AWARD NUMBER: W81XWH-15-1-0101

TITLE: Phase 1B Clinical Trial of a Candidate Breast Cancer Prevention Vaccine

PRINCIPAL INVESTIGATOR: William E. Gillanders

RECIPIENT: Washington University
St. Louis, MO 63110-1010

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Phase 1B Clinical Trial of a Candidate Breast Cancer Prevention Vaccine

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Washington University, The
One Brookings Drive
Saint Louis MO 63130-4862

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Materiel Command
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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT This project involves a phase 1b clinical trial in breast cancer patients undergoing neoadjuvant endocrine therapy. Sixty subjects with mammaglobin-A-expressing breast cancer will be randomized in a 1:1 ratio to neoadjuvant endocrine therapy alone, or neoadjuvant endocrine therapy plus mammaglobin-A DNA vaccination. The primary objective is to assess the safety of the mammaglobin-A DNA vaccine. The secondary objective is to assess the ability of the mammaglobin-A DNA vaccine to induce an immune response to mammaglobin-A. During the first year of the project most efforts focused on optimizing patient awareness and accrual. Several protocol amendments were implemented earlier in the year to improve accrual. Additionally, we implemented screening of both medical and surgical oncologists' clinic schedules, and added Dr. Bisi Ademuyiwa, a Breast Cancer Medical Oncologist, to the trial team. To increase awareness a patient information package was prepared that explains the goal and details of the clinical trial. To date, a total of 12 patients signed the screening consent. Of these, 5 patients were eligible and 4/5 were randomized to the trial.

15. SUBJECT TERMS Phase 1b, neoadjuvant, endocrine, DNA vaccine, mammaglobin-A, immune response

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	31	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

This project involves a phase 1b clinical trial in breast cancer patients undergoing neoadjuvant endocrine therapy or chemotherapy. Forty six subjects with mammaglobin-A-expressing breast cancer will be randomized to neoadjuvant endocrine therapy alone (n=8); neoadjuvant endocrine therapy plus mammaglobin-A DNA vaccination (n=15), neoadjuvant chemotherapy (n=8) or neoadjuvant chemotherapy plus mammaglobin-A DNA vaccination (n=15). The primary objective is to gain additional information about the safety of the mammaglobin-A DNA vaccine. Safety will be closely monitored after injection with eight or more clinical and laboratory assessments in the first 24 weeks of the trial. The secondary objective is to assess the ability of the mammaglobin-A DNA vaccine to induce an immune response to mammaglobin-A. The immune response will be measured in the peripheral blood (ELISPOT analysis, multi-parameter flow cytometry), and in the primary tumor (imaging mass cytometry, IHC and RT-PCR).

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Breast, cancer, neoadjuvant, therapy, mammaglobin-A, DNA, vaccine, endocrine, chemo, phase 1b, ELISPOT, T-cells, microenvironment

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Subtask1: Manufacture mammaglobin-A DNA vaccine. Complete manufacture, product release tests, and other IND –enabling studies of the mammaglobin-A DNA vaccine.
Subtask 2: Obtain regulatory approval for phase 1b clinical trial. Obtain FDA approval, RAC approval, Institutional Biosafety Committee approval, PRMC approval and IRB approval. All approvals have been obtained.
Subtask 3: Patient enrollment. Enroll patients to the phase 1b clinical trial.
Subtask 4: Screening studies. Complete screening studies including HLA type and mammaglobin expression levels.
Subtask 5: Assessment of safety. The primary endpoint is safety of the mammaglobin-A DNA vaccine. Safety will be closely monitored after injection and toxicity will be graded according to the NCI CTCAE version 4.0.
Subtask 6: Immune monitoring. The secondary objective is immune response in the peripheral blood. PBMC will be analyzed for the presence of mammaglobin-A-specific T cells by ELISPOT and multi-parameter flow cytometry. Tetramer staining will also be combined with intracellular cytokine staining (IFNγ, TNFα).
Subtask 7: Manuscript Preparation. Safety and immune response to the mammaglobin-A DNA vaccine are the primary and secondary objectives of the trial and will be published together.

What was accomplished under these goals?

The clinical trial was modified earlier and currently has four cohorts: neoadjuvant endocrine therapy alone (n=8), neoadjuvant, neoadjuvant endocrine therapy + mammaglobin-A DNA vaccine (n = 15), neoadjuvant chemotherapy alone (n = 8), and neoadjuvant chemotherapy + mammaglobin-A DNA vaccine (n=15). We completed recruitment to the neoadjuvant endocrine therapy group (n=8).

After discussion with the Grants Officer (Ms. Jamie Shortall) and Science Officer (Ms. Julia Huiberts) in early December, we submitted a request in early January 2020 for a no-cost extension and a proposal to open the trial at Ohio State University Comprehensive Cancer Center (see below). We have experienced challenges in the last several months related to the COVID-19 pandemic. Our expansion of the trial to OSU has been significantly delayed as all new trials were suspended through May. We hope to have the trial active at this new site in the next quarter, barring any additional restrictions at OSU. Although the trial has remained open here at Siteman Cancer Center, our patient visits have been primarily conducted as telemedicine visits from March through most of May. Concerns about additional in-person visits for vaccinations has resulted in a few patient withdrawls. We do expect accruals to the trial will continue to be impacted in the coming quarter(s), although this should improve as patients resume in-person visits.

Accrual and treatment: During the past year of the project most efforts focused on optimizing patient accrual to the trial. **Appendix #1** presents an overview of all patients consented to date; changes from last year are highlighted and reflect a total of 16 new patients and a status update in 1 additional patient. Of the 16 new patients, 6 patients were eligible for treatment and 5 of those received neoadjuvant endocrine therapy plus mammaglobin DNA vaccine. The other patient received chemotherapy + vaccination (WU-075). Five patients preferred an alternative treatment. The other 5 new patients dropped out because of a mammaglobin-negative tumor. One patient (WU-69) completed endocrine + vaccine therapy during the past year. In total, 21 patients have completed therapy; 8 patients with neoadjuvant endocrine therapy alone; 12 patients with neoadjuvant endocrine therapy plus vaccine, and one patient with chemotherapy + vaccine. Neoadjuvant endocrine therapy plus vaccine is ongoing in an additional patient.

A recent modification to the trial is the addition of Ohio State University (OSU) as a second site for the trial. Dr. Robert Wesolowski expressed great enthusiasm to open the trial, and the clinical trial protocol has been approved by the Ohio State University Comprehensive Cancer Center Clinical Scientific Review Committee. We are currently waiting for approval of the contract agreement and completion of a site visit prior to opening the trial. The plan is for OSU to accrue 10 patients onto the trial, and forward tissue and peripheral blood mononuclear cells (PBMC) from patients to the Gillanders laboratory at Washington University for immune monitoring studies.

Safety/toxicity: In all 21 patients that completed therapy to date, toxicity was restricted to mostly grade 1/2, and none of the patients experienced toxicity that required discontinuation of treatment.

Immune monitoring: We have initiated immune monitoring assays using peripheral blood samples collected at various time points to perform pre-versus post-treatment comparison of the immune response to mammaglobin-A. A library of 15-mer peptides overlapping by 11 amino acids spanning the entire mammaglobin-A protein is used to screen for initial T cell responses to mammaglobin-A by interferon-gamma (IFNγ) ELISpot assay. A total of 20 peptides were divided into 5 pools of four peptides each. PBMC collected at each time point were tested against individual peptide pools. PBMC were then harvested from the ELISpot plates and cultured in the presence of IL-2 for 12 days, followed by a repeat of the ELISpot assay. Data on the first four patients and further experimental details are presented in **Appendix 2.**

A second assay that is being explored is an intracellular cytokine assay (ICC) in which PBMC are stimulated with mammaglobin-A peptides but analyzed by flow cytometry using fluorescent antibodies for T cell markers and cytokines (see **Appendix 3** for details and preliminary results). Lastly, we have initiated studies using imaging mass cytometry (IMC) to characterize the tumor immune microenvironment before and after treatment (see **Appendix 4**).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report." Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We continue to emphasize patient screening and enrollment. We have initiated a subaward agreement to OSU and a site initiation meeting to open the trial is scheduled for 7/22/2020. Additionally, we will continue with the correlative studies. Specifically, we will perform additional immune monitoring assays using peripheral blood samples from trial patients collected at multiple time points and integrate imaging mass cytometry and potentially T cell receptor sequencing. It should be noted, however, that Washington University, and by extension the School of Medicine and Department of Surgery, are carefully monitoring the COVID-19 pandemic, and have instituted defined phases of research activity. We are currently in the "Yellow Phase" which permits 60-80% research activity, but still has additional limitations in place regarding the total number of personnel allowed and distancing of personnel. It is not clear yet if and when the university will transition into the next phase of activity.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Please see above for discussion regarding delays

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:	
Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

William Gillanders
Principal Investigator
PM: 1
No change

S. Peter Goedegebuure
Co-Investigator
PM: 1
No change

Nancy B. Myers
Staff Scientist
PM: 5
no change

Rashmi Mishra
Postdoc Research Associate
PM: 12

Kathleen Harris
Clinical Research Coordinator
PM: 1
No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

WILLIAM GILLANDERS

NEW AWARDS

R01CA240983 (Gillanders/Schreiber) 8/1/2019-7/31/2024 1.2
National Institutes of Health $404,968 Calendar
Targeting Neoantigens in Triple Negative Breast Cancer
The overall hypothesis is that enhancing neoantigen-specific T cell responses can improve clinical outcomes in TNBC.

Specific Aims

Aim 1. Test the hypothesis that neoantigen vaccines +/- anti-PD-L1 can induce and/or enhance neoantigen-specific T cell responses.
Aim 2: Test the hypothesis that targeting tumor-associated macrophages (TAM) can enhance eoantigen-specific T cell responses in TNBC.
Aim 3: Test the hypothesis that a neoantigen simian Ad vector vaccine "prime" followed by a neoantigen DNA vaccine "boost" can enhance the response to breast cancer neoantigens.

Program Official: Admin Official:
Min-kyung H Song Sabrina Oasan

P19-00559 (Perlmutter) 4/1/2019-3/31/2022 0.12
Centene Corporation $387,500 Calendar
Research in the Field of Personalized Medicine - Cancer (Breast and Pancreatic), Alzheimer's Disease,Obesity and DiabetesB101 Personalized Breast Cancer Vaccines (Gillanders)
The Centene Corporation contract supports the Washington University-Centene Personalized Medicine Initiative (PMI) which awards funding to internal Washington University (WU) research projects in four disease specific areas. This project is funded under the WU-Centene PMI Breast Cancer Program.
The goal of this proposal is to validate an in vivo model to evaluate human tumors in the context of an intact human immune system in a completely personalized and autologous fashion.

Specific Aims

Aim 1: Test innovative neoantigen vaccine platforms in a preclinical model of breast cancer.
Aim 2: Optimize neoantigen vaccines.
Aim 3: Multidimensional profiling of the tumor microenvironment following neoantigen vaccine therapy.

Program Official: Admin Official:
Rebecca Evans / evansb@wustl.edu n/a

P19-00559 (Perlmutter) 4/1/2019-3/31/2022 0.12
Centene Corporation $387,500 Calendar
Research in the Field of Personalized Medicine - Cancer (Breast and Pancreatic), Alzheimer's Disease, Obesity
and DiabetesB103 Platform Trial to Evaluate Personalized Breast Cancer Therapies (Gillanders)
The Centene Corporation contract supports the Washington University-Centene Personalized Medicine Initiative (PMI) which awards funding to internal Washington University (WU) research projects in four disease specific areas. This project is funded under the WU-Centene PMI Breast Cancer Program.
The goal of this proposal is to validate an in vivo model to evaluate human tumors in the context of an intact human immune system in a completely personalized and autologous fashion.

Specific Aims

Aim 1: Multidimensional profiling of the tumor microenvironment following chemoimmunotherapy.
Aim 2: Perform a clinical trial of neoadjuvant chemotherapy + anti-PD-L1 in patients with clinical stage II/III triple negative breast cancer.
Aim 3: Test novel chemoimmunotherapy strategies in patient-derived models.

Program Official:
Admin Official:
Rebecca Evans; n/a

NIT-SRA (Gillanders) 5/8/2019-5/7/2020 0.12
NeoImmune Tech $99,360 Calendar
Preclinical Studies of Hyleukin-7 as a Vaccine Adjuvant
The main objective of this project is to evaluate the sponsor's product, Hyleukin-7, a recombinant form of interleukin-7, as an adjuvant for DNA vaccine therapy for the treatment of breast cancer in a preclinical model.

Specific Aims

Experiment 1: Identify optimal timing of Hyleukin-7 administration in combination with DNA vaccine therapy, and test the hypothesis that combination Hyleukin-7 and DNA vaccine therapy induces a more potent neoantigen-specific immune response compared to DNA vaccine therapy alone.
Experiment 2: Identify the optimal dose of Hyleukin-7 to be used as an adjuvant for DNA vaccine therapy.
Experiment 3: Test the hypothesis that Hyleukin-7 enhances memory neoantigen-specific immune responses following DNA vaccine therapy.
Experiment 4: Test the hypothesis that Hyleukin-7 enhances the antitumor response of DNA vaccine therapy.

Program Official: Admin Official:
Byung Ha Lee n/a

P19-00559 (Perlmutter) 4/1/2019-3/31/2022 0.12
Centene Corporation $100,000 Calendar
Research in the Field of Personalized Medicine - Cancer (Breast and Pancreatic), Alzheimer's Disease, Obesity and DiabetesP102A Next Generation of Personalized Vaccines for PDAC

(Hawkins/Goedegebuure/Gillanders)
The Centene Corporation contract supports the Washington University-Centene Personalized Medicine Initiative (PMI) which awards funding to internal Washington University (WU) research projects in four disease specific areas. This project is funded under the WU-Centene PMI Pancreatic Cancer Program.
The overall objective of this project is to test second generation personalized vaccines incorporating both neoantigens and immune modulatory molecules in mouse models of pancreatic cancer.

Specific Aims

Aim 1: Develop and credential a neoantigen vaccine preclinical model.
Aim 2: Optimize the neoantigen vaccine.
Aim 3: Multidimensional profiling of the tumor following neoantigen vaccine therapy.

Program Official: Admin Official:
Rebecca Evans; n/a

EXPIRED AWARDS

R01CA130988 (Hahn) 8/11/2014-7/31/2019 0.3
National Institutes of Health $49,836 Calendar
Regulation and Function of IKKE in Breast Cancer Initiation and Maintenance
The Gillanders Laboratory at WUSM will be primarily responsible for investigating the effects of inhibiting IKK-epsilon in breast cancer cell lines as detailed in Specific Aim 3A, determining the effect of inhibiting IKK-epsilon of tumor maintenance as detailed in Specific Aim 3B, and characterizing inhibitor resistant IKK-epsilon alleles as detailed in Specific Aim 3C.

Specific Aims

Aim 1: Investigate the regulation of IKKε by ubiquitination.
Aim 2: Interrogate signaling pathways essential for IKKε-induced cell transformation.
Aim 2: Interrogate signaling pathways essential for IKKε-induced cell transformation

Program Official: Admin Official:
Athorized Official @ DFCI: Tina DaSilva Financial Contact: Deborah Brown

R01CA190700 (Schreiber) 4/1/2015-3/31/2020 0.6
National Institutes of Health $292,319 Calendar
Development of Genomics Based Personalized Cancer Immunotherapy
The work proposed in this application seeks to use a preclinical mouse model of cancer to critically test (a) the ability of our method to identify antigenic mutant proteins in cancer cells; (b) whether our method can distinguish between cancers that express strong versus weak mutant antigens thus making individuals carrying those cancers good- versus badcandidates for cancer immunotherapy, respectively; and (c) whether we can generate cancer vaccines based on an individual's tumor specific mutant antigenic proteins to enhance the success rates of current cancer immunotherapies.

Specific Aims

Aim I: Identify tumor-specific mutational antigens eliciting CD8+ T cell responses to MCA sarcomas and B16-F10 melanoma with differential sensitivities to checkpoint blockade therapy.
Aim 2: Determine whether vaccines targeting tumor-specific mutational antigens, either alone or in combination with checkpoint blockade, can therapeutically control growth of MCA sarcomas or B16-F10 melanoma.
Aim 3: Define the characteristics of tumor-specific CD8+ T cells that specify their therapeutic effectiveness.

Program Official: Susan McCarthy

Admin Official: Alania Foster

SIP (Gillanders/Schreiber) 1/1/2018-12/31/2019 0.24
Siteman Cancer Center $87,095 Calendar
Siteman Cancer Center Breast Cancer SPORE
Targeting Neoantigens in Triple Negative Breast Cancer (Gillanders/Schreiber)
The overall hypothesis of Research Project 1 is that neoantigen-specific T cell responses contribute to antitumor immunity in TNBC.

Specific Aims

Aim 1: Test the hypothesis that neoantigen vaccines, +/- anti-PD-L1, can induce and/or enhance neoantigen-specific T cell responses.
Aim 2: Test the hypothesis that neoadjuvant chemotherapy +/- anti-PD-L1 can enhance neoantigen-specific
T cell responses.

Program Official: not yet available

Admin Official: not yet available

S. PETER GOEDEGEBUURE

NEW AWARDS

R01CA240983 (Gillanders/Schreiber) 8/1/2019-7/31/2024 0.6
National Institutes of Health $404,968 Calendar
Targeting Neoantigens in Triple Negative Breast Cancer
The overall hypothesis is that enhancing neoantigen-specific T cell responses can improve clinical outcomes in TNBC.

Specific Aims

Aim 1. Test the hypothesis that neoantigen vaccines +/- anti-PD-L1 can induce and/or enhance neoantigen-specific T cell responses.
Aim 2: Test the hypothesis that targeting tumor-associated macrophages (TAM) can enhance neoantigen-specific T cell responses in TNBC.
Aim 3: Test the hypothesis that a neoantigen simian Ad vector vaccine "prime" followed by a neoantigen DNA vaccine "boost" can enhance the response to breast cancer neoantigens.

Program Official: Min-kyung H Song

Admin Official: Sabrina Oasan

P19-00559 (Perlmutter) 4/1/2019-3/31/2022 0.6
Centene Corporation $100,000 Calendar
Research in the Field of Personalized Medicine - Cancer (Breast and Pancreatic), Alzheimer's Disease, Obesity and DiabetesP102 Next Generation of Personalized Vaccines for PDAC (Hawkins/Goedegebuure/Gillanders)
The Centene Corporation contract supports the Washington University-Centene Personalized Medicine Initiative (PMI) which awards funding to internal Washington University (WU) research projects in four disease specific areas. This project is funded under the WU-Centene PMI Pancreatic Cancer Program.
The overall objective of this project is to test second generation personalized vaccines incorporating both neoantigens and immune modulatory molecules in mouse models of pancreatic cancer.

Specific Aims

Aim 1: Develop and credential a neoantigen vaccine preclinical model.
Aim 2: Optimize the neoantigen vaccine.
Aim 3: Multidimensional profiling of the tumor following neoantigen vaccine therapy.

Program Official: Admin Official:
Rebecca Evans; not yet available

R01CA248277 (Fields/Flavell) 3/1/2020-2/28/2025 0.96
National Institutes of Health $431,069 Calendar
Advancing Precision Oncology in a Humanized, Fully Autologous Mouse Model
The overarching goal of this proposal is to evaluate a comprehensively HuMo system to faithfully model the innate and adaptive human immune system in order to deliver on the promise of precision medicine by evaluating immunotherapy modalities in vivo.

Specific Aims

Aim 1: Validate the ability of hMISTRG mice bearing autologous melanoma tumors to serve as models for ICB treatment response and toxicity.
Aim 2: Validate the ability of hMISTRG mice bearing autologous melanoma tumors to serve as models for peptide vaccine-based therapy.
Aim 3: Establish humanized hMISTRG mice and evaluate tumor growth and lymphocyte development in patients with PDAC or CRC.

Program Official: Admin Official:
Susan A. Mccarthy Rebecca Brightful

P19-00559 (Perlmutter) 4/1/2019-3/31/2022 1.2
Centene Corporation $387,500 Calendar
Research in the Field of Personalized Medicine - Cancer (Breast and Pancreatic), Alzheimer's Disease, Obesity and DiabetesB101 Personalized Breast Cancer Vaccines (Gillanders)
The Centene Corporation contract supports the Washington University-Centene Personalized

Medicine Initiative (PMI) which awards funding to internal Washington University (WU) research projects in four disease specific areas. This project is funded under the WU-Centene PMI Breast Cancer Program.
The goal of this proposal is to validate an in vivo model to evaluate human tumors in the context of an intact human immune system in a completely personalized and autologous fashion.

Specific Aims

Aim 1: Test innovative neoantigen vaccine platforms in a preclinical model of breast cancer.
Aim 2: Optimize neoantigen vaccines.
Aim 3: Multidimensional profiling of the tumor microenvironment following neoantigen vaccine therapy.

Program Official: Rebecca Evans; not yet available

Admin Official:

EXPIRED AWARDS

R01CA204115 (Fields) 1/9/2017-12/31/2019 0.84
National Institutes of Health $460,459 calendar

Towards True Precision Oncology: Validation of a Comprehensively Humanized, Autologous Mouse Model

The purpose of this project is to model human cancers in the context of an autologous competent immune system.

Specific Aims

Aim 1: Validate the ability to establish humanized MISTRG (hMISTRG) mice from patients with melanoma.
Aim 2: Evaluate tumor growth and lymphocyte development in autologous human melanoma tumors in hMISTRG mice.
Aim 3: Validate the ability of hMISTRG mice bearing autologous melanoma tumors to serve as models for cancer immunotherapy treatment response and toxicity.

Program Official: Susan A. McCarthy

Admin Official: Alania Foster

(Dmitriev) 6/27/2018-6/26/2019 0.36
DNAtrix, Inc. $75,000 calendar

A Universal Cell Line for Targeted Adenovirus Virotherapy Agents

Immune checkpoint blockade is one of the most promising approaches achieving spectacular results in cancer therapy. Recent studies also suggested that its therapeutic efficacy in cancer patients could be enhanced by tumor inflammation. In this regard, programmed death-1 (PD-1) represents a potent inhibitory receptor of T cells which binds to two different ligands, namely PD-L1 and PD-L2, and upon binding, it inhibits T cell activation, differentiation, and proliferation, leading to a state of immune tolerance (Fig. 1 A). Blocking these interactions recently emerged as a 'game changer' approach in immunotherapy. Keytruda, a humanized antibody (Ab) derived to block PD-1 and prevent ligand binding is currently used in cancer immunotherapy to induce an antitumor immune response. Our current hypothesis is that

engineering DNX-2401 to express single-chain fragment variable (scFv) of antibody against PD1 could brake immune tolerance and allow efficient T cell-mediated killing of tumor cells (Fig. 1A) while translating to superior outcomes of oncolytic virotherapy.

Specific Aims

Aim 1: Evaluate each of the following viruses in the C57BL/6:ID8 model, a syngeneic immunocompetent orthotopic mouse model of carcinoma of the ovary- DNX-2401, DNX-mPD-1, DNX-2440 (OX40L), and 1 selected combination. Parameters to be evaluated include safety, survival, and effects on tumor growth and immune populations compared to DNX-2401E3e-GFP control.

Aim 2: Utilizing strategies employed in Project 1, generate a new DNX-2401 derived virus expressing 2 inserts based on results of Aim 2.

Admin Official:

not available

OC170200 (Curiel) 6/1/2018-11/30/2019 1.2

Department of Defense $155,000 calendar

Novel Ovarian Cancer Therapy

These studies will test a hypothesis regarding the biologic basis of virotherapy action that is of field-wide relevance. In addition, we will realize the database rationalizing translational development of a novel virotherapy agent for carcinoma of the ovary.

Specific Aims

Aim 1: To construct an ovarian cancer CRAd based upon gorilla adenovirus.

Aim 2: To characterize the tumor selectivity of the gorilla CRAd in vitro and in vivo.

Aim 3: To evaluate the ability of CRAd-based virotherapy to induce anti-tumor immunity in a syngeneic immunocompetent murine model of carcinoma of the ovary.

Program Official: Admin Official:

not yet available not yet available

SIP (Gillanders/Schreiber) 1/1/2018-12/31/2019 0.12

Siteman Cancer Center $87,095 calendar

Siteman Cancer Center Breast Cancer SPORE

Targeting Neoantigens in Triple Negative Breast Cancer (Gillanders/Schreiber)

The overall hypothesis of Research Project 1 is that neoantigen-specific T cell responses contribute to antitumor immunity in TNBC.

Specific Aims

Aim 1: Test the hypothesis that neoantigen vaccines, +/- anti-PD-L1, can induce and/or enhance neoantigen-specific T cell responses.

Aim 2: Test the hypothesis that neoadjuvant chemotherapy +/- anti-PD-L1 can enhance neoantigen-specific
T cell responses.

Program Official: Admin Official:

not yet available

not assigned (Fields) 10/1/2017-9/30/2019 0.6

Barnes-Jewish Hospital Foundation $100,000 calendar

The Washington University PDX Development and Trial Center (U54 Supplement)
The goal is to support Dr. Fields for the generation of animal models regarding the multi-PI (Govindan, Ding, Li) award U54CA224083: WASHINGTON UNIVERSITY PDX DEVELOPMENT AND TRIAL CENTER

Specific Aims

x

Program Official:
not yet available

Admin Official:
not yet available

FOLUSO ADEMUYIWA

NEW AWARDS

R01CA240983 (Gillanders/Schreiber) 8/1/2019-7/31/2024 0.24
National Institutes of Health $404,968 calendar
Targeting Neoantigens in Triple Negative Breast Cancer
The overall hypothesis is that enhancing neoantigen-specific T cell responses can improve clinical outcomes in TNBC.

Specific Aims

Aim 1. Test the hypothesis that neoantigen vaccines +/- anti-PD-L1 can induce and/or enhance neoantigen-specific T cell responses.
Aim 2: Test the hypothesis that targeting tumor-associated macrophages (TAM) can enhance eoantigen-specific T cell responses in TNBC.
Aim 3: Test the hypothesis that a neoantigen simian Ad vector vaccine "prime" followed by a neoantigen DNA vaccine "boost" can enhance the response to breast cancer neoantigens.

Program Official:
Min-kyung H Song

Admin Official:
Sabrina Oasan

EXPIRED AWARDS

CCR-16-110 (Ademuyiwa) 1/25/2017-6/30/2020 3.60
Rising TIde Foundation $98,480 calendar
A Phase II Neoadjuvant Study of Palbociclib in Combination with Letrozole and Trastuzumab as Neoadjuvant Treatment of Stage II-III ER+ HER2+ Breast Cancer (PALTAN)

In this study, we will conduct a clinical trial in patients with HR+ HER+ breast cancer evaluating single HER2 blockade combined with anti-hormonal therapy and CDK4/6 inhibition, as CDK4/6 inhibition leads to enhanced activity of anti-hormonal therapy. The goal will be to reduce the toxicities and cost of HR+ HER2+ breast cancer treatments by de-escalating therapy.

Grant officer:
Shawn Stephenson
grants@risingtide-foundation.org

R01 EB002136 (Zhu) 9/1/2016-8/31/2019 0.24
National Institutes of Health $274,464 calendar

Near Infrared Diffused Light Imaging with Ultrasound Guidance: Predicting Neoadjuvant Chemotherapy Response
Specific Aims:
1) Upgrading NIR imaging systems and validating imaging algorithms optimized for imaging large lesions by compensating for depth-dependent absorption mapping and by simultaneously reconstructing tumor absorption and scattering maps;
2) Validating the initial findings from a larger patient pool of approximately 80 neoadjuvant chemotherapy patients who will be recruited to the study from the Hartford Hospital, the University of Connecticut Health Center, and the Waterbury Hospital; and
3) Perform data analysis 1) to determine the best time-window to assess response based on cycle 1 %tHb for different treatment regimens; 2) validate the prediction model developed from pilot data based on tumor pathological variables (tumor type, grade and mitotic count), tumor molecular markers of estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu, and pretreatment NIR functional parameters as well as response rate based on one cycle of %tHb.

Program Official:	Admin Official:
Randy L. King	Angela Eldridge

Siteman Investment Program (Ma) 7/1/2018-6/30/2020 0.36
Barnes Jewish Hospital Foundation $195,373 calendar
NEK9-MAP2K4: A Novel Signaling Axis Promoting Breast Cancer Growth and Chemotherapy Resistance
Specific Aims:
1) To test the hypothesis that NEK9-MAP2K4 axis is important for mitotic cell cycle progression and tumor cell growth in breast cancer cells, particularly TNBC;
2) To test the hypothesis that NEK9-MAP2K4 contributes to chemotherapy resistance and to determine the cell cycle checkpoint(s) that NEK9-MAP2K4 participates in;
3) To establish the clinical relevance of NEK9 and MAP2K4 signaling axis in TNBC.

Program Official:	Admin Official:
n/a	Donald Buckner FBJH_grants@bjc.org

FENG GAO

NEW AWARDS

R01CA240983 (Gillanders/Schreiber) 8/1/2019-7/31/2024 0.60
National Institutes of Health $404,968
Targeting Neoantigens in Triple Negative Breast Cancer
The overall hypothesis is that enhancing neoantigen-specific T cell responses can improve clinical outcomes in TNBC.
Specific Aims
Aim 1. Test the hypothesis that neoantigen vaccines +/- anti-PD-L1 can induce and/or enhance neoantigen-specific T cell responses.
Aim 2: Test the hypothesis that targeting tumor-associated macrophages (TAM) can enhance

neoantigen-specific T cell responses in TNBC.
Aim 3: Test the hypothesis that a neoantigen simian Ad vector vaccine "prime" followed by a neoantigen DNA vaccine "boost" can enhance the response to breast cancer neoantigens.

Program Official:
Min-kyung H Song

Admin Official:
Sabrina Oasan

P50CA244431 (Brownson/Colditz) 9/18/2019-8/31/2024 0.96
National Institutes of Health $191,409 PM
Washington University Implementation Science Center for Cancer Control Implementation Laboratory (James)
The Implementation Lab, hereafter called the Innovation Incubator, through Incubator Membership, a Data Management Unit, and a Practice Surveillance Unit, will work closely with the WU-ISCCC Research Program to facilitate implementation science research by connecting investigators to implementation sites in the Membership, convening community and clinical partners to review data and identify needs and priorities and support evaluation of program outcomes.

Specific Aims
Aim 1: Establish leadership structure and functions that enable and support rapid-cycle and innovative implementation science in cancer control.
Aim 2: Sustain and develop additional research partnerships with a range of healthcare systems and organizations/agencies committed to innovative implementation science projects that address social determinants of health and reduce cancer disparities.
Aim 3: Support data systems and resources to manage multi-site projects, collect rigorous data, and produce timely reports to the project leaders and the WU-ISCCC leadership.
Aim 4: Cultivate a surveillance unit to report on progress toward reducing cancer disparities in the populations across Siteman Cancer Center's 82-county catchment area.
Aim 5: Draw on real-time clinical service data from health systems within BJH Healthcare and the BJC Collaborative network to monitor policies, provider practices, patient outcomes and progress in reducing cancer disparities.

Program Official:
Cynthia Vinson

Admin Official:
Viviana Knowles

R01CA248277 (Fields/Flavell) 3/1/2020-2/28/2025 0.24
National Institutes of Health $431,069
Advancing Precision Oncology in a Humanized, Fully Autologous Mouse Model
The overarching goal of this proposal is to evaluate a comprehensively HuMo system to faithfully model the innate and adaptive human immune system in order to deliver on the promise of precision medicine by evaluating immunotherapy modalities in vivo.

Specific Aims
Aim 1: Validate the ability of hMISTRG mice bearing autologous melanoma tumors to serve as models for ICB treatment response and toxicity.

Aim 2: Validate the ability of hMISTRG mice bearing autologous melanoma tumors to serve as models for peptide vaccine-based therapy.
Aim 3: Establish humanized hMISTRG mice and evaluate tumor growth and lymphocyte development in patients with PDAC or CRC.

Program Official:	Admin Official:
Susan A. Mccarthy	Rebecca Brightful

EXPIRED AWARDS

P50CA196510 (Hawkins)	7/28/2016-6/30/2021	1.2
National Institutes of Health	$78,666	PM

Washington University SPORE in Pancreatic Cancer Core C: Biostatistical Core (Colditz)
The goal of the Biostatistics Core (Core C) of the Washington University SPORE in Pancreatic Cancer is to provide the statistical and computational support for all pancreas cancer SPORE investigators. The Core will support consultation and collaboration on all aspects of study design, database development and quality control, and analysis, interpretation, and presentation of data.

Specific Aims
Aim 1: Provide ready access to statistical expertise and computing consultation to the Pancreas Cancer SPORE program.
Aim 2: Provide biostatistical/epidemiological expertise for the planning, analysis, and reporting of laboratory experiments, epidemiology studies, and clinical trials as well as links to the Bioinformatics Core resources.
Aim 3: Advise and support SPORE investigators and their data collectors (e.g., technicians, nurses, data managers) in the areas of data form design, data collection, record abstraction, computerization, database design and management, and data quality control.
Aim 4: Provide the scientific computing expertise required to meet the data management and analytical needs of the Pancreas Cancer SPORE investigators, and support the interpretation and presentation of data.

Program Official:	Admin Official:
Steven Nothwehr	Alania Foster

R01CA204115 (Fields)	1/9/2017-12/31/2019	0.3
National Institutes of Health	$460,459	PM

Towards True Precision Oncology: Validation of a Comprehensively Humanized, Autologous Mouse Model
The purpose of this project is to model human cancers in the context of an autologous competent immune system.

Specific Aims
Aim 1: Validate the ability to establish humanized MISTRG (hMISTRG) mice from patients with melanoma.

Aim 2: Evaluate tumor growth and lymphocyte development in autologous human melanoma tumors in hMISTRG mice.
Aim 3: Validate the ability of hMISTRG mice bearing autologous melanoma tumors to serve as models for cancer immunotherapy treatment response and toxicity.

Program Official: Admin Official:
Susan A. McCarthy Alania Foster

not assigned (DiPersio/Cooper) 11/1/2017-10/31/2019 0.12
Alvin J. Siteman Cancer Research Fund $300,000
Clinical Development of CRISPR/Cas9 Gene Edited CAR-T for the Treatment of T Cell Hematologic Malignancies and Mitigation of Cytokine Release Syndrome
The goals of this project is to further the development of CAR-T therapy against T cell malignancies and provide the framework for performing the first clinical trial using CAR-T therapy against T cell malignancies.

Specific Aims
n/a

Program Official: Admin Official:
not yet available

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

Appendix 1: Overview of trial patients through June 30, 2020

Patient ID	Eligible	Treatment Arm	Treatment Status
WU-001	No (MGB negative)		
WU-002	Yes	Endocrine+vaccine	discontinued
WU-003	No (MGB negative)		
WU-004	No (BMI)		
WU-005	No (Ki-67 too high)		
WU-006	No (MGB negative)		
WU-007	No (MGB negative)		
WU-008	No (MGB negative)		
WU-009	Yes	Other*	
WU-010	No (ineligible for neo-adj therapy)		
WU-011	No (MGB negative)		
WU-012	No (MGB negative)		
WU-013	No (MGB negative)		
WU-014	No (MGB negative)		
WU-015	Yes	Endocrine	completed
WU-016	Yes	Endocrine+vaccine	completed
WU-017	No (Ki67 too high)		
WU-018	No (MGB negative)		
WU-019	No (MGB negative)		
WU-020	Yes	Other*	
WU-021	Yes	Endocrine	completed
WU-022	Yes	Endocrine	completed
WU-023	No (Ki67 too high)		
WU-024	No (patient not compliant)		
WU-025	No (patient chose alternate trial)		
WU-026	No (patient chose surgery)		
WU-027	Yes	Endocrine	completed
WU-028	No (patient chose surgery)		
WU-029	Yes	Endocrine+vaccine	completed
WU-030	Yes	Other*	
WU-031	No (MGB negative)		
WU-032	Yes	Endocrine+vaccine	completed
WU-033	Yes	Endocrine	completed
WU-034	No (MGB positive, Ki67 not tested)	Other*	
WU-035	No (patient chose surgery)		
WU-036	No (MGB negative)		
WU-037	No (MGB negative)		
WU-038	No (MGB negative)		
WU-039	No*		
WU-040	No (MGB negative)		
WU-041	No (MGB negative)		
WU-042	Yes	Endocrine+vaccine	completed
WU-043	No (MGB negative)		
WU-044	No (MGB negative)		
WU-045	No (Ki67 too high)		

1

Patient ID	Eligible	Treatment Arm	Treatment Status
WU-046	Yes	Endocrine	completed
WU-047	No (MGB negative)		
WU-048	Yes	Endocrine	completed
WU-049	Yes	Endocrine	completed
WU-050	Yes	Other*	
WU-051	No (MGB negative)		
WU-052	No (MGB negative)		
WU-053	No*		
WU-054	Yes	Other*	
WU-055	No*		
WU-056	Yes	Endocrine+vaccine	completed
WU-057	Yes	Other*	
WU-058	No (MGB negative)		
WU-059	No (Ki67 too high)		
WU-060	No (MGB negative)		
WU-061	Yes	Endocrine+vaccine	completed
WU-062	No	Other*	
WU-063	No (MGB negative)		
WU-064	Yes	Other*	
WU-065	Yes	Endocrine+vaccine	completed
WU-066	No	Other*	
WU-067	No	Other*	
WU-068	Yes	Other*	
WU-069	Yes	Endocrine+vaccine	completed
WU-070	No (MGB negative)		
WU-071	Yes	Endocrine+vaccine	completed
WU-072	No (MGB negative)		
WU-073	Yes	Endocrine+vaccine	completed
WU-074	Yes	Endocrine+vaccine	completed
WU-075	Yes	Chemo + vaccine	completed
WU-076	No*		
WU-077	No (MGB negative)		
WU-078	Yes	Endocrine+vaccine	completed
WU-079	Yes	Other*	
WU-080	No*		
WU-081	No (MGB negative)		
WU-082	No*		
WU-083	Yes	Endocrine+vaccine	ongoing
WU-084	Yes	Other*	
WU-085	No (MGB negative)		

*Patient declined to be on trial

Changes from the previous Annual Report are highlighted

Appendix 2. Mammaglobin-A immune responses in patient PBMC detected by ELISpot analysis

Patient peripheral blood was collected at various time points; for patients receiving endocrine therapy only, three blood draws were performed at baseline, at the time of surgery, and at one year post surgery. All PBMC samples were tested for recognition of mammaglobin-A peptides at the same time using an IFNγ ELISpot assay. Cells were harvested after the ELISpot assay and cultured for 12 days in the presence of IL-2, and subsequently retested against the same peptide pool used for stimulation, as well as the individual peptides comprising that pool, dependent on cell numbers obtained. Controls included anti-CD3 antibody and a standardized pool of common viral peptides, CEF, purchased from CTL Technologies. The data suggest that in two patients mammaglobin-A specific T cells were detectable in the peripheral blood, even in the absence of active vaccination. This appears mostly evident at the surgery and week 52 time points for both patients. Earlier reports have described similar observations, and even some healthy individuals have mammaglobin-A-specific T cells detectable by ELISpot.

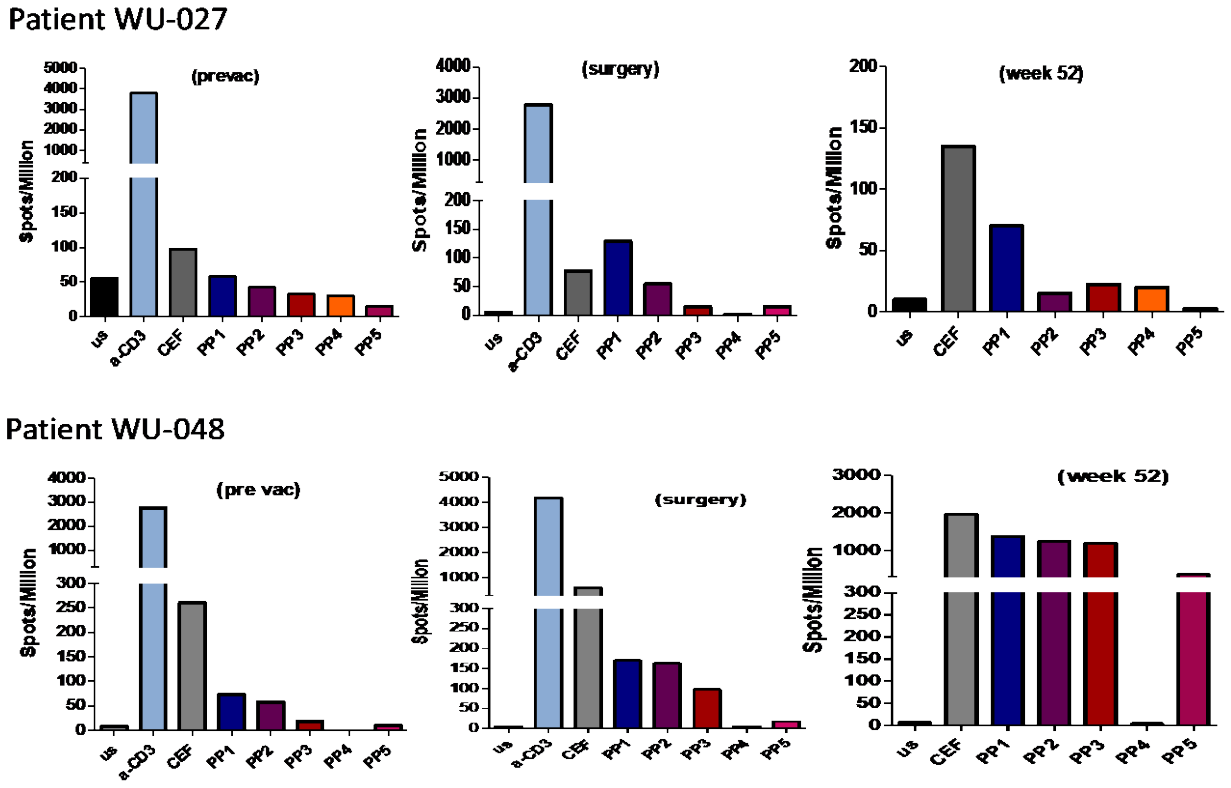


Figure 1. Analysis of T cell responses to mammaglobin-A by IFNγ ELISpot assay. PBMC were plated in duplicate at 200,000 cells per well in medium (unstimulated, "US"); stimulated with anti-CD3 antibody, or CEF/mammaglobin-A peptide pools (pp1-pp5). Plates were developed 48 hrs later and data are displayed as the number of spots/million cells plated. The anti-CD3 controls for the two 52-week time points were highly positive and have been omitted for clarity of the data.

While both the anti-CD3 and CEF peptide pool elicited IFNγ in the PBMC of the other two patients at all three time points, no mammaglobin-A-specific responses were detectable.

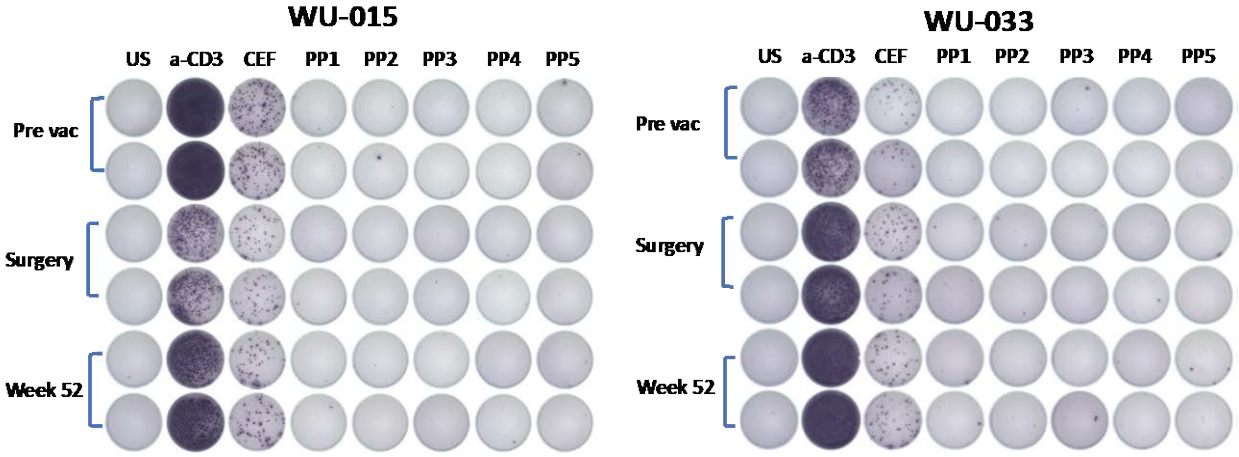


Figure 2. Analysis of T cell responses to mammaglobin-A by IFNγ ELISpot assay. PBMC were plated in duplicate at 200,000 cells per well in medium (unstimulated, "US"); stimulated with anti-CD3 antibody, or CEF/mammaglobin-A peptide pools (pp1-pp5). Plates were developed 48 hrs later. Images of the ELISpot plates are shown and indicate no responses to any of the mammaglobin-A peptide pools.

T cell responses were generally more robust after expansion of stimulated cells for 12 days. Overall responses against individual peptides were fairly similar, but a few appeared especially strong, e.g WU-015 against p21 and WU-027 against p6 and p10. Additional experiments will be performed to characterize the T cell response and confirm peptide specificity.

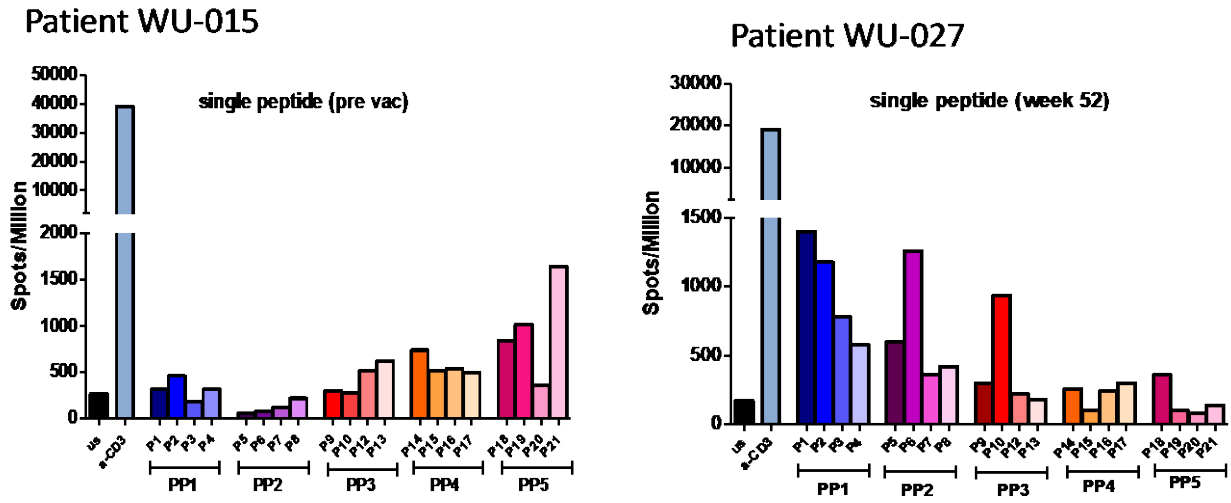
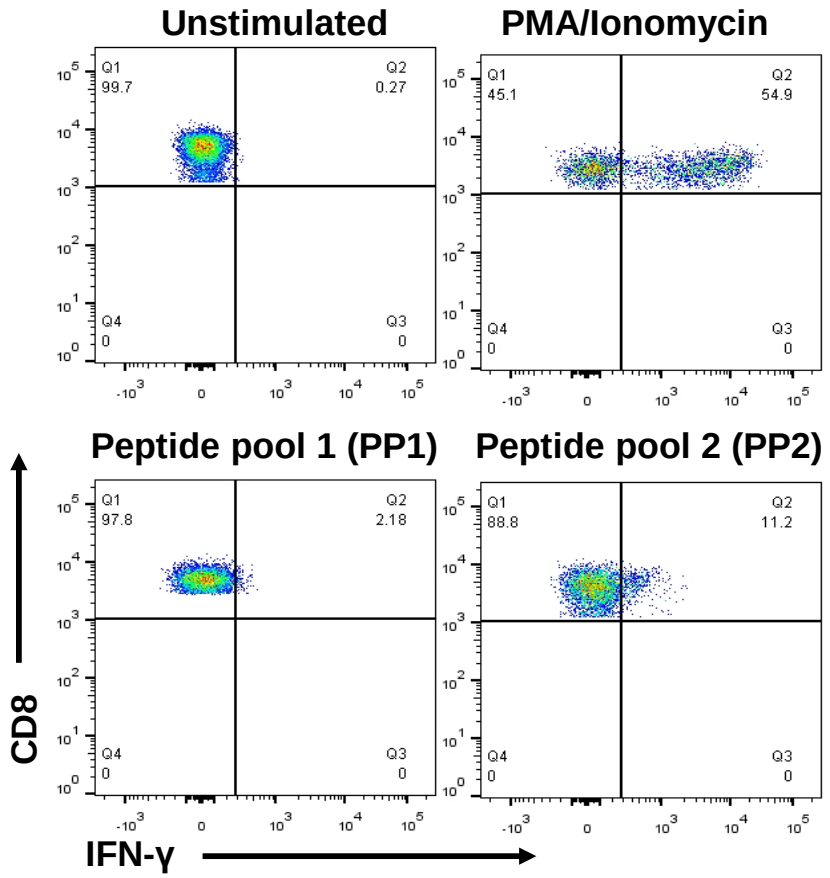
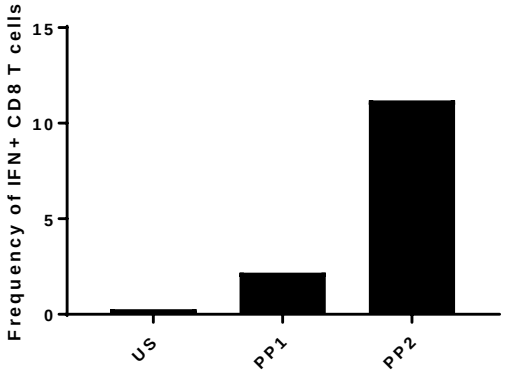


Figure 3. Analysis of T cell responses to mammaglobin-A by IFNγ ELISpot assay. T cells were plated in duplicate at 50,000 cells per well in medium (unstimulated, "US"); stimulated with anti-CD3 antibody, or individual mammaglobin-A 15-mer peptides. Autologous PBMC were irradiated and either pulsed with peptide or not before plating 50,000 cells/well. Plates were developed and analyzed as described above.

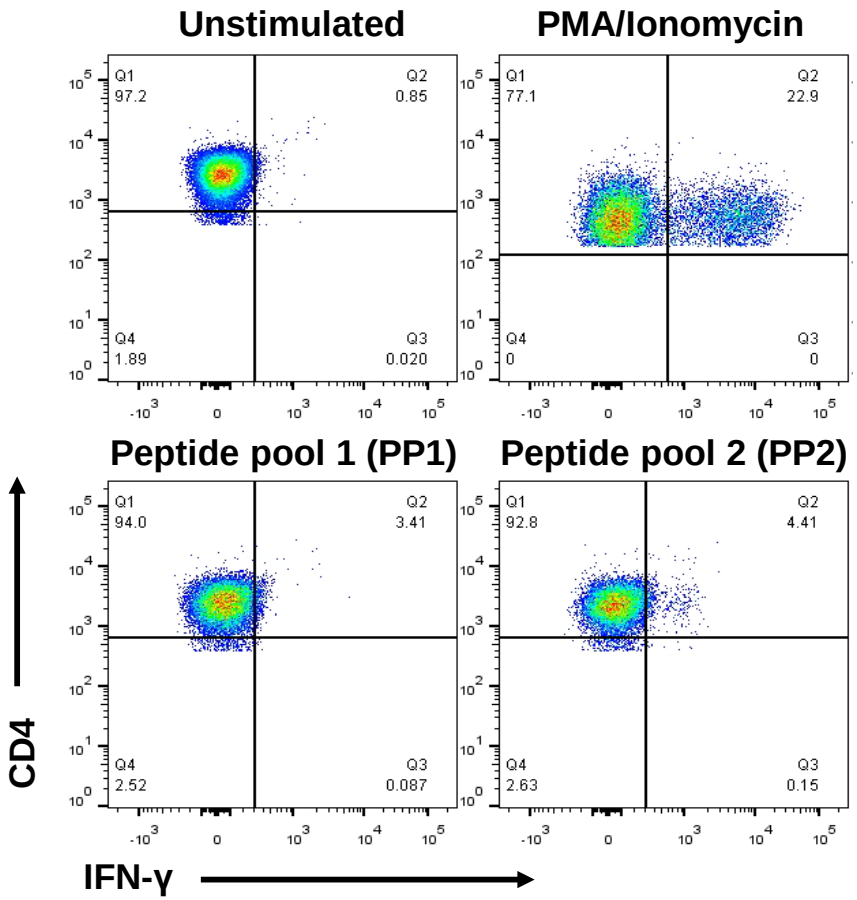
Mammaglobin-A induced IFN-γ production by CD8 T cells

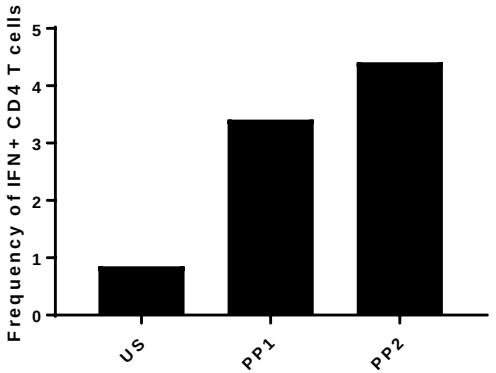




PBMC were stimulated with mammaglobin-A peptides (peptide pools 1 and 2) in the presence of IL2 for 12 days. Cells were subsequently harvested and restimulated with either PMA/ionomycin (positive control) or the peptide pools for 5 hours and 20 hrs respectively. Brefeldin-A was added for the last 4 hours to block secretion of cytokines. Cells were stained for CD4,CD8, and IFN-γ using fluorescent antibodies, and analyzed by flow cytometry. Data show subtle but detectable IFNγ production by a fraction of the CD8 T cells. The bar graph on the right shows the percentage of CD8+IFNγ+ cells detected by flow cytometry.

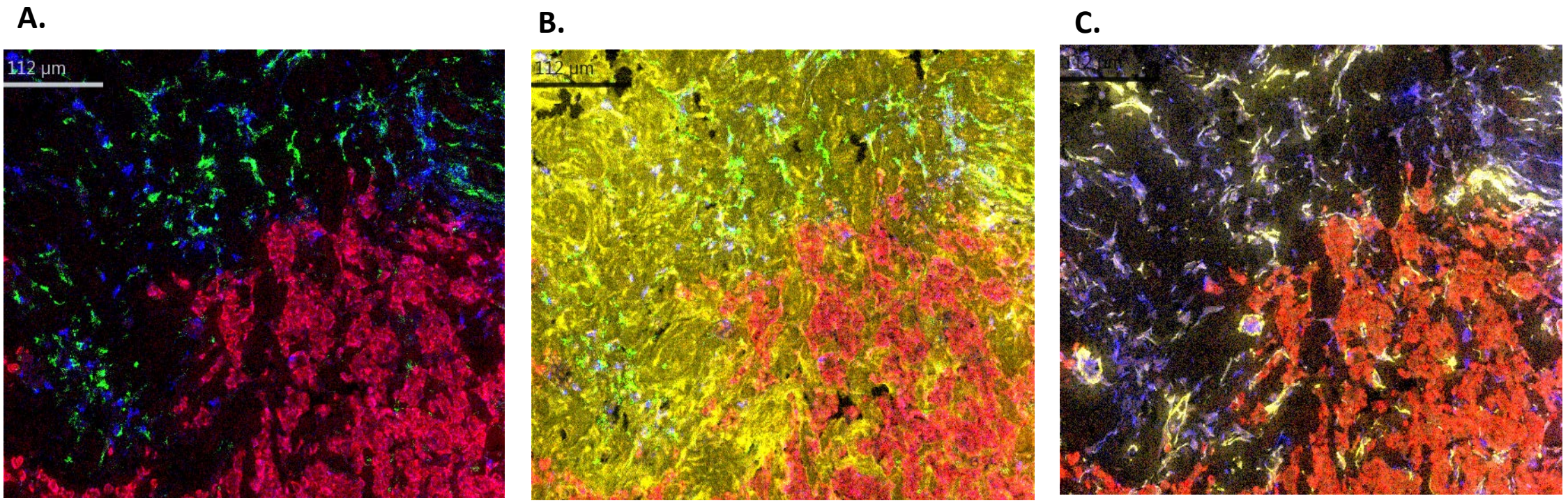
Mammaglobin-A induced IFN-γ production by CD4 T cells





PBMC were stimulated with mammaglobin-A peptides (peptide pools 1 and 2) in the presence of IL2 for 12 days. Cells were subsequently harvested and restimulated with either PMA/ionomycin (positive control) or the peptide pools for 5 hours and 20 hrs respectively. Brefeldin-A was added for the last 4 hours to block secretion of cytokines. Cells were stained for CD4,CD8, and IFN-γ using fluorescent antibodies, and analyzed by flow cytometry. Data show subtle but detectable IFNγ production by a fraction of the CD4 T cells. The bar graph on the right shows the percentage of CD4+IFNγ+ cells detected by flow cytometry.

Appendix 4: Representative example of a human breast cancer tissue sample analyzed for immune markers by imaging mass cytometry



Representative mass cytometry images of an invasive lobular carcinoma with tumor cells stained by Pan-Keratin (red). Additional staining includes (A.) CD8 (blue) and macrophages (CD163, green); (B.) collagen (yellow), and (C.): vimentin (blue) and α-smooth muscle actin (yellow).