AWARD NUMBER: W81XWH-19-1-0340

TITLE: Enhanced Melanoma Vaccine Against Neoantigens and Shared Antigens by CD40 Activation and TLR Agonists

PRINCIPAL INVESTIGATOR: Timothy Bullock, PhD.

CONTRACTING ORGANIZATION: University of Virginia
Charlottesville, VA 22908

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6. AUTHOR(S)

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5d. PROJECT NUMBER

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

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Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project will test the hypotheses that vaccination with CD40 Ab/polyICLC will be safe and will induce strong and durable T cell responses to the peptides in the vaccine, and that a mutated neoantigen peptide ($BRAF_{585\text{-}614\_V60E}$) will be immunogenic and will support infiltration and regression of BRAF-mutant nevi. We also hypothesize that the vaccine regimen will activate DC and induce tertiary lymphoid structures in the vaccine-site microenvironment. This will be accomplished through a clinical trial (NCT04364230). All approvals are in place. We expect to open to enrollment in September or October 2020.

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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

This project will test the hypotheses that vaccination with CD40 Ab/polyICLC will be safe and will induce strong and durable T cell responses to the peptides in the vaccine, and that a mutated neoantigen peptide ($BRAF_{585\text{-}614\_V60E}$) will be immunogenic and will support infiltration and regression of BRAF-mutant nevi. We also hypothesize that the vaccine regimen will activate DC and induce tertiary lymphoid structures in the vaccine-site microenvironment.

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

Melanoma, vaccine, helper T cell, neoantigen, toll like receptor, CD40, peptide, dendritic cell

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

Aim 1. To test the safety and immunogenicity of vaccination against melanoma with helper peptides + CD40 antibody CDX-1140 + TLR3 agonist polyICLC.

Aim 2. To determine whether vaccination with peptides in CDX-1140 + polyICLC enhances dendritic cell activation and maturation, Th1 dominant microenvironment, and induces tertiary lymphoid structures (TLS).

Aim 3: To evaluate whether vaccination against a mutant BRAF peptide induces T cells that recognize BRAF-mutant melanomas, infiltrate nevi and are associated with regression of nevi.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Our effort has been directed to opening the clinical trial that is the basis for the work of this project. Progress includes:

PREPARATION OF neoAg-mBRAF PEPTIDE FOR USE IN THE CLINICAL TRIAL.

1) In prior reporting periods, we had:
    a. synthesized the new peptide for the proposed vaccine: neoAg-mBRAF. This peptide, representing a mutated sequence in BRAF-V600E, was synthesized in a GMP laboratory and received in October 2019.
    b. Determined optimal conditions for solubilization of neoAg-mBRAF peptide.
    c. Sterile filtered and vialed the neoAg-mBRAF peptide (600 vials) in our cGMP laboratory at the University of Virginia. December 2019.
2) We have received results, for vials of neoAg-mBRAF, on quality assurance testing including mass spectrometry (for accurate mass and amino acid sequence), amino acid analysis (for peptide concentration), HPLC (for purity), and sterility testing. They all met lot release criteria.

FINALIZATION OF CLINICAL TRIAL PROTOCOL (Mel66 protocol) AND HRPO REVIEW

3) In prior reporting periods, we had:
    a. Revised and completed clinical trial protocol that forms the basis of this project.
    b. Submitted protocol to HRPO for pre-review 9/26/19, responded to HRPO pre-review provided 12/19/19.
    c. We submitted the protocol and associated requested documents to HRPO 1/3/20 for full review - HRPO Log Number E00804.1a
    d. On 31 Jan 2020, we were authorized to proceed with the FDA and IRB submissions.
4) IRB approval obtained at the University of Virginia April 30th, 2020.

DRAFTING OF IND APPLICATION TO THE FDA

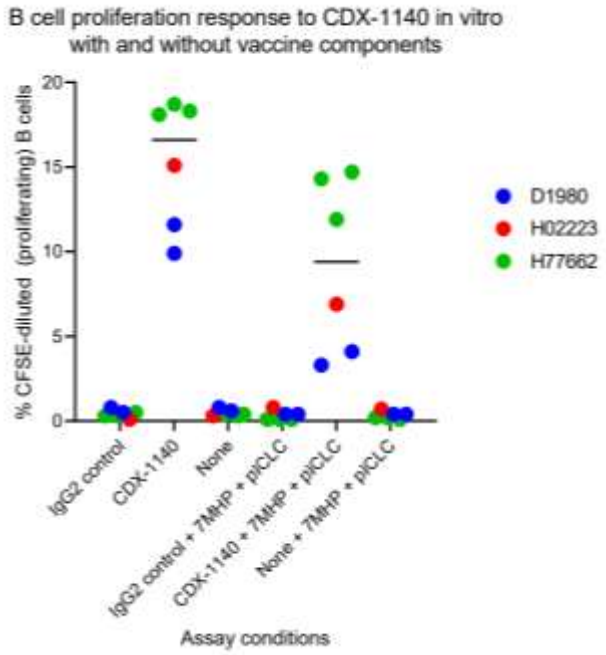
5) In the current reporting period, we have:
    a. Completed the IND application as an amendment to IND #10825 (Amendment 070), and submitted it to the FDA March 5th, 2020. That amendment adds the neoAg-mBRAF peptide and the clinical trial protocol for this project.
    b. The 30 day waiting period has passed, and there no comments or critiques from the FDA reviews; so, the study is cleared to open.

CONTRACTING WITH CELLDEX FOR USE OF THEIR CDX-1140 ANTIBODY

12) Contract with Celldex written approved for use of the CDX-1140 antibody in this clinical trial.
13) Material transfer agreement written and approved with Celldex for provision of a sample of the CDX-1140 antibody for preclinical testing to ensure that it remains functional when mixed with polyICLC and peptides. The CDX-1140 antibody was received in the laboratory at the end of January 2020. Those studies have been completed:

Peripheral blood mononuclear cells from 3 normal donors were cultured 5 days with media, CDX-1140, 7MHP+polyICLC, or both, and evaluated for proliferation in vitro, measured by dilution of CFSE. There was no proliferation with peptides alone or media alone, but there was proliferation for all 3 normal donors with CDX-1140 alone or CDX-1140 plus 7MHP+polyICLC. The proliferation was slightly less when combined with peptide and polyICLC, but the persistent effect when combined with peptides and polyICLC confirms that the antibody activity is not blocked by mixing with the other agents. In discussion with collaborators at Celldex on 7/2/20, they agreed that it was reasonable to proceed with the mixing of CDX-1140, 7MHP (6MHP + mBRAF peptide), and polyICLC as planned.

The data are shown here:



These data enabled proceeding with the opening the protocol as intended, where the CDX-1140 antibody is to be delivered mixed with the peptides and polyICLC.

FINAL LOGISTIC ARRANGEMENTS:

- We have developed a system for choosing the biopsy dates for each of the nevi selected for biopsy (day 0 or day 85) on protocol, by use of a random number generator system, and arranged logistics with the department of dermatology at UVA for skin exams, nevus selection and biopsy.
- Have made plans for work-flow for processing of nevus biopsies, with review by the pathologist co-investigator at UVA and for research purposes.
- Case report forms have been developed for use at each site and should be finalized within August 2020.
- Prepared shipping containers for blood samples to be send from Cleveland Clinic to UVA.
- Formal mixing instructions for the vaccine preparations have been completed, reviewed by staff, approved, and Jennifer Bryant has trained in the preparation methods.
- Investigator Brochure prepared for the peptides and other components of the vaccines.

**** We expect to open to enrollment by the end of September 2020 or in early October 2020. ****

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.

No dissemination of results yet, as the focus has been on preparing to open the clinical trial.

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.

1) Initiation of enrollment on the Mel66 clinical trial protocol at the University of Virginia. Expected by late August 2020 or early September 2020.
2) Initiation of enrollment at the Cleveland Clinic soon after opening at UVA, under Dr. Brian Gastman's leadership.
3) Begin to collect samples and to provide them for immunologic studies under Dr. Timothy Bullock's leadership in his portion of this project.

4. **IMPACT:** D*escribe 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).

None yet, pending the performance of the trial, which is expected to open soon.

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.

None yet, pending the performance of the trial, which is expected to open soon.

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 yet.

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.*

None yet, pending the performance of the trial, which is expected to open soon.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

None.

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.

The process of writing the protocol, obtaining HRPO approval, ordering and receiving the neoAg-mBRAF peptide, and GMP vialing and solubilization of the neoAg-mBRAF peptide took time earlier, which prevented submission to the FDA as early as intended. However, IRB and FDA clearance have been obtained.

There were some processes to finalize to manage logistics of the trial, including working with dermatology and pathology on the sample collections. These have been worked out for the most part and final steps should be finished in the month of September.

The COVID-19 crisis and some personnel changes slowed progress because we needed to validate the function of the CD40 antibody after combining with the peptides and polyICLC. The staff member who was ideal for these experiments (Walter Olson PhD) had retired in December, but has returned part-time but the paperwork for that return took time, By the time he returned, the COVID-19 crisis had effectively closed the laboratory. We have been able to resume limited laboratory activities, and Dr. Olson did complete the necessary studies, which we have since discussed with staff at Celldex and are discussed above.

The COVID-19 slowdown also prevented Jenn Bryant from being in the lab earlier, but she is now active in the lab and has trained on the vaccine preparation steps and has done a careful review of the mixing instructions which have now been finalized.

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.

The cost of the cGMP peptide (mBRAF peptide) was greater than what was budgeted. The difference has been covered with institutional funds.

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

No significant changes.

Significant changes in use or care of vertebrate animals

None.

Significant changes in use of biohazards and/or select agents

None.

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.

  The clinical trial design is listed on Clinicaltrials.gov: ClinicalTrials.gov Identifier: NCT04364230

  Otherwise, nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  The mutated BRAF peptide, modified to enhance solubility is a new technology.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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.

  An invention disclosure has been submitted to the UVA Licensing and Ventures group for the vaccine preparation including the new neoAg-mBRAF peptide plus 6 other melanoma helper peptides.

  The neoAg-mBRAF peptide has been prepared as a clinical-grade reagent under GMP conditions.

- **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;*
  - *physical 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.*

No others yet.

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.)*

Name: *Craig Slingluff*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): *ORCID ID: 0000-0002-6664-4373*
Nearest person month worked: *1*
Contribution to Project: *Dr. Slingluff has overseen and been intimately involved with the protocol writing and revision and contracts, selecting the vendor to synthesize the neoAg mBRAF peptide, determining optimal solubilization of the neoAg mBRAF peptide. Drafted SOP for GMP solubilization and vialing of mBRAF peptide.*

Name: *Timothy Bullock*
Project Role: *collaborating PI*
Researcher Identifier (e.g. ORCID ID): *ORCID ID:* 0000-0001-6141-3261
Nearest person month worked: *1*
Contribution to Project: *Supported analysis of the immunogenicity of formulated vaccine. Planned flow cytometry and IHC work flows for study.*

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.

Dr. Bullock has had the following changes in Other Support:

New grants

R01EB030409-01 (Price and Bullock) 9/1/19- 8/31/24 2.4 calendar NIH/EB Focused Ultrasound Energy Regimes for Melanoma Immunotherapy Goals of Project: The goals of this application are to understand how characteristics of mechanical and thermal ultrasound influence anti-tumor immunity and activate adaptive resistance mechanisms. Direct costs: $450,000 p.a.

R21NS118278 (Price) 7/1/20-6/30/22 0.6 CM NIH/NINDS ImmunoPET Assessment of anti-CD47 Immunotherapy Delivery to Glioblastoma with Focused Ultrasound Goals of Project: The major goals of this project are to (i) use anti-CD47 immunoPET analyses to optimize the timing of drug administration with respect to focused ultrasound-mediated bloodbrain barrier opening and (ii) utilize this information to develop optimized protocols for controlling glioblastoma with focused ultrasound and anti-CD47 immunotherapy. Direct Costs: $125,000. p.a.

Research Grant (Bullock, T) 10/01/19 – 09/30/20 0.3 calendar Focused Ultrasound Foundation "Targeting immunosuppressive myeloid cells to increase efficacy of thermally ablative FUS in breast cancer" Goals of Project: We have found that thermally-ablative focused ultrasound cooperates with gemcitabine to substantially improve the immunogenicity of a murine model of breast cancer. The current studies will help understand whether this synergy is a function of ablating immunosuppression or promoting immunogenicity. Direct costs: $100,000 total Overlap: None

R21NS116518-01(Bullock, T)) 09/01/20-08/31/22 0.6 calendar NIH/NNCI "Leveraging MR-guided Focused Ultrasound to Potentiate Immunotherapy for GBM" Goals of Project: Here we propose to test a novel approach using the energy of sound (ultrasound) to open these barriers, and test whether strategies that are designed to help the activation and targeting of the immune response to GBM can work together with ultrasound to help control GBM outgrowth in a pre-clinical setting. Direct costs: $125,000 p.a. Overlap: None

Expiring grant:

1R01CA197111-01 (Price R, Bullock T, Engelhard V, co-PI) 4/1/15- 5/31/20 1.8 calendar NIH/NCI
Immunotherapeutic Nanoparticle Delivery to Melanoma with MR-Guided Focused Ultrasound
Goals of Project: The goals of this study are to develop focused ultrasound to delivery genetic material to the TME that augments tumor antigen expression or limits inhibitory mechanisms in order to promote tumor immunity

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.*

The Cleveland Clinic is a partner on this project. One of the coPIs is Dr. Brian Gastman, at the Cleveland Clinic.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.