

# Thomas Jefferson University

## Annual Progress Report: 2009 Nonformula Grant

### Reporting Period

June 1, 2010 – June 30, 2010

### Formula Grant Overview

The Thomas Jefferson University received \$4,500,000 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

*Therapeutic Vaccine Bridging the Gap in Racial Disparities in Colorectal Cancer* – This Center of Excellence for Cancer Immunotherapy will focus on therapeutic vaccines bridging the gap in racial disparities in outcomes in colorectal cancer. The purpose of this Center is to develop new vaccine strategies specifically targeted to patients at excess risk associated with race in colorectal cancer. Center goals include (1) advancing a novel vaccine paradigm for secondary prevention of recurrent colorectal cancer into phase I clinical trials in African American and Caucasian patients, (2) defining barriers and race-sensitive solutions to improve patient participation in cancer vaccine trials, (3) mechanism-based optimization of this novel colorectal cancer vaccine to maximize immunotherapeutic efficacy, and (4) developing the next generation of investigators from under-represented minorities for careers in biomedicine.

### Anticipated Duration of Project

6/1/2010 - 5/31/2014

### Project Overview

The Center of Excellence in Cancer Immunotherapy will develop a new vaccine paradigm that prevents disease recurrence and reduces stage-specific racial disparities in colorectal cancer. There is an unmet need for improved therapeutics in colorectal cancer, the third leading cause of cancer and second leading cause of cancer mortality worldwide. In Pennsylvania, colorectal cancer incidence and mortality rates are higher than those expected in the nation with ~15,000 cases treated each year associated with a total in-patient annual cost of >\$200M. Mortality reflects metastatic disease: ~50% of patients present with clinically apparent metastases, while ~30% present with occult metastases. Moreover, there is a disparity in outcomes in stage I and II African American patients, who exhibit ~40% excess mortality compared to Caucasians, reflecting occult metastases. This project advances an emerging paradigm in colorectal cancer detection and eradication, employing guanylyl cyclase C (GCC) as a prognostic marker and immunological target. GCC is a protein whose expression normally is restricted to intestinal

epithelial cells, but universally over-expressed by metastatic colorectal tumors. GCC is a marker of occult metastases in lymph nodes, which disproportionately burdens African American patients. Also, GCC is the index example of a new class of vaccine targets, cancer mucosa antigens, whose expression normally is restricted to mucosae, but extends to the immunologically naive systemic compartment upon metastasis of mucosal tumors. Advantages of these antigens include systemic immunoreactivity profiles supporting durable antitumor immunity, with limited immune cross talk between compartments restricting autoimmunity. This Center will translate these advances in molecular diagnostics and immunotherapy into new vaccines that bridge racial disparities in colorectal cancer. Center objectives will be accomplished through: (1) the Translational Research Program, which will define the safety and immunological efficacy of a GCC-based vaccine in stage I and II African American and Caucasian colon cancer patients variably burdened by occult metastases identified by GCC; (2) the Health Services Research Program, which will identify barriers that prevent African Americans and Caucasians from participating in vaccine trials, and strategies to increase informed participation; (3) the Biomedical Research Program, which will define mechanisms shaping responses to cancer mucosa antigens that inform clinical strategies to maximize antitumor efficacy; and (4) the Training Program, a collaboration of regional universities to recruit new scholars from under-represented minorities for careers in biomedicine.

### **Principal Investigator**

Scott A. Waldman, MD, PhD  
Professor and Chairman  
Pharmacology and Experimental Therapeutics  
Thomas Jefferson University  
1025 Walnut Street, Room 901  
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### **Other Participating Researchers**

Vitali Alexeev, PhD, Laurence C. Eisenlohr, VMD, PhD, Terry Hyslop, PhD, Ronald E. Myers, PhD, Elizabeth B. Rappaport, MD, Takami Sato, MD, PhD, Michael Mastrangelo, PhD – employed by Thomas Jefferson University  
Steven G. Hughes, PhD – employed by Cheyney University  
David Weinberg, MD – employed by Fox Chase Cancer Center  
Judith A. W. Thomas, EdD, James L. DeBoy, PhD – employed by Lincoln University  
David S. Zuzga, PhD – employed by St. Joseph's University  
Robert E. Schoen, MD, Nathan Bahary, MD, PhD – employed by University of Pittsburgh

### **Expected Research Outcomes and Benefits**

Outcomes from this program will: (1) Define the safety and immunological efficacy of adenoviral (AV)-GCC in Caucasian and African American stage I and II colon cancer patients with defined occult tumor burden in a phase I clinical trial. It is anticipated that AV-GCC will induce immune responses in African American and Caucasian patients variably burdened by occult metastases, without autoimmunity. This study will provide the first critical step in

developing an immunotherapeutic approach to secondary colorectal cancer prevention that can bridge racial disparities in disease outcome. (2) Identify barriers underlying racial disparities in participation in cancer vaccine trials. Processes underlying decision-making, racial differences in those processes, and methods to facilitate informed decisions with respect to participation in cancer vaccine trials have not been defined. Here, we will design patient education materials, adapt decision counseling materials, and revise survey data collection instruments to define barriers to participation in cancer vaccine trials through decision counseling, producing culturally-sensitive strategies to assist patients in making informed decisions about trial participation. (3) Define mechanisms shaping responses to cancer mucosa antigens that inform clinical strategies. The utility of cancer vaccines ultimately will reflect an understanding of mechanisms modulating immune responses that can be exploited to maximize efficacy. Studies here will identify tolerance landscapes that oppose vaccine therapy, define strategies to overcome that immunoinhibition, and identify receptor-dependent approaches to maximize immunotherapeutic efficacy, to optimize future clinical trials of AV-GCC. (4) Prepare trainees from under-represented minorities for careers in biomedicine. Here, we will provide training opportunities for students from under-represented minorities interested in careers in biomedicine through a summer internship program at Thomas Jefferson University that will include didactic and experiential research components.

### **Summary of Research Completed**

#### Safety And Immune Efficacy Of Adenoviral (AV) GCC In Caucasian And African American lymph node negative ( Pn0 ) Colon Cancer Patients With Defined Occult Tumor Burden

The hGCC<sub>1-429</sub>-PADRE-AV seed virus has been produced in the laboratory. The stock was tested in the laboratory for Replication Competent Adenovirus (RCA) and was confirmed to be RCA-negative. Aliquots of the seed virus were also sent to WuXi AppTec and passed testing for Sterility B/F, Immersion Sterility Testing, and Mycoplasma Detection. Therefore, the seed stock is suitable for manufacturing by the Center for Cell and Gene Therapy (CAGT) at the Baylor College of Medicine. Service agreements will be finalized shortly and virus will be transferred to CAGT.

Non-GLP animal studies are also being initiated at Thomas Jefferson University (TJU) to confirm the utility of the seed AV to produce human GCC antigen *in vitro* and *in vivo*, produce human GCC-specific antibody and CD4+ and CD8+ T cell responses in mice, and produce PADRE-specific CD4+ T cell responses in mice.

An initial assessment of preliminary data and plans for vaccine manufacturing, preclinical vaccine testing and the vaccine clinical trial has been performed by David J. Pepperl, Ph.D., Stephen D. Litwin, M.D., and Andra Miller, Ph.D. from Biologics Consulting Group. They are currently coordinating manufacturing with CAGT and TJU, assisting in chemistry, manufacturing and controls (CMC) preparation, and developing a regulatory strategy and tentative timelines for interacting with the Center for Biologics Evaluation and Research (CBER).

#### Mechanisms Shaping Responses To Cancer Mucosa Antigens That Inform Clinical Strategies

These studies employ the use of a novel transgenic mouse to test hypotheses on tolerance to intestinal antigens, employing the model antigen Tac. This transgenic mouse will possess Tac downstream of a ubiquitously expressed promoter and intervening floxed-stop cassette, such that the Tac will not be expressed. However, expression of Cre-recombinase in a tissue-specific fashion will result in tissue-specific stop excision and Tac expression. The targeting construct to insert a single copy of Tac downstream of a floxed-stop has been produced and sequencing has been performed to confirm the integrity of the construct. Linearized construct DNA has been transferred to the Transgenic and Gene Targeting Facility at the Kimmel Cancer Center at TJU. They are currently performing embryonic cell electroporation and selection. Embryonic cell clones will be screened shortly to identify those suitable for injection and production of chimeric mice.

In the process of transgenic development, recombinant adenovirus producing Tac has been generated. Preliminary assays have been performed to confirm the utility of the Tac-AV to produce immune responses to the model epitopes. Assays to measure antibody responses to the HA (B cell) epitope and Ova<sub>323-339</sub> (CD4+ T cell) epitope have been developed and combined with the well-established assays for Ova<sub>257-264</sub> (CD8+ T cell) epitope. A retroviral construct containing Tac has also been produced and stable cell lines (B16 and MC38) are currently being generated. The B16-Tac model cell line has been finalized and will be used to support experiments performed with MC28-Tac.

#### Preparation Of Trainees From Under-Represented Minorities For Careers In Biomedicine

Five underrepresented minority students began Summer Research Internships in Cancer Immunotherapy on June 1, 2010. Three of these students were recruited with assistance from faculty liaisons at Cheyney and St. Joseph's Universities. One is a third-year student at Jefferson's School of Nursing. The fifth, a graduate student in the MPH program at Drexel University who had worked with Dr. Ron Myers during the previous academic year, also was recruited. Each of these students is engaged in a project that is part of one of the program objectives. Two students are working with Dr. Myers developing pilot materials for decision counseling on participation in colon cancer vaccine trials. Two students are involved in laboratory research in cancer biology and immunology. One student is working with Dr. Terry Hyslop in biostatistics. This young man, a computer science major from Cheyney University, participated in the 2009 summer internship in biostatistics supported by a previous CURE non-formula grant (Center of Excellence for Research in Obesity, PI B. Falkner, M.D.). He applied to participate in the second summer internship and is planning to pursue an advanced degree in biostatistics following graduation from Cheyney in 2011.

The didactic program, designed by Dr. Elizabeth Rappaport with input from the Principal Investigator, other co-investigators and members of the Training Oversight Committee, will have provided 20 hours of lectures by June 30, on topics including laboratory safety, animal care and use, research ethics and protection of human subjects in research, principles of clinical research and clinical trial design, patient decision-making about clinical trial participation, a review of cell biology, an introduction to cancer biology, and instruction from library staff on media literacy, use of Internet-based search tools, and reference management. All students have full access to TJU Library services and facilities. The following topics will be covered in subsequent

didactic sessions during July: fundamentals of immunology, pathobiology of cancer, tumor immunobiology and immunotherapy, and additional topics in biostatistics.

The Training Oversight Committee met on May 11, 2010 and reviewed student recruitment and program elements. Student recruitment for the current summer interns could not begin until funding was secured and thus was limited to the final weeks of the academic year. Nevertheless, Dr. Rappaport and faculty liaisons from collaborating universities were able to identify and enroll suitable candidates. The group agreed that recruitment for the 2011 summer intern group would begin during the fall semester of the 2010-2011 academic year and the application deadline would be February 2011. The group will consider developing a website to provide information and facilitate applications. A faculty member from Lincoln University, Dr. James DeBoy, Chair, Department of Health, Physical Education and Recreation, is in discussion with Dr. Ron Myers regarding a collaboration related to African-American patient decision-making regarding clinical trials participation.