University of Pennsylvania

Annual Progress Report: 2009 Nonformula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Formula Grant Overview

The University of Pennsylvania received $4,620,420 in nonformula funds for the grant award period June 1, 2010 through May 31, 2014. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Novel Adjuvants for Cancer Vaccine Immunotherapy - The purpose is to conduct a series of thematically related projects to test new cancer vaccine approaches. The projects will include a clinical trial in patients with lung and ovarian cancer, and basic, translational and pre-clinical investigation at three institutions to encourage collaboration. A programmatic effort to promote technology transfer in cancer vaccine research in Pennsylvania is included. Finally, there will be an innovative program to develop a pipeline of new scientists and clinicians trained in cancer research in the Commonwealth which will involve outreach in Philadelphia and Lincoln University.

Anticipated Duration of Project

6/1/2010 - 5/31/2014

Project Overview

The overall research objectives of this program are:

1. To prolong survival and reduce mortality of patients with ovarian and lung cancer by enhancing T cell activation in the tumor microenvironment.
2. To support cancer vaccine research and training throughout eastern Pennsylvania.
3. To provide a training and mentoring program in translational cancer research for underrepresented minorities.
4. To promote technology transfer and the potential for job growth within the Commonwealth in the biotechnology of cancer vaccines.

We propose the following specific research aims to accomplish the general objectives of this program:

1. Establish the safety, antitumor activity and optimum biologic dose of a bispecific antibody that targets CD326 (epithelial cell adhesion molecule (EpCAM)) with the first arm and anti-CD3 (MT110) with the second arm in a phase I/II clinical trial. (Project 1: "CD326 (EpCAM) based

immunotherapy in patients with ovarian and lung cancer")
2. Determine whether or not the inclusion of specificities for costimulatory molecules or antagonists of inhibitory receptors will enhance the activity of the bispecific T cell engager platform. (Project 2: "Engineering Second Generation BiTE antibodies")
3. Explore the role of tumor endothelial marker-1 (TEM1) vaccination targeting the tumor microvasculature with combination therapies that antagonize vascular endothelial growth factor (VEGF). (Project 3: "Enhancing Cancer Immunotherapy by Immune Attack of the Tumor Vasculature")
4. Determine whether or not the inclusion of adjuvants that augment the Th17 arm of the cellular immune system enhances antitumor effects. (Project 4: "ICOSL based tumor vaccines")
5. Establish an educational program for undergraduate and graduate level training in translational cancer research. (Translational Research Fellowship Program)

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Other Participating Researchers

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Hossein Borghaei, DO, Gregory P. Adams, PhD, Matthew K. Robinson, PhD – employed by Fox Chase Cancer Center
John O. Chikwem, PhD – employed by Lincoln University

Expected Research Outcomes and Benefits

This program in cancer vaccines will produce both short term and long term benefits. In the near term, the program will enable the conduct of a promising clinical trial for patients with lung and ovarian cancer, thereby providing scientific information on the ability of CD326 to serve as a vaccine target using bispecific T cell engager antibody technology. As a result of the inter-institutional collaborative research efforts, long term benefits will continue to accrue for patients in eastern Pennsylvania who will benefit by improved access to state of the art trials in cancer vaccine research. At the same time, the development of new approaches to harness the power of the immune system to attack tumor cells and the tumor microenvironment will be pursued in the laboratory. A world-class group of clinicians and scientists has been assembled to address this vital problem and will synergize to develop new cancer therapies. In summary, this Program will allow outstanding scientists, educators and clinicians to leverage the power and potential of the immune system to develop new cancer vaccines with improved efficacy and reduced toxicity.

In addition to research outcomes for cancer therapy, this project contains a significant focus on teaching and education in order to produce a durable result for years to come. We will reach out to the City of Philadelphia, and to college students at Lincoln University to enhance the pipeline of new investigators trained in this promising scientific field, while providing role models and unparalleled opportunities for aspiring under-represented minority students.

Summary of Research Completed

Specific Aim 1. The scientific goal of Project 1 is to find and clinically evaluate new antibody constructs that target tumors for a vaccine effect, thereby redirecting the force of polyclonal T cell populations against tumor cells in patients with advanced breast, lung, and ovarian cancer. Our initial efforts during the past year were to develop the CD326/CD3 bispecific antibody construct from Micromet for this purpose (and a clinical protocol was written); however, further results from the phase I study of this reagent in Europe became available and demonstrated poor clinical efficacy and importantly raised questions of liver toxicity. Although considerable effort was expended in collaboration with the company to develop alternative dose and schedule scenarios to mitigate these concerns, intellectual property issues became highly complex and jeopardized the scientific goals of this project such that collaborations with the company were terminated.

In the interim, we became highly interested in the tumor target c-met as the focus of this project. Like CD326, c-met is overexpressed on the surface of solid tumor malignancies, but unlike CD326, a role for c-met as a receptor tyrosine kinase in tumor growth and development is clearly established, increasing its value as a potential immune target. During the past year, we have (i) developed mRNA gene transfer technology to express a c-met antibody moiety on the surface of human T cells that redirects these T cell against c-met expressing tumors; (ii) written and submitted a clinical protocol to test c-met redirected T cells in patients with newly diagnosed breast cancer (Figure 1). This protocol, entitled "A clinical trial of autologous met redirected T cells administered intratumorally and intravenously in patients with operable triple negative breast cancer" carries the primary objective of establishing safety and feasibility and secondary objectives of determining immunological impact of this therapy, including establishing direct cytotoxicity of the T cell product vs. epitope spreading (i.e. "vaccine effect); and (iii) evaluated the feasibility to apply the Quantigene platform to formalin-fixed and paraffin embedded (FFPE) samples as a novel technology that allows for the multiplexed quantification of mRNA expression in an attempt to identify the panel of antigens expressed on tumor cells as part of our larger programmatic effort to develop patient specific targeted therapeutics. We have initiated efforts to apply the Quantigene platform and evaluate c-met expression in 40 primary breast tumors that will confirm the high level of c-met expression in these tumor types and establish the foundation for qualifying this assay for clinical use in the proposed trial.

Specific Aim 2. The goals of this project are to generate and validate 2nd generation bispecific T cell engagers (BiTEs) that engage co-stimulatory receptors (e.g. ICOS) or co-inhibitory receptors (e.g., PD-1 and CLTA-4) on T cells. Our focus during the past year has been on cloning and expressing the target receptors and initiating the process of isolating single chain antibodies (scFvs) that engage these targets. The genes encoding each of the three target receptors' extracellular domains (ECDs) were synthesized and cloned into pSec/Fc vectors to create in-

frame fusion proteins with a human IgG1 Fc. The expressed fusion proteins were designed to contain an optimized thrombin cleavage site for liberation of the target ECDs and a 6X-HIS tag at the C-terminus of the Fc domain for purification and detection. HEK-293T cells were transiently transfected with vectors containing each ECD gene and the resulting fusion proteins were isolated and purified. Two of the fusion proteins, CTLA-4 and ICOS, exhibited very poor production yields and after multiple attempts the decision was made to purchase these proteins that had recently become available from a commercial vendor. The third target ECD, PD-1 exhibited significantly better production from transiently transfected cells with approximately 3.5 mg of protein recovered from 100 ml of media (Figure 2). Phage display selection was initiated using the PD-1 ECD as a target. Briefly, $1x10^{17}$ pfu of the Nissim scFv combinatorial phage display library (Nissim et al EMBO J 1994) was depleted against Immunotubes coated with 100 μg/mL human IgG1 to reduce Fc-specific clones in the input phage. The depleted library was then used to select binders against immobilized PD-1/Fc. Eluted phage were used to infect TG1 bacteria and enriched phage libraries were generated by infection with M13K07 helper phage for iterative rounds of panning (Table 2). After the 3rd round, 534 clones were analyzed by ELISA for binding to PD1 and human IgG1 Fc. Fifteen clones exhibited increased binding to PD1 as compared to Fc. An additional 8 clones exhibited binding to the Fc domain. PD1-specific clones are currently undergoing further evaluation.

Specific Aim 3. In the past year, we focused on optimizing TEM1 vaccination. First, we characterized anti-TEM1 responses in the murine model (~150 animals), both C57BL/6 and BALB/c haplotypes. Epitope characterization was assessed with a Mimotope library of murine TEM1 (mTEM1) peptides. We designed a library of 15-amino acid peptides overlapping by 11 amino acids that span the entire length of mouse TEM1, which was used to stimulate splenocytes of mTEM1-DOM immunized mice and control groups. T cell responses were assessed by IFNγ ELISPOT and intracellular cytokine staining (ICS). Using mathematical set theory from 4 pools of peptides, matrices were designed and immunodominant tem1 epitopes were identified. To improve expression of the mTEM1 protein, a new version (mTEM1-SL, "SL" as short length) was designed, in which the wild type leader sequence was replaced by tissue plasminogen activator leader sequence, and the intracellular and transmembrane domains were deleted. We completed the construction of all the predicted optimized vectors and compared their level of expression in CHO cells (Fig 3A). Next, we tested the potency of the new vectors in vivo (~210 animals); none of the DNA modifications applied to the original clone mTEM1-DOM resulted in the expected increase of antitumor activity (Fig 3B). We also evaluated the mTEM1 specific immune response using IFNγ ICS and ELISPOT and found that the applied molecular engineering did not improve the number of tumor-reactive T cells in immunized animals.

An alternative for vaccine optimization was the construction of DNA vectors which simultaneously express mTEM1-DOM and siRNA targeting endogenous NF-κB inhibitors. We initially designed two distinct shRNAs. The screened vectors were tested in vivo (~ 210 animals); we demonstrated that co-expression of shRNA results in significant increase in mTEM1-reactive CD3+ CD8+ IFNγ+ antitumor T cells. In vivo, TEM1-DOM/shRNA (TD-C1) resulted in a significant increase in antitumor efficacy compared to the original mTEM1-DOM clone (Fig. 3C).

Specific Aim 4. The main goal of this Aim is to determine if Th17 cells can be augmented in order to enhance antitumor effects. The first set of experiments was initiated by testing conditions to polarize and expand normal human peripheral blood and cord blood cells for possible use as a cell based approach to deliver Th17 cells to the tumor site. In experiments that were initiated with other funds, and then completed with this project, we found that the inducible costimulator (ICOS) was critical for the differentiation and expansion of human Th17 cells. Cells grown in vitro with ICOS stimulation had prominent inflammatory properties, including increased secretion of IL-21, IL-17, and IFNγ compared with cells stimulated with CD28. In contrast, cells stimulated with CD28 grew equally, but were less potent in tumor models than the ICOS stimulated cells. A mechanism for this effect was suggested by the fact that ICOS stimulation induced c-MAF, RORC2, and T-bet expression, all of which are markers of Th17 cells. This work was published (Paulos et al. The inducible costimulator ICOS is critical for the development of human TH17 cells. Science Transl Med 2(55) Oct 27, 2010) and a News and Views discussed the significance of these findings (J. Garaude. ICOStomizing Immunotherapies with TH17. Sci Transl Med 2, 55ps52 (2010).

We have tested during last year whether these results could be extended by genetically providing the ICOS signal in T cells. Our hypothesis is that redirection of Th17 cells with chimeric receptors containing the ICOS intracellular domain in tandem with TCR-ζ signal-transduction domain will promote and retain the Th17 phenotype after antigen recognition. In order to test this hypothesis, we generated a chimeric receptor containing a single-chain variable fragment that binds mesothelin (SS1 scFv) linked to the signaling domains of ICOS and the CD3ζ chain (SS1-ICOS-z). Activation of Th17 cells through SS1-ICOS-z resulted in significantly higher production of IL-17A, IL-17F and CCL20, consistent with a predominant Th17 phenotype. By contrast, cells expressing SS1-CD28-z secreted significantly higher amounts of IL-2 and IFNγ cytokines (Figure 4). In summary, these preliminary results suggest that redirection of Th17 cells with a chimeric antigen receptor containing the ICOS intracellular domain retains Th17 cell function and phenotype and can be a highly promising approach in the treatment of cancers expressing mesothelin, or c-met, as described in Specific Aim 1.

Specific Aim 5. The objectives of the training core are to provide undergraduate and graduate students with an introduction and the basic tools necessary to pursue careers in translational research. As part of this goal, we established both a Summer Internship Program and a monthly seminar series for undergraduate students at Lincoln University during the academic year. The first year of the Summer Internship (2010), we had one student enrolled. Ms. Bianca Montalmont completed a project entitled "Adoptive Immunotherapy for Peripheral T cell Lymphoma" in the laboratory of Dr. Nicole Aqui at the University of Pennsylvania (UPenn), attended several workshops and seminars, and the culmination of her internship was an oral presentation at the CTSA Research Symposium. The seminar series covered various aspects of translational research being conducted by investigators involved in all projects of this grant. The seminars were extremely well-received as evidenced by the increase in applications to our Summer Internship Program (2011). Of four applications, Ms. Shauna Ebanks, was accepted and is currently performing research at UPenn under the mentorship of Dr. Michael Kalos. Her project is entitled "Q-RT-PCR Analysis of the Immunological Milieu in Tumors after T cell Immunotherapy." Ms. Ebanks will have the opportunity to continue doing research in the laboratory of Dr. Karen Baskerville, an Associate Professor at Lincoln University, during the

upcoming academic year. Dr. John Chikwem, Co-Director of the Summer Internship Program nominated Dr. Baskerville, to lead the research component of the program at Lincoln University. Also as part of this training effort, a scientific colloquium focused on cell-based therapies was sponsored by both the PA grant and the UPenn Clinical and Translational Science Award. The colloquium, held on April 1, 2011 was attended by students from Lincoln, UPenn and other area colleges.

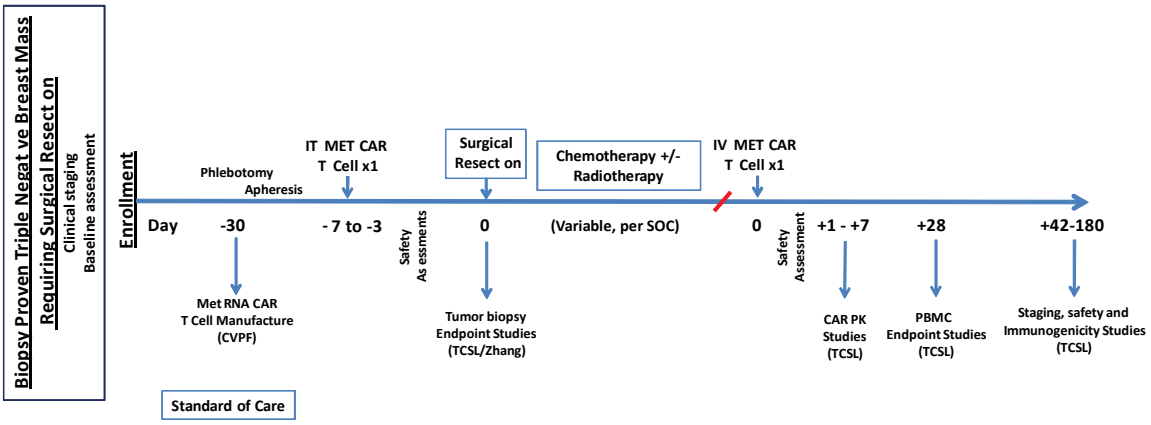
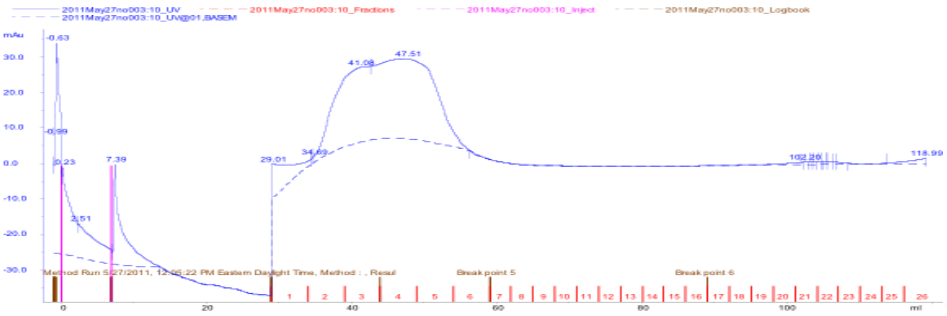


Figure 1. Clinical trial schema for c-met redirected T cell therapy in breast cancer.

A.



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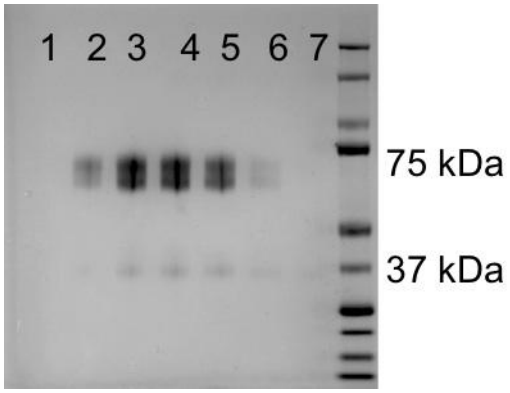

Figure 2. Purification of fusion protein. (A) Following expression from HEK cells transiently transfected with PD-1, the fusion protein was purified by Immobilized Metal Affinity and size exclusion chromatography over a HiPrep 16/60 Sephacryl S-100 column. (B) Western blot analysis revealed a fusion protein of the expected molecular weight - approximately 75 kDa.

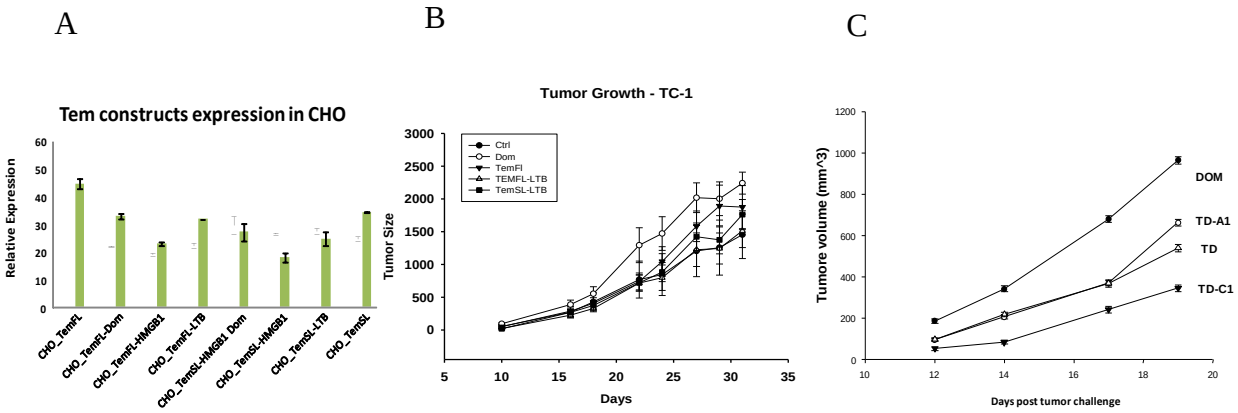

Figure 3. Analysis of predicted optimized TEM1 vectors. A) Expression of fusion proteins caring the full length wild type leader sequence (TemFL) and the new construct (TemSL) on CHO cells by mRNA quantification; B) In vivo comparison of the antitumor effects of DNA TEM1-DOM full length and the engineered version of TEM1-DOM short length (TemSL); C) In vivo antitumor effects of TEM1-DOM/shRNA constructs (TD-A1, TD-C1) compared to TEM1-DOM construct (TD) and control (DOM).

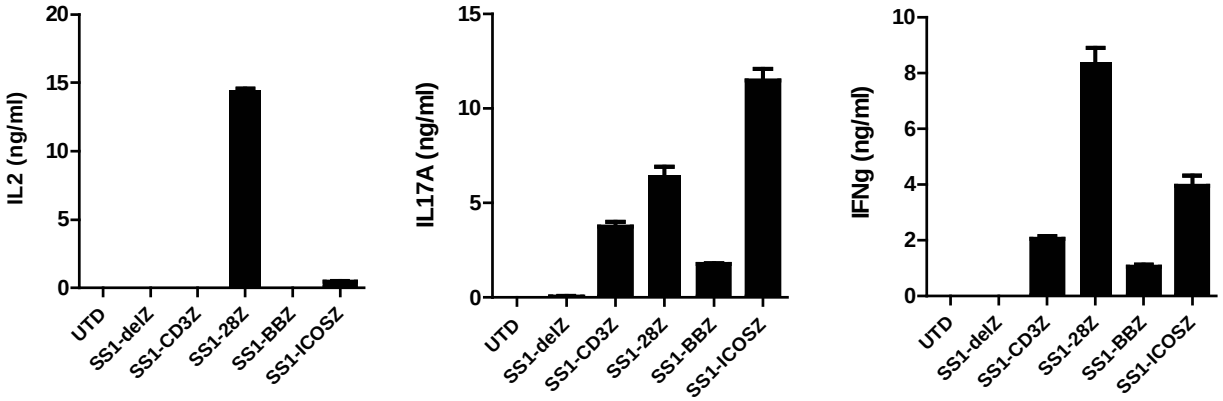

Figure 4. Redirected Th17 cells containing CD28 or ICOS signaling domains were cocultured with antigen presenting cells expressing mesothelin recognized by the SS1 antibody. After 24h, supernatants were collected and cytokine production was analyzed by ELISA.