Cellular Production of MSCs for vocal fold regeneration

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Having a voice and the production of speech is uniquely human. Impairments in vocal production have far-reaching social, emotional, and occupational consequences such that persons with voice problems have nearly as many days of short-term disability claim and work productivity losses as those with chronic conditions like asthma, heart disease, and depression. Voice disorders secondary to scarring of the vocal folds are difficult to treat effectively with current surgical paradigms and available biomaterials. Accordingly, there is a clinical need for the development of advanced treatments that appreciate the extracellular matrix structure and biomechanical properties of the vocal folds. Novel cellular products are being investigated to fill this treatment gap by providing advanced treatment modalities using a specialized hydrogel formulation containing bone marrow mesenchymal stem cells (BM MSC) or BM MSC alone. Products that could be utilized to treat scarring of the vocal folds would ideally be nonimmunogenic, nontoxic, noninflammatory and easily injectable. The long-term aim of our work is to translate our bioengineered injectable product, seeded with BM MSC and shown in animal studies to induce tissue regeneration of the vocal fold, into a human clinical product for the treatment of vocal fold scarring. Over the past ten years, promising reports of BM MSC based therapies for regeneration of scarred vocal fold tissue in animal models have been published. Further, a scientific rationale for BM MSC-mediated vocal fold scar attenuation is similar between vocal fold fibroblasts (hVFF) and BM MSCs. The most abundant cells in the vocal fold lamina propria are fibroblasts. During a wound healing response in the vocal fold, fibroblasts migrate to the site of injury and remodel the tissue by generating an ECM (extra cellular matrix), however a native supply of healthy hVFF is not available for therapeutics because the tissue source (normal vocal fold from live donors) is virtually impossible to obtain. Our group has previously demonstrated similar cell surface markers, immunophenotype, and differentiation potential between hVFFs and BM MSCs. Specifically, two hVFF primary cell lines (p59 and p21) expressed MSC markers CD73, CD90 and CD105, and were negative for CD14, CD34 and CD45. hVFFs were able to differentiate toward osteogenic, adipogenic and chondrogenic lineages and showed similar immunological phenotypes as BM-MSCs. The similarity between these two cell types provides support for the investigative use of BM-MSCs in the vocal fold.

Our cell therapy product includes the use of a hyaluronic acid (HA) hydrogel produced by Biotime -- HyStem-VF and MSCs. HyStem-VF has undergone rigorous biomechanical, biocompatibility, safety and toxicity testing in vitro and in vivo over the past ten years by our laboratory. Most relevant to this therapeutic approach, it
has been documented that the restorative effects of MSCs can be amplified when delivered to scarred vocal fold tissue within an appropriate scaffold such as HyStem-VF. The synergistic effects of this combination therapy are promising. One underlying mechanism supporting the anti-inflammatory profile of this therapeutic is macrophage activation. It was recently reported that macrophages cultured with BM MSCs/HyStem-VF *in vitro* are more likely to have an anti-inflammatory immunophenotype (lower expression of CD16 and HLA-DR and higher expression of CD206) than macrophages cultured on tissue culture plastic or those cultured on HyStem-VF without BM MSCs.9

Work in collaboration with the NHLBI Production Assistance for Cellular Therapies (PACT) group at the University of Wisconsin - Madison (Contract No. HHSN268201000010C) has included development of final product formulation, assay development, and delivery methods to support the translation of this technology from the research environment into the clinic. Quality Control assays were developed to support in-process and final product lot release testing. In an effort to create an 'off the shelf' product for commercial use, product formulation and delivery has been optimized and specifications developed so that the treatment is standard across various conditions/subjectsclinical sites. Assays have determined a favorable protocol for consistent cell mixing and uniform dispersion throughout the gel and identified an ideal gelation time for a clinically relevant number of cells within HyStem-VF or in isolation. Because the ideal treatment of vocal fold scarring requires direct injection into the tissue, the effect of shearing and thinning on the viability of the BM MSC was evaluated using a variety of clinically relevant delivery devices. The University of Wisconsin Madison PACT produced bone marrow-derived, allogeneic human MSCs from a fully characterized and tested Master Cell Bank. Final dosages formulation included cryopreserved human clinical doses using qualified materials and reagents to support human clinical studies. Lastly, to ensure quality control, the development of a quantitative potency assay was crucial for monitoring the biological activity and ensuring the consistency of the MSC product. Identification of surrogate markers for the relevant anti-inflammatory, immunomodulatory and paracrine activity of the MSC will ensure consistent quality of clinical grade material; the markers are based on the attributes of the product that have been linked to treatment efficacy in preclinical studies. This critical development effort is necessary to perform pre-clinical safety studies using clinical-grade material to support an IND application which is required to translate our work into a human Phase I clinical trials for patients with vocal fold scarring.

While our research questions specifically address the clinical problem of vocal fold defects, we expect that our research and resultant findings will be valuable to others investigating tissue engineering of connective tissue in other physiological systems. Because we are studying the problem of scarring and the permanent restoration of appropriate tissue biomechanics, our BM MSC construct tissue engineering approach will be applicable to a wide range of medical fields. More specifically our cell production manufacturing, processing and delivery method refinement will be of interest to a large number of investigators who anticipate utilization of MSC in similar tissue engineering constructs including those whose areas are under the auspice of the National Heart, Lung, and Blood Institute.

References
PI Highlight: Ann Leen, PhD

PACT Center: Center for Cell and Gene Therapy (CAGT), Baylor College of Medicine, Texas Children’s Hospital
Adrian Gee, PhD - Principle Investigator
Cliona Rooney, PhD - Co-Investigator

Website links:
Hospital page: https://www.bcm.edu/research/centers/cell-and-gene-therapy/faculty-leen
Assistant Professor
Department of Pediatrics - Hematology and Oncology
Center for Cell and Gene Therapy

PACT Projects:
- Allogeneic multivirus-specific CTL for infusion post hematopoietic stem cell transplant (HSCT)
- In vitro expanded virus-specific cytotoxic T lymphocytes (CTLs) as prophylaxis/treatment for infections associated with EBV, CMV, Adenovirus, BK virus and HHV6.

Q: Please describe your clinical and research interests.
One of the primary goals of my laboratory is to develop and test novel T-cell based therapies for the prevention and treatment of viral infections, which remain a major cause of mortality and severe and prolonged morbidity in the allogeneic hematopoietic stem cell transplant (HSCT) setting. We and others have previously demonstrated that the adoptive transfer of in vitro generated virus-specific T cells (VSTs) specific for Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenovirus (Adv) antigens can treat infections that are impervious to conventional therapies, but broader implementation and extension to additional problematic viruses has been limited by competition between viral antigens and time-consuming and laborious manufacturing procedures – issues that PACT funding helped us to address.

Q: Can you describe the projects in which PACT has supported product manufacturing?
In the allogeneic stem cell transplant setting we have produced virus-specific T cells for the prophylaxis and treatment of virus-associated malignancies and to date, we have infused T cell lines to more than 200 patients. However, traditionally our method for generating VSTs relied on multiple rounds of in vitro T cell stimulation using virus/viral vector-transduced antigen presenting cells (APCs) to stimulate T cell specificity which was expensive, time consuming and labor intensive, limiting applicability. Further, we saw evidence of “antigenic competition” in our trivirus (EBV+CMV+Adv) VSTs, which limited the range of viruses that could be recognized and thus reduced the overall potency of the effector cells that we produced. To address these issues we developed a rapid manufacturing process using dendritic cells nucleofected with plasmids encoding immunogenic antigens from EBV, CMV and Adv as APCs to stimulate virus-specific T cells, followed by a 10-day expansion in a novel cell culture device (G-Rex). Using all preclinical assessment tools available, we were able to show that these “rapid” rVSTs were equivalent to conventionally generated VSTs. However, the clinical testing of this product – the ultimate test of safety and activity – proved impossible to fund via traditional (NIH) routes since the work was concerned to be process development and lacking in novelty. PACT, however, saw the potential of this work and the direct impact that it could have in allogeneic HSCT recipients and thus they supported the clinical manufacture of rVSTs. To date, we have treated eleven patients with active Adv, EBV or CMV infections, and achieved complete responses in nine - the manuscript describing the complete study results was recently published in Molecular Therapy (Gerdermann et al, 2013). However, to summarize the impact of this work, we took a process which entailed approx. 3 months of manufacturing with exposure to biohazards to make a VST line and streamlined it to a 17-day process, which when tested clinically performed as well as the lines made using our traditional strategies. This work has received substantial recognition from investigators in the stem cell transplant field including a Best Abstract Award at the American Society for Blood and Marrow Transplantation annual meeting in 2013.

In the meantime we continued to optimize our T cell manufacturing approaches. We wondered whether we could replace plasmids by directly exposing peripheral blood mononuclear cells to clinical grade overlapping peptide libraries, thus eliminating the requirement for dendritic cells is our cultures and further decreasing the production time from 17 to 10 days. In addition, we investigated approaches to minimize activation-induced cell death and found that culture of T cells in the presence of the cytokines IL7 and IL4 increased the frequency and repertoire of antigen-specific cells in our cultures, allowing us to accommodate additional antiviral specificities within our VSTs. Again, PACT stepped in to allow us to clinically make and test the clinical activity of T cell cultures with simultaneous specificity for Adv, EBV, CMV, BK and HHV6 (pentavalent VSTs). To date we have infused pVSTs to 11 allogeneic HSCT recipients and have seen no immediate infusional toxicities. Three patients received the cells as viral prophylaxis while the other eight received the cells as treatment for one or more active infections. The results of this study will be presented for the first time as an oral presentation at ASH this year (Papadopoulos et al) but to summarize we have seen that the pVSTs were able to control infection with all five targeted viruses. So essentially we can now infuse a single T cell product with broad-spectrum specificity that is substantially more cost-effective and less toxic that administering multiple antiviral agents, even if these were able to deliver the same breadth of protection.

Q: How has your partnership with the PACT team at CAGT impacted your clinical research?
The low toxicity and long term protection provided by virus-specific T cells compares favorably with the significant toxicities and short-term effects of most anti-viral drugs. However, complex and lengthy manufacturing strategies have been major barriers preventing the broader
**PI Highlight: Continued...**

Implementation of this approach. PACT funding has allowed us to develop and test novel, robust and scalable protocols for the generation of broad-spectrum VSTs. We hope, based on the promising clinical results that have been achieved to date, that these simplified strategies will help to advance T cell based therapies beyond specialized centers.

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**PACT Announcement**

The current contract award period for the PACT program will end on January 14, 2015. PACT will continue to accept and evaluate new applications until March 15, 2014. The timeline and scope of each application will be considered on an individual basis. PACT representatives will work with applicants to revise the timeline and milestones to determine how much work can be completed before the end of the contract. Proposed work that cannot be completed by the end of the contract will need to be funded through other mechanisms. Further updates will be provided as they become available.

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**PACT Cell Processing Facilities:**

- **Baylor College of Medicine Center for Cell and Gene Therapy**
  - Contract Number: HHSN268201000007C

- **University of Minnesota Molecular and Cellular Therapeutics Facility**
  - Contract Number: HHSN268201000008C

- **Center for Human Cell Therapy Boston**
  - Contract Number: HHSN268201000009C

- **University of Wisconsin - Madison Waisman Biomanufacturing Facility**
  - Contract Number: HHSN268201000010C

- **City of Hope Center for Applied Technology Development**
  - Contract Number: HHSN268201000011C

**PACT Coordinating Center:**

- **The EMMES Corporation**
  - Contract Number: HHSN268201000006C

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Ovine Models of Acute Lung Injury for Testing Novel Therapeutics

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The acute respiratory distress syndrome (ARDS) is a life-threatening condition associated with multiple organ dysfunctions and a high mortality rate. Clinically, ARDS is characterized by severe pulmonary edema leading to impaired pulmonary gas exchange (PaO2/FiO2 <300mmHg) (1). In the past decade, considerable advances have been made in the understanding of the pathophysiologic mechanisms, diagnostics and therapeutics of ARDS. Improved supportive care with lung protective ventilation (2, 3) for severe ARDS has improved survival. However, new therapies that decrease the magnitude of lung injury and hasten lung repair are needed.

Several clinical disorders have been associated with the development of ARDS, including pneumonia, sepsis, gastric aspiration, and major trauma (4). Injury to the lung endothelium and alveolar epithelium is mediated by activated neutrophils, monocytes and platelets as well as coagulation abnormalities, pro-inflammatory cytokines, oxidants, arachidonic acid metabolites, proteases and toxins released by bacteria, fungi and viruses (5). Many basic science and animal studies have been addressed those pathological changes, but no therapy has yet been advanced for use in clinical practice.

The multifaceted pathophysiology of ARDS and the failure of promising therapies relate to specific etiologic factors that require single or combined etiologically specific interventions. It is possible that the site and the severity of injury may vary depending on the etiology. For example, toxic inhalation or gastric aspiration may predominantly cause local airway or alveolar epithelial damage, whereas systemic causative factors such as sepsis may target mostly the lung endothelium.

To date, there have been nearly 150 clinical trials performed to modulate inflammatory responses in critically ill patients, and all of these trials have failed (6, 7, 8, 9). Most of these clinical trials have been translated directly from rodent studies. Seok et al (10) in their recent paper described substantial differences in the genomic responses of patients with trauma (burns and sepsis) and murine models of trauma and sepsis. Although there are many possible explanations for the failures of those clinical trials, on possible common factor is the lack of evidence from well-designed, clinically relevant studies in large animal models that confirm promising data from rodent studies. Such large animal studies should help investigators to better understand pathogenesis of lung injury and anticipate the effects of novel therapeutic interventions in patients.
In this regard, the Investigational Intensive Care Unit (IICU) (Department of Anesthesiology, the University of Texas Medical Branch, Galveston, Texas) provides a unique opportunity. In 2008, the IICU moved into a new facility that was designed especially as a large animal intensive care unit. It consists of three completely-equipped hospital-type operating rooms, 24 intensive care bays and a necropsy room. In addition, an isolated suite with 7 intensive care stations allows performance of BSL-2 level studies. The IICU offers multiple clinically relevant and highly translatable large animal studies: 1) ovine ALI/ARDS and pneumonia/sepsis model induced, while under anesthesia, by instillation of various Gram-negative and Gram-positive bacteriological pathogens into the lungs, then permitted to awaken for an interval of intensive care and assessment of novel therapeutic interventions (11, 12); 2) ovine ALI/ARDS model induced, while under anesthesia, by toxic gas inhalation such as cotton smoke and chlorine gas, then permitted to awaken for an interval of intensive care and assessment of novel therapeutic interventions (13, 14); 3) ovine ALI/ARDS model induced, while under anesthesia, by combined cutaneous burn and smoke inhalation, then permitted to awaken for an interval of intensive care and assessment of novel therapeutic interventions (15); and 4) ovine (16), porcine (17) and rabbit (18) models of wound healing and metabolism. Additionally, there are fully-equipped intensive care units for rodents and support from cell biology and molecular biology laboratories.

The IICU functions 24 hours per day with three shifts of highly trained personnel caring the animals while obtaining and recording various cardiopulmonary, renal and other necessary data. Animals with ALI/ARDS are mechanically ventilated and studied in a conscious state for various periods up to 24 or 48 hours or even for a few weeks. The availability of new, state-of-art equipment such as Hamilton GS ventilators (Hamilton Medical, Bonaduz, Switzerland) with various gentle modes of ventilation enables investigators to investigate the pathophysiology of ALI/ARDS with minimal mechanical ventilation-related barotrauma or volutrauma.

Among the various etiologies of ALI/ARDS, pneumonia/sepsis is the most common and lethal cause of ARDS (2, 6). ARDS and sepsis together account for 400,000 deaths per year in the U.S. To date, there is no specific treatment for the treatment of patients with sepsis. Current supportive care of septic patients with ARDS is often ineffective when faced with antibiotic resistant organisms, vasodilatory shock refractory to aggressive fluid resuscitation, hypo-responsiveness to vasopressors, and severe systemic and lung vascular injury. These critical issues must be addressed in clinically relevant animal models. Over the past two decades however, IICU investigators have studied the pathophysiology of pneumonia/sepsis using their well-established ovine models and have proposed various novel therapeutic options (12, 13, 19). The close parallels between ovine model ARDS/sepsis and its clinical progression facilitates successful translation of IICU findings to the clinical use. For example, recent ovine ARDS/sepsis studies at the IICU led to the successful completion of a Phase II clinical trial on the safety and efficacy of the arginase vasopressin V1a agonist Seлепressin (Ferring Pharmaceuticals, San Diego, CA) for treatment of septic patients.

More recently, collaborative ovine studies at the IICU with the colleagues from University of San Francisco (Drs. Jae-Woo Lee and Michael Matteey) have resulted in another multi-site clinical trial to test the safety and efficacy of allogenic human bone marrow-derived mesenchymal stem cells (MSCs) for treatment of patients with severe ARDS. In the latter study, we infused approximately 5-10 million human bone marrow-derived clinical-grade, MSCs (stromal) per kg over 1 hour into a central vein of severely septic or healthy, control sheep. The cells were kindly provided by the NIH Production Assistance for Cellular Therapy Group at The University of Minnesota (Director, Dr. David McKenna, Division of Transfusion Medicine, Department of Laboratory Medicine and Pathology, University of Minnesota). The infusion of one dose of MSCs was well tolerated in both injured and healthy (control) sheep in terms of pulmonary arterial and systemic hemodynamics (pulmonary artery, central venous, left atrial, and mean arterial pressures as well as cardiac output) (20). This therapy with allogeneic MSCs significantly improved pulmonary gas exchange, and reduced the peak pressures during mechanical ventilation. It also significantly reduced lung water content, pulmonary transvascular fluid flux as determined by measuring lung lymph flow and fluid retention (fluid net balance), thereby suggesting a potential favorable impact on the pulmonary and systemic endothelial permeability (20). Although the exact mechanisms are not completely understood, the results from our studies strongly suggest potential anti-inflammatory and anti-microbial properties of MSCs because the bacterial and neutrophil numbers were significantly reduced in the bronchoalveolar lavage fluid in sheep treated with MSCs (20). In addition, the investigators in the IICU are currently performing ovine studies testing the safety and the efficacy of aerosolized or intravenously administered adipose-derived auto and allogeneic ovine MSCs on the toxic inhalation-induced ALI/ARDS. Topical application of adipose-derived ovine MSCs has also been tested on grafted burn wounds.

Although, since their initial characterization, MSCs have been shown to be beneficial in number of diseases such as myocardial infarction (21), diabetes (22), sepsis (23), hepatic failure (24), and acute renal failure (25), the precise mechanisms by which MSCs exert these salutary effects are incompletely understood. There is an ongoing debate among investigators as to whether the therapeutic effects of MSCs are attributable to engraftment in tissues, or to secretion by MSCs of anti-inflammatory and growth factors. Krause et al (26) reported that
PI Highlight: David A. Williams, MD

Leland Fikes Professor of Pediatrics, Department of Pediatrics, Harvard Medical School Chief, Division of Hematology/Oncology; director of Translational Research at Boston Children’s Hospital; Associate Chairman, Department of Pediatric Oncology, Dana-Farber Cancer Institute.

PACT Center: Center for Human Cell Therapy (CHCT), Boston Children’s Hospital
Leslie Silberstein, MD-Director
Jerome Ritz, MD-Co-director
Myriam Armant, PhD-Technical director

Website links:
Hospital page: http://www.childrenshospital.org/cfapps/research/data_admin/Site2613/mainpageS2613P0.html
Laboratory: www.williamslaboratory.org

PACT Projects:
- Autologous CD34+ hematopoietic progenitor cells transduced with GALV pseudotyped (SIN) gammaretroviral vector (pSRS1.1.EFS.IL2RGpre*) for the treatment of X-linked severe combined immunodeficiency (X1-SCID)
- Pilot and feasibility study of hematopoietic stem cell gene transfer for Wiskott-Aldrich syndrome

Q: What are the challenges of gene therapy trials for pediatric blood diseases?
There are three main challenges that we face in dealing with pediatric blood diseases. By and large, the first one is the complication of treating rare diseases. These are blood diseases that are very rare; in some cases only one in one million children are affected, making the expense to treat each patient extremely high. There is also regulatory disharmony across multiple countries and agencies, as these children are often times being treated using the same protocol all over the world. Differences in health care insurance across states and countries can add additional financial burden and difficulty in treating rare disease patients.

The second challenge revolves around the ethics of doing this kind of research in children. Often times the idea of testing new therapies on children brings about a certain unsettling feeling. However, as a Pediatrician, I remind others that children have the same rights to new types of medicine and innovative treatments as anyone else.

The third challenge is putting together the resources that are needed to perform these trials. Many of these new technologies are dependent on GMP facilities and the personnel trained to do this type of work. In Boston, we are fortunate to have such a facility at the Dana Farber Cancer Institute. You also need sophisticated regulatory oversight, which is provided by our Clinical and Translational Investigation Program at Boston Children’s Hospital.

Q: How important is it to have the NHLBI-funded Production Assistance for Cellular Therapies (PACT) support cell and gene therapy initiatives?
One word comes to mind – critical. The support we have received from PACT has been absolutely critical in getting these new trials open. Without PACT and the Center for Human Cell Therapy-Boston, it would have taken a lot longer to launch these trials as our research lab and community do not have the expertise required to develop and validate SOPs. PACT allows for the smooth transition of work out of the research lab and into a clinical setting. The attitude of PACT which can be summarized as: “Our job is to help you do this” is so very important.

Q: What are the new gene therapy clinical trials in development at Boston Children’s Hospital?
We have several gene therapy trials at different stages of development and the pipeline looks like this:
- CD19 CAR T cells in pediatric acute lymphocytic leukemia
- Chronic Granulomatous Disease
- Childhood Cerebral Adrenoleukodystrophy
- Sickle cell disease
"Academic to Industry Translation in Cell Therapies: The Evolution of Concepts into Practice"

ISCT North America Regional Meeting
Hyatt at Penn’s Landing

PACT will be conducting a Scientific Session (Workshop 6) on Sunday, September 8, 2013 3:15-4:45 pm ET

Session Title: Optimizing Bench to Bedside Translation of Research – An Update from PACT

Session Description: This session will provide an overview of projects with significant translational development that have reached the clinical trial stage. The session will include at least two case studies with specific product development experience. Speakers from academic research and the PACT cell therapy facilities will discuss the pathways for bringing promising therapies into clinical trials.

Click HERE for more information on the ISCT NA Regional Meeting

20% of bone marrow-derived MSC were engrafted into lung tissue, including epithelial cells. It has also been shown that application of human MSCs improved alveolar fluid clearance via keratinocyte growth factor in an ex vivo lung preparation following endotoxin challenge (27). We believe that clinically relevant large animal models will help to properly address these circumstances and translate these exciting basic science discoveries into clinical use.

The advantages of ovine models of sepsis and ALI/ARDS are: 1) similarity of septic responses (hyperdynamic sepsis) to humans; 2) relatively easy continuous monitoring of cardiopulmonary and other variables; 3) fluid resuscitation to clinically relevant physiological endpoints; 4) clinically relevant acute study intervals of 96h or longer; 5) comfortable tolerance of mechanical ventilation without sedation through a tracheostomy, thereby facilitating monitoring of cardiopulmonary variables without confounding sedation effects; 6) similarity of ovine and human mucus glands and bronchial circulation; and 7) ease of intermittent blood, urine and lymph samplings. Finally, it is important to note a close similarity between ovine and human genomics. Although the ovine, in comparison to human genomic profile, especially under disease conditions such as sepsis required more detailed characterization, many previous studies demonstrate important similarities. Iannuzzi et al (28) reported a high degree of conserved human chromosome regions in the ovine genome and further confirm the high degree of chromosome similarity among related bovids. Vaccarelli et al (29) demonstrated that the T cell receptor gamma locus is more highly conserved between ovine and human than between mice and human, suggesting that immune responses in sheep might be more similar than mice to the immune responses of humans. Of particular interest, the sensitivity of sheep to lipopolysaccharide (LPS) is similar to that in humans, i.e., intravenous continuous infusion of LPS at doses of 10-20ng/kg/min (30, 31) produces in sheep cardiopulmonary responses that closely resemble those produced by comparable doses in humans (32); in contrast, rodents require LPS doses that are one million-fold greater. As mentioned, there are no studies comparing the immune responses between humans and sheep in disease conditions such as sepsis or polytrauma, but future studies in that direction are undergoing in the ICU.

In conclusion, based on several small and large animal studies, MSCs therapy represents a promising modality for patients with sepsis and ARDS. The mechanisms of therapeutic benefit from MSC therapy appear to depend on both cell-dependent and cell-independent pathways, including the release of paracrine molecules that can reduce lung injury and enhance lung repair. Nevertheless, the safety and efficacy of the stem cell-based therapy requires more extensive testing in clinically relevant translational large animal models.

Click HERE for a list of citations

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Contract Number: HHSN268201000006C

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Gene Therapy for the Wiskott-Aldrich syndrome

Myriam Armant, Center for Human Cell Therapy, Boston Children’s Hospital

Wiskott-Aldrich syndrome (WAS) is a rare life-threatening X-linked immunodeficiency caused by mutations in the WAS gene coding for the Wiskott-Aldrich syndrome protein (WASp). WASp is a hematopoietic-specific cytoskeletal regulator that is essential for multiple cellular functions in leukocytes like adhesion, migration and chemotaxis, phagocytosis and immune synapse formation. WASp also functions as a scaffold protein for assembly of effective signaling complexes downstream of surface receptor engagement (1).

WAS clinical presentation varies from patient to patient. Mutations abolishing the expression of WASp are associated with full-blown form of WAS characterized by recurrent infections, eczema, thrombocytopenia, autoimmunity and a higher risk of lymphoproliferative malignancy (2). At the other end of the spectrum, patients expressing low levels of a mutated protein have a less severe phenotype predominantly described as X-linked thrombocytopenia (XLT) (3).

Therapeutically, hematopoietic stem cell transplantation (HSCT) is considered a treatment of choice for WAS patients with an HLA-identical sibling donor available (4). For patients without a matched donor, alternative approaches are needed. For more than a decade, autologous transplantation of gene-corrected hematopoietic stem cells has been used as an alternative to HLA-mismatched HSCT, to treat patients with primary immunodeficiencies like X-linked severe combined immunodeficiency (SCID-X1) or adenosine deaminase deficient SCID (5). A recent clinical trial using a conventional gammaretroviral vector also demonstrated the feasibility and efficacy of gene therapy in WAS (6). However, these proof-of-concept studies have also highlighted the risk of genotoxicity observed in some patients as a consequence of retroviral integration near proto-oncogenes like LMO2 (5). These severe adverse events prompted a change in gene transfer vector design. A collaborative effort has led to the development of a recombinant HIV-derived lentiviral vector that is replication-defective, self-inactivating and encodes the human WAS cDNA driven by its endogenous 1.6kb promoter (w1.6_hWASP_WPRE (VSVg) lentiviral vector) (7). Preclinical studies have demonstrated the safety and efficacy of the WAS rHIV vector (8-12). GENETHON has manufactured the vector under cGMP conditions to support the initiation of phase I/II gene therapy trials for WAS in London, Paris and Boston (13). A similar study is also ongoing in Milan to test the same vector produced by a different manufacturing facility. This multicenter approach is meant to accelerate the development of alternative therapies for rare diseases like WAS (14).

Successful gene transfer in WAS will require stable engraftment of gene-corrected cells in multiple lineages in order to see a clinical improvement (leukocyte function restored, platelet number increase, less eczema, less bleeding and bruising, lower infection rate and correction of thrombocytopenia). The clinical trial is ongoing and the first patients treated in Paris (Dr. Marina Cavazzana-Calvo) and London (Dr. Adrian Thrasher) show encouraging results. The trial is now also open at Boston Children’s Hospital (Drs. Sung-Yun Pai, Luigi Notarangelo and David Williams) and enrollment has...
started. The development of standard operating procedures for the manufacturing of WAS HIV-transduced CD34+ was supported by the Production Assistance for Cellular Therapies (PACT) program and an overview is shown in figure 1. Product characterization is typical of gene therapy products: in addition to standard cell therapy quality control testing (sterility, endotoxin, mycoplasma, gram stain, cell counts, viability and phenotype), there are specific tests like WASp expression by flow cytometry or Western blot, vector copy number determination in bulk and in individual colonies, integration site analysis and RCL testing. The patients will be followed up on study at 1 month, 6 weeks, 3, 6, 9, 12, 18 and 24 months post gene therapy. The patients will then be reviewed annually off study for a further 3 years.

Autologous HSC gene therapy for pediatric immunodeficiencies has become an attractive therapeutic option. Recent advances in vector design and gene transfer technology has helped overcome some of the hurdles that have stood in the way of progress (15). More time is needed to measure the success of ongoing clinical trials but it is an exciting time to be part of this field where clinical translation is thriving.

**Figure 1**: WAS trial manufacturing and conditioning timeline

![Figure 1: WAS trial manufacturing and conditioning timeline](image)

**Legend to Fig 1**: Autologous CD34+ hematopoietic stem cells are collected from bone marrow or mobilized peripheral blood and positively selected by Miltenyi Clinimacs®. Cells are stimulated in culture with a cocktail of cytokines (SCF, TPO, Flt3L, IL-3) and subjected to 2 rounds of transduction with the w1.6_hWASP_WPRE (VSVg) lentiviral vector. The total cell culture time is ≤72 hours. During the manufacturing of gene-corrected cells, patients will receive myeloablative conditioning with busulphan and fludarabine over 3 days prior to infusion of transduced cells as illustrated in Fig. 1. Transduced cells are immediately infused after final harvest.

Dr. Wu is the Co-Director of the Stanford Cardiovascular Institute and Associate Professor of Medicine/Cardiology & Radiology at the Stanford University School of Medicine in Stanford, California.

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Please describe your clinical and research interests.

My clinical focus is heart disease. I am interested in hereditary cardiovascular disorders as well as treatment of ischemic heart disease, which is the leading cause of morbidity and mortality in the Western World. My research program at Stanford has 2 main focuses: 1) use of adult and pluripotent stem cells as a cell therapy for treatment of ischemic cardiovascular disease (i.e., heart attack and heart failure), and 2) development of "clinical trial in a dish" drug screening models using our patient-specific iPSC libraries, which we have constructed over the past 5 years. We currently have over 100+ patient-specific and disease-specific lines. We have used "disease in a dish" models to study hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). We are currently using these models to identify novel disease pathways for pharmaceutical inhibition as well as drug development. On the cell therapy front, we have received a grant from the California Institute of Regenerative Medicine to work on human embryonic stem cell-derived cardiac cell therapy.

How has PACT been a resource for you in pursuing a clinical trial in cell therapy?

PACT has been an amazing partner in our efforts to produce a pluripotent stem cell product that can be used to treat heart disease. We are working with Larry Couture and his team to produce GMP grade pluripotent stem cell-derived cardiomyocytes. Our protocols are extremely robust and we anticipate with PACT's help moving toward clinical trial within the next 5 years.

How has your partnership with the PACT team at the CATD impacted your clinical research?

Working with the PACT team at the CATD has really been great exposure to industrial grade research.
PACT will be hosting a session on Sunday, September 8, 2013 3:15-4:45 pm ET

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Optimizing Bench to Bedside Translation of Research – An Update from PACT

Session Description:
This session will provide an overview of projects with significant translational development that have reached the clinical trial stage. The session will include at least two case studies with specific product development experience. Speakers from academic research and the PACT cell therapy facilities will discuss the pathways for bringing promising therapies into clinical trials.

PACT can provide general facility SOPs upon request to assist you in developing your own cell processing facility SOPs.

SOP Categories available for request:
- Cleaning Procedures
- Deviation Management
- Environmental Monitoring
- Personnel Training
- Quality Assurance/Quality Control
- Quality Management
- Regulatory/Clinical
- Standard Operating Procedures (SOP): Development and Management
- Validation

PLEASE NOTE that these SOPs are for INFORMATIONAL PURPOSES ONLY and therefore require validation by your own facility.

To see a full list of SOPs available for request go to the PACT Website and look under the Resources tab.

https://www.sctweb.org/public/meetings/2013/home.cfm

SCT 34TH ANNUAL MEETING (2013)
BOSTON, MASSACHUSETTS, USA - MAY 19-22, 2013
Invited Session V - Session 22 Tuesday, May 21 from 2:15-3:45pm
Cell Therapy Clinical Trials: What is unique about the conduct of clinical trials using cell and gene therapies?

Objective: This session will describe clinical trials using cell and gene therapies and what is unique about the conduct of those clinical trials.

Robert Lindblad, MD, FACEP
The EMMES Corporation
PI for the Production Assistance for Cellular Therapies (PACT)
Topic: Overview of clinical trials using cellular therapies

Sung-Yun Pai, MD
Assistant Professor of Pediatrics, Harvard Medical School
Topic: A clinical trial using gene therapy

Corey Cutler MD, MPH, FRCP
Associate Professor of Medicine, Harvard Medical School Dana-Farber Cancer Institute Boston
Topic: A clinical trial using umbilical cord blood

PACT Cell Processing Facilities:
Baylor College of Medicine Center for Cell and Gene Therapy
Contract Number: HHSN268201000007C

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Contract Number: HHSN268201000006C

This project has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services.
Through The Valley Of Dearth

Cliona Rooney and Adrian Gee,

*Center for Cell and Gene Therapy, Baylor College of Medicine, The Methodist Hospital and Texas Childrens’ Hospital, Houston, Texas

The rationale used for almost every NIH grant application is “the ultimate alleviation of human suffering”. Many cell therapy applications can realistically test this goal within their Specific Aims. However the realization of clinical efficacy usually requires iterative phase I clinical trials in which small steps are taken to improve an initially promising therapy. This may mean the addition of a novel gene that alters the biological behavior of the cells, but more often is more mundane for example, the addition of an new antigen specificity for cytotoxic T lymphocytes (CTLs), or modifications to the manufacturing process that shorten or simplify the procedure. These changes are essential to pave the way for pivotal late phase clinical trials that could be supported by industry, but between the “innovative” first trial and the industry-supported phase 2/3, there is a dearth of funding, since when these projects hit the study section they score highly for clinical significance but show a dismal lack of “innovation”. PACT is ideally suited to shepherd these important studies across the “valley of funding dearth” and has allowed the clinical evaluation of cell therapy products that might not otherwise be tested.

An example of this has been the development of virus-specific T cells to prevent and treat viral infections after hematopoietic stem cell transplantation HSCT. T cells specific for CMV, EBV and adenoviruses had proved effective for the prevention and treatment of those viruses after HSCT, but their wide spread use was severely limited by a long (> 10 weeks) and complex manufacturing procedure and the requirement for live virus (EBV) and adenoviral vectors as a source of antigen. T cells that were functionally and phenotypically similar could be prepared within 17 days using dendritic cells as antigen presenting cells and nucleofected DNA plasmids as source of antigen, but testing of the resultant T-cells was not considered novel by several funding agencies. NHLBI-PACT enabled their clinical evaluation and early results show that these are effective in HSCT recipients with viral disease. The further shortening of CTL manufacture to less than 14 days and extension of protection to two additional viruses, BK and HHV6 by pulsing peripheral blood mononuclear cells with overlapping peptides spanning antigens from all five viruses has also been approved by PACT who will sponsor their manufacture and clinical testing.

This type of support will increasingly be required for effective cell therapies that require further optimization if they are not to stagnate for lack of support and if years of innovative preclinical development are not to be wasted.

* An NHLBI, Production Assistance for Cell Therapy (PACT) Center

Reference List
PACT Related Manuscripts


Product Related Manuscripts


For additional information on the PACT Bibliography go to www.pactgroup.net

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pactinfo@pactgroup.net
NHLBI Programs

PACT is only one of many programs offered by NHLBI. We would like to highlight other programs that the investigators we serve may utilize.

SMARTT

The National Heart, Lung, and Blood Institute (NHLBI) Science Moving TowardS Research Translation and Therapy (SMARTT) Program supports the translation of novel discoveries into successful new therapies for heart, lung, and blood diseases by providing free and confidential preclinical development services to investigators. SMARTT provides tailored pharmacology and toxicology testing, manufacturing services, and regulatory support to investigators to expedite the transition of their discoveries to the clinic.

To date, the SMARTT program has helped 11 investigators move their lead candidates forward in preclinical development. Investigators interested in SMARTT may register to receive program updates, including the SMARTT newsletter, and may apply for SMARTT services at [https://www.nhlbismartt.org](https://www.nhlbismartt.org).

SAVE THE DATE

Cell Therapy for Lung Indications

Presenters will examine the current state of cell therapy research and product development and the resources available to support novel cell therapy approaches in the treatment of lung diseases. Navigating the regulatory pathway for and how to prepare an IND submission will be discussed.

-Speakers-

Carol Blaisdell, MD  
Medical Officer  
Division of Lung Diseases NHLBI, NIH

Michael Matthay, MD  
Senior Associate and Professor  
University of California San Francisco

Derek Hei, PhD  
Senior Scientist, Waismann BioManufacturing  
University of Wisconsin-Madison

Marlowe Eldridge, MD  
Associate Professor  
University of Wisconsin School of Medicine and Public Health

-PACT Mission-

Provide assistance for cellular therapy translational research and the manufacture of cellular therapy products

-PACT Facilities-

Baylor College of Medicine, Center for Cell and Gene Therapy  
Center for Human Cell Therapy Boston  
City of Hope, Center for Applied Technology Development  
University of Minnesota, Molecular and Cellular Therapeutics Facility  
University of Wisconsin - Madison, Waismann BioManufacturing

The EMMES Corporation serves as the Coordinating Center for PACT

Please Join Us!

PACT Web Seminar  
Wednesday, February 20, 2013  
12-1:15 pm ET

Registration opens January 21, 2013

Intended Audience:  
Clinical Investigators, Scientists, Researchers, and Technologists specializing in cell therapy

Registration Fee: No Charge

http://www.pactgroup.net
Save the Date
The University of Wisconsin is holding “Back-2-Back” meetings on April 9-10th, 2013

PACT Workshop
April 9, 2013

Developing Cellular Therapies: From Preclinical Safety to Clinical Evaluation

Host site: University of Wisconsin Waisman Biomanufacturing (WB)
Location: Wisconsin Institutes for Discovery (on UW campus) Madison, Wisconsin

No fee to attend. Register to open in February
Visit www.pactgroup.net for updates

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Telephone: 301.251.1161
Fax: 240.306.2527
Email: pactinfo@pactgroup.net

http://www.pactgroup.net

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8th Annual Wisconsin Stem Cell Symposium:
April 10, 2013
Cell-Based Therapy for Heart and Vascular Disease: Pathways to Clinic

Host sites: University of Wisconsin Stem Cell and Regenerative Medicine Center and BTC Institute
Location: BioPharmaceutical Technology Center Institute (BTCI)
5445 Cheryl Pkwy Madison, WI (approximately 7 miles south of UW campus)

Registration fee: $90*
*A discounted fee of $45 is available to students and post-doctoral candidates

To register for the 8th Annual Wisconsin Stem Cell Symposium visit http://www.btci.org/stemcell/default.html

Contact PACT Workshop
April 9, 2013

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