CONNIE FRANK AND EVAN THOMPSON PROGRAM FOR RESTORATIVE TRANSPLANTATION RESEARCH

2021-2022 ANNUAL SUMMARY

DAVID GEFFEN SCHOOL OF MEDICINE AT UCLA

UCLA Health | David Geffen School of Medicine

With the far-sighted and munificent support of Connie Frank and Evan Thompson, Kodi Azari, M.D., continues to lead an unparalleled group of researchers in advancing the field of vascularized composite allotransplantation (VCA). In 2011, under the leadership of Dr. Azari, the UCLA Health Hand Transplant Program became the fourth center to perform such procedures in the United States. While still rare, these multi-tissue hand, face, and abdominal wall transplants hold great promise in improving quality of life for wounded warriors and others suffering from diseases and injuries necessitating such transplants. While substantial barriers to success remain, Dr. Azari's team, comprising some of the top scientists in the field of immunology, continues to make progress in surmounting the obstacles of immunosuppression, ischemia-reperfusion injury, and transplant vasculopathy.

After a lull in research created by the COVID-19 pandemic, the Program for Restorative Transplantation Research has made major advances since the last annual report, in 2019-2020. The investigators' innovative research has led to their securing additional funding from institutions such as the National Institutes of Health (NIH) and U.S. Department of Defense to take their studies to the next stage.

Dr. Azari and his team are planning a retreat for spring 2023, where they will present full research updates at the conclusion of the program. Below is a brief update on the team's progress since the last report.

Project One: Immunomodulation

Immunosuppressive drugs currently must be taken during a transplant recipient's lifetime to prevent rejection of the graft and can have significant side effects. Immunosuppression therapy is not specific to the transplant organ, and therefore the overall lowered immunity may allow cancer or infection to occur. Gay M. Crooks, M.D., Rebecca Smith Chair in Molecular and Cellular Pathology, and Director of the Immunity, Inflammation, Infection, and Transplantation (I3T) research theme in the David Geffen School of Medicine at UCLA, has continued to make great strides in utilizing T-cell immunotherapy to develop a cell-based approach to prevent transplant rejection while maintaining the patient's natural immunity.

The thymus is an organ that generates T-cells with specialized functions, such as conventional T-cells (Tconv) that react to infections and foreign tissue, and regulatory T-cells (Tregs), which react against Tconv to suppress inflammation and provide immune tolerance. In a healthy immune system, the two are balanced. However, following VCA, absent immunosuppression, the Tregs are overwhelmed, resulting in inflammation and transplant rejection. Immunosuppressive drugs suppress Tconv, which control cancer and viral infections, as well as Tregs. The Crooks lab has developed an artificial thymic organoid (ATO) system that can produce T-cells from blood stem cells, which are immature cells that have the potential to develop into any type of blood cell, but typically turn into Tconv cells. This research has far-reaching implications for cancer treatment. In VCA, the team has engineered the ATO to produce Treg cells from a patient's own blood stem cells, which can then be stored before a transplant and utilized afterward to fight inflammation and rejection. The goal now is to increase the number of Treg cells the ATO can produce.

The key to the Crooks lab's approach is to engineer stem cells to produce T-cells that express FOXP3, the key gene responsible for development of Tregs. However, they have found that if FOXP3 is expressed too early during T-cell development, all T-cell production halts. The team has discovered a way to use gene editing (CRISPR) to regulate the time and place of the onset of FOXP3, inserting it into stem cells at genomic sites that are active at the optimal stage of T-cell differentiation. The team is currently experimenting to define the best time and level of FOXP3 expression to bring Treg cells to maturation.

Using these innovative approaches, Dr. Crooks and her team are excited to begin testing the ability of the FOXP3-engineered stem cells to differentiate normally into T-cells, and then to determine if these T-cells can function to suppress inflammation and graft rejection.

Support from the Connie Frank and Evan Thompson award led to the Crooks lab's ability to gather the data essential for a grant application to the California Institute for Regenerative Medicine in November

2022. Awardees will be notified around March 2023. In addition, the data demonstrating that FOXP3+ cells can be produced in ATOs was included in the lab's paper "In Vitro Recapitulation of Murine Thymopoiesis from Single Hematopoietic Stem Cells," published in the journal *Cell Reports* in 2020.

Project Two: Ischemia-Reperfusion Injury

Ensuring the quality of tissues at the time of transplant is essential to the long-term success of transplants. The harmful effects of ischemia/reperfusion injury, the tissue damage caused when blood supply returns to tissue after a period of ischemia, or lack of oxygen, during organ harvesting and periods of extended cold preservation, can lead to cellular dysfunction and organ death, resulting in a failed transplant.

Jerzy Kupiec-Weglinski, M.D., Ph.D., and his team continue their in-depth research into the molecular signaling pathways in VCA that develop after ischemia and reperfusion of tissues. Dr. Kupiec has forged a partnership with researchers at Johns Hopkins University School of Medicine who perform hindlimb grafts in both genetically similar and disparate scientific models. They then send related tissues to Dr. Kupiec's group for analysis and comparison. Dr. Kupiec's team compares cells from the skin, muscle, and bone marrow and their interactions, and studies both in vivo and in vitro samples to see how different modalities affect the activation of those cells. Their work has been recognized by the prominent American Transplant Congress, where they were invited to present papers in both 2020 and 2022, as well as at the 2020 joint meeting of the Congress of the European Society for Surgical Research and the Congress of the Austrian Society for Surgical Research.

The studies performed under the Program for Restorative Transplantation Research also provided a base for further funding for Dr. Kupiec and his team. Based on the success of this work, the group was awarded funds from the U.S. Department of Defense to continue research using a large animal model; early results are now available. The group also recently received a three-year, $1.5 million grant from the Department of Defense to improve outcomes in hand and face transplantation. In particular, the investigation will focus on the largely unexplored role of the microbiome in VCA, where multiple structures—such as skin, muscles, blood vessels, and nerves—are involved. These bacteria, both from the transplant recipient and the donated tissue, may affect recipients' immune systems and rejection responses.

Project Three: Transplant Vasculopathy

Human leukocyte antigens (HLAs) are proteins (or markers) that are found on most cells in the body. The immune system uses these markers to identify which cells belong in a person's body and which do not. One of the main causes of chronic transplant rejection is a recipient's development of donor-specific antibodies in response to mismatched donor HLA. Recipients of VCAs and solid organ

transplants who exhibit donor-specific antibodies develop a progressive vascular disease known as transplant vasculopathy.

After a transplantation, the recipient's donor-specific HLA antibodies bind to the endothelial cells (ECs) inside of the new tissue's blood vessels, triggering an immune reaction that activates ECs to recruit macrophages, which then accumulate in the vessels. Gradually, this inflammatory response causes thickening of the vessels, leading to transplant vasculopathy and transplant rejection. The problem is made more challenging by the fact that the mechanisms for this chronic immune injury have not yet been identified.

Elaine F. Reed, Ph.D., Director, UCLA Immunogenetics Center, and Daljit S. and Elaine Sarkaria Endowed Chair in Diagnostic Medicine; and Enrique Rozengurt, D.V.M., Ph.D., A.G.A.F., Distinguished Professor in the UCLA Vatche and Tamar Manoukian Division of Digestive Diseases, and Ronald S. Hirshberg Chair in Translational Pancreatic Cancer Research, have partnered with Robert Fairchild, Ph.D., in the Department of Inflammation and Immunity at the Cleveland Clinic, to investigate the effects of and possible treatments for transplant vasculopathy in both VCAs and solid organ transplants. The team has identified signaling pathways implicated in the process of donor-specific, antibody-mediated cell proliferation and migration, and are exploring two related hypotheses.

Drs. Reed and Rozengurt and their team believe that they have identified two coactivators in this process and that drugs in the statin family could be the key to blocking these signals and preventing transplant vasculopathy. The team anticipates that novel targets will emerge for developing new and potent drugs for preventing chronic allograft injury in VCA and solid organ transplants induced by donor-specific antibodies.

As a direct result of funding from the Connie Frank and Evan Thompson Program for Restorative Transplantation Research, Drs. Reed, Rozengurt, and Fairchild received two-year NIH funding for their R21 grant proposal, through December 2022.

THANK YOU

Thanks to the visionary support of Connie Frank and Evan Thompson, vascular composite allography is on the path to more successful outcomes and greater accessibility to more patients. Dr. Azari and his team of leading-edge researchers want to thank Ms. Frank and Mr. Thompson for their continued partnership. Their generous gift to the Program for Restorative Transplantation Research and investment in these projects gives hope that VCA can move from a rare procedure to a safe and successful surgery benefiting many.