University of Pennsylvania

Annual Progress Report: 2009 Nonformula Grant

Reporting Period

July 1, 2013 – May 31, 2014

Nonformula 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.

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)

Principal Investigator

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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. During the last year, three subjects were enrolled and treated in Cohort 1 of the clinical trial testing feasibility and safety of cMet RNA chimeric antigen receptor (CAR) T cells administered by intratumoral (IT) injection in patients with breast cancer. For increased safety, the cMet CAR in the T cell product was designed to have a transient expression by using the Maxcyte technology for RNA electroporation in a GMP validated manufacturing process.

The enrolled patients had metastatic breast cancer with accessible target metastatic tumor, cutaneous, subcutaneous, or superficial, not close to a major blood vessel and/or palpable adenopathy/mass expressing cMet by immunohistochemical (IHC) analysis. The treatment regimen consisted of one IT injection of $3x10^7$ cells expressing cMet CAR at 3 days before the target tumor excision. The excised tumor was analyzed for evidence of cMet directed responses. Preliminary IHC analysis indicates occurrence of tumor necrosis along the needle track associated with the presence of lymphocytes and macrophages. Additional correlatives studies testing the presence of cMet CAR in the T cell in tumor tissue and peripheral blood indicate presence of redirected T cells in the excised tumor tissue and lack of cells leaking into the circulation. To date, no dose limiting toxicity was noted allowing progression of the study to the next cMet CAR dose level.

Specific Aim 2. The overarching goal of this aim is to evaluate the therapeutic impact of 2nd generation BiTE (bispecific T-cell engager) molecules that recruit and activate T cells through interactions with one of three different receptors (ICOS, PD-1, CTLA4) present on the surface of T cells. Work in the past year has focused both on the expression\ characterization of the T cell binding single chain Fv (scFV) antibodies and on construction\expression of the BiTE molecules. A panel of single-chain Fv antibodies expressed in mammalian systems to prevent the introduction of endotoxin was generated against ICOS, PD1 and CTLA4. These reagents were expressed and sent to colleagues at UPenn for analysis of biological function. While the PD1 antibodies failed to exhibit appropriate activities in the assay systems employed (Data not shown), one anti-ICOS antibody exhibited activities equivalent to positive control antibodies (Figure 1). Assays are being established to measure anti-CTLA-4 function.

We have begun constructing a novel bispecific antibody platform (Figure 2) for use in developing next generation BiTE molecules.

Specific Aim 3. In this last year, we have been mostly involved in the work required for the final rebuttal to the first submission of our manuscript presenting data on TEM1-specific DNA vaccination targeting tumor vasculature. The manuscript was submitted and accepted for publication in Journal of Clinical Investigation (Facciponte et al., JCI 2014). Data generated was used to apply for and obtain additional funding from Basser Research Center for BRCA to

continue and expand the research in the clinical field. The toxicology studies and manufacturing process for clinical investigation development were initiated. In the clinical proposal, we plan to conduct a standard first in human phase I dose escalation study to determine safety and tolerability, clinical and immunogenic response of TEM1-TT DNA vaccine in BRCA positive recurrent ovarian cancer patients who are platinum sensitive after completing secondary treatment with platinum-based therapy and are in remission. A 3+3 design will be employed whereby the following doses will be tested respectively: 0.3 mg/dose, 1.5mg/dose and a 3 mg/dose. Four injections will be given in each cohort on Day 0, week 4, week 12, and week 24. Patients will receive a dose of cyclophosphamide infusion of 200 mg/m^2 the day before each vaccination. Cyclophosphamide has been shown to augment anti-tumor immune response in patients via downregulation of regulatory T-cells. Patients who have no evidence of disease will continue receiving maintenance vaccine every month until progression. A total of 15-18 patients will be enrolled, 3 in each cohort followed by an additional 6 patients allocated to the maximally tolerated dose. The primary objective of this study is to determine the safety and feasibility of TEM1-TT while the secondary objectives are immune and clinical response. Immune response will be measured by interferon gamma response while the clinical response will be assessed looking at PFS at 6 and 12 months and by RECIST criteria. The strategy for clinical development of the vaccine will move in line with previous trials with DNA cancer vaccines. The steps include: a) manufacturing GLP vectors for toxicology studies, and GMP vectors for the human trial; b) complete toxicology studies in small and large animals; c) submit an IND application; d) initiate a phase I study.

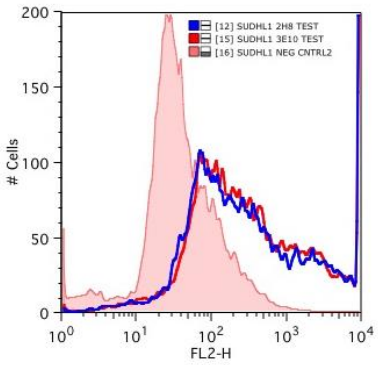
Specific Aim 4. Long-term persistence of adoptively transferred CAR redirected T cells correlates with antitumor effects in clinical trials performed at the University of Pennsylvania. To test *in vivo* persistence of CAR redirected T cells, we used a mesothelioma tumor mouse model based on NSG mice. A panel of mesothelin (ss1)-specific CAR bearing various signaling domains (BBζ, 28ζ, ICOSζ) was used to transduce either polarized Th17 or non-polarized Th1 which were then injected into tumor bearing mice. Our results suggests that T cells redirected with BBζ showed a relatively slow tumor decline, with all mice showing significant tumor shrinkage after treatment, while 28ζ and ICOSζ treatments showed a rapid tumor decline. Mice were sacrificed 51 days after treatment due to graft-versus-host disease. At this time, the Th17 cells redirected with ICOSζ showed an enhanced persistence; the average number of T cells in peripheral blood was more than 4-fold higher when compared with 28ζ and BBζ groups. To confirm that T cell persistence was due to tumor recognition, and not to recognition of xenoantigens by the endogenous TCR, we performed a similar experiment in which NSG mice were implanted with non-small cell lung tumors. This experiment was done in duplicate in mice with or without tumors (empty mice). Th17 cells redirected with ICOSζ showed an increased *in vivo* expansion and persistence that was significant when compared with 28z and BBz groups. Importantly, ICOSζ T cell expansion was dependent on tumor antigen encounter, as empty mice showed significantly less numbers of ICOSζ cells when compared with mice with tumors. In summary, these results suggest that Th17 cells redirection with an ICOS-based CAR retains Th17 cell function and phenotype and enhances antitumor effect and T cell persistence in mice. These results were recently published (Guedan et al., Blood 2014).

Specific Aim 5. The objectives of this core are to provide undergraduate and graduate students with an introduction and the basic tools necessary to pursue careers in translational research.

Each student participates in an intensive, supervised research experience with mentors involved in some aspect of translational research, from bench work to clinical trials. As part of this goal, we established both a Summer Undergraduate Internship and a monthly seminar series for undergraduate students at Lincoln University during the academic year.

The seminar series at Lincoln University has been completed for this academic year. Our undergraduate program hosted two students in the summer of 2013. Danielle Mathis from Lincoln University completed a project in the lab of Dr. Andrea Facciabene. The goal of her project was to improve the immunogenicity of a DNA vaccine (Tumor Endothelial Marker-1, TEM1) by a gene expression modifying approach. Danielle sub-cloned the mouse vaccine into a new vaccine that will be able to target the human form of TEM1. These vaccines will be subsequently tested in humanized HLA-A2 mice to assess efficacy, before being put into clinical trials. Conner Paez from UPenn worked with Dr. Bruce Sachais to study heparin-induced thrombocytopenia (HIT). Dr. Sachais has previously developed, optimized and published a retrospective study on several assays that may have improved diagnostic parameters for HIT. The goal of Conner's project was to optimize and prospectively validate a novel test to diagnose HIT. He modified this test to improve robustness and reproducibility of the assay. He then examined over 100 prospectively collected samples with this modified assay. He has continued this work during the past academic year, and now ~200 patients have been examined. This work is ongoing and Connor will once again spend the summer in Dr. Sachais' laboratory. Both students participated in the CTSA Summer Undergraduate Internship Program's workshops and seminars, and are expected to present their research at the CTSA Research Symposium. Ms. Ebanks, our undergraduate student from 2011, continued her research in the laboratory of Dr. Karen Baskerville, an Associate Professor at Lincoln University, with funding for supplies provided by the PA DOH.

A.



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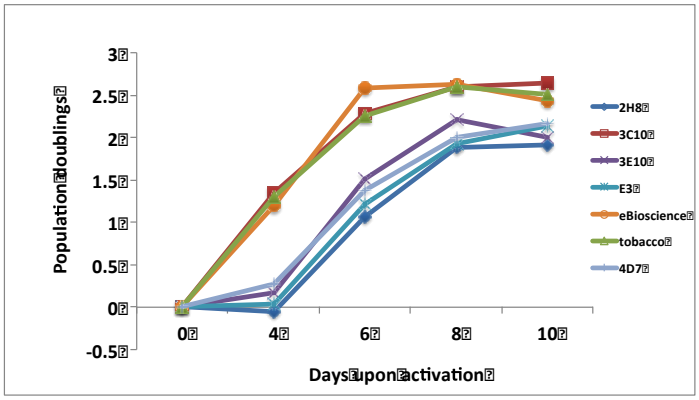

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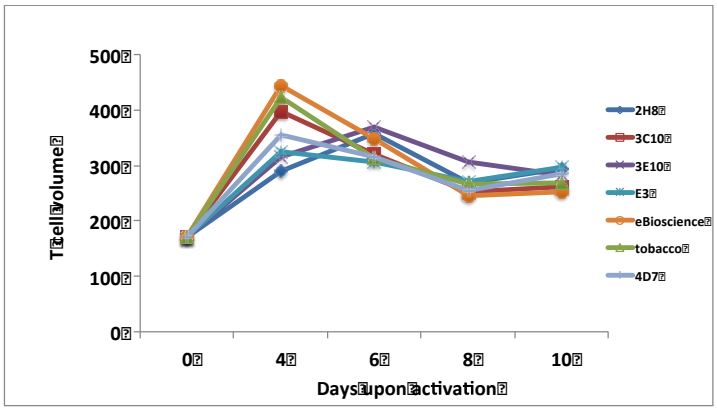

Figure 1. Binding and Impact of anti-ICOS antibodies on receptor function. The ability of newly-developed anti-ICOS antibodies (2H8, 3E10) to bind to ICOS expressing SUDHL1 cells (A). The ability of anti-ICOS antibodies (2H8, 3C10, 3E10, 4D7) positive control anti-ICOS antibodies (eBioscience, JMAB 52 [tobacco]) and a negative control antibody (E3) and to stimulate receptor function were measured using population doubling (B) and T Cell volume (C).

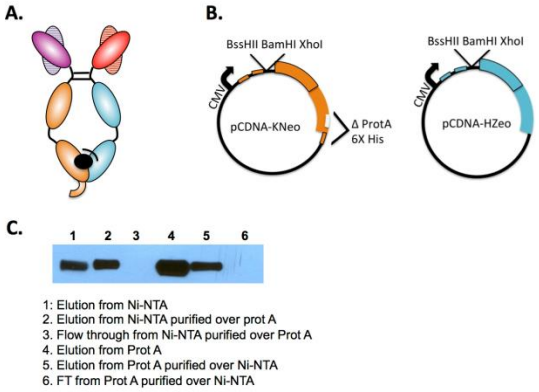

Figure 2. Schematic and Vector Map of new bispecific antibody construct. A depiction of our new bispecific antibody format (A), vector maps of the light and heavy chain domains (B) and elution profiles of test molecules verifying the behavior of the expression system are presented.