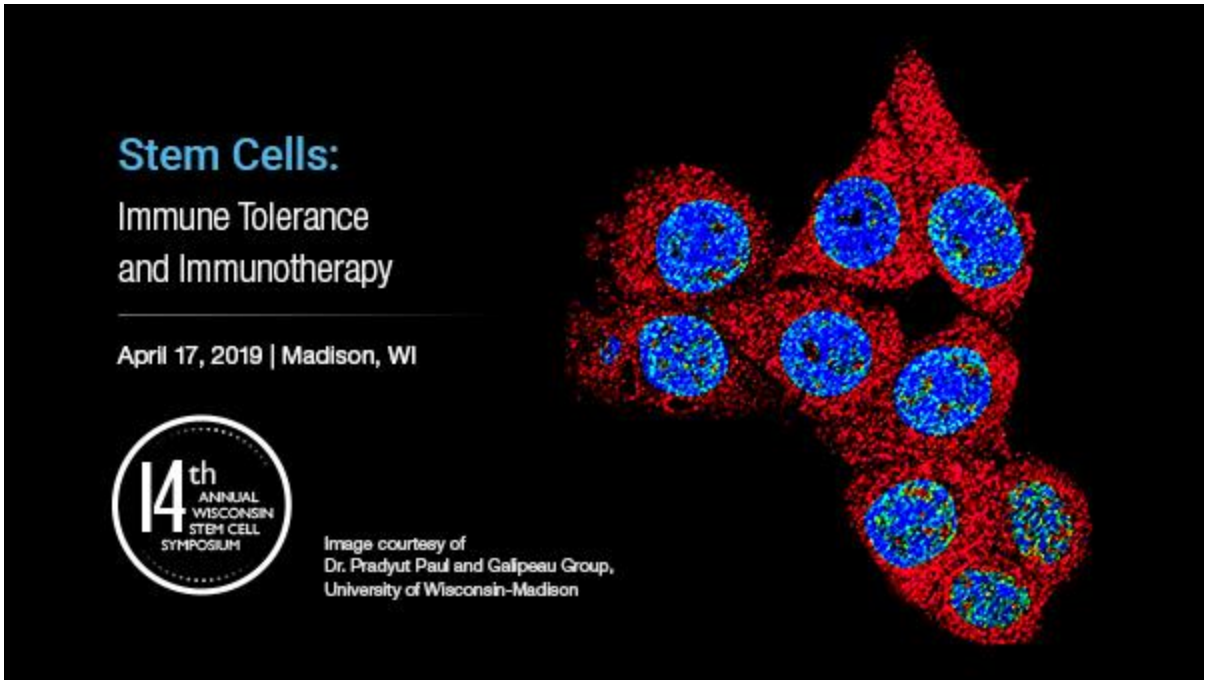

SPEAKER ABSTRACTS

Achieving durable immune tolerance of curative pluripotent stem cell Therapies, in the absence of immunosuppressive drugs
Matthew E. Brown, Ph.D.

Pluripotent stem cells (PSCs) have the potential to enable curative, patient-specific cellular therapies for cardiovascular pathologies and other diseases affecting millions of Americans. In recent years, tremendous progress has been made in improving PSC differentiation protocols to yield promising cell products that can mimic the function of normal primary cells. However, even a perfectly functional transplant graft can be swiftly rejected by a patient's immune system. It is therefore crucial that we gain an in-depth understanding of the mechanisms of PSC immunogenicity, in order to devise effective strategies to avoid rejection and achieve durable tolerance of these promising therapies. Humanized mice and non-human primates are two very useful models for evaluating the in vivo immune response to transplanted primary tissues and PSC-derived grafts. This presentation will describe the recently developed NeoThy humanized mouse model, which incorporates neonatal, rather than fetal, tissue for human immune cell reconstitution in the mouse host. Additionally, a recently completed arterial transplant study in MHC-haplotyped Mauritian Cynomolgus Macaques will be discussed. These in vivo models, in conjunction with in vitro assays of immunogenicity, will be crucial experimental tools for translating PSC discoveries to the clinic.

Who is orchestrating repair – immune cells or stem cells?
Jennifer H. Elisseeff, Ph.D.
[TBA]

Multipotent adult progenitor cells suppress T cell activation in vivo
Karen E. English, Ph.D.

In the setting of transplantation, T cells are deliberately depleted to prevent allograft rejection. However, this IL-7 activates the accelerated proliferation of effector memory T cells, which are the predominant mediators of graft rejection. Multipotent adult progenitor cells (MAPC) have been previously shown to modulate the response of T cells to IL-7, *in vitro* but not *in vivo*. Here, for the first time, we demonstrate that MAPC suppress IL-7 and anti-thymocyte (ATG) driven pro-inflammatory cytokine production by both $CD4^+$ and $CD8^+$ T cells in the spleen. While, MAPC significantly suppressed T cell proliferation in the IL-7 driven model, MAPC did not suppress T cell proliferation in the ATG model when administered on day 4. The effects of routes of administration on the bio-distribution of MAPC in our model of homeostatic proliferation was further clarified using novel 3D whole animal Cryo-imaging technology. MAPC administered i.v. were observed in the spleen 48 hours post injection. However, following i.p. administration, MAPC do not gain access to the spleen but reside in the omentum tissue suggesting that MAPC mediate their effects in the spleen through trophic signalling. Importantly, we demonstrate that MAPC mediate their suppression of pro-inflammatory cytokine production in a PGE-2 dependent manner. Given the importance of successful reconstitution of the immune cell compartment to provide a functional immune system, the fact that MAPC do not suppress this reconstitution but dampen pathogenic cytokine production is a very positive finding. Together this data supports the idea that MAPC may be useful in controlling immune dysregulation via suppression of T cell cytokine production while sparing T cell reconstitution in lymphodepleted patients.

Mesenchymal Stromal Cells – pre-clinical science, clinical development and pathway to FDA marketing approval
Jacques Galipeau, MD FRCP(C)

Mesenchymal Stromal Cells have been the subject of clinical trials for more than a generation and the outcomes of advanced clinical trials have fallen short of expectations raised by encouraging pre-clinical animal data in a wide array of disease models. Important biological and pharmacological disparities in pre-clinical research and human translational studies are highlighted and analysis of clinical trial failures and successes provide a rational pathway to MSC regulatory approval.

Improved cancer therapy using engineered human pluripotent stem cells
Dan Kaufman, M.D., Ph.D.

Production of therapeutic blood cells such as transplantable hematopoietic stem cells (HSCs) from hESCs and iPSCs remains a key goal. However, despite intensive research efforts by our group and many others, most studies have not been able to use hESCs/iPSCs to produce HSCs capable of long-term multi-lineage engraftment when transplanted into immunodeficient mice, unless the hESCs/iPSCs are genetically modified. In contrast to production of transplantable HSCs, the ability to use hESCs/iPSCs to produce functional

lymphocytes with anti-tumor and anti-viral activity has been quite successful. Our group routinely utilizes a 3-stage process to efficiently produce natural killer (NK) cells that can be expanded to clinical scale. These hESC/iPSC-derived NK cells have phenotype and genetic profile similar to NK cells isolated from peripheral blood. Additionally, these hESC/iPSC-NK cells are able to kill diverse tumor cells in vitro and in vivo. The hESCs/iPSCs also serve as an important platform to engineer genetic enhancements to produce NK cells with improved anti-tumor activity. For example, we have produced hESC/iPSC-derived NK cells that express novel chimeric antigen receptors (CARs) that are able to better target tumors that are more refractory to NK cell-mediated killing. This NK-CAR construct utilizes the NKG2D transmembrane domain, 2B4 co-stimulatory domain, and the CD3ζ signaling domain to activate key NK cell-specific intracellular signaling pathways and increase NK cell survival and expansion in vivo. We have engineered other modifications into iPSC-NK cells to enhance NK cell targeting, proliferation, expansion and survival that are all key qualities to improve in vivo anti-tumor activity. These studies demonstrate that hESC/iPSC-provide an ideal platform to produce standardized, targeted, “off-the-shelf” cellular immunotherapies to treat refractory hematological malignancies and solid tumors. Finally, iPSC-derived NK cells have now been produced at clinical scale under current good manufacturing practices (cGMP) conditions with clinical trials recently initiated.

Off-the-shelf iPS derived NK cells to treat cancer

Jeffrey S. Miller, MD

Natural killer (NK) cells are capable of immune surveillance mediated by a balance of activating and inhibitory receptors. We have shown that adoptive transfer of NK cells can induce complete remissions in patients with refractory acute myelogenous leukemia (AML) when combined with lymphodepleting chemotherapy and IL-2, however; one of the limitations is that IL-2 can stimulate regulatory T cells (Treg) resulting in immune suppression. We have studied the ability of IL-2/DT to eliminate Treg and more recently, IL-15, because it does not bind Treg. Using these approaches, 30-50% of patients with refractory AML attain clinical remissions as a bridge to transplant. Yet, NK cells are limited by longevity after adoptive transfer and the lack of antigen specificity. To address longevity, we have discovered that “adaptive” NK cells induced by CMV are long-lived, highly functional and exhibit properties of immune memory. In addition, we have explored IL-15/IL-15R□-Fc (ALT-803), an IL-15 superagonist complex, which may be more optimal to present IL-15 to the immune system. Clinically, ALT-803 induces potent stimulation of NK and CD8+ T cells in vivo and clinical responses including a complete remission lasting 7 months. This has led to a relapse prophylaxis study with ALT-803 to promote immune reconstitution after reduced intensity conditioning transplantation. To make NK cells antigen specific, we have developed trispecific killer engagers (TriKEs). We have previously shown that bispecific killer engagers (BiKEs) are capable of creating immunologic synapses between NK cells and CD33 antigens on AML and MDS targets leading to NK cell signaling through the highly potent CD16 (FcγRIII) receptor. We observed that although CD16 engagement leads to enhanced killing and cytokine production by NK cells, there is no effect on proliferation. Because IL-15 is the homeostatic factor for NK cells, we developed a TriKE that includes a modified human IL-15 crosslinker sandwiched between single chain Fv against CD16 and CD33. The 161533 TriKE was highly specific to CD33+ targets and induced NK cell specific proliferation in vitro and in mice in vivo. This has been the motivation to brings these TriKEs to the clinic upon FDA approval, expected this summer. For those settings where endogenous NK cells are absent or suppressed, use of TriKEs will require

adoptive transfer of healthy NK cells. Our data suggests that adaptive NK cells can be enriched from CMV+ donors after culture with IL-15 and a GSK3 inhibitor, a novel NK cell product in phase I clinical trials. Lastly, new strategies using off-the-shelf NK cells from induced pluripotent stem cells (iPSC) are being developed and will be in the clinic by the end of the year. This will allow multiple dosing of cryopreserved "living drugs" to treat patients with cancer. We have shown that iPS derived NK cells have potent activity and alter the homing of T-cells. Adaptive NK cells and genetically modified iPSC NK cells expressing a high affinity ADAM17 cleavage resistance CD16 should be optimal for targeting with TriKEs or other already approved anti-cancer antibodies. In summary, NK cell based immune therapies offer great potential for off-the-shelf cell therapy strategies alone and in combination against hematologic malignancies and solid tumor cancers.

Immune rejection of allogeneic cell transplants derived from iPSCs is prevented by genetic engineering

Sonja Schrepfer, M.D., Ph.D.

Autologous induced pluripotent stem cells (iPSCs) constitute an unlimited cell source for patient-specific cell-based organ repair strategies. However, their generation and subsequent differentiation into specific cells or tissues entail cell line–specific manufacturing challenges and is a lengthy process which precludes acute treatment modalities. These shortcomings could be overcome by using prefabricated allogeneic cell or tissue products, but the vigorous immune response against histo-incompatible cells has prevented the successful implementation of this approach. Here we show that both mouse and human iPSCs lose their immunogenicity when major histocompatibility complex (MHC) class I and II genes are inactivated and CD47 is over-expressed. These hypo-immunogenic iPSCs retain their pluripotent stem cell potential and differentiation capacity. Endothelial cells, smooth muscle cells, and cardiomyocytes derived from hypo-immunogenic mouse or human iPSCs reliably evade immune rejection in fully MHC-mismatched allogeneic recipients and survive long-term without the use of immunosuppression. These findings suggest that hypo-immunogenic cell grafts can be engineered for universal transplantation.

Biomaterials strategies to promote brain repair after stroke

Tatiana Segura, Ph.D.

[TBA]