University of Pennsylvania

Annual Progress Report: 2009 Nonformula Grant

Reporting Period

July 1, 2011 – June 30, 2012

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.

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 interinstitutional 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. We have developed a novel therapeutic strategy to target cMet-positive breast cancer cells using autologous T cells transiently expressing the chimeric anti-Met immunoreceptor scFv using the RNA electroporation technology. Since the time of our last progress report, we have generated preclinical data indicating the safety profile, specificity of target recognition, and anti-tumor efficacy of cMet redirected T cells, data supporting development of the clinical protocol and the IND application. The primary objective of this study is to determine the safety of treating patients with newly diagnosed non-metastatic triple negative breast cancer (TNBC) with cMet RNA autologous T cells. The clinical protocol was approved by NIH RAC and FDA allowed opening the IND. Following regulatory revisions by University of Pennsylvania (UPenn) committees, the protocol has been amended several times and is awaiting final approval.

Recently, we reported the prospect of mesothelin as a second breast cancer surface target for engineered T cell (Tchou J, Wang LC, Selven B, Zhang H, Conejo-Garcia J, Borghaei H, Kalos M, Vonderheide RH, Albelda SM, June CH, Zhang PJ: Mesothelin, a novel immunotherapy target for triple negative breast cancer. Breast Can Res Treat, 2012; 133:799-804). Screening 99 primary breast cancer samples by immunohistochemistry analysis confirmed that mesothelin is over expressed in the majority of TNBC (67%) but only rarely (<5%) in other breast cancers. A significantly higher anti-tumor cytotoxicity by such T cells compared to control was observed in vitro, suggesting for the first time that mesothelin has promise as a novel immunotherapy target for TNBC for which effective targeted therapy is lacking to date.

Specific Aim 2. The overarching goal of this project is to develop 2nd generation BiTE (bispecific T-cell engager) molecules in an effort to redirect a patient’s T cells against their cMet expressing cancer cells. BiTEs are comprised of two scFv antibody fragments, one specific for a tumor-associated antigen and a second specific for a regulatory receptor on the surface of T cells. The BiTEs being developed target the cMet tumor-associated antigen and one of the T cell markers (ICOS, PD-1, or CTLA4) using both phage-display and antibodies identified from the literature to isolate the respective scFv. We currently have isolated 4 classes of anti-ICOS scFv (Figure 1), 4 classes of anti-PD1, and one class of anti-CTLA4 scFv. Three rounds of phage-display pannings have been completed against cMet scFv; results from those screens show that 0.0007%, 0.0112%, and 0.4% of phage were recovered from each round, consistent with the expected enrichment of cMet specific clones during each round of selection. Tests are underway to determine the classes of anti-cMet scFv isolated in this screen.

Initial plans called for expression of the BiTE molecules in an E. coli-based expression system. Three anti-ICOS scFv along with a negative control scFv were conjugated to CD3 beads and assayed for T cell activation phenotype in comparison to either CD3/ICOS or CD3/CD28
costimulation. An unexpected activation phenotype was associated with the negative control scFv which was attributed to a low level of endotoxin contamination despite efforts to remove this contaminant from the preps. This has led to a revised plan for expressing scFv and subsequent BiTE molecules in mammalian expression systems. To date, genes encoding 3 anti-ICOS, 2 anti-PD1, and the single anti-CTLA4 scFv have been cloned into mammalian expression vectors for initial testing to determine the lead scFv that will be moved forward into the 2nd generation BiTEs. We have also constructed a pSECTag2-based vector for expression of BiTEs in mammalian cells. Versions of this vector currently contain anti-Her2 scFv as tumor targeting moieties that will be used as proof-of-concept until lead anti-cMet scFv are identified.

Specific Aim 3. During the second year, we have gained further understanding of TEM1 vaccination by studying the effects on tumor vasculature, dissecting the role of cross priming, and exploring prime/boosting strategies to improve vaccination protocols; additionally, we initiated characterization of human TEM1.

Following confirmation of TEM1 expression in blood vessels of CT26 tumor (by FISH technique), we analyzed changes in the blood perfusion of the hyper-vascularized CT26 tumors collected from mice immunized with TEM1-DOM or control DOM vaccine. Our analysis of blood perfusion (hemoglobin levels by ELISA) and blood flux (Doppler) indicated a statistically significant decrease in TEM1-DOM treated tumors compared to control treatment. We hypothesized that the reduction in blood perfusion from TEM1-DOM vaccinated mice resulted from an immune mediated endothelial/pericyte cell lysis and vessel damage followed by increased tumor hypoxia. Using immunohistochemical staining and qRT-PCR analysis of the hypoxia-related marker carbonic anhydrase IX (CAIX), we observed an increase of CAIX signal in TEM1-DOM treated compared to control treated mice (Figure 2).

In the previous report, we showed that vaccination against tumor vasculature resulted in induction of immune responses against tumor-associated antigens (TC1 model). To confirm this finding, we analyzed the CT26 colon adenocarcinoma mouse model; CT26 cells express an endogenous retroviral gene product, gp70, processed intracellularly to express the class I epitope gp70423-431 known as the AH1 peptide. We found that CT26 tumor-bearing BALB/c mice vaccinated with TEM1-DOM mounted specific responses against both TEM1516-530 epitope and AH1 peptide, but the control DOM vaccine did not induce any response. The magnitude of the TEM1 and AH1-specific T cell responses were inversely correlated with tumor volume on day 30 (Figure 3). These data suggest that TEM1-DOM vaccination generates initially a potent anti-TEM1-specific T cell response which disrupts the tumor vasculature followed by antigen spreading and additional tumor specific responses (illustrated here by the AH1 specific response) that contribute to improved control of tumor growth. In an attempt to improve the vaccination potency, we tested differential in vivo prime/boosting strategies using the TEM1-DOM expression cassette as DNA or as adenovirus vaccine formulations; however, none of the strategies utilized boosted the anti-TEM1 response.

Finally, we initiated in vivo studies on immunogenicity of human TEM1 protein; splenocytes of immunized C57b6 mice were stimulated in vitro with 4 peptide pools of 15-mers (overlapped by 10 amino acids) covering the entire human TEM1 protein. An immunodominant response was
Specific Aim 4. Adoptive transfer of Th17 polarized cells expanded in vitro is an attractive therapy for cancer treatment. The inducible costimulator ICOS has been shown to be critical for sustained expansion of human Th17 cells. We analyzed whether incorporation of ICOS intracellular domain in a chimeric antigen receptor (CAR) specific for mesothelin (ss1) tumor antigen can promote the Th17 phenotype after antigen encounter and enhance the anti-tumor activity of redirected T cells. Th17 polarized cells were engineered to express ss1 scFv fused with either ICOS+CD3ζ (ICOSz), CD28+CD3ζ (28z), or 41BB+CD3ζ (BBz) signaling domains. When stimulated in vitro with ss1-expressing tumor cells, ss1-ICOSz T cells secreted high amounts of IL-17 and CCL20 and nominal levels of IL-2; by contrast, ss1-28z T cells secreted high amounts of IL-2 and IFNγ and nominal levels of IL-17. The in vivo antitumor efficacy was studied upon T cells transfer into NSG mice with large vascularized pre-established mesothelioma. NSG mice were injected with the human mesothelioma cell line M108 (5 x 10⁶ cells/mice) and 8 weeks later when the tumors reached 500 mm³, mice were treated with 2 intratumoral injections of 10⁷ Th17 and Tc17 mixed cells (1:1 ratio, 80% CAR expression for CD4+ T cells and 60% of CAR expression for CD8+ T cells) redirected with ss1-28z, ss1-BBz, or ss1-ICOSz CARs on days 61 and 67 (n=9 for all groups). A potent antitumor effect was observed in all T cell treated groups compared to the control group treated with PBS (Figure 4A), and 70% of mice treated with ss1-ICOSz CAR showed complete remission. Importantly, ss1-ICOSz CAR CD4 T cells had a significantly increased persistence post-infusion compared to ss1-28z and ss1-BBz CAR T cells (Figure 4B), which correlated with severe graft versus host disease (Figure 4C). This data indicate that the design of novel ICOS-based CARs has the potential to augment the antitumor effect on clinical trials.

Specific Aim 5. The objectives of this Minority 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 Undergraduate Internship and a monthly seminar series for undergraduate students at Lincoln University during the academic year. The seminar series has been completed for this academic year. University of Pennsylvania (UPenn) speakers from a wide range of departments covered diverse topics in translational research, including pharmacology, neuroscience, and cancer immunology. New this year, we instituted an evaluation form that captures demographic information as well as a critique of the speaker. For the summer 2012 undergraduate program, we received 3 applications. All applicants were accepted for internships at UPenn laboratories. Two applicants (Ms. Anup Misra and Ms. Jhoneil Cooper) are funded through this grant, while the third (Ms. Jodi-Ann Foster) is funded through the UPenn Clinical and Translational Science Award (CTSA). Jhoneil Cooper is studying genetic regulation of lipid and lipoprotein metabolism and its molecular relationship to atherosclerosis in the laboratory of Dr. Dan Rader; Anup Misra is studying genetic engineering of patients T cells in the laboratory of Dr. Carl June; and Jodi-Ann Foster is studying therapeutics for type 1 and type 2 diabetes in children with Dr. Steven Willi. All 3 applicants will participate 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 last summer, is continuing her research in the laboratory of Dr. Karen Baskerville, an Associate Professor at Lincoln University, with funding for supplies provided by this grant.
The graduate program received interest primarily from students of the UPenn Perelman School of Medicine. Four students were interviewed and an additional five students submitted applications. Ms. Ernestina Nyarko was accepted and she will begin her independent study project with Dr. Jun Mao in September 2012, after completing her electives. Ms. Nyarko will be working on two projects: 1. cancer survivor’s perceptions of survivorship care from primary care physicians, and 2. immunological markers in acupuncture as treatment of althralgia in breast cancer patients on aromatase inhibitor therapy.

Figure 1. Analysis of purified anti-ICOS scFv for T cell engagement. Flow cytometry analysis of 4 anti-ICOS scFv clones (shaded area) binding to ICOS-positive SUDHL cells; control samples are stained with secondary antibody alone (open area).

Figure 2. TEM1-DOM vaccination increases tumor hypoxia. Tumors from mice vaccinated with either TEM1-DOM or control DOM vaccine were analyzed by immunohistochemistry (A) and qRT-PCR (B) to evaluate the CAIX expression; n=5.
Figure 3. TEM1 immunization induces epitope spreading. (A) Splenocytes from tumor bearing mice vaccinated with TEM1-DOM were tested for the capacity to respond to the specific TEM1<sub>516-530</sub> epitope (TEM1), and to the gp70<sub>423-431</sub> epitope (AH1) by ELISA. (B) Both anti-TEM1 and anti-AH1 immune responses inversely correlate with tumor volume.

Figure 4. Potent antitumor effect of T cell redirected with ss1-ICOSz CAR. NSG mice were implanted with the human mesothelioma cell line M108 (5 x 10<sup>6</sup> cells/mice); when the tumors reached 500 mm<sup>3</sup>, mice were treated with intratumoral injections of 10<sup>7</sup> T cells (1:1 CD4 to CD8 ratio) redirected with ss1-28z, ss1-BBz, or ss1-ICOSz CARs on days 61 and 67 (n=9). (A) Antitumor effect was determined by measuring the tumor volume at multiple time points following treatment. (B) CD4 and CD8 T cell persistence in peripheral blood at the end of the experiment was measured by flow cytometry for each treated group (white= ss1-28z; black= ss1-BBz; red= ss1-ICOSz). (C) Graft-versus-host-disease was scored at the end of the experiment for each mouse.