# Thomas Jefferson University

# Annual Progress Report: 2009 Nonformula Grant

**Reporting Period**

July 1, 2010 – June 30, 2011

**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**

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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
Catalina Arango, 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**

Translational Research Program: Vaccine to prevent recurrent colorectal cancer. Activities focused on GMP (Good Manufacturing Practices) manufacturing of clinical-grade Ad5-hGCC-PADRE vector at the Baylor College of Medicine, Center for Cell and Gene Therapy. Completion of manufacturing, originally estimated at ~12-18 months, is on schedule. In that regard, a pre-PreIND dossier was submitted to the Center for Biologics Evaluation & Research, Office of Cellular, Tissue and Gene Therapies (CBER-OCTGT) in November 2010, anticipating a pre-PreIND conference. The submitted development plan was considered exemplary and CBER-OCTGT recommended the project move directly to a PreIND meeting. We are preparing the formal PreIND meeting package.

*GLP Preclinical Testing*. According to regulatory specifications, the murine equivalent of the human vaccine (Ad5-mGCC-PADRE expressing mouse GCC) underwent GLP manufacturing at Baylor for Preclinical Safety Testing. As part of this process the following steps have been initiated or completed:

PILOT SPLENOCYTE CRYOPRESERVATION. Preclinical testing will include studies of safety, immunogenicity and biodistribution. WuXi AppTec, Inc. will perform safety and biodistribution studies, and ship splenocytes to Thomas Jefferson University (TJU) for immunogenicity studies. Cryopreservation of murine splenocytes for shipment is non-standard. Thus, Baylor performed a pilot study in which WuXi administered Ad5-mGCC-S1 to BALB/c mice, cryopreserved splenocytes and shipped them to TJU. GCC- and Ad5-specific responses were equivalent in cryopreserved and fresh samples, validating the process for GLP Preclinical Studies.

MAXIMUM TOLERATED DOSE (MTD). Three doses of Ad5-mGCC-PADRE were administered to C57BL/6 mice based on the anticipated clinical dose of $10^{11}$ viral particles (vp) per patient

(Table 1) and safety data collected (Table 2). At all doses, Ad5-mGCC-PADRE had no specific adverse systemic toxicity. Therefore, the MTD of vaccine following one intramuscular (IM) injection over a 14 day period in C57BL/6 mice was $10^{11}$ vp.

90-DAY SAFETY, IMMUNOGENICITY AND BIODISTRIBUTION. C57BL/6 mice (n=240) were assigned to 3 groups, where Groups 2 and 3 were administered vaccine by IM injection and Group 1 were administered control vehicle (Table 3). Animals will be terminated on Day 14, Day 30 ± 1 or Day 90 ± 1, blood collected for hematology and clinical chemistry, immunogenicity analysis and biodistribution analysis, and tissues collected for histology, splenocyte harvesting, or biodistribution. Studies were initiated in June 2011.

Health Services Research Program: Barriers underlying racial disparities in cancer vaccine trials. Phase 1 of this Aim is nearly completed, including:

*Literature Review.* A review of the literature identified factors positively associated with patient participation in clinical research, including white race, education, trust in study personnel, knowing others who have participated in research, and social support for participation. Factors negatively associated include lack of knowledge about the study, concerns about being part of an experiment, restrictive eligibility criteria, onerous participation requirements and costs, limited accessibility, fear of side effects, and fatalism. Decision aids have been reported to help patients make informed decisions about complex health care choices, such as participating in a vaccine trial. The most effective decision aids provide easy to understand information, facilitate values and preference clarification, and involve interaction with a trusted health professional.

*Surveys of the Research Team and Patients.* A draft informational booklet on a hypothetical Colorectal Cancer (CRC) vaccine trial was distributed to 14 research team members to assess whether it clearly explained the purpose of the trial, contained accurate content, was easy to read, presented both pros and cons associated with trial participation, explained important keywords and concepts, covered appropriate topics, and included sufficient information to allow informed decision making about participation. Thirteen respondents from all three participating institutions provided feedback. Respondents recommended inclusion of more information about trial procedures and requirements for trial participation. The booklet was revised in accordance with these recommendations. The revised booklet is being reviewed by colon cancer patients at all study sites. To date, 12 patients have reviewed the booklet and have completed a brief survey. Responses show that patients think the booklet is easy to read (n=11); describes the purpose of the vaccine trial (n=12); clearly explains trial eligibility criteria (n=11); gives enough information about trial procedures and requirements related to trial participation (n=10); and adequately addresses safety and confidentially concerns (n=10). All 12 respondents reported that they would recommend the booklet to patients who are asked to consider participating in the trial.

*Modified Decision Counseling Program.* We developed an on-line interactive decision counseling application that is designed for use in helping patients clarify personal preference for or against available health care options, including participation in clinical trials. The final version of the booklet will be made available as a pdf file through this application. Inclusion of the booklet will render the application available for use in recruiting patients to the vaccine trial.

*CRC Vaccine Trials Scenario and Survey.* A written scenario was developed that will be included in a survey questionnaire being developed for Phase 2 of this Aim. The scenario will be presented to colon cancer patients who agree to participate in the survey. Items included will ask respondents to report the extent to which they view themselves as susceptible to colon cancer recurrence, believe that participation in the vaccine trial is important, have concerns about participating in the trial, think that participation in the trial will help to develop a successful vaccine, and have support from health professionals and family members for trial participation.

Biomedical Research Program: Optimization of vaccine to maximize therapeutic efficacy. Aim 3.A will define systemic immune responsiveness and mechanisms underlying tolerance to a model cancer mucosa antigen (Aim 3.A.1). Additionally, the kinetics of tolerance induced by ectopic extra-mucosal antigen expression may reveal a therapeutic window for patients to optimize immunization prior to tumor-induced tolerance by occult metastases (Aim 3.A.2).

*Transgenic mouse mode.* The primary model system for testing hypotheses in Aim 3.A is a transgenic mouse model in which a model antigen (Tac) is inserted at a single locus for expression by an endogenous promoter following Cre/lox-mediated induction. We developed a strategy targeting the ROSA26 locus (Fig. 1). C57BL/6 embryonic stem cells were targeted using the pRosa26-DEST plasmid modified to insert Tac by homologus recombination. PCR screening of clones revealed 2 with homologous recombination of the Tac into Rosa26 (Fig. 1). Establishing transgenic mice from these clones is ongoing.

*Tumor immunotherapy models.* We established B16 melanoma cells expressing Tac, and recombinant adenoviruses containing Tac or Tac-PADRE. Ad5-Tac produced Tac-specific immune responses in C57BL/6 mice (not shown) and anti-tumor immunity (Fig. 2). To determine if Tac-expressing metastases could induce Tac-specific tolerance, subcutaneous B16-Tac tumors were established (Fig. 3). Control mice received no tumor challenge or parental B16 tumor challenge. Seven days later, all mice were immunized with Ad5-Tac and ELISpot was performed 10 days later to examine SIINFEKL CD8+ T cell responses. Surprisingly, mice with B16 tumors produced responses ~50% better ($p>0.05$) than naïve mice, suggesting that tumors may adjuvanate Tac-specific responses. More importantly, mice with B16-Tac tumors produced lower SIIINFEKL-specific responses suggesting that they drive antigen-specific tolerance. Experiments will examine CD4+ T cell responses, utilizing lung metastases, and examine responses to control Ad5 vectors to confirm the antigen-specificity of B16-Tac tolerance induction.

Training Program: Prepare under-represented minorities (URM) for careers in biomedicine.

*Program Progress.* In summer 2010, five URM students completed internships (Table 4). During spring 2011, 22 URM undergraduates from Lincoln, Cheyney, and St. Joseph's Universities and two TJU URM medical students applied to this program. Two undergraduates from St. Joseph's University are continuing work from their 2010 internships. Two new undergraduates from Cheyney and Lincoln Universities, and one TJU medical student, also were accepted. By July 1, these students will have initiated laboratory work and received 20 hours of curricular instruction.

*Curriculum Development.* The didactic program for 2011 was modified based on input from 2010 interns. Lectures on basic cell biology have been replaced by sessions on the pathobiology of colon cancer. In addition, two minority faculty members have been added: Dr. Hector Lopez, Assistant Professor, Pathology, Anatomy & Cell Biology and Dr. Nancy Brisbon, Assistant Professor, Family and Community Medicine. Moreover, Dr. Elizabeth Brooks, Director of TJU Admissions, was added and will meet with each student individually to discuss the application process for medical school and other postgraduate education and training.

*Program Administration.* The Training Oversight Committee met September 23, 2010, November 30, 2010, and March 10, 2011 to review student evaluations of the 2010 summer internship, recommend revisions to the didactic program, and implement recruitment strategies. Dr. David Zuzga left St. Joseph's University in September 2010 and Dr. Catalina Arango, Assistant Professor, Department of Biology replaced him as co investigator. Consistent with the faculty development component of this Aim, Dr. James DeBoy, Chair, Department of Health, Physical Education and Recreation, Lincoln University is collaborating with Dr. Myers on Aim 2. Dr. DeBoy has conducted literature reviews, contributed to team meetings, provided feedback on scenario narratives and made suggestions for content and format of the booklet that will be used in the decision counseling component of the project.

Translational Research Program: Vaccine to prevent recurrent colorectal cancer.

**Table 1. MTD dose strategy**

| Dose | Rationale |
|---|---|
| 0 | Vehicle Control |
| $1.7x10^{10}$ vp/kg | 10x scaled human dose |
| $4.25x10^{11}$ vp/kg | 250x scaled human dose |
| $10^{11}$ vp[a] | Proposed Human Clinical Dose (~2500x scaled human dose) |

*[a]$10^{11}$ vp is the maximum human dose. In a 60 kg patient, this dose represents $1.7x10^{9}$ vp/kg. In the mouse (0.025 kg), this dose represents $4.25x10^{12}$ vp/kg, which is 2500x the human dose.*

**Table 2. MTD summary**

| Dose | Weight Change (g) | | Feed Consumption (g/day) (during 1st 7 days post-vaccine) | | Hematology and Clinical Chemistry | |
|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females |
| 0 | 1.3 | -0.1 | 4.7 | 5.5 | Normal | Normal |
| $1.7x10^{10}$ vp/kg | 0.1 | -0.3 | 4.8 | 5.4 | Normal | Normal |
| $4.25x10^{11}$ vp/kg | 0.3 | 0.1 | 4.6 | 5.5 | Normal | Normal |
| $10^{11}$ vp | 0.8 | -0.3 | 4.7 | 5.6 | Normal | Normal |

**Table 3. Safety, immunogenicity, and biodistribution of Ad5-mGCC-PADRE over 90 days**

| GROUP | ARTICLE | DOSE | # ANIMALS (MALE/FEMALE) | IN-LIFE DURATION (DAYS) | | | SAMPLE COLLECTION AT TERMINATION |
|---|---|---|---|---|---|---|---|
| | | | | 14 | 30 ± 1 | 90 ± 1 | |
| 1 | Control | 0 | Safety: 5M/15F | 5M/5F | 5M/5F | 5M/5F | Blood: hematology, clinical chemistry; Tissues: histology |
| | | | Immunogenicity: 15M/15F | 5M/5F | 5M/5F | 5M/5F | Blood for Sponsor; Spleen for cell harvest |
| | | | Biodistribution: 15M/15F | 5M/5F | 5M/5F | 5M/5F | Blood for PCR; Tissues for PCR |
| 2 | Ad5-mGCC-PADRE | 4.25 x $10^{11}$ vp/kg | Safety: 15M/15F | 5M/5F | 5M/5F | 5M/5F | Blood: hematology, clinical chemistry; Tissues: histology |
| | | | Immunogenicity: 15M/15F | 5M/5F | 5M/5F | 5M/5F | Blood for Sponsor; Spleen for cell harvest |
| 3 | Ad5-mGCC-PADRE | 1.0 x $10^{11}$ vp | Safety: 15M/15F | 5M/5F | 5M/5F | 5M/5F | Blood: hematology, clinical chemistry; Tissues: histology |
| | | | Immunogenicity: 15M/15F | 5M/5F | 5M/5F | 5M/5F | Blood for Sponsor; Spleen for cell harvest. |
| | | | Biodistribution[a]: 15M/15 F | 5M/5F | 5M/5F | 5M/5F | Blood for PCR; Tissues for PCR. |

*[a]Biodistribution with Ad5-mGCC-PADRE is only required at the 1.0 X $10^{11}$ (maximum) dose.*

Biomedical Research Program: Optimization of vaccine to maximize therapeutic efficacy

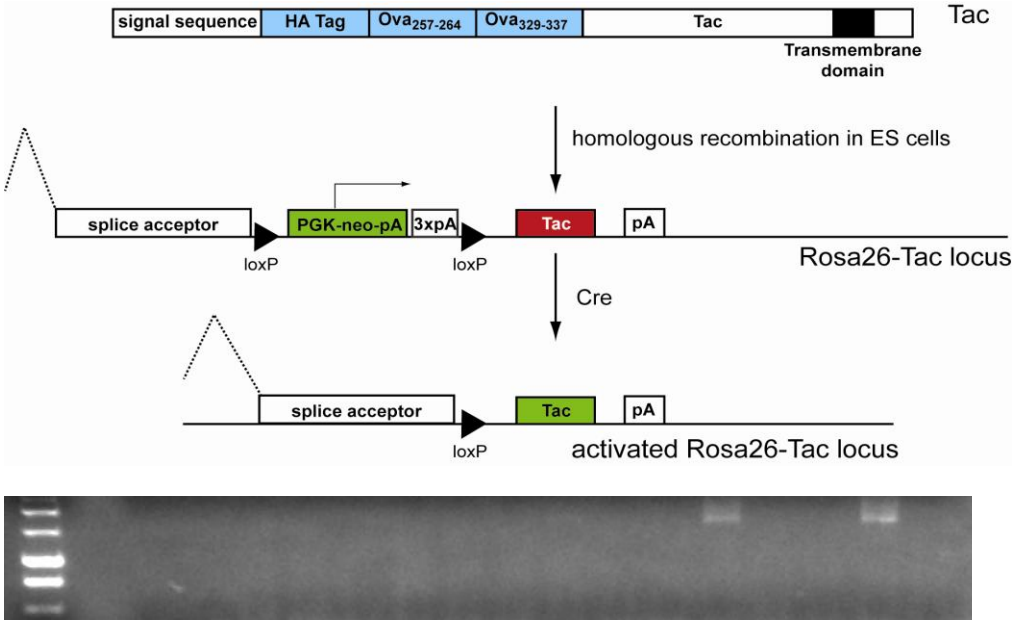


**Figure 1. Rosa26-targeting.** The Tac molecule possessing the defined epitopes was inserted into the Rosa26 locus in which Tac is inserted into the intron of the non-coding Rosa26 mRNA downstream of a splice acceptor site. An intervening Neomycin expression cassette (PGK-neo-pA) blocks Tac expression (red). Upon Cre expression, the neomycin cassette will be excised and Tac will be expressed (green). The gel image identifies 2 ES clones possessing correctly targeted Rosa-Tac by PCR. Transgenic mice are currently being established from these clones.

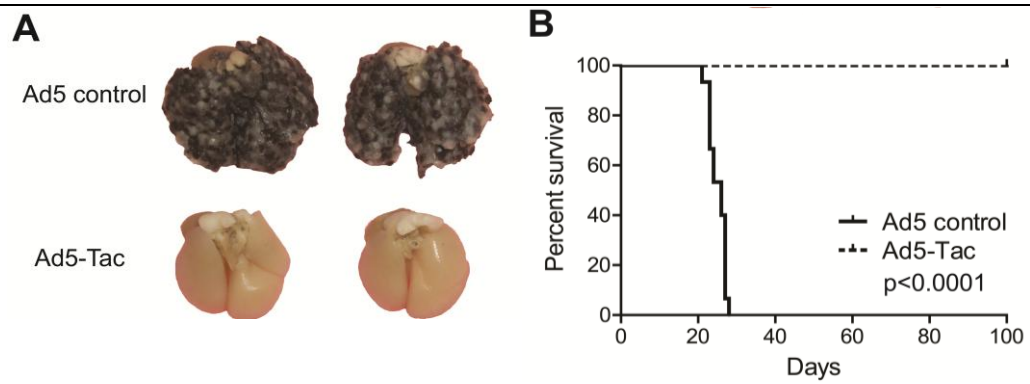


**Figure 2. B16-Tac tumor immunotherapy**. C57BL/6 mice were immunized with $10^8$ IFU of control Ad5 or Ad5-Tac and challenged 7 d later with $5x10^5$ B16-Tac cells by tail vein injection. A) Gross enumeration of tumors revealed an average of 213 metastases in control mice and 0 in Ad5-Tac-immunized mice. B) Survival analysis also revealed a pronounced protective effective by Ad5-Tac.

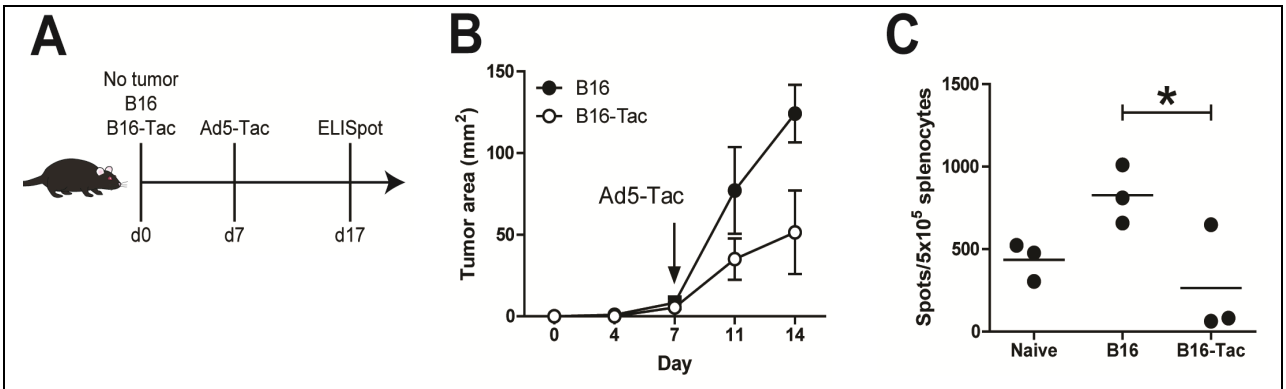


**Figure 3. Metastasis-induced antigen-specific tolerance.** A and B) C57BL/6 mice were naïve or challenged via subcutaneous injection with $1x10^5$ B16 or B16-Tac cells and tumor area was quantified. Seven days later, tumors were palpable and mice were immunized with $10^8$ IFU Ad5-$Tac_{ECD}$ IM. Splenocytes were collected 10 days later and SIINFEKL-specific responses were analyzed by ELISpot. B) SIINFEKL-specific responses were nearly eliminated in 2/3 mice with growing B16-Tac tumors compared to robust SIINFEKL-specific responses in mice with growing B16 parental tumors (* $p<0.05$).

## Training Program: Prepare trainees from under-represented minorities for careers in biomedicine.

**Table 4. Summer 2010 Interns in Cancer Immunotherapy**

| Institution | Race/Sex | Mentor | Project | June 2011 Outcomes |
|---|---|---|---|---|
| St. Joseph's | African American/ Female | L. Eisenlohr, DVM, PhD | Using TAC as a model antigen to examine immunologic tolerance | Returned for second summer internship in cancer immunotherapy |
| St. Joseph's | African American/ Female | T. Sato, MD PhD<br>V. Alexeev, PhD | IL-10 regulation of immune response to tumors | Returned for second summer internship in cancer immunotherapy |
| Cheyney | African American/ Male | T. Hyslop, PhD | A comparison of analytical methods to assess change in tumor volume over time | BS, May 2011<br>will enter Masters in Applied Statistics program Fall 2011 |
| TJU Nursing | African American/ Female | R. Myers, PhD | Factors affecting patients' decisions to participate in colorectal vaccine trial | BSN, May 2011 |
| Drexel | Hispanic/ Male | Ronald Myers, PhD | Factors affecting patients' decisions to participate in colorectal vaccine trial | MPH, June 2011, plans to apply to medical school.<br>Other[§] |

[§] This student continued to work with Dr. Amy Leader in the Department of Medical Oncology, Division of Population Science during the 2010-2011 school year as part of his MPH capstone project work. This collaboration resulted in two abstracts accepted to the 2011 national meeting of the American Public Health Association. He was principal author on one abstract and co-author on the second abstract.