



# ANNUAL DAY IN TRANSPLANTATION

Monday, December 12, 2011  
MaRS Auditorium  
0800h-1600h

Presented by the:  
University of Toronto Transplantation Institute

Hospital For Sick Children  
St. Michael's Hospital  
Sunnybrook Health Sciences Centre  
University Health Network

## SYMPOSIUM AGENDA

- 0715–0800h Registration and Breakfast
- 0800–0805h Welcome – Dr. Michael Ratcliffe
- 0805-0815h Introductory Remarks - Dr. Gary Levy and Dr. Jeffrey Zaltzman
- Chair – Heather Ross*
- 0815– 0845h Shaf Keshavjee - An opportunity to build ‘super organs’ for transplantation
- 0845-0915h Darren Yuen - Developing novel therapies targeting graft fibrosis
- 0915-0930h Hyunhee Kim - Mechanisms of Hypothermic Ischemia-Reperfusion-Induced Acute Lung Injuries in Human Lung Epithelial Cells
- 0930-1000h Tom Hudson - Cancer Genomics and Personalized Medicine
- 1005h Audience response
- 1005-1015h Break
- 1015-1045h Richard Weisel - Cardiac regeneration by cell transplantation
- 1045-1100h Arash Ghashghai - Cyclosporin-A inhibits nuclear localization of the transcription factor Nrf2 in human coronary artery endothelial cells
- 1100-1145h **STATE OF THE ART PRESENTATION - Doris Taylor, PhD**  
**Bioartificial Organs: Cures for the 21<sup>st</sup> Century**
- 1145h Audience Response
- 1150-1230h Lunch
- Chair – Jeff Zaltzman*
- 1230– 1300h Seema Mital - Pharmacogenetic Trial of Tacrolimus after Solid Organ Transplantation
- 1300-1330h Ian McGilvray - Gene profiling HCV infection pre- and post-transplantation outlines new aspects of disease pathogenesis
- 1330-1345h Liza Grosman-Rimon - Inflammatory Markers and Cardiopulmonary Exercise Testing in Continuous Flow Left Ventricular Assist Device Recipients
- 1345-1415h Markus Selzner – Normothermic Acellular Ex Vivo Liver Perfusion (NEVLP) for the Storage, Assessment, and Repair of Marginal Liver Grafts
- 1415h-1445h Gary Levy – Biomarkers in Tolerance
- 1445-1500h Andrzej Chruscinski - Non-HLA Antibodies and Risk of Rejection after Heart Transplantation: Profiling with Antigen Microarrays
- 1500-1545h **STATE OF THE ART PRESENTATION - Daniel Salomon, MD**  
**Functional Genomics Applied to Transplantation Biology: From Discovery to Biomarkers**
- 1545-1600h Closing Remarks – Dr. Michael Ratcliffe
- 1605h Audience Response

# An Opportunity to Build 'Super Organs' for Transplantation

Shaf Keshavjee MD MSc FRCSC FACS  
Surgeon-in-Chief, UHN  
James Wallace McCutcheon Chair in Surgery  
Director, Toronto Lung Transplant Program  
University of Toronto

Transplantation is life saving therapy for many patients with end stage organ failure. Standard techniques of organ preservation for transplantation have relied on cold static flush preservation to slow down metabolism and hence slow down the dying process of the organ. This has been effective in maintaining the organs in a relatively static state with sufficient time to preserve, transport and successfully transplant all of the major organs.

Transplantation today however is limited by the shortage of donor organs, primary graft dysfunction and chronic graft dysfunction. Many organs are not used either because they have some element of injury already, or because of fear of primary graft dysfunction. In addition, the early injury related to the transplantation process, followed by persistent immune attack in the host, leads to further injury, chronic graft dysfunction and ultimately failure of the organ.

In order to overcome the current limitations in our ability to safely and effectively provide life saving transplants to more patients, we need to re-evaluate our strategy. Our understanding of the biology of organ injury and technological advances have progressed to the point where we can truly envision the creation of organs that are superior in condition and function to the natural state in which we found them. We need to *concentrate on repairing and regenerating the organ, rather than simply focusing on slowing down the processes of dying.*

We have developed a technique that allows us to maintain a lung at normothermic temperature, outside the body for extended periods (Toronto-XVIVO<sup>R</sup>). This allows us to *diagnose* the injury and *treat* the organ using therapies targeted to the specific problem. In a sense, this is the beginning of "*personalized medicine for the organ.*" Diagnose the specific problem with the organ and treat it with specific therapy. This also provides the unique opportunity to immunologically pre-prepare the organ for the implantation before it is exposed to the recipient, the chance to locally down-regulate the innate and acquired (alloimmune) immunological responses – to make the organ look more like "self" - ultimately towards creating organs to which the recipient host will be tolerant.

The future of transplantation will see organs being carefully and accurately diagnosed using sophisticated molecular diagnostic techniques. The organs will then be processed - treated, repaired and regenerated using pharmacologic, molecular, gene and cell therapies. In "*Organ Repair Centers*", we will engineer organs that are superior in function and immunologically prepared for the transplant event. This strategy will not only allow us to use more organs for transplantation, but will also improve the safety, short-term and long-term outcomes of transplantation.

## References

1. Cypel M, J. Yeung, M. Liu, F. Chen, W. Karolak, K. Yasufuku, M. DePerrot, A. Pierre, T. Waddell, C.-W. Chow, M. Hutcheon, C. Chaparro, L.G. Singer, **S. Keshavjee**. Normothermic Human Ex Vivo Lung Perfusion (EVLP) for Improved Assessment of Extended Criteria Donor Lungs for Transplantation. *The J Heart and Lung Transplantation*, Volume 28, Issue 2, Supplement 1, February 2009, Page S126.
2. Cypel M, Rubacha M, Yeung J, Hirayama S, Torbicki K, Madonik M, Fischer S, Hwang D, Pierre A, Waddell TK, de Perrot M, Liu M, **Keshavjee S**. Normothermic Ex Vivo Perfusion Lungs Injury Compared to Extended Cold Preservation for Transplantation. *Am J Transplantation*. 2009; 9(10):2262-9.
3. Cypel M, Liu M, Rubacha M, Yeung J, Hirayama S, Anraku M, Sato M, Medin J, Davidson B, de Perrot M, Waddell T, Slutsky A, **Keshavjee S**. Functional Repair of Human Donor Lungs by 1L-10 Gene Therapy. *Sci Transl Med*. 2009;1(4) :4ra9.
4. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, Sato M, Laratta J, Azad S, Madonik M, Chow C, Chaparro C, Hutcheon M, Singer LG, Slutsky S, Yasufuku K, De Perrot M, Pierre A, Waddell TK, **Keshavjee S**. Normothermic Ex vivo lung Perfusion in Clinical Lung Transplantation. *New England Journal of Medicine*. 2011; 364(15):1431-1440.

## Developing Novel Therapies Targeting Graft Fibrosis

Darren Yuen MD, University of Toronto

Chronic allograft dysfunction is one of the leading causes of renal graft loss. Despite significant advances in the treatment of native chronic kidney disease, very few therapies attenuate progression of chronic allograft dysfunction. A key pathophysiological contributor to the progression of both native and allograft chronic renal injury is kidney fibrosis. Early outgrowth cells (EOCs) are a novel bone marrow-derived cell population that we and others have recently shown to significantly attenuate renal fibrosis in experimental models of native chronic kidney disease. Importantly, this structural protection is associated with improved organ function.

Recent studies in our lab have identified that EOCs act by releasing soluble factors with potent renoprotective activity. Using high throughput fractionation and mass spectrophotometry techniques, we have identified 4 putative molecules that may account for this anti-fibrotic renoprotective activity. As kidney dysfunction itself can impair EOC function, these studies have raised the possibility of using novel EOC-based, cell-free strategies for the treatment of chronic renal injury, avoiding the ethical and practical challenges of implementing a cell therapy approach in the clinic.

## Mechanisms of Hypothermic Ischemia-Reperfusion-Induced Acute Lung Injuries in Human Lung Epithelial Cells

Hyunhee Kim, Xiaohui Bai, Shan-Yu Fung, Wenxi Gao, and Mingyao Liu

Ischemia-reperfusion (IR) induced lung injuries are serious complications of lung transplantation. Understanding the cellular and molecular mechanisms of IR induced lung injury is crucial in developing effective therapies. To examine the intrinsic changes occurring in lung stromal cells, we designed a protocol to simulate the lung transplantation procedure in cell cultures.

To simulate IR induced lung injury, human lung epithelial (BEAS-2B) cells were initially cultured in DMEM with 10% fetal bovine serum, then replaced with ice-cold Perfadex® and incubated at 4°C containing 100% oxygen for 6 to 18 h (cold ischemic time, CIT, simulated lung preservation). The lung preservation solution was then replaced with culture medium mentioned above and incubated at 37°C for 4 h (simulated reperfusion). Cell morphology, viability and cytokine secretion were then examined.

Cell-to-cell contact was reduced after 6 h and 18 h CIT; cell shrinkage created substantial gaps between each other, suggesting that significant cytoskeletal rearrangement had occurred. When cells underwent 6 h CIT, cell morphology recovered after reperfusion. In contrast, when CIT was prolonged to 18 h, cells were detached from the bottom surface. Significant cell death occurred after 18 h CIT, compared to 6 h CIT as analyzed by flow cytometry. Multiple proinflammatory cytokines were significantly increased during reperfusion after 6 h CIT and reperfusion, as indicated by cytometric beads array and ELISA. The most dramatically increased cytokines included IL-6, IL-8, IFN $\gamma$ , Fractalkine, and IL-7.

Findings from this study demonstrate that pro-inflammatory responses and cell death occur in human lung epithelial cells. The simulated IR injury model has important features of actual lung transplantation and it will be useful for examining the molecular mechanisms of IR induced lung injury.

## Cancer Genomics and Personalized Medicine

Thomas Hudson, MD, Ontario Institute for Cancer Research

Personalized cancer medicine is based on a rapidly emerging knowledge of the cancer mutation repertoire, the unique patterns of mutations in human tumors that is continually evolving, and the increased availability of anti-cancer agents that target altered genes or pathways. Transforming actionable mutations into actionable cancer gene panels is an important step toward using comprehensive molecular analysis of tumors in the clinical setting to help guide physicians in selecting therapies. Given advances in cancer genetics, technology and therapeutics development, the timing is right to develop a clinical trials and research framework that may benefit patients and also build a long-term repository of knowledge linking mutation profiles with clinical interventions and outcomes, such that future clinical decisions can move from heuristic to evidence-based decisions.

In my presentation, I will present concepts and experiences gained from a pilot study involving patients with advanced metastatic cancers from five cancer centers in Ontario who are potential candidates for early phase clinical trials of targeted agents. The study includes rapid mutation detection in a set of genes deemed to be actionable, validation in a clinical molecular diagnostics laboratory, and reporting of actionable mutations to clinicians and patients.

# Cardiac Regeneration by Stem Cell Transplantation

Richard Weisel

Survival following a myocardial infarction (MI) has improved with aggressive percutaneous coronary interventions (PCI). However, the incidence of heart failure has continued to increase and has become an epidemic. Despite early reperfusion, patients who have suffered an extensive infarct frequently have thinning of the infarct scar and progressive left ventricular dilatation (LV remodeling) which can result in congestive heart failure (CHF). New therapies are urgently needed to stem the avalanche of heart failure.

Current therapies following an extensive MI are largely aimed at counteracting the abnormal neuro-humoral balance. Despite advances in treatment options, the mortality of CHF remains unacceptably high, with greater than 50% of all patients succumbing within 5 years of the initial diagnosis of heart failure. The advent of stem cell therapy offered the promise to reverse ventricular dysfunction and prevent heart failure.

Coronary artery bypass grafting (CABG) improves survival, reduces angina and increases exercise tolerance. However, for patients who have suffered an extensive infarction and have minimal reversible ischemia in the infarcted area, CABG will reduce the risks of recurrent ischemic events, but the procedure will not restore ventricular function. To repair or regenerate the damaged myocardium will require new therapeutic approaches. Cell transplantation has been proposed to induce angiogenesis, prevent matrix remodeling and recruit stem cells to prevent progressive infarct scar thinning, ventricular dilatation and to restore cardiac function. Preclinical studies demonstrated dramatic improvements in cardiac function following stem cell implantation and these results encouraged the initiation of clinical trials.

Bone marrow cells containing both hematopoietic and mesenchymal stem cells were employed in the initial clinical trials. Lin<sup>-</sup>, *c kit*<sup>+</sup> (hematopoietic), mesenchymal stem cells or a mixture have been injected into the borderzone surrounding an infarct and have been demonstrated to induce healing of the cardiac scar. The most plausible mechanism to explain the beneficial effects of bone marrow stem cells is the paracrine release of regenerative cytokines by the engrafted cells. Bone marrow stem cells secrete a wide array of bioactive agents which stimulate angiogenesis, inhibit apoptosis and improve cardiac function. However, recent studies suggest that the stimulation of cardiac resident stem cells is required to achieve restoration of ventricular function.

Unfortunately, the initial clinical trials of cell therapy did not report the dramatic improvements in ventricular function which had been reported in the preclinical animal studies. Despite the disappointing results, the improvements in LV ejection fraction with stem cell therapy after a myocardial infarction was at least as good as the benefits achieved with thrombolysis, ACE inhibitors and  $\beta$ -blockers.

The limited improvement with cell therapy was the result of the old age of the recipients and their extensive comorbidities. Older patients have diminished stem cell function and a decreased responsiveness of their bone marrow and cardiac resident stem cells. These patients require rejuvenation of both the injected cells and the stem cells which respond to cell therapy.

To enhance the benefits of cell therapy, the paracrine effects of the implanted cells from older patients may be enhanced by restoration of the production of beneficial cytokines. Cell based gene therapy increases the survival and paracrine benefits of the

engrafted cells. Transmyocardial laser revascularization (TMR) has been demonstrated to induce angiogenesis, increase cell engraftment and improve ventricular function. Finally, we recently reported that ultrasound mediated bubble destruction provided a minimally invasive method to deliver cytokines to the ischemic myocardium, increase angiogenesis and augment cell therapy.

To increase the number of cells engraft in the heart, the application of preformed cell sheets to the epicardial surface of the infarcted myocardium has been evaluated. Tissue engineering approaches may dramatically improve cell survival after implantation.

Finally, older patients with diminished stem cell function may benefit from the injection of allogeneic cells from healthy young donors. Mesenchymal stem cells (MSCs) are not rejected after engraftment in the heart and improve heart function. However, recent studies suggest that MSCs become immunogenic when they differentiate resulting in recurrent ventricular dysfunction. In the future, rejuvenation of the stem cells from older patients or the induction of prolonged tolerance of allogeneic stem cells may restore the promise of cardiac regeneration.

In summary, cell therapy was as beneficial as thrombolysis, ACE inhibitors and  $\beta$  blockers following an extensive MI. However, the results of stem cell therapy during CABG were not as beneficial in patients as it was in preclinical animal studies. Enhanced cell therapy may restore the benefits in older patients. Either rejuvenated autologous stem cells or allogeneic stem cells rendered tolerant may be required to fulfill the promise of cardiac regeneration by stem cell therapy.

## Cyclosporin-A inhibits nuclear localization of the transcription factor Nrf2 in human coronary artery endothelial cells

Ghashghai A<sup>1,2</sup>, Badiwala MV<sup>1,2</sup>, Tumiaty LC<sup>2</sup>, Wallen J<sup>2</sup>, Joseph JM<sup>1,2</sup>, Xu F<sup>2</sup>, Rao V<sup>1,2</sup> <sup>1</sup> Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, ON; <sup>2</sup> Division of Cardiovascular Surgery, Peter Munk Cardiac Centre, Toronto General Hospital, Toronto, ON

Cyclosporine (CsA)-induced endothelial injury mechanisms have not been fully elucidated. We investigated the effect of CsA on Nrf2, which is the master regulator of antioxidant defence systems. It has been suggested that high nitric oxide (NO) enhance Nrf2 activity. We assessed the hypothesis that CsA-exposure impairs Nrf2 cellular localization in the presence of endothelial NO-enhancer drugs such as tetrahydrobiopterin (BH4) and exogenous endothelin-1 (ET-1).

Cells were incubated with BH4, ET-1, or DMSO in the presence or absence of CsA. Incubation with *tert*-Butylhydroquinone (tBHQ) was used as positive control. Confocal microscopy was used to obtain images and the data quantified. We analyzed Nrf2 cellular localization as measured by the nuclear/cytosolic ratio (NCR).

Compared to control, treatment with tBHQ significantly increased Nrf2 NCR ( $0.61 \pm 0.04$  vs.  $3.95 \pm 0.47$ ,  $p < 0.001$ ). Incubation with CsA did not significantly change NCR compared to control ( $0.97 \pm 0.94$  v.s  $0.61 \pm 0.04$ ,  $p = \text{NS}$ ). NCR significantly increased with either BH4 or ET-1 treatment compared to control ( $1.76 \pm 0.07$  and  $3.23 \pm 0.22$  respectively vs  $0.61 \pm 0.04$ ,  $p < 0.001$  for both). However, co-incubation with CsA significantly attenuated nuclear localization of Nrf2 in cells treated with BH4 or ET-1 ( $0.91 \pm 0.07$ , and  $0.84 \pm 0.22$  respectively,  $p < 0.001$  for both).

Our study reveals the novel observation that CsA inhibits Nrf2 nuclear translocation. Furthermore, we found that CsA-induced Nrf2 nuclear localization inhibition is persistent despite increased levels of endothelial NO suggesting that CsA may act to impair endothelial cells ability to detect increased levels of cytosolic electrophiles. CsA-mediated endothelial-injury incurred after heart transplantation may be in part due to inhibition of Nrf2 nuclear translocation.

## Bioartificial Organs: Cures for the 21<sup>st</sup> Century

Doris Taylor, PhD  
University of Minnesota

The definitive treatment for end stage organ failure remains allograft transplant, which in turn is limited by the number of donor organs. Developing innovative therapies for the treatment and cure of cardiovascular disease is the goal of the University of Minnesota's Center for Cardiovascular Repair. Our first approaches almost 17 years ago involved the transplantation of single populations of cells or genes into damaged myocardium – to prevent or reverse negative remodeling. This has progressed to clinical studies that currently involve a myriad of bone marrow-, blood- and tissue-derived stem or progenitor cells delivered into injured or failing heart. The status of cell therapy will be briefly reviewed.

Despite its use as an intervention, cell therapy is unlikely to be sufficient to restore myocardial function in end-stage heart failure. Instead, rebuilding whole or partial organs is likely to be required. Doing so requires, in most cases, supplying new cells, new vascular input and even a new microenvironment to regions of injury. Perfusion decellularized organ matrix provides a substrate for doing so. Not only does it provide a 3D testbed where the potential for regeneration and repair can be evaluated *in vitro* and *in vivo*, it is an architecturally correct 3-D perfusable microenvironment. As a result, the complex interplay between cells, their microenvironments, and the vascular network can be evaluated. More importantly, decellularized matrix provides a novel therapeutic tool that can be used alone or with cell therapy to promote repair. The ultimate goal is a transplantable organ. In addition, the *in vivo* use of acellular matrix as a cardiac repair tool and its implications clinically will be described.

# Pharmacogenetic Trial of Tacrolimus after Solid Organ Transplantation

Seema Mital, MD

Associate Professor of Pediatrics, Staff Cardiologist  
Hospital for Sick Children, University of Toronto

Tacrolimus is a common maintenance immunosuppressive agent used after solid organ transplantation. Achievement and maintenance of therapeutic levels is challenging. Subtherapeutic levels in the early post-transplant period increase the risk of rejection, while levels above the target range result in tacrolimus-related adverse events. Tacrolimus is metabolized primarily by the *CYP3A* enzymes in the liver particularly the *CYP3A5*. Age and *CYP3A5* genotype account for a third of the variation in tacrolimus dosing requirements and ~50% of the variation in the concentration/dose ratio with fast metabolizers i.e. *CYP3A5* expressors requiring significantly higher doses compared to slow metabolizers i.e. *CYP3A5* non-expressors to attain target therapeutic levels. The purpose of the proposed trial is to determine if age and *CYP3A5* genotype-guided dosing will result in early attainment and maintenance of therapeutic drug levels and reduce the frequency of out-of-range levels in the early post-transplant period. This will demonstrate the potential of personalized medicine to improve post-transplant outcomes.

## Ian McGilvray, MD

University Health Network

Assistant Professor of Medicine, University of Toronto

The attraction of genomics-based analysis is discovering new aspects of old diseases. Hepatitis C virus (HCV) infection is the single most common indication for liver transplantation, and recurs in all of the transplanted livers. Although new treatments are under investigation, how the host (the infected individual) responds to the virus is likely to be critical to how the patient responds to treatment. Treatment is based, to a greater or lesser degree, on stimulating aspects of the innate immune response with interferon (IFN) complemented with other agents, such as ribavirin or the newer direct acting antivirals. We have used genomics tools to identify a dichotomous host response to chronic HCV infection. This response is characterized by upregulated IFN-stimulated gene (ISG) expression in patients who then respond poorly to IFN-based therapy. The gene expression pattern is driven by individual cellular subsets in the liver (hepatocytes vs macrophages), and the cellular pattern of ISG expression is the most predictive of treatment response. Individual members of the targeted genes play surprising roles in fostering HCV infection. In particular, the ISG15/USP18 pathway appears to be central to the innate immune response and to a pro-HCV environment. The unfavourable pattern of gene expression is found in virtually all patients post-transplantation, highlighting the difficulty of treating patients in the post-transplantation setting. Overall, while whole organ gene expression patterns can point to new aspects of disease pathogenesis and be clinically useful, it is critical to identify the cellular source of the patterns in order to identify specific mechanisms of disease.

# Inflammatory Markers and Cardiopulmonary Exercise Testing in Continuous Flow Left Ventricular Assist Device Recipients

Liza Grosman-Rimon, David Cherney, Stacey Pollock Bar-Ziv, Laura Tumiasi, Michael McDonald, Ira Jacobs, Vivek Rao,

**BACKGROUND:** Continuous-flow left ventricular assist devices (CF-LVADs) have become standard of care for mechanical circulatory support in end stage heart failure. Although CF-LVADs restore cardiac output and end organ perfusion, impaired exercise capacity has persisted in these patients. Reduced exercise capacity is linked to elevated levels of the inflammatory markers in patients with heart failure. Accordingly, we hypothesize that in CF-LVAD recipients high levels of inflammatory markers are associated with impaired exercise capacity.

**METHODS:** The levels of inflammatory markers were compared between 12 CF-LVAD recipients and 12 healthy controls. Cardiopulmonary exercise testing was performed prior to CF-LVAD implantation and at least 3 months following implantation. The relationships between exercise capacity and inflammatory markers were examined in CF-LVAD recipients.

**RESULTS:** Following CF-LVAD implantation, improvement was observed in peak VO<sub>2</sub> (13.6±0.5 vs. 8.9±1.1 ml/kg/min, P < 0.05), % predicted VO<sub>2</sub> max (51.1± 2.3 vs. 32.9± 2.7, p<0.01), and heart rate recovery (22.1± 3.1 vs. 9.8 ± 3.2 beats/min, p<0.01) compared to heart failure baseline. Compared with healthy controls, in CF-LVAD recipients we found significantly higher levels of the inflammatory markers CRP, TNF- $\alpha$ , IP-10, MCP-1 and MDC. No significant difference was observed in the inflammatory markers IL-6, IL-1 $\beta$ , IL-8 and GM-CSF. Peak VO<sub>2</sub> is significantly negatively correlated with CRP levels in CF-LVAD recipients (r=-0.61, p=0.05).

**CONCLUSION:** This is the first study to find an association between impaired exercise capacity and high levels of inflammation in CF-LAVD recipients. Further studies should investigate the mechanisms in which inflammation contributes to impaired exercise capacity in patients supported with CF-LVADs.

# Normothermic Acellular Ex Vivo Liver Perfusion (NEVLP) for the Storage, Assessment, and Repair of Marginal Liver Grafts

Markus Selzner, MD

Markus U Boehnert, Jonathan Yeung, Mathias Knaak, Fateh Bazerbachi, Nazia Selzner, Shaf Keshavjee, Gary A Levy, David R Grant, Markus Selzner  
Multi Organ Transplant Program, Toronto General Hospital, Toronto, Canada

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

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

**Results:** 12hr acellular normothermic ex vivo liver perfusion followed by 12hr whole blood reperfusion was not associated with liver injury. Serum ALT (mean 27U/L) remained normal, and histology did not show any evidence of necrosis (<1%). Bile production (mean 3cc/hr), oxygen consumption (400mmHg), and BUN synthesis (1.8mmol/l) were within normal limits. In a second approach, NEVLP was compared to cold static storage in a DCD model using 1hr warm ischemia and 12hr preservation. NEVLP was associated with significantly decreased serum AST (26U/L vs 285U/L), decreased necrosis (10% vs 35%, p<0.05), and increased oxygen consumption (400mmHg vs 230mmHg, p<0.05). Cold static storage after DCD retrieval was associated with loss of peripheral arterial blood supply, while arterial blood flow was maintained in NEVLP preserved grafts. Accordingly, UW preserved grafts had massive biliary necrosis (90%), while bile ducts were normal in NEVLP preserved livers.

**Conclusion:** NEVLP allows prolonged organ storage with normal liver metabolism without inducing preservation injury. Bile duct injury after DCD liver retrieval develops in cold static, but not NEVLP preserved organs. NEVLP is a novel preservation technique for the assessment of marginal grafts.

## Biomarkers in Tolerance

Gary Levy, MD, FRCP(C)

Director, University of Toronto Transplantation Institute

Transplantation is now recognized as one of the major medical achievements of the last half century and in many instances serves as the only therapy for patients with end stage organ failure. Despite outstanding short term patient and graft survival rates, solid organ transplantation continues to face several major challenges including a shortage of organs and need for long term immunosuppressive therapy which limits its usefulness.

Understanding mechanisms of rejection which involve both innate and adaptive immunity would allow for novel therapeutic approaches to eliminate or avoid the use of toxic immunosuppressive agents. The evolving era of functional genomics in organ transplantation has been supported by advances in gene profiling, sequencing, proteomics, antibody profiling and bioinformatics, thus, heralding a new era of intelligent and personalized monitor and therapy. Molecular and cell based biomarkers are now emerging which may be useful to monitor the immune status of the patient and it is anticipated that over the next several years these will detect rejection for immune events before a transplanted organ or cell is damaged. Patterns of genomic biomarkers are also being developed which may predict patients who achieve tolerance which may not only be useful in the setting of transplantation, but also in patients with autoimmune disease. The ultimate goal of future studies will be to identify markers with sufficient predictive value to improve graft survival, limit graft injury from under immunosuppression and reduce patient morbidity from over immunosuppression.

In both experimental and human transplantation, we and others have demonstrated that monitoring the graft expression of a novel biomarker gene set using the multiplex RT-PCR GeXP analysis system may allow differentiation between rejection and tolerance. These studies may also allow approaches to induce tolerance in patients undergoing solid organ transplantation.

## **Non-HLA Antibodies and Risk of Rejection after Heart Transplantation: Profiling with Antigen Microarrays**

**Andrzej Chruscinski** MD, PhD, Flora Huang, Kathryn Tinckam MD., Vivek Rao MD, PhD, Heather Ross MD, Gary Levy MD - Multi-Organ Transplant, University Health Network – Toronto General Hospital, Toronto, Ontario, Canada

### **Introduction:**

Non-HLA antibodies have previously been implicated in heart transplant rejection. In order to more fully profile non-HLA antibodies, we generated custom antigen microarrays comprising 60 autoantigens and probed the microarrays with pre-transplant serum.

### **Hypothesis:**

Non-HLA antibodies are elevated in heart transplant recipients who experience significant rejection as compared to recipients who do not experience rejection.

### **Methods:**

Patients who developed significant rejection (two or more episodes of ISHLT 2R rejection over the first year) were classified as rejectors (n=8) while patients who did not experience rejection were classified as non-rejectors (n=16). Antigen Microarrays were constructed by spotting proteins onto FAST slides using a microarrayer. After blocking the slides, they were probed with diluted serum. They were then probed with a fluorescently labelled secondary antibody. The SAM algorithm was used to detect significant changes in antigen reactivities.

### **Results:**

Patients in the rejector group were younger (37.3 vs. 51.2 years), more likely to be female (75% vs. 44%) and were more likely to have a non-ischemic cardiomyopathy (100% vs. 62%) as compared non-rejectors. After antigen microarrays were performed, SAM analysis revealed eight antigen reactivities that were significantly elevated (Q value < 0.05) in the rejector group. Interestingly, we identified enhanced reactivity to cardiac myosin in the rejector group. In addition, we observed reactivity to other antigens such as troponin I, single-stranded DNA, and Ribosomal Protein P to be elevated in the rejector group.

### **Conclusions:**

Patterns of non-HLA antibodies in pre-transplant serum may identify patients at increased risk of rejection after heart transplantation.

# Functional Genomics Applied to Transplantation Biology: From Discovery to Biomarkers

Daniel R. Salomon, MD

Professor, Molecular and Experimental Medicine

The Scripps Research Institute

We started with two challenges. The remarkable advances in technology for interrogating the information contained and flowing constantly from the human genome generates such a complex, global view of biology that it is presently beyond our ability to reduce to simple, functional mechanistic explanations of events in health and disease. However, this technology and its compelling new views of biology begs the question of how it can be applied to change the practice of medicine. In effect, what tools and experimental strategies must be developed to systematically reduce this complexity to a sufficient reductionist level that patients will benefit and scientists will be guided to the next generation of insights. For focus, we chose transplantation biology and immunology. We believe that discovery informed by a pragmatic understanding of our clinical discipline will guide biomarker development in transplantation and drive both direct applications to practice and hypothesis-driven mechanistic studies. We also believe that functional genomics and systems insights must be pursued with multiple technologies and the results integrated to achieve the objectives. The discussion will relate two evolving stories from our efforts that are chosen to illuminate how we are using functional genomics to go from discovery to biomarkers. One story involves the epigenetics of transplantation immunity and the other story describes the discovery of a novel intersection between chronic rejection and the microbiome. I will also briefly review the progress we have made in identifying blood gene expression and proteomic biomarkers for acute and chronic kidney transplant rejection. These are all work in progress but we are beginning to see the shape of how functional genomics will change medicine.

This conference has been generously sponsored by:



The guest speakers have been sponsored by:

