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

PRINCIPAL INVESTIGATOR: Brian Gastman, MD.

CONTRACTING ORGANIZATION: Cleveland Clinic, 9500 Euclid Ave, Cleveland, Ohio, 44195

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Brian Gastman
e-mail:gastmab@ccf.org

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project will test the hypotheses that vaccination with CD40 Ab/TLR3 agonist is safe and induces strong and durable T cell responses to the peptides in the vaccine, and that a mutated neoantigen peptide (BRAF585-614-V600E) is immunogenic. There have been no findings during this reporting period as the study is not yet open at our site.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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TABLE OF CONTENTS

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/TLR3 agonist is safe and induces strong and durable T cell responses to the peptides in the vaccine, and that a mutated neoantigen peptide (BRAF585-614–V600E) is immunogenic.

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

Melanoma, Vaccine, Peptides, Local adjuvants

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 CD40Ab + polyICLC enhances DC activation and maturation, Th1 dominant microenvironment, and 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.

Major Task 1: Open the Mel66 clinical trial testing CDX-1140 + polyICLC + 7MHP vaccine
Major Task 2: Assess the safety of vaccination with 7MHP + CDX-1140 +polyICLC
Major Task 3: Test whether vaccination with 7MHP + CD40Ab + polyICLC induces strong and durable T cell responses to 6MHP and to Neo-Ag-mBRAF
Major Task 4: Test whether vaccination with CDX-1140 + polyICLC increases DC activation and maturation in the VSME and SIN compared to peptide vaccines in IFA
Major Task 5: Determine whether CD40 + poly ICLC induces an inflammatory microenvironment at the VSME and SIN that is more Th-Dominant than vaccines with IFA
Major Task 6: Determine whether vaccination with peptides in CDX-1140-pICLC induces tertiary lymphoid structures and decreased expression of integrins in the VSME
Major Task 7: Determine whether vaccination with a mutated BRAF peptide induces T cell responses that selectively recognize BRAF-mutant melanoma cells across a range of class II MHC
Major Task 8: To test whether vaccination with 7MHP induces T cells reactive to mBRAF or to melanocytic proteins that will selectively infiltrate BRAF-mutant or wild-type nevi.
Major Task 9: To test whether vaccination with 7MHP induces clinical regression of melanocytic nevi associated with BRAF mutation status and immune infiltration.

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.

Under Major Task 1: CCF & UVA: Finalized the clinical trial protocol for Mel66 trial, submitted the protocol to Cancer Center Protocol Review Committees, received protocol approval from UVA IRB then submitted it to the CCF IRB, wrote Initial New Drug (IND) Application for Mell6 trial and neoAg-mBRAF peptide. UVA only: Submitted IND application for Mel66 trial and neoAg-mBRAF peptide to FDA, received HRPO approval.

Under Major Task 2: CCF & UVA: Enrolled patients on protocol (*CCF accrual goal – 12, UVA accrual goal – 28*), identified treatment groups, preformed study procedures (dermatologic assessment and selection of nevi for biopsies, study biopsies and blood collection, monitored data and site compliance with protocol, clinical follow up). UVA only: Reviewed any DLTs and determined maximum tolerated dose.

Under Major Task 3: CCF & UVA: Analyzing data over the study and compare to prior vaccines with IFA, discuss with team. UVA only: Evaluated T cell responses by ELISpot assay, Evaluated data for immune response measures

Under Major Task 4: UVA only: Flow cytometric evaluation of DC in SIN for DC maturation and function, including CD70 expression and multiparameter immunohistochemistry of vaccine site biopsy samples for DC infiltrates

Under Major Task 5: UVA only: Gene expression analysis (by RNAseq) of VSME for measures of immune activation (eg: Tbet, GATA3, STAT1, Th1 cytokines), multiparameter immunohistochemistry of vaccine site biopsy samples: costaining for CD4 (and CD8) and T cell transcription factors (Tbet, GATA3, RORgt, FoxP3), and Ki67, and flow cytometric evaluation of T cells (Th1, Th2, Th9, Th17, Treg) in SIN for function and Th1 dominance

Under Major Task 6: CCF & UVA: Preparation summary data and review with study team, *Milestone Achieved: Impact of vaccine regimen and adjuvant on vaccine site microenvironment. Prepare summary publications.* UVA only: mIFH of VSME tissues for markers TLS, including CD8 T cells, CD20 B cells, PNAd, mature DCs, Tregs, and Ki67, mIFH of VSME tissues for Th1 dominance- Density of CD8 and CD4 T cells, Staining T cells for retention integrins CD49a and CD103, mIFH of VSME tissues for presence of tertiary lymphoid structures, Gene expression analyses for markers of DC activation, Th1 dominance, TLS, and prepared summary data for Major Tasks 3-6.

Under Major Task 7: CCF & UVA: Identify epitope and determine if it varies for different MHC II. Use 60 melanoma cell lines from the UVA Melanoma Master Cell Line Bank and 24 PDX (40% BRAF-mutant), with and without siRNA knockdown of BRAF-V600E. UVA only: Expand patient T cells. Evaluate proliferation via CFSE dilution and production of IFNy and IL-2 via intracellular staining in response to BRAF mutant or BRAF WT peptide.

Under Major Task 8: UVA only: Evaluate selected nevi histologically. Stain for BRAF V600E. Assess for necrosis, fibrosis, or other changes that suggest regression. Stain for melanocytes, T cell markers, cell division, B cells, density of T cell subsets (6-10 nevi per patient x 20-40 patients). Extract DNA from nevus sections and perform high-throughput TCR sequencing to identify clonally expanded TCRs 20 – 40 patients: two samples each (prevaccine and day 22). Determine TCRs of T cells expanded in circulation after vaccination for patients whose nevi have increased T cell infiltrates (and assess if TCRs expanded in nevi are those expanded in circulation by vaccination) in approximately 15 - 20 patients.

Under Major Task 9: CCF&UVA: Document presence, size, and appearance of all nevi. Review images for clinical evidence of nevus regression. Assess changes in nevi over time. Record new nevi and enlargement of nevi, excised nevi will be imaged with high resolution photography. Assess for proportion of patients with new halo nevi, clinical regression/disappearance of nevi by day 85. *Milestone Achieved: impact of CDX-1140 and polyICLC and vaccine on nevi in patients with melanoma. Review and reporting of findings.*

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.

Currently CCF is at three active patients, we plan to continue to accrue at this rate.

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).

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

- *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 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:*

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.

Nothing to report

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

Nothing to report

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.

https://clinicaltrials.gov/ct2/show/NCT04364230?term=mel66&draw=2&rank=1

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

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.)*

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

Name: *Kate Caputo*
Project Role: *Research Coordinators*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*

Contribution to Project: *Kate Caputo has worked on the regulatory items for this project.*

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.

Nothing to report

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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.*

Organization Name: University of Virginia
Location of Organization: (if foreign location list country) Charlottesville, VA
Partner's contribution to the project (identify one or more) Sponsor/ Collaboration (e.g., partner's staff work with project staff on the project)

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.