CIRTA XVIII Basic Science Postgraduate Course

Friday, June 30th, 2023

Course Directors:

Tomoaki Kato (Columbia Univ, USA)

James Mathew (Northwestern Univ, USA)

Learning Objectives & Talk Description

Presenter: **Alexander Kroemer (Georgetown Univ, USA)**
Time: 1:10pm – 1:35pm
Title: Basics of Immunology in Intestinal Transplant

Learning Objectives:

1. To provide an understanding of the key immune system components and their roles in intestinal transplant rejection and tolerance.

2. To explore the immunological challenges specific to intestinal transplantation and the strategies used to overcome them.

3. To discuss the latest advancements in immunosuppressive therapies and their implications for improving intestinal transplant outcomes.

Talk Description:

This presentation will provide an overview of the fundamental concepts in immunology as they pertain to intestinal transplantation. Attendees will gain an understanding of the immune system's key components and their roles in transplant rejection and tolerance. The talk will highlight the unique immunological challenges associated with intestinal transplantation, such as the gut's high lymphoid content, and continuous exposure to external antigens. The presentation will also cover the strategies employed to overcome these challenges. Additionally, the latest advancements in immunosuppression, such as the use of novel biologics, will be discussed in the context of their potential to improve intestinal transplant outcomes. The aim of this talk is to provide a solid foundation in the basics of immunology in intestinal transplantation, enabling attendees to better understand the complex interplay between the immune system and graft survival.

Presenter: **Joseph Leventhal (Northwestern Univ, USA)**
Time: 1:35pm – 2:00pm
Title: Chimerism and Tolerance

Learning Objectives:

1. Recognize the treatment burden associated with chronic post-transplant immunosuppression.

2. Review scientific basis for investigational cell therapies with the potential to induce tolerance in kidney and liver transplant recipients .

3. Review outcomes in translational approaches to tolerance induction in solid organ transplant.

Talk Description:

This talk will provide an overview of approaches to use therapeutic cell transfer to achieve transplantation tolerance in solid organ transplantation. The need for minimization of immunosuppression related to the long term limitations of drug based IS will be reviewed. Mechanisms of chimerism and non chimeric operational tolerance will be reviewed. The current state of translational research approaches to tolerance induction will be provided.

Presenter: **James Mathew (Northwestern Univ, USA)**
Time: 2:00pm – 2:25pm
Title: Immunity and Regulation by Chimerism-Inducing Cells

Learning Objectives:

1. To understand the immune reactions brought about by chimerism inducing cells.

2. To learn about the anti-donor and anti-recipient immune responses possibly taking place in the recipient because of donor hematopoietic stem cell (DHSC) infusion.

3. To understand the different immunoregulatory properties of DHSC and cells that develop and differentiate from them.

Talk Description:

Donor hematopoietic stem cells (DHSC) are the principal component of cellular infusions used for clinical transplant tolerance induction. DHSC when infused in to patients can cause several immunological effects in the recipient: (i) DHSC can respond to the recipient causing GVH reactivities, (ii) DHSC can modulate this anti-recipient immunity thus attenuating the GVH responses, (iii) DHSC can stimulate immune responses thus sensitizing the recipient against the donor, and above all, (iv) DHSC and cell subsets developed from them can inhibit the anti-donor immune responses, i.e., induce immune unresponsiveness or tolerance in the recipient. The presentation will review currently available results, particularly from our laboratory on these paradoxical immune reactivities.

Presenter: **Adriana Zeevi (Univ of Pittsburgh, USA)**
Time: 2:25pm – 2:50pm
Title: Biomarker Updates

Learning Objectives:

1. Why there is an increasing need to examine non-invasive biomarkers to improve risk stratification and prediction of allograft rejection.

2. The mechanistic basis for Pleximmune™ blood test and how it is used for clinical management.

3. How donor-specific HLA antibodies (DSA) are detected and characterized to evaluate the risk for antibody mediated rejection and to monitor efficacy of intervention.

Talk Description:

Improvements have been achieved in short-term graft and patient outcomes following intestinal transplantation, however long-term survival is still a significant problem. There is increased interest in the implementation of non-invasive biomarkers to reduce the risks associated with invasive biopsies to monitor allograft injury.

Pleximmune™ blood test measures the immune response of recipient T cells to the donor in co-cultures of lymphocytes from both sources. The frequency of recipient T-cytotoxic memory cells that express the inflammatory marker CD154 (CD154+TcM) is assessed by flow cytometry. Results are expressed as a proportion of CD154+TcM induced by a reference cell that is different from the recipient and donor. An increased immunoreactivity index (IR) ≥1.1 has been shown to predict allograft rejection with a sensitivity and specificity of 84% and 80%, respectively.

Donor-specific anti-HLA antibodies (DSA) present pre- or de-novo developed post-transplantation contribute to inferior graft survival and have been associated with a broad spectrum of allograft damage. However, not all HLA antibodies are equally detrimental and certain characteristics, such as HLA-DQ specificity, titer and complement binding, have been associated with pathogenesis and deleterious effects. Incorporating the characteristics of DSAs can provide a better approach to risk stratify sensitized transplant candidates pre- transplant and to assess the risk for antibody mediated rejection post-transplant.

Donor-specific cell free DNA (ddcfDNA) shows promise as a biomarker for detection of acute allograft injury in solid organ transplants with >90% negative predictive value (NPV). Steady

state of ddcfDNA differs based on organ type and is highest in liver and lung. Very little information is available in intestinal transplantation.

Ongoing goals of exploring biomarkers include the prediction of allograft injury due to cellular and humoral alloreactivity induced by HLA disparity but also to avoid overimmunosuppression associated with infection and drug toxicity.

Presenter: **Jianing Fu (Columbia Univ, USA)**
Time: 2:50pm – 3:15pm
Title: 101 of Immunogenomics

Learning Objectives:

1. Understanding what immunogenomics is and its application in clinical medicine, especially human organ transplantation.

2. Get familiar with 1) common techniques, such as high throughput T and B cell repertoire sequencing, single cell RNA sequencing and multiomics; 2) frequently used algorithms to determine repertoire diversity (clonality, R20, JSD, abundance plot slope), TCR structural similarity (GLIPH2, tcrdist3) and visualize transcriptomic profiles (tSNE, UMAP); and 3) publicly accessible databases (TCRdb, VDJdb, and PIRD) involved in immunogenomic studies in transplantation field and beyond.

3. Understand how to apply immunogenomics in deciphering allograft rejection and tolerance after human organ transplantation through example studies in human kidney transplantation and intestinal transplantation.

Talk Description:

This presentation will touch base on immunogenomics and its application in clinical medicine, especially human organ transplantation. With the nature of information science, immunogenomics is an emerging field that brings together experts in genomics, immunology, computational biology, and clinical research to tackle the complexity of disease etiology and treatment outcomes. Recent advances in next-generation sequencing technologies have enabled new opportunities to provide a precise picture of the tremendously diverse immune repertoire, including T cell receptors and B cell receptors, and to resolve fundamental questions of alloimmunity. Common techniques, such as high throughput T and B cell repertoire sequencing, single cell RNA sequencing and multiomics, frequently used algorithms to determine repertoire diversity (clonality, R20, JSD, abundance plot slope), TCR structural similarity (GLIPH2, tcrdist3) and visualize transcriptomic profiles (tSNE, UMAP), and publicly accessible databases (TCRdb, VDJdb, and PIRD) involved in immunogenomic studies in transplantation field and beyond will be introduced. By providing example studies in real world, the audience will get understand how to apply immunogenomics in deciphering allograft rejection and tolerance after human organ transplantation, including kidney transplantation and intestinal transplantation.

Presenter: **Mihai Oltean (Univ of Gothenburg, Sweden)**
Time: 3:40pm – 4:05pm
Title: Intestinal Preservation for Transplantation: A Primer

Learning Objectives:

1. Understand the various mechanisms involved in the development of intestinal preservation injury.

2. Learn about the main strategies aimed to mitigare the development of intestinal preservation injury explored so far.

3. Learn about the most recent developments and research trends on intestinal preservation injury.

Talk Description:

The intestine is very susceptible to ischemia and tolerates the shortest cold preservation time (less than 10 hours) among the transplantable abdominal organs. The current practice for the intestinal preservation (IP) consists of an in-situ vascular flush with cold organ preservation solutions followed by static cold storage at 4°C. Mucosal injury develops within 1 hour and rapidly progresses to mucosal breakdown; tissue injury worsens upon reperfusion and further impairs the mucosal barrier, favoring bacterial translocation and sepsis. Several alternative approaches have been tested as alternatives to the static storage including ischemic preconditioning, gasseous interventions, pharmacologic interventions and additional luminal introduction of various solutions. Recently, more dynamic approaches including luminal or vascular perfusion have been explored. The greatest limitation of all these approaches is that virtually all research has been performed in rodents, with no or limited translational research in large animal models or humans. This lecture will summarize and discuss the underlying pathophysiological mechanisms of intestinal preservation injury, the research performed so far and the potential windows of opportunity for protective interventions.

Presenter: **Jenny Zhang (Northwestern Univ, USA)**
Time: 4:05pm – 4:30pm
Title: Surgical Techniques for Rodents

Learning Objectives:

1. Demonstrate greater understanding of key characteristics of intestinal transplant models in rodents including heterotopic intestinal transplantation vs orthotopic intestinal transplantation.

2. Be able to set up necessary lab equipment, surgical instruments and practice the microsurgical techniques for small bowel transplantation, independently.

3. Get familiar with post-transplant monitoring, identify surgical complications, clinical sign of allograft rejection and pattern of intestinal allograft rejection in different genetical background of animal strain combination.

Talk Description:

Intestinal transplantation has emerged as an effective treatment for intestinal failure, but its relatively high graft rejection rate and mortality rate when compared to those of other transplanted organs has led to difficulties in post-transplantation treatment management. Rodent models have been used to study the unique interaction between graft derived mucosal immunity and host rejection response. However, the techniques needed to perform small bowel transplant procedures in rodents, especially in mice, require skilled microsurgeons, as well as specific dedicated equipment and instruments. The proficiency in experimental microsurgery is essential and profoundly affects consistency and reproducibility of research results. This course will start with introduction of general features of commonly used small bowel transplant models and its variants; will discuss surgical techniques in great details, including step by step surgical maneuvers, key elements, technique challenges and surgical complications, learn curves as well as pre-transplant care and post-transplant monitoring. In addition, this course will discuss preparation of microsurgical instrument and lab set up including space and supplies. Finally, this talk will briefly discuss pattern of allograft rejection and animal straining differences and applications/implications.

Presenter: **Francisco Hernandez (Hospital La Paz, Spain)**
Time: 4:30pm – 4:55pm
Title: How to Set Up A Large Animal Model

Learning Objectives:

1. To acknowledge the importance of the anatomic and physiologic features of different experimental models.

2. To understand the importance of multidisciplinary team, chronogram and resources organization in experimental research.

3. To be able to choose the appropriate experimental model according to the aim of the project.

Talk Description:

The selection of the proper animal model is crucial for the success of a research project. Large animal models are variable in anatomy and physiology and requires different facilities. A common feature to all of them is that large animal models cannot be managed by the researcher with just little assistance like rodent models, on the contrary, a multidisciplinary team is usually required. This presentation will describe briefly the main large animal model that has been employed in transplant research, with particular focus on intestinal transplant research. The differences in anatomy and physiology will be discussed. A real experience in launching a large animal model for DCD will be presented. Finally, the attendees will be invited to propose a large animal model for some topic related to intestinal rehabilitation and transplantation.

Presenter: **Flavio Galvao (Univ of São Paulo, Brazil)**
Time: 4:55pm – 5:20pm
Title: Xenotransplantation

Learning Objectives:

1. Xenotransplantation or interspecies transplantation is a prospective method for the shortage of human donors and to reduce the mortality rate on the waiting list.

2. Advancements in genetic engineering swine technology have demonstrated remarkable potential overcoming swine-to-human xenotransplantation complications such hyperacute rejection, porcine endogenous retrovirus infection and physiological incompatibilities.

3. Xenotransplantation using genetically modified swine may solve the shortage of organs for transplantation; however, bioethical issues concerning this prcedure, mainly related to the undefined risk of xenozoonotic infection, have endured and persisted.

Talk Description:

The great success of allotransplantation increased organ donation demand that outpaced the available supply. This situation contributes to an important mortality rate in the transplantation waiting list worldwide. Xenotransplantation is a potential method to solve the shortfall of human organ donors. Recent advances in gene-editing technology including CRISPR/Cas9 systems, allowed the creation of genetically modified swine by deleting several genes related to the hyperacute rejection and by inserting human complement- and coagulation-regulatory transgenes to induce xenograft immune tolerance. Genetic engineering swine may also overcome the physiological incompatibilities and epidemiological concern (porcine endogenous retrovirus infection) related to swine-to-human xenotransplantation. In January 2022, a heart from a swine with 10 individual gene edits was transplanted into a 57-year-old man. This historic milestone brought great hope to thousands of patients waiting for transplantation. The xenograft functioned well for more than 40 days, but the patient died on day 60 probably due to graft rejection and porcine cytomegalovirus pulmonary infection. Despite the setback, this pioneer xenotransplantation demonstrated great potential and provided valuable insights for future researches in this field; however, bioethical issues such the undefined risk of xenozoonotic infection, have endured and persisted today. Further studies are necessary to overcome the remaining challenges and ensure the safety and efficacy of xenotransplantation for clinical use.