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To: The O'Neill & Rancic Families

From: Richard Childs, Senior Investigator, Hematology Branch, NHLBI

Through: The Foundation for the National Institutes of Health

Subject: Post-Baccalaureate Student Progress Report

Over the past year, your generous funding supported Claire Scrivani's post-baccalaureate appointment with Dr. Childs' lab. Claire is originally from Earlysville, Virginia and earned a Bachelor of Science in biochemistry from Virginia Tech. Starting in August 2015, Susan Doh will be supported by this gift fund. She is from Chicago, Illinois and graduated with a Bachelor of Arts in biology from the University of Chicago. Susan has already had one successful year with Dr. Childs' lab, and we're excited to have her stay on for another year. Below is summary of the work that both of these young scientists have helped and will continue to help advance.

Significance and Background

The most common type of kidney cancer is renal cell carcinoma (RCC), which begins in the cells that line the small tubes within the kidneys. Kidney cancer cells may also spread (metastasize) outside the kidneys to nearby organs as well as to more distant sites in the body. We recently identified a tumor antigen that was derived from a newly discovered gene that is expressed in about 80% of RCC tumors. This gene turns out to be a human endogenous retrovirus type E (HERV-E), a virus that is part of the human genome and located on the long arm of chromosome 6. This virus likely integrated into the human genome as a retrovirus millions of years ago but was subsequently silenced as a consequence of genetic mutations. We found that this virus is indeed silent or "turned off" in normal tissues but surprisingly is "turned on" or expressed in kidney cancer cells. HERVs exist widely within the human genome as proviruses, with most being transcriptionally inactive. Although more than 50 HERV-E's are estimated to exist in the human genome, this is only the second study to identify a HERV-E transcription product

expressed in a tumor cell and the first report to identify a HERV-E that is selectively expressed in RCC with virtually undetectable levels of expression in normal tissues, potentially making this antigen an ideal target for tumor immunotherapy.

This new RCC antigen was discovered by using T-cells from a patient with kidney cancer who had tumor regression following an allogeneic bone marrow stem cell transplant. Following the transplant, we isolated a T-cell clone that killed tumor cells in vitro. Using molecular biology techniques, we were able to identify the target antigen of these killer T-cells to be a HERV-E derived antigen (a peptide antigen called CT-RCC-1). Remarkably, it appears that this HERV-E antigen is "immunogenic", inducing the transplanted donor's immune system to expand T-cells that were able to kill the patient's kidney cancer tumors. To the best of our knowledge, this is the first report to identify a T-cell population recognizing a HERV-derived antigen with expression restricted to tumor cells. This finding is of great importance as new tumor antigens are urgently needed to develop more effective immunotherapy approaches for RCC and other solid tumors.

What has been accomplished?

The CT-RCC-1 antigen derived from HERV-E is restricted to the "HLA-A11" human leukocyte antigen. Only 15-18% of the human population possesses HLA-A11, meaning that immunotherapy approaches targeting the CT-RCC-1 antigen through tumor peptide vaccination would be limited to only a minority of patients with metastatic kidney disease. In the last couple of years, we have been exploring other immunogenic peptides derived from this HERV-E that are expressed on more common HLA class I molecules. HLA-A2 is expressed in almost 50% of humans, and the identification of an HLA-A2 restricted HERV-E antigen would greatly expand the percentage of RCC patients that could potentially benefit from immunotherapy targeting this peptide. After using an original protocol that did not yield any data, Claire developed a new stimulation protocol which lead to the development of eight different groups of cytotoxic "killer" T cells that specifically target one of three proteins derived from HERV-E in the context of HLA-A2. She showed that these cytotoxic T cells can target HERV-E expressing clear cell RCC

tumors for killing.

Susan's work has focused on two different overlapping peptide libraries, or peptide pools, derived from the HERV-E envelope sequence. Both peptide pools contain small peptides overlapping by 11 amino acids that span the entire length of the predicted HERV-E envelope proteins. The pools allow the lab to identify T cells that recognize additional peptides other than the ones that are currently being evaluated. The peptide pools are used to identify kidney cancer peptides presented in different HLA contexts to generate HERV-E reactive T cells. Susan is testing cells from an HLA-A2 donor with one of the peptide pools derived from the HERV-E specific T cells clones. She will then screen the clones with individual peptides derived from these pools to identify the specific peptide recognized by the clones. Ideally, these experiments will result in the identification of the most immunogenic peptides to generate a diverse group of "killer" T cells.

What are the next steps?

Moving forward, we will work to determine a method to illicit the strongest killer T cell response, isolate and expand the most responsive T cell population, then sequence their T Cell receptors in order to use them therapeutically in a clinical trial for HLA-A2 positive patients with metastatic clear cell RCC.

Previously, the lab created a clonal population of cytotoxic T cells that target both the HERV-E virus and the clear cell RCC expressing HERV-E tumors in the context of HLA-A11. Dr. Nishimura from Loyola University is working on cloning the T cell receptors that target these tumor cells, an integrating them into a clinical vector. These would then be used in a T cell receptor gene transfer approach to treating kidney cancer patients. The goal for our lab is to develop a phase I clinical trial for HLA A11 positive patients with metastatic kidney cancer to explore the ability of gene modified autologous HERV-E T cell receptor transduced T cells to induce remission in their metastatic cancer. Claire has been involved with the writing of a grant

for this clinical trial.

Eventual Goals of Research

Recently we identified that the envelope (*env*) component of the HERV-E contains two genomic regions called open reading frames (ORFs) that would be predicted to be expressed within the RCC tumors. One of these regions was found to contain an ITAM sequence (immunoreceptor tyrosine-based activation motif). Some viral ITAM-containing proteins have been shown to cause transformation of "healthy" cells to cancer. We are currently investigating whether expression of the HERV-E *en*v gene might somehow promote or even cause kidney cancer. If true, we would begin to explore for new drug therapies to silence or turn off the HERV-E in the tumor as a method to inhibit the growth of RCC. Another major focus is to develop anti-envelope antibodies that could be used to for diagnostic purposes as well as therapeutic purposes. Antibody therapies targeting antigens expressed on the surface of tumors have now become the standard of care for many malignancies, prolonging survival in patients with lymphomas, breast cancer, colon cancer and other tumors. The development of an antibody targeting the HERV-E envelope protein expressed on the surface of kidney cancer cells could potentially provide a new method to effectively treat patients with metastatic RCC. Our lab is now actively pursuing the development of a monoclonal antibody to target HERV-E proteins expressed on the surface of kidney cancer tumors.