research ethics challenges

Platform 3: Health Economics and Economic Evaluation

- Conduct contemporary high-quality health care costing, using a population-based provincial dataset;
- Conduct a high-level economic evaluation to determine the utility and scope of future investments in transplantation such as: novel strategies of organ perfusion and repair; increasing numbers of donors through strategies to recruit living donors, DCD and marginal donors; use of novel biomarkers that may reduce rejection and ameliorate the consequences of graft-vs-host disease; interventions at the level of the health care system or patient that improve medication adherence

Platform 4: Public Representations and Engagement

- Examine how the popular press, including print and online media, as well as new media (e.g. blogs, social networking websites), represents 1) organ donation issues, 2) incentive policies, 3) our relationship with tissue/organs, and 4) developments in neuroscience that bear upon questions of awareness, prognosis and suffering in unconscious or dying patients, among other topics.

- Build a meaningful and sustainable partnership between patients and researchers within the CNTRP.

Changes:

- Research on the issue of family override of consent for deceased donation has expanded in scope to include multiple studies with numerous investigators across the CNTRP.
- The CNTRP's Patient Engagement Strategy has, up until this point, been encompassed by Platform 4; however, moving forward, this branch of Core 1 will operate independently as Core 4.
- Additional planning of cost effectiveness analyses done in conjunction with CNTRP researchers to determine the cost effectiveness of organ donation with provision of eCPR.

Major accomplishments in Year 3

Platform 1: Legal Frameworks and Ethical Norms

CAULFIELD: At the 2015 CNTRP Annual Scientific Meeting, research on the issue of family override of consent for deceased donation, led by Caulfield's team was presented. Following

the meeting, this project attracted a lot of interest and has since expanded in scope to include multiple studies with numerous investigators across the CNTRP, Canadian Blood Services, and abroad. Branches of this project include: (i) Legal and policy analysis, including an international comparison of law and policy across Canada, the US, the UK, and Australia; (ii) Media studies, including separate studies examining media portrayal of this issue in Canada and the U.S (supports Platform 4 below), (iii) Surveys, including a public survey administered in Alberta in 2015 (supports Platform 2) and a survey being developed for the critical care and donation communities; (iv) Quantitative analysis, in which we have worked with Trillium Gift of Life Network and BC Transplant to collect data on the occurrence of family override in both the NDD and DCD contexts. Investigators working on these projects include Maeghan Toews (Core 1), Timothy Caulfield (Core 1), Samantha Anthony (P2), Linda Wright (P2), Matthew Weiss (P2), Sonny Dhanani (P2), Sam Shemie (P2/CBS), Jehan Lalani (CBS), Roseanne Dawson (CBS), Amber Appleby (CBS), and Bernadette Richards (University of Adelaide).

Caulfield's team has also furthered the work being done on the issue of incentives for donation, with various high-level presentations and participation at the AST's recent Cutting Edge of Transplantation meeting in Phoenix, Arizona (see below).

CHANDLER: Over the past year Chandler's team has been active in the analysis of the law and ethics of organ donation in the context of physician-assisted death. This analysis has been shifting as her team has monitored government policy and legislative responses for their impact on organ donation - most notably the recent federal Bill C-14 released in April 2016. Since the submission of the last report, trainee Sherri Yazdani completed her Masters in Law degree on this topic under the supervision of Chandler; a paper on point (reflecting Bill C-14) has been drafted ready for submission (spring 2016), and numerous presentations related to this work were given (see below).

Chandler's team's research on family experiences of donation after cardiac death represent a collaboration between Core 1 and Project 2's DEPPART study on the physiology of dying after withdrawal of ventilation, led by Dr. Sonny Dhanani. Chandler's qualitative study recruits families who are participating in DEPPART at 5 Ontario hospitals and one Halifax hospital. Although slow to start due to both contract and technical complexities, recruitment is underway, with several families indicating their willingness to be re-contacted for interview purposes. Despite the slow start, Chandler's team implemented several adjustments to supplement recruitment strategies, including: 1) addition of QEII hospital in Halifax (including the recruitment of a collaborator based in Nova Scotia, and obtaining REB approval); 2) addition of Sacre Coeur Hospital (Montreal) (Core 1 Fortin has joined, and Chandler's team is in the process of arranging the inter-institutional agreement and REB approval); 3) the development of a strategy (which involved collaboration with CBS) to recruit families through Canadian OPOs, an initiative led by Vanessa Gruben.

Platform 2: Research Ethics Challenges

CAULFIELD: Caulfield's team is hosting a two-day workshop in Banff, Alberta, September 2016, to explore research ethics challenges as they relate to organ donation and transplantation. Specifically pressing issues pertaining to consent for research in the deceased donation, biobanking, and clinical trial design contexts will be explored. This event will involve members from across various CNTRP Cores and Projects.

Under this platform, Caulfield's team has also collaborated with Dr. Marcello Tonelli through the Interdisciplinary Chronic Disease Collaboration (ICDC). This research initiative involves mapping out a wide range of legal and ethical norms and professional standards that are implicated in novel clinical trial designs. This work will be useful for the many CNTRP

researchers involved in deceased donation research, clinical trials, and biobanking, as it will provide an up-to-date Canadian perspective on these issues.

CAULFIELD AND ZARZECZNY: Caulfield and Zarzeczny are producing a fast facts document on physicians' obligations in the context of transplant tourism, as well as producing a commentary on point. Additionally, they have submitted a paper (titled *Curbing Transplant Tourism: Canadian Physicians and the Law)* exploring options aimed at gathering better data that reflects more accurately what is taking place with regards to transplant tourism in Canada to the Canadian Medical Association Journal. Specifically it considers mandatory reporting scheme approaches, registry systems, and public health based reporting tactics, with the goal of providing better evidence for policy development. Additionally, Zarzeczny will be working closely with members of Caulfield's team on the analysis of survey results related to this platform noted above.

Platform 3: Health Economics and Economic Evaluation

KLARENBACH: Klarenbach's team is close to completing the scoping review of strategies to increase organs for transplantation. Data tables are being finalized and the manuscript is in draft form. In addition to this high-level scoping review, select areas will be explored in greater depth, with future publications expected. Klarenbach's team's systematic review of the cost effectiveness of solid organ transplantation is also largely complete (i.e. search, article screening, data abstraction, data presentation, draft of manuscript) and will be widely disseminated in user-friendly format to CNTRP members. Additionally, Klarenbach made several invited lecture to medical professionals, decision makers, and the general public; delivered the CNTRP webinar presentation "Health Economics of Donation and Transplantation"; provided key information and input to the CBS System Progress Report on economics of donation and transplantation; and Co-Chairs the Alberta Kidney Strategic Clinical Network, the objective of which is to harmonize best practices and increase living donation in Alberta.

Platform 4: Public Representations and Engagement

CAULFIELD: As noted above, in the Fall of 2015 Caulfield's team administered a public survey to over 1200 Alberta residents to gather public opinion data on a range of organ donation and transplantation topics. The survey (which included questions provided by Caulfield, Toews, Zarzeczny, Wright and Chandler) encompassed issues such as (i) knowledge of the donation registry/consent process, (ii) knowledge and opinion of consent issues such as family override and presumed consent, (iii) public solicitation for living organ donation, (iv) financial incentives for organ donation, (v) organ trafficking and transplant tourism, (vi) use of pre-mortem interventions in DCD, and (vii) consent to participate in decased donation research. The results of this extensive survey are currently being written up for publication.

FORTIN: Over the past year, Fortin's team has continued with their patient engagement activities. Specifically, they completed a national survey administered to more than 500 respondents (patients, caregivers, healthcare professionals and researchers) and held a national workshop on point in Toronto in November 2015. At this event, participants identified pressing research priorities in transplantation as well as in organ donation, as well as how to integrate and partner with patients within the CNTRP structure. Semi-directed interviews were also conducted with researchers pre and post national workshop. Analysis of focus groups, nominal group techniques and interviews are almost completed, with articles in preparation. Fortin's team has also launched the Patient/Research Partnership Pilot Study, the objective of which is to build a sustainable partnership between patients and researchers, and assess its impact. Funding for this initiative has been provided by a CIHR SPOR collaboration grant (30K) (04/2016-03/2017) and an Astellas grant (50K for one year).

Interactions within the project/core

Members of Core 1 work together on many projects to utilize their expertise and knowledge base from across the country and incorporate multiple perspectives in their work. For example, as noted above, multiple Core 1 members contributed to the development of the Alberta public survey, contributing questions relating to their various fields of expertise to strengthen the survey and analysis of the results. Other areas of collaboration between Core 1 members include the many Fast Facts that have been produced, as well as projects examining organ trafficking (Zarzeczny and Caulfield), pre-mortem interventions in DCD (Chandler and Toews), and family override (Toews and Caulfield). New collaborations have also been formed between Chandler and Fortin, with Fortin interviewing families in Quebec about DCD experiences and on the issue of compatible pair participation in kidney exchanges.

Core 1's close collaboration also allows them to maximize opportunities to share and disseminate other's work. For example, the Health Law Institute, directed by Caulfield, hosts an annual lecture series, with at least one lecture devoted to topics on organ donation each year. This year, the HLI hosted Chandler to present on her work considering organ donation in the context of physician-assisted dying. This lecture series has also featured various Core 1 and CNTRP investigators including Klarenbach, Gill, Beed, West, and Toews.

Interactions across the CNTRP

Core 1 has engaged with a variety of CNTRP members from several other Cores and Projects. In particular, the family override project and the development of the public survey have involved close collaboration with Project 2 (Linda Wright, Samantha Anthony, Sonny Dhanani, Sam Shemie, Matthew Weiss).

Core 1 was also able to respond to ethical and legal issues arising in relation to the work being done by other Projects and Cores. For example, the issue of organ donation in the context of extended CPR (eCPR) protocols was brought to Core 1 by members of Project 2 (John Gill) to analyze potential ethical and legal issues. In addition, as donation from HIV positive donors is starting to occur, we have also been approached by members of Project 2 (John Gill) to contribute a legal and ethical analysis of this issue.

Project 2 also requested Core 1 members to consider issues of privacy and confidentiality in the context of communicating personal information about prospective donors to ODOs in the deceased donation referral process. As a result of this request, Caulfield's team is preparing a Fast Facts on point, performing a Canada-wide legal review, and participating in an upcoming Donor Identification and System Accountability Workshop with CBS in Ottawa. Additionally, Chandler's project exploring the family experience of DCD is a sub project of Project 2's DEPPART study (a study on which she sits on the steering committee).

Klarenbach's team has also done considerable work to support other investigators within the CNTRP including: background work and the development of an integrated economic evaluation for the ex vivo liver perfusion study (PI Shapiro) which has been funded; teleconference meetings among aim leads to discuss and develop approaches for the capture of important data elements and design of economic evaluations (as noted above); economic evaluation support for extended CPR currently in the development phase (Klarenbach will be attending the research planning meeting in May 2016 in Toronto).

Lastly, the patient partnership pilot study led by Fortin has also resulted in considerable collaboration within the CNTRP. For example, Project's 2 Greg Knoll, is one of the patient partnership research team members. Additionally, co-leaders of Project 3 (Marie-Josée Hébert and Claude Perreault), Project 5 (Atul Humar, Lee-Ann Tibbles and Jeans-Sébastien Delisle) and Project 7 (Sunita Mathur) agreed to co-lead their projects with patients. Finally, Dr. Dhanani in the DePPaRT study (project 2) and Dr Janaudis-Ferreira (project 7) in CAN-RESTORE study have integrated patients as co-researchers.

New research & collaborations

Core 1 has collaborated very closely with Canadian Blood Services. In particular, Toews and Chandler (as current and former recipients of CBS's James Kreppner Fellowship respectively) are members of CBS's Donation Legal Research and Health Policy Working Group. This group allows for communication and coordination of work being done through CBS and the CNTRP to avoid duplication of efforts and to work collaboratively together on a range of legal and policy issues. In addition, through this group, Toews has been asked to Chair the Family Override Steering Committee, which is comprised of CNTRP and CBS members who are working on this issue, with the overall goal of achieving practice and policy change. Toews has also been invited to participate in a CBS initiative directed at opt out/presumed consent frameworks for donation. Additionally, Chandler joined the Ethics Working Group for the Canadian Society for Transplantation, and worked with the group on the development of guidelines on public solicitation of live donors. She also assembled a new group to look at the ethics and law of premortem interventions (Chandler, Yazdani, Shemie, Weiss, and Toews).

Caulfield's team is also working closely with colleagues in Australia. In particular, Dr. Bernadette Richards of the University of Adelaide, Faculty of Law, hosted Toews as a visiting research scholar, where connections were forged with the Australian ODT community, including Dr. Stewart Moodie, the State Medical Director of DonateLife South Australia, and Dr. Paolo Ferrari, the Clinical Director of the Australia Kidney Exchange Programme. Toews is currently collaborating with these individuals on the family override project and on the topic of incentives. Caulfield's team is also collaborating with the ICDC project (noted above), and will bring together CNTRP members and ICDC members at the upcoming research ethics workshop in Banff, September 2016.

Finally, Fortin is working closely with Dr. Antoine Boivin, director of the Partnership Lab. This collaboration will allow testing assessment tools in order to quantify the impact of the patient-researcher partnership.

Next Steps (Year 4 plan)

In conjunction with CBS and the Family Override Steering Committee, Caulfield's team is working toward organizing a national meeting in the Fall of 2017 of legal, ethical, medical, and donation stakeholders to discuss the research that has been done on this issue and strategies for moving forward. Prior to this meeting, Caulfield's team (plus select CNTRP members) will complete the survey of the critical care community, the international legal and policy comparison, and the U.S. media study.

Caulfield's team will also continue our work on the issue of incentives for organ donation, specifically examining the meaning of "valuable consideration" in this context, and the associated implications for the paired kidney exchange program. This will encompass an

international comparison, in collaboration with colleagues in Australia. Additionally, they, in collaboration with Zarzezcny's team, will continue with their collaborative exploration of transplant tourism and additional key research ethics issues.
Chandler's team will produce research papers on the ethics and law or premortem interventions in Canada (with Yazdani, Shemie, Weiss, and Toews), and on legal regulation of public solicitation of live organ donors (with Porkonjak). Her team will also continue with their work exploring the participation of
compatible pairs in kidney exchange programs (with Fortin), as well as that related to the qualitative study of family experiences of DCD (in conjunction with Project 2's DEPPART study, Gruben).

Fortin plans to implement the Patient-Researcher Partnership Strategy within the CNTRP, which will involve hiring some patients, and Klarenbach with continue with the economic evaluation research activities noted above.

Core 2 - Research infrastructure and registries support platform

Leads: Dr. Kirk Schultz, University of British Columbia
Co-Leads: Dr. Donna Wall, University of Manitoba and
Dr. Kristjan Paulson, University of Manitoba
Core 2 Manager: Peter Subrt

Original aims/sub-aims and changes

There have been no changes in the aims from last year.

1. Develop the foundation for national transplantation registries to evaluate transplant outcomes in Canada, starting with patients involved in CNTRP studies.
   - Aim 1A. Consensus building on Canada's future transplant registry
   - Aim 1B. CNTRP Outcomes Database (COD)
   - Aim 1C. Develop a biostatistical core for registry research in Canada

2. Create national methodology standards and standardized national 'core' resources and services for interventional clinical trials
   - Aim 2A. Develop national infrastructure strategies for the performance of large interventional clinical trials that involve multiple transplantation types and communities.
   - Aim 2B. Facilitate project development and performance by creating a national resource system for interventional clinical trials studies

3. Create national methodology standards and standardized national 'core' resources and services for biorepositories
   - Aim 3A. Build a national consensus regarding necessary methodologies and standard operating procedures for correlative biology studies in transplantation in Canada
   - Aim 3B. Develop a national virtual biorepository network of biological samples for transplant research studies

- Aim 3C. Evaluate the feasibility of using standardized biological cassettes in CNTRP research

4. Establish a training program for personnel essential to the future of transplantation in Canada. (***Moved to Core 3***)

Major accomplishments in Year 3

Aim1: Develop the foundation for national transplantation registries to evaluate transplant outcomes in Canada, starting with patients involved in CNTRP studies.

Aim 1A. Consensus building on Canada's future transplant registry
This aim has been difficult to develop due to factors outside our control. The CBMTG registry continues to develop and the CBS established solid organ registry does not have sufficient funding to support interaction with the CNTRP. Although a number of attempts have been initiated to further a long-term solid organ and HSCT registry, there has not been sufficient support from outside agencies for a research role for the CNTRP.

Aim 1B. CNTRP Outcomes Database (COD)
The CNTRP Patient Registration Database (PRD) will launch shortly, after initial hurdles with funding and contract development. This comprehensive database will track research subjects enrolled across all CNTRP clinical studies, and will have the capacity for linkage with administrative databases for both long term follow-up and correlative health utilization studies. The PRD is also in the process of being expanded to track deceased donors. For the foreseeable future this aim has had to be suspended.

Aim 1C. Develop a biostatistical core for registry research in Canada
Progress has been made on protocols for pilot studies linking clinical and administrative databases. The first study, understanding patterns in geographic variation in transplant utilization, has a draft protocol and will be completed in the next year. Additional studies, understanding the prevalence of chronic diseases following transplant and the incidence of malignancies following transplant will follow.

Aim 2 – Create national methodology standards and standardized national 'core' resources and services for interventional clinical trials

Aim 2A. Develop national infrastructure strategies for the performance of large interventional clinical trials that involve multiple transplantation types and communities.
Clinical trials infrastructure support is accelerating and diversifying to meet the needs expressed by various projects. The Montreal Health Innovations Coordinating Centre (MHICC) partnership is in place and in use by Aim 1 and Project 1. We have, in the process of coordinating the opening of the Project 4 CARE trial, defined the most pressing needs of the transplant community for successful multi-center, including multinational, trials. Through this process the foundations for a multiuser Data Safety Monitoring Board (DSMB) and a resource for managing regulatory requirements is well along the way to full implementation. This has required intensive stakeholder input and is at the stage of final approvals and implementation.

A European-style advisory DSMB is under development. This was selected over a US/NIH style regulatory body after vigorous discussions over several months of Core 2 meetings with pediatric, adult, BMT, and solid organ transplant investigators. This DSMB will provide reports

directly to study PI with recommendations. Terms of reference have been drafted by Dr. Donna Wall and circulated for input. A CNTRP Health Canada regulatory support service has been proposed by Dr. Kirk Schultz to assist with the activation of existing US/European clinical trials to open with local CNTRP investigators, as well as to assist in the start-up of investigator driven clinical trials. This regulatory service would share coverage with the C17 Childhood Cancer group based in Edmonton, having already launched and administered over 230 clinical trials, and handle all Health Canada correspondence for the studies at minimal cost. This service will be established by year 4.

Aim 2B. Facilitate project development and performance by creating a national resource system for interventional clinical trials studies

The large CaPITAL (Canadian Patient-oriented Immune balance in Transplantation and Autoimmunity to improve Lives) SPOR application (NPA – Kirk Schultz) was selected as one of 17 applications for final consideration with the application deadline in October 2015. The CaPITAL SPOR for a total of $32M (with $20 million matching funding) leveraged off of Core 2 as well as projects 3 and 4. The project included 78 investigators with strong patient engagement. It sought to expand the mandate of the CNTRP to include autoimmunity. Although application was unsuccessful, it resulted in new projects that are being pursued with other funding sources (thymic Treg therapy – BioCanRx) and chronic rejection biomarkers (SPOR clinical application October 2016).

Aim 3 Create national methodology standards and standardized national 'core' resources and services for biorepositories

Aim 3A. Build a national consensus regarding necessary methodologies and standard operating procedures for correlative biology studies in transplantation in Canada

An informal survey of participating laboratory PIs has revealed only a minimal level in correlative research laboratories of interest in developing such standards. Because of this the aim was deferred for later development. A consensus meeting for year 4 is under development with future dialogue on the needs of the research community.

Aim 3B. Develop a national virtual biorepository network of biological samples for transplant research studies

The *CNTRP National Virtual Biorepository* has expanded beyond initial pilot testing with three sites in **Project 3**, and is now located at 7 labs across Canada with interest from a number of other labs and external organizations. More than 13,000 samples have already been indexed into the biorepository, with work underway to increase batch entry into the database to allow the system to more easily incorporate existing specimen collections. A request has already been made to see how a 10 year BMT repository could be indexed and transferred to a different CNTRP lab for preservation (pending ethics review) due to local funding difficulties. Clinical data collection support has not been needed by any projects; however assistance with survey data collection has been provided in kind for Project 4 to explore current practices in kidney transplantation in Canada.

Aim 3C. Evaluate the feasibility of using standardized biological cassettes in CNTRP research

The CNTRP immune monitoring committee worked with Ryan Brinkman to establish automated analyses pipelines for the first generation DuraClone panels tested in phase I and the results from central manual analyses were compared to automated analyses. A phase 2 study was initiated to test the standardized flow cytometry using samples from HSC recipients 3 months post-transplant. This study will recruit 10 subjects in Vancouver and 10 in Montreal and will compare fresh whole blood and 24 h frozen/thawed PBMC (recruitment ongoing). As a test of

standardization, Edmonton will run the PBMC samples from both sites. The immune monitoring committee is also currently working with Beckman-Coulter to establish a $CD4^+$ T cell subset panel and a granulocyte panel that will be sold as DuraClone tubes. Using the techniques and knowledge gained in our work with the immune monitoring committee we have set up immune monitoring for the project 4 CARE trial (see the project 4 report for details).

Challenges

Overall challenges in Core 2 have been primarily related to administrative, legal, and ethical delays that have caused timelines in a number of aims and projects to slip. There was much debate on the nature and responsibilities of the Core 2 services that spoke to the question of whether the CNTRP is a semi-centralized partnership across a diverse array researchers or a directory of individual researchers who at times collaborate based on cost and mutual self-interest.

With respect to Aim 1, a significant challenge over the past year has been availability of funding, and legal contracts. After multiyear delays between University legal teams, first funding from the CNTRP was only released to the sub lead, Dr. Kristjan Paulson in February 2016. Additional delays related to data sharing agreements also were a challenge, but have been overcome. Within the patient registration database a number of project aims are either already recruiting, or have completed recruitment, necessitating additional discussion on how patients can be retrospectively integrated into the PRD based on existing consent language and collected data. Technical discussions with the MHICC are also underway to see how this data can be batch entered into the database several hundred at a time, as opposed to individual retrospective patient entry.

The creation of the Health Canada regulatory support service will be targeted to smaller project that are primarily performed by individual sites of PIs whereas the MHICC will support larger CNTRP trials.

The Biorepository has grown by 5 sites and over 13,000 samples in the last year, but is still at only a fraction of the size it has the potential to be. The initial database structure setup and sample entry method is not straight forward, facilitating intensive one-on-one training for a site to be able to start up with confidence.

The Immune Monitoring aim is experiencing very slow recruitment of phase 2 patients, delaying the submission of the phase 1 data for publication (which is to include both studies). Part of the problem is caused by exclusion of ATG-treated recipients and the exclusion criteria are now being eased to increase enrolment rates.

Interactions across the CNTRP

Interactions within Core 2 continue to offer services across the CNTRP. Core 2 aims to facilitate networking and provide services that allow for ‘plug and play’ integration into projects while providing value through economy of scale and access to experience from across the CNTRP researchers.

The DSMB will be available to all clinical studies requiring it across the CNTRP on a request basis. Currently only Project 4 is requesting a DSMB to monitor the CARE trial. The Health Canada regulatory support service has piqued interest across many projects encompassing

both BMT and solid organ transplant researches, and it remains in the planning stages pending further feedback.

Core 2 is contacting all project and aims regarding participation in the Patient Registration Database (PRD), and to ensure that standard PRD consent language is being implemented across all projects within the CNTRP, and working to have active projects enter patients into the PRD as soon as it goes live. This will also allow researchers to follow subjects across provinces and track long-term outcome data. As well, the Biorepository has expanded to include Project 1, 3, and 4 across 7 different sites, and is ready to ship a repository kit to an 8th site.

The main interaction of the Immune Monitoring group has been with Project 4 (CARE trial) and Project 6 (POSITIVE trial). As part of the CARE trial immune monitoring we are collecting plasma, urine and PBMC samples for the use of other researchers in Projects 3 and 5.

New research & collaborations

As described within the major accomplishments, Core 2 is in the process of developing a Health Canada Regulatory support services with the C17 Council, which is a non-profit organization, composed of the institutionally appointed heads of the sixteen pediatric hematology, oncology, and stem cell transplantation programs in 17 institutions across Canada. This service would function as a go-between for all Health Canada submissions and communications and assist with regulatory compliance documentation, while leaving operations in the hands of the PI and host institutions. There has been a high level of interest in this service from a number of researchers wishing to open investigator initiated trials, as well as expanding international trials into Canada.

The standardized immune monitoring has been widely presented (see section X) and is attracting some attention from other investigators. The ITN are using the first generation DuraClone tube on a phase I/II clinical trial of Ustekinumab for new onset pediatric T1D. We are also testing the CD4+ T cell subset panel (developed in collaboration with Beckman-Coulter) on some of these trial participants. The same tubes, as well as the granulocyte tubes, will be used in a pilot study of patients with skin pathology due to Th17 imbalance.

Next Steps (Year 4 plan)

Aim 1 Develop the foundation for national transplantation registries to evaluate transplant outcomes in Canada, starting with patients involved in CNTRP studies.

Aim 1A. Consensus building on Canada's future transplant registry

As already mentioned this aim has been deferred indefinitely.

Aim 1B. CNTRP Outcomes Database (COD)

The PRD will launch within the next several weeks, providing a means and mechanism to assign patients a unique CNTRP ID number, and provide long-term follow-up of research subjects using linkage with administrative databases. In addition, two draft protocols have been developed to link data contained in the CBMTG Registry (maintained and developed by the Canadian Blood and Marrow Transplant Group) with the Canadian Organ Replacement Registry (CORR) and administrative databases held by Canadian Institute for Health Information (CIHI).

Aim 1C. Develop a biostatistical core for registry research in Canada

We shall complete the first pilot project linking clinical databases (the Canadian Blood and Marrow Transplant Group database) with administrative databases (the Discharge Abstract Database, maintained by the Canadian Institute for Health Information). This study will aim to understand variations in transplant access and utilization across Canada. The second project reviews the development of chronic diseases following transplant, using administrative databases to review the incidence of chronic diseases (heart disease, diabetes, chronic lung disease, etc) in transplant recipients, and would be linked to a future Project 7 on improving outcomes for transplant patients.

Aim 2 Create national methodology standards and standardized national 'core' resources and services for interventional clinical trials

Aim 2A. Develop national infrastructure strategies for the performance of large interventional clinical trials that involve multiple transplantation types and communities.
We continue the support for the CARE trial and continue to support development of large multicenter, multidisciplinary trials. Recently, we have offered to support the application for the current RFA for SPOR clinical trials due October 25, 2016. Currently an adaptive clinical trial design for translational available prognostic and diagnostic biomarkers is being planed with CNTRP investigators. Dr. Schultz is the NPA and it will involve both HSCT and solid organ transplantation chronic rejection (heart and kidney) with close collaboration with project 3. Core 2 is also offering support for development of a two center trial using the OrganOx technology in high risk decreased liver donor transplants (Project 1).

Aim 2B. Facilitate project development and performance by creating a national resource system for interventional clinical trials studies
We shall finalize and implement the CNTRP interventional trial Manual of Procedures, and leading to the creation of the Clinical Trials Operations Committee, as well as active the DSMB (for which Dr. Paul J. Martin at the University of Washington has been recently recruited as a Chair). We will finalize and launch the Health Canada Regulatory support services in collaboration with the C17 Council with continuous services available as a 0.5 FTE equivalent shared across 3 highly qualified personnel located in Edmonton.

Aim 3 Create national methodology standards and standardized national 'core' resources and services for biorepositories

Aim 3A. Build a national consensus regarding necessary methodologies and standard operating procedures for correlative biology studies in transplantation in Canada
We are planning a consensus meeting to occur in the later part of year 4 to further develop standards for assays based on what has been accomplished in aims 3A and 3C. An initial oversight committee meeting will be planned for the CNTRP Annual Meeting taking place the fall of 2016 to create the standards for minimal non-identifiable clinical data, database linkage between labs/projects, and to develop guidelines on how to review access requests to already banked samples across the CNTRP National Virtual Biorepository.

Aim 3B. Develop a national virtual biorepository network of biological samples for transplant research studies
The Biorepository is ending its pilot phase having deployed systems in 7 labs across Canada. We will now begin to aggressively advertise its availability as well as target select CNTRP researchers who are known to collect many biospecimens. The next phase of biorepository development is an active linkage of the lab/PI spaces maintained on the CNTRP Biorepository server in Edmonton. Training videos and a printable manual are under development, and will be produced this upcoming year to help streamline this process. Work with the Canadian

Biosample Repository software engineers is also underway to increase the batch tube import image recognition from about 50-60% of a 10x10 freezer box to hopefully 80-90%, requiring much less user effort in scanning barcodes as samples are indexed into the database.

Aim 3C. Evaluate the feasibility of using standardized biological cassettes in CNTRP research

The Immune Monitoring group is working with The Transplantation Society's Global Virtual Laboratory to make all protocols associated with immune monitoring available for everyone.

Core 3 - Academic and Career Training Platform

Leads: Dr. Lee Anne Tibbles, University of Calgary
Co-Leads: Dr. Silvy Lachance, Université de Montréal and
Dr. Sonny Dhanani, Children's Hospital of Eastern Ontario

Original aims/sub-aims and changes

CNTRP, spanning the breadth of investigation from basic biology through health systems, economics and policies, offers the ideal training environment for cross-pollination of clinical and basic science trainees on a national basis, together with all CNTRP investigators and participants. A variety of comprehensive training initiatives across the program serve to develop a new generation of highly qualified personnel for the transplantation field, from physicians and surgeons, Masters & PhD scientists, allied health and technical personnel to trainees in ethics and law. The CNTRP Core 3 Academic and Career Training Platform consists of two aims:

Aim 1: The Academic Training Program (Curriculum and Mentoring)
Aim 2: The Professional and Technical Development Program.

Academic Training Program

Over the first three years, the objectives of the CNTRP Academic Training Program were to set standards for admission to the program, select appropriate candidates, provide each trainee with an individual training plan with requirements and timelines, ensure mentorship relationships are set up and effective, and provide web-based modular learning, professional development and networking opportunities.

Professional and Technical Development Program

The goal of this aim was to provide all members of the CNTRP with educational modules to ensure development of a common language and scientific understanding amongst the diverse participants from the three research communities (SOT, BMT, Donation). Additionally we sought to ensure common approaches to biobanking of samples, clinical trial case report forms, common data management protocols, and standard operating procedures for technology used throughout the CNTRP.

Changes: Originally conceived as part of Core 2, the Training Program became an independent entity in March 2013 under the leadership of Drs. Tibbles, Dhanani and Lachance (SOT, Donation and BMT leads)

Major accomplishments in Year 3

Academic Training Program

- Core 3 partnered with Astellas Canada and the Alberta Transplant Institute to develop the CNTRP Astellas Training Award Competition - www.cntrp.ca/training_award. This competition provided two national grants and two Alberta grants, maximally $50,000 each over two years, with the stated objectives:
  - to support the training and development of future researchers in the field of organ and cell donation and transplantation
  - to advance CNTRP's objective to increase organ and tissue donation in Canada and enhance the survival and quality of life of Canadians who receive transplants with the ultimate goal of improving patient care
- Core 3 received 22 applications to the training grant competition and following a peer review process four grants were awarded to the following top ranked trainees:
  - Kyla Naylor (Astellas funded) (supervisor Dr. Kim)
  - Craig Hasilo (Astellas funded) (supervisor Dr. Paraskevas)
  - Anne Halpin (ATI funded) (supervisor Dr. West)
  - Minal Borkar (ATI funded) (supervisor Dr. Tibbles)
- Core 3 accepted 13 new trainees into the CNTRP Academic Training Program in October 2015. The CNTRP now has 29 trainees in total participating across the country.
- Core 3 organized and recorded 13 new training webinars since June 2015 (see appendix 3). These webinars cover a broad range of topics across the field of donation and transplantation and the recorded webinars are available for all CNTRP members to view on the private CNTRP website.
- The CNTRP Academic Training Curriculum was finalized and approved by the CNTRP Executive on December 1, 2015 and published at www.cntrp.ca/training. (see appendix 4). This curriculum also included a description and template for the Individual Training Curriculum that each trainee was required to complete. These individual curriculums help to define the personal learning objectives and strategies for each trainee (in consultation with their supervisor and CNTRP mentor) and include a key topic that the trainee will present as a webinar to the other trainees over the summer of 2016.
- Core 3 partnered with the **Toronto Transplant Institute** to help record and promote their course "Principles in Immunology", which took place on April 9th, 2016. The recordings from this session are available to all CNTRP trainees - https://piicourse.eventbrite.ca.
- Core 3 partnered with the **Health Law Institute at the University of Alberta** to record a public lecture by Prof Jennifer Chandler on November 24, 2015 on the topic of "Organ Donation in the Context of Physician Assisted Dying". The recording is available at http://www.cntrp.ca/#!Watch-Prof-Jennifer-Chandler-discuss-organ-donation-in-the-context-of-physician-assisted-dying/cbmd/5696813e0cf263fc5a891573
- Core 3 fostered professional development with sessions on:
  - Pecha Kucha Presentation format, (theory, group work and presentation at 2015 CNTRP Annual Meeting) – June 2015
  - Peer review of abstracts for national and international scientific meetings (group and individual work with feedback) – March 2015
- Core 3 created a new CNTRP Trainee Facebook Group to encourage dialogue and community building between the CNTRP Trainees (at the request of several CNTRP Trainees)

Professional and Technical Development Program

- Core 3 hosted and organized a CNTRP Partners Forum on April 4 and April 8 2016 for all the current and potential partners of the CNTRP to present an update on progress and highlight opportunities for collaboration. The sessions were attended by over 80 partners and the recording is available at https://youtu.be/XAKgHfsmARs
- The CNTRP partnered with the **Canadian Critical Care Trials Group (CCCTG)** to develop and offer a "principles in clinical trial design and operations" webinar series. The CNTRP provided technical expertise and the use of our webinar platform for the CCCTG (first session took place on Feb 17, 2016) and Core 3 will continue to host these webinars with the CCCTG over the course of 2016/2017.
- Core 3 worked with the **Project 7 CanRestore** team to develop a space to promote and share the recordings from their October 2015 patient training workshop, which are available at http://www.cntrp.ca/#!cr---workshop-resources/rvyfr
- Core 3 worked with the new **CNTRP Core 4 – Patient-Researcher Partnership Platform** to host training webinar sessions for patients and researchers across the CNTRP that are participating in the CNTRP patient partnership strategy.
- Core 3 organized, hosted and recorded several general CNTRP sessions to support and promote important new developments across the CNTRP, including the following sessions:
    - Core 2's Immune Monitoring Platform – Dr. Sabine Ivison
    - Core 4's Patient Engagement Research in Transplantation – Dr. Marie-Chantal Fortin and Hélène Campbell -
    - Core 2 - Showcasing our new CNTRP Patient Database and Virtual Biorepository – Drs. Kristjan Paulson, Kirk Schultz, Peter Subrt, Core 2 -
    - Project 4 - Tolerance Induction Protocols in the Canadian Context – Drs. Stephan Busque, Tony Jevnikar, Kirk Schultz

Interactions within the core

The Core 3 team meets on average twice a month to develop and plan the academic training program. All trainees also meet twice per month on the live webinars. All trainees participate in the Annual CNTRP Meeting for in-person sessions. There are opportunities at every live webinar for questions regarding the presentation, the Training Program, and any obstacles to advancement of their learning. We have also created the CNTRP Trainee Facebook Group.

Interactions across the CNTRP

Trainees are participating from all CNTRP Projects.

For the 2015 Annual CNTRP Meeting, all of the trainees participated in learning the Aims of all of the different Projects, as part of their 'Pecha Kucha' presentations. This presentation style (20 slides, each displayed for 20 seconds automatically progressing) was introduced in a webinar by one of the trainees who had prior experience with the format. The trainees were formed into groups including students from 2-4 projects. They were assigned to develop and present actual and potential interactions between projects, from the point of view of a Project that was not their own. To accomplish this they were required to contact the Leads of the Project, understand the interactions between this project and all others, and construct and present at a plenary session their Pecha Kucha presentation.

Next Steps (Year 4 plan)

- Continue development of live and archived webinars

- Increase partnerships with industry, professional associations, public to provide support to more trainees
- Assess mentorship meetings and increase the participation of mentors in the trainees' development
- Assess individual curricula for all trainees
- Liaise with the Canadian Society of Transplantation "Research Fellows in Transplantation" session at the upcoming CST/CNTRP/SQT meeting in October 2016 in Quebec City.
- Encourage more participation from CNTRP investigators to partake in the live and archived webinar series.

Trainees and other HQP

Name	Institution	Supervisor	Mentor	Project/ Core
Ibrahim Adam	University of Alberta	Lori West	Marie-Josée Hébert	Project 4
Chantal Allen	University of Alberta	Lori West	Sunita Mathur	Project 7
Minal Borkar	University of Calgary	Lee Anne Tibbles	Lori West	Project 5
Julie Boucquemont	McGill University Health Center	Bethany Foster	Heloïse Cardinal	Project 6
Mariusz Bral	University of Alberta	James Shapiro	Darren Freed	Project 1
Antonio Bruni	University of Alberta	James Shapiro	Jean Buteau	Project 1
Rahul Chanchlani	Sick Kids	Rulan Parekh	Atul Humar	Project 6
Ryan Cunnington	University of Calgary	Lee Anne Tibbles	Marie-Josée Hébert	Project 5
Nick Dawson	UBC	Megan Levings	Raewyn Broady	Project 4
Esme Dijke	University of Alberta	Lori West	Megan Levings	Project 4
Juan Echeverri	University of Toronto	Markus Selzner	Atul Humar	Project 1
Boris Gala-Lopez	University of Alberta	James Shapiro	Norman Kneteman	Project 1
Qingdong Guan	University of Manitoba	Donna Wall	Megan Levings	Project 4
Steven Habbous	Western University	Amit Garg	Patricia Birk	Project 2
Anne Halpin	University of Alberta	Lori West	Marie-Josée Hébert	Project 3 & 4
Craig Hasilo	McGill University Health Center	Steven Paraskevas	Marie-Josée Hébert & James Shapiro	Project 3
Sanaz Hatami	University of Alberta	Darren Freed	Jayan Nagendran	Project 1
Romy Hoeppli	UBC	Megan Levings	Lori West	Project 4
Qianni Hu	Dalhousie	Ian Alwayn	Eric Boilard	Project 1

Moritz Kaths	University of Toronto	Markus Selzner	Lisa Robinson	Project 1
Caroline Lamarche	University de Montréal and UBC	Jean-Sébastien Delisle	Marie-Josée Hébert	Project 4 & 5
Alvin Li	Western University	Amit Garg	Gregory Knoll	Project 2
Ivan Linares-Cervantes	University of Toronto	Markus Selzner	Atul Humar	Project 1
Kyla Naylor	Western University	Amit Garg	Gregory Knoll	Project 2
Andrew Pepper	University of Alberta	James Shapiro	Gregory Korbutt	Project 1
Alissa (Ali) Rutman	McGill University Health Center	Steven Paraskevas	Marie-Josée Hébert	Project 1 & 3
Chia Wei Teoh	University of Toronto	Christoph Licht	Lisa Robinson	Project 1
Ananda Venkatachalam	Dalhousie	Ian Alwayn	James Shapiro	Project 1
Bing Yang	University of Montréal	Marie-Josée Hébert	Lakshman Gunaratnam	Project 3

Appendix 1 – CNTRP Produced and Recorded live Webinars between June 2015 and May 2016

1. **Tolerance Induction Protocols in the Canadian Context**
   Stephan Busque, Tony Jevnikar, Kirk Schultz
   https://cntrp.adobeconnect.com/p8rvxav12pk/

2. **Tissue Transplantation: Past, Present and Future**
   Jelena Holovati – University of Alberta
   https://cntrp.adobeconnect.com/p7ntw1ui6d4/

3. **Metagenomic Approaches to Virus Discovery in Transplant Recipients with Idiopathic Disease**
   Andrew Mason - Professor of Medicine and Director of the Applied Genomic Core at the University of Alberta. CNTRP Project 5 Researcher
   https://cntrp.adobeconnect.com/p1g5rhx3hb4/

4. **Deceased Donation, Solid Organ and Bone Marrow Transplantation - exploring research synergies for the future**
   Maureen Meade – Project 7 and Project 2
   https://cntrp.adobeconnect.com/p3nk84s6sx6/

5. **The CNTRP Standardized Immune Monitoring Initiative**
   Sabine Ivison - Core 2
   https://cntrp.adobeconnect.com/p8hlu3kaaoa/

6. **Patient Engagement Research in Transplantation**
   Marie-Chantal Fortin and Hélène Campbell - Core 1

https://cntrp.adobeconnect.com/p3nk60dybew/

7. **Showcasing our new CNTRP Patient Database and Virtual Biorepository**
   Kristjan Paulson, Kirk Schultz, Peter Subrt, Core 2
   https://cntrp.adobeconnect.com/p7a05nf7ne0/

8. **Experiences as a Donation Support Physician: Dead or Not Dead?**
   Dr. Sonny Dhanani - Project 2 Researcher and Core 3 Co-Lead
   https://cntrp.adobeconnect.com/p2rawsgs707/

9. **Economic Research in the field of donation and transplantation**
   Dr. Scott Klarenbach - Core 1 Co-Lead
   https://cntrp.adobeconnect.com/p4dshexh3kd/

10. **Clinical change on the wings of science - the saga of the first ABO incompatible heart transplant**
    Dr. Lori West - Director, CNTRP
    https://cntrp.adobeconnect.com/p68film645c/

11. **Showcasing our new CNTRP Patient Database and Virtual Biorepository -Kristjan** Paulson, Kirk Schultz, Peter Subrt, Core 2
    https://cntrp.adobeconnect.com/p7a05nf7ne0/

12. **ABO antigens - The intersection between transplantation and glycomics**
    Dr. Todd Lowary and Dr. Lori West – Project 4 Researchers and CNTRP Director
    https://cntrp.adobeconnect.com/p2go136uwo9/

13. **Exercise Rehabilitation Therapy Research in Solid Organ Transplant Patients**
    Dr. Sunita Mathur - Project 7 Researcher
    https://cntrp.adobeconnect.com/p4zrjj8xya6/

Appendix 2 –Webinars from Partner Training Programs Supported by Core 3

- Principles in Immunology Course - with the **Toronto Transplant Institute** - April 9th, 2016. The recordings from this session are available to all CNTRP trainees - https://piicourse.eventbrite.ca.
- "Organ Donation in the Context of Physician Assisted Dying" - Prof Jennifer Chandler - with the **Health Law Institute at the University of Alberta** on November 24, 2015. The recording is available to all CNTRP members at http://www.cntrp.ca/#!Watch-Prof-Jennifer-Chandler-discuss-organ-donation-in-the-context-of-physician-assisted-dying/cbmd/5696813e0cf263fc5a891573
- The **Project 7 CanRestore** recordings from their October 2015 patient training workshop are available at http://www.cntrp.ca/#!cr---workshop-resources/rvyfr