Thomas Jefferson University

Annual Progress Report: 2009 Nonformula Grant

Reporting Period

July 1, 2013 – May 31, 2014

Nonformula 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.

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
Philadelphia, PA 19107

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

Aim 1. Translational Research Program: Vaccine to prevent recurrent colorectal cancer. Good Manufacturing Practices (GMP) production of clinical-grade Ad5-hGCC-PADRE was completed at the *Baylor Center for Cell and Gene Therapy*. *Preclinical testing* to evaluate the safety, efficacy and biodistribution of the vaccine was completed January 2013 by *WuXi AppTec*, demonstrating the absence of vaccine-related toxicities. *Regulatory approval* of the vaccine focused on two parallel tracks: federal and institutional. Because our vaccine involves administration of recombinant DNA to humans, review by the Recombinant DNA Advisory Committee (RAC) of the NIH, Office of Biotechnology Activities was required. Our protocol was unanimously approved by the RAC at their quarterly meeting June 11th, 2013. Subsequently, institutional approvals (Clinical Cancer Research Review Committee; Institutional Biosafety Committee; and the Institutional Review Board) were obtained. The Investigational New Drug application was submitted to the FDA on October 16, 2013 and approved November 10, 2013. *Clinical Trial* enrollment initiated immediately after FDA approval and the first subject was vaccinated on November 20, 2013. Currently, a total of 10 subjects have been vaccinated (Fig. 1) and ***zero*** adverse reactions have occurred. Each subject will return to Thomas Jefferson University for 3 follow-up visits at 30, 90 and 180 days after vaccination. Each visit includes clinical assessment by physical exam and blood work and includes collection of blood samples to monitor vaccine activity (immunomonitoring). The study design included two primary endpoints: safety and immune activity and the study will be stopped if the vaccine is unexpectedly toxic or unexpectedly inactive. For the purposes of this clinical trial, immune activity is defined as GCC-specific antibody immune responses at 30-days post vaccination. If 1 or more of the first 10 subjects produce an antibody response at 30 days, enrollment will continue and 12 additional (22 total) subjects will be enrolled. Day 30 samples were collected from subject #10 on July 3, 2014 and assessment of day 30 antibody responses for the 10 enrolled subjects is ongoing. Preclinical studies suggest that T cell responses to GCC and to the

PADRE component of the vaccine are critical to the antitumor activity of the vaccine. Thus, full immunomonitoring studies will be carried out on all subjects to include antibody responses to GCC and the Ad5 vector and T-cell responses to GCC, PADRE and Ad5 at 30, 90 and 180 days post vaccination. Full assay validation (Fig. 2-3) including those for sample processing, ELISA (antibody) and ELISpot (T cell) were performed prior to analyzing samples from vaccinated subjects. Full immunomonitoring assessment is ongoing for the 3 subjects that have completed all follow-up visits and will be conducted for the remaining subjects following their final visit.

Aim 2. Health Services Research Program: Barriers underlying racial disparities in cancer vaccine trials. In **Phase 3**, the research team adapted a novel online decision support intervention (DSI) known as the Decision Counseling Program© (DCP) for use in facilitating patient participation in the vaccine trial. The DCP is a vehicle to deliver informational content to patients about available options related to any designated health decision. Here, we introduced the vaccine trial informational booklet as a decision aid. The DCP also is a tool that can be used to guide patients through a values elicitation and personal preference clarification exercise related to available options. The research team configured the DCP for use in clarifying whether patients preferred to join the vaccine trial (Option 1) or not to join the trial (Option 2). A total of 70 potential participants were identified, and 56 (80%) were successful contacted. These contacts resulted in the following resolutions related to the trial: 19 completed a decision counseling session, 8 were found to be ineligible for the trial, and 29 declined participation in decision counseling and the trial. Research staff members attempted to contact the 19 patients who completed a decision counseling session in order to administer an endpoint survey, and were successful in completing the survey with 16 patients. Three patients were lost to follow-up. Frequency distributions of survey variables were generated for the respondents, and univariable analyses were performed to assess the associations between independent variables (i.e., demographic background, knowledge about the vaccine trial, and vaccine trial preference score) and the outcome variable (i.e., actual trial participation). Of the 19 individuals who completed a decision counseling session, 14 preferred to join the vaccine trial, 3 were undecided, and 2 preferred not to join the trial. All individuals who completed a decision counseling session agreed to receive a follow-up call from the vaccine trial coordinator to discuss participation further. The trial coordinator succeeded in contacting 15 patients, but could not reach 4 patients. Overall, 16 individuals completed both a decision counseling session and an endpoint survey. Background characteristics of 16 individuals with complete endpoint survey responses and decision counseling session results were as follows: < 65 years of age (38%), white (81%), female (50%), > high school education (75%), and married (44%). In terms of knowledge about the vaccine trial, 75% of these individuals answered at least 9 out of 10 knowledge questions correctly. We also found that 75% of individuals preferred to participate in the vaccine trial. Univariable analyses were conducted to determine if patient sociodemographic factors, knowledge about the trial, and trial participation preference were associated with actual participation in the vaccine trial. We determined that 75% of patients with a preference score that favored participation actually participated in the trial; while only 25% of those who a score indicating they were unsure about or did not favor participation did so. Thus, preference score was positively associated ($p=0.118$) with actual participation. The small number of individuals in this part of the study serves as a limitation to statistical inference. All 16 of individuals with complete endpoint survey and decision counseling data reported on the survey that that the vaccine informational booklet was easy to read. Most participants also agreed that the booklet

described the purpose of the vaccine sufficiently (94%), eligibility criteria related to trial participation were explained well (94%), vaccine safety concerns were adequately addressed (100%), and sources of further information were clearly presented (88%). Finally, 94% of respondents said that they would recommend the booklet to others.

Aim 3. Biomedical Research Program: Optimization of vaccine to maximize therapeutic efficacy. The External Scientific Advisory Committee re-prioritized the laboratory-based aims, focusing on *Chemokine-potentiated heterologous vaccine strategies that amplify GCC-targeted responses* (Aim 3.C) prior to examining T_{reg}-dependent tolerance and GCC responses (Aim 3.B). Thus, studies in Aim 3.C were initiated in 2011 while studies in Aim 3.B began in 2012. Aim 3.C examines the utility of chemokine-adjuvanated DNA vaccines in combination with adenoviral vaccines to produce robust GCC-specific immune responses. Homologous and heterologous combinations of DNA vaccines and adenovirus vaccines were administered to mice (Fig. 4). Immunity against GCC-expressing colorectal metastases in lung was quantified following each immunization (Fig. 4A), revealing DNA-priming, followed by adenovirus boosting (D+A) as the most effective combination, producing ~50% overall survival compared to <10% overall survival following all other vaccinations. The number (Fig. 4B) cytokine profile (Fig. 4C) and functional avidity (Fig. 4D) of GCC-specific T-effector cells were quantified following each immunization. Surprisingly, D+A produced only ~2-fold increase in GCC-specific T cell quantity (Fig. 4B) and produced no improvement in polyfunctional cytokine T-cell responses (Fig. 4C) compared to Ad5-GCC alone. However, D+A produced a 10-fold increase in T-cell avidity for GCC (Fig. 4D), superior to that of any other vaccination regimen, suggesting that the superior efficacy of D+A immunization reflected enhanced T-cell avidity, rather than T-cell number or cytokine profiles. To confirm the importance of TCR avidity enhancement in D+A antitumor synergy, we produced an Ad5 construct possessing only the $GCC_{254-262}$ T-cell epitope (Ad5-$GCC_{254-262}$). We have previously shown that vaccines possessing epitopes requiring no proteasomal processing induce high peptide-MHC complex levels and low T-cell avidity. As expected, Ad5-$GCC_{254-262}$ produced T-cell quantity (Fig. 5A) and polyfunctional cytokine (Fig. 5B) responses comparable to D+A vaccination, but produced T cells with ~20-fold lower avidity than D+A (Fig. 5C), resulting in no survival benefit for mice with metastatic colorectal cancer (Fig. 5D). Comparing survival benefit to T-cell quantity ($p>0.05$), T-cell cytokine polyfunctionality ($p>0.05$) and T-cell avidity ($p<0.0001$) across all tested vaccination combinations revealed only T-cell avidity predicting tumor outcomes. These studies reveal the previously unrecognized mechanism of functional T-cell avidity enhancement mediating synergy of DNA-Ad5 prime boost vaccinations. Those results direct vaccine design for future GCC vaccine combinations in patients. More importantly, these results suggest that the magnitude and polyfunctional cytokine profiles of cancer vaccine-induced T-cell responses are poor predictors of outcomes following prime-boost immunizations. Rather, functional T-cell avidity correlates with outcomes and should be explored as a biomarker of cancer vaccine efficacy. Moreover, strategies that specifically enhance T-cell avidity may have the greatest efficacy in cancer vaccination. A manuscript describing the results of the *Chemokine-potentiated heterologous vaccine strategies that amplify GCC-targeted responses* aim has been submitted for publication.

Aim 4. Training Program: Prepare trainees from under-represented minorities for careers in biomedicine. *Trainees:* During July and the first two weeks in August 2013, the five

underrepresented minority students chosen to participate in the 2013 Summer Research Internships in Cancer Immunotherapy Program at Jefferson completed their program. The students were provided an opportunity to work with mentors at Thomas Jefferson University for a 10-week summer session. *Didactic Program:* The didactic program, originally designed by Dr. Elizabeth Rappaport with input from the Principal Investigator, other co-investigators and members of the Training Oversight Committee, provided lectures on cancer genetics, fundamentals of immunology, hematopoiesis, pathobiology of cancer, tumor immunobiology and immunotherapy, and additional topics in biostatistics. The students were provided with a copy of the textbook "How the Immune System Works" by Lauren M. Sompayrac. This summer the students also participated with the Jefferson first year medical students in the Jefferson Medical College Summer Seminar Series which included topics on 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 were given full access to Jefferson Library services and facilities. This part of the program provided the students with an excellent opportunity to interact with the medical students and receive coaching on what they would need to do to be ready for careers in biomedicine. *Program Administration:* The Training Oversight Committee met on September 10, 2013 and reviewed the evaluations of the Summer Internship Program; venues and opportunities for students to present their summer research projects and discussed tracking students post internships. The faculty participants from Cheney, Lincoln and Saint Joseph's Universities expressed their regret that the program was ending and agreed that this was an excellent program which greatly benefitted their students.

Translational Research Program: Vaccine to prevent recurrent colorectal cancer.

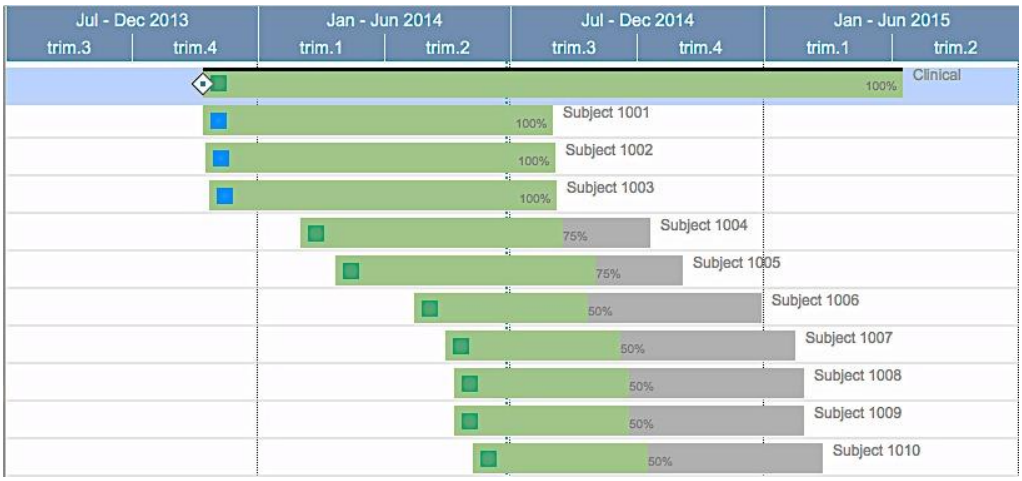

Figure 1. Progress on follow-up for the phase I trial. Ten subjects have been vaccinated. Three subjects have completed all visits (30, 90 and 180 days post-vaccination). Remaining subjects have completed 1-2 visits each.

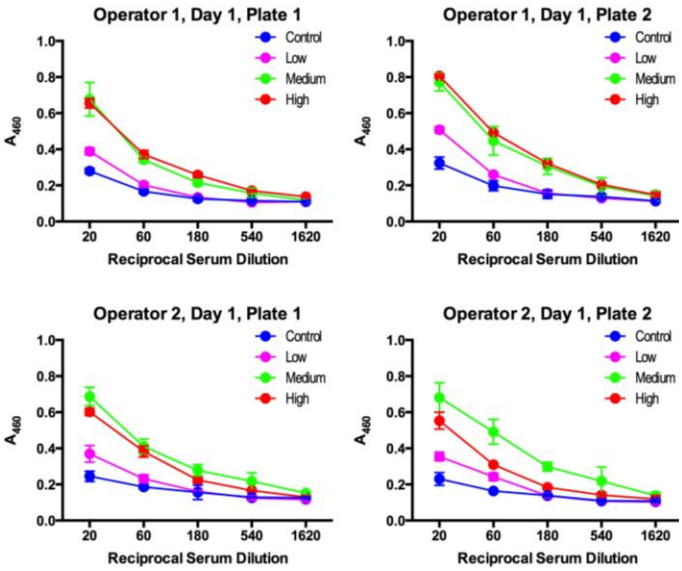

Figure 2. ELISA Assay Development and Validation. GCC-specific immune responses have never been measured in humans so a novel hGCC ELISA was developed and validated. A total of 23 development/validation experiments were carried out. A representative validation study is shown here to determine the inter-operator, inter-plate, inter-day reproducibility. Negative control serum was spiked with GCC monoclonal antibody at high, medium and low concentrations corresponding to the magnitude of responses observed in preclinical mouse experiments. Overall, a robust, sensitive and reliable assay was validated for assessing antibody responses to hGCC in human serum samples.

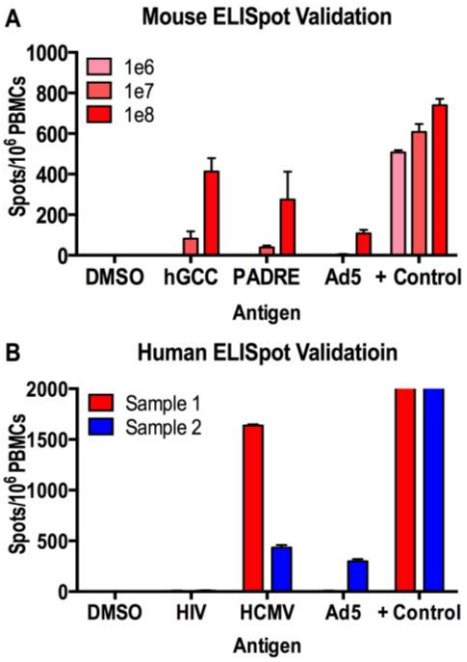

Figure 3. ELISpot Assay Development and Validation. A) A library of peptides spanning hGCC was synthesized. Mice were vaccinated with $1x10^6$ to $1x10^8$ IFU of Ad5-hGCC-PADRE and reactivity to the hGCC peptide library was confirmed. The hGCC peptide library will be used to quantify patient T-cell responses. **B**) Human ELISpot assay was validated on two human samples employing HIV and HCMV as positive and negative antigens, respectively. Ad5 responses were also confirmed. Overall, a robust, sensitive and reliable assay was validated for assessing T-cell responses to hGCC in human samples.

Biomedical Research Program: Optimization of vaccine to maximize therapeutic efficacy.

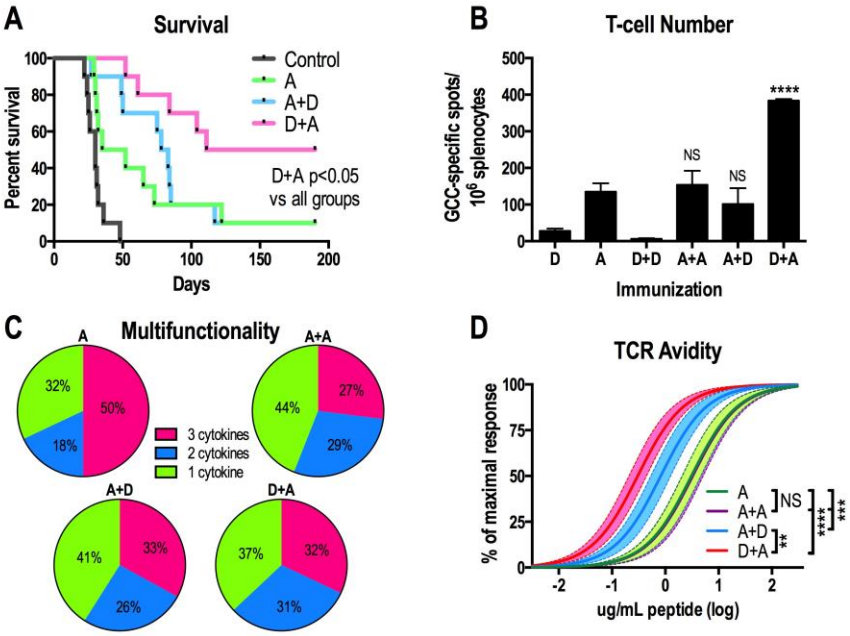

Figure 4. DNA-Ad5 Prime-Boost Vaccination Maximizes GCC-Specific Antitumor Immunity. A-D) Mice were immunized with control vaccine (Control), Ad5-GCC (A), DNA-GCC (D), or homologous and heterologous combinations of A and D. **A**) Following immunization, mice were challenged with GCC-expressing CT26 colorectal cancer cells to establish lung metastases and survival was monitored. **B-D**) T cells were collected from immunized mice and GCC-specific T-cell number (avidity (**D**) were quantified.

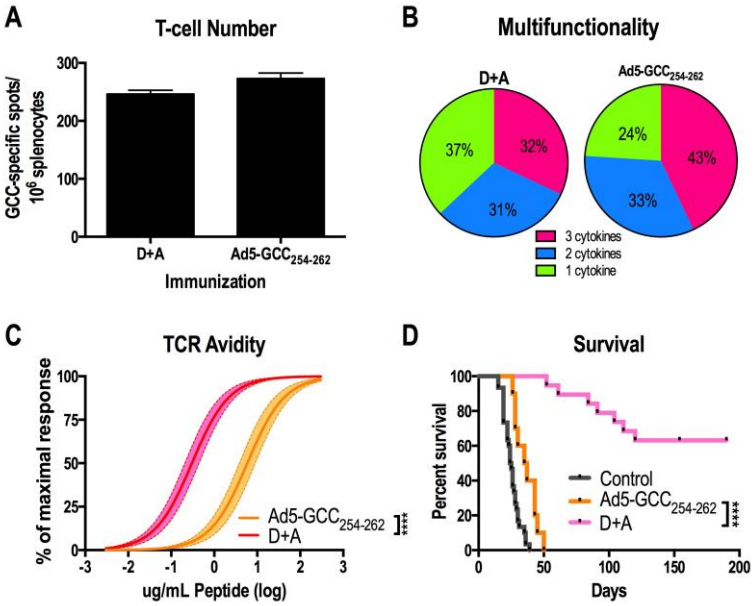

Figure 5. DNA-Ad5 Prime-Boost Synergy Reflects TCR Avidity Enhancement. A-D) Mice were immunized with control vaccine (Control), Ad5-GCC$_{254-262}$ or heterologous prime-boost with DNA-GCC followed by Ad5-GCC (D+A). **A-C**) T cells were collected from immunized mice and GCC-specific T-cell number (**A**), cytokine multifunctionality (**B**) and TCR avidity (**C**) were quantified, confirming that Ad5-GCC$_{254-262}$ produces comparable quantity and cytokine profiles of GCC-specific T cells, but >20-fold lower TCR avidity. **D**) Following immunization, mice were challenged with GCC-expressing CT26 colorectal cancer cells to establish lung metastases and survival was monitored.