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TITLE: Improving Mesothelioma Therapy by Boosting Immune Responses to Mutations by Vaccination and by Immunogenic Chemotherapy

PRINCIPAL INVESTIGATOR: Bruce Robinson

CONTRACTING ORGANIZATION: University of Western Australia

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This is the second annual report for award CA190450. The project examines neoantigen immune responses in (a) clinical samples and (b) mouse models. We have recruited 24 of the proposed 30 subjects for this study and continue to perform genomic and immunoproteomic analysis on tumour samples. Neoantigens have been bioinformatically predicted for six cases with immunological screening and analysis commenced for four. Neoantigens can be classified as 'simple' and 'complex' depending upon the origin of the tumour-associated alteration. In mouse studies we have experienced difficulties with analysis of complex neoantigens. In the mouse model only a restricted set of simple neoantigens induce T cell responses. To generate a protective anti-cancer vaccine we found that a combination of endogenously recognised neoantigens and a strong exogenous neoantigen is required. Vaccination with individual components is not protective. Unraveling the mechanisms underlying these differences in vaccination efficiently will be crucial to the development of neoantigen vaccines.

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

Mesothelioma is an aggressive cancer caused by exposure to asbestos. New checkpoint blockade therapies have demonstrated that a person's own immune system can eradicate the tumour. However, this only occurs in the minority of cases. In this project we look at an alternative approach to stimulate the immune system to specifically attack a patient's own tumour by vaccinating against tumour derived mutated new or "neo"-antigens. Our objective is to study neo-antigen immune responses in mesothelioma patients and use mouse model systems to evaluate the most effective neo-antigen vaccination strategy.

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

mesothelioma, neoantigen, chemotherapy, immunotherapy, asbestos, treatment

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 identify candidate mesothelioma neo-antigens and evaluate T cell responses to them

Milestone(s)	*Target Date*	*Completion Date*
Local IRB/IACUC Approval	01-Jul-20	100%
HRPO review and approval of IRB protocols	01-Oct-20	09-Jul-20
Recruit 8 patients	01-Jan-21	100%
Recruit 20 patients	01-Jul-21	60%
WGS, and RNAseq (n=20)	01-Jul-21	60%
Proteomic studies (subset of patients estimate 21)	01-Jul-21	5%
Neo-antigen identification	01-Jul-21	20%

AIM 2 To examine the effect of mesothelioma chemotherapy on neo-antigen specific T cell responses (n=30).

Milestone(s)	*Target Date*	*Completion Date*
Testing of neo-epitopes from pools	01-Jul-23	5%
Immunogenicity of simple or complex neo-epitopes	01-Jul-23	5%
Responses following chemotherapy	01-Jul-23	0%
Does chemotherapy enhance T-cell activation	01-Jul-23	0%
Does chemotherapy alter T-cell frequency	01-Jul-23	0%
Transcriptional profile of T cells	01-Jul-23	0%
Define clonality of epitope specific T cells	01-Jul-23	5%
Confirm TCR antigen specificity	01-Jul-23	0%

AIM 3 Pre-clinical evaluation of neo-antigen vaccination

Aim 3.1: Neoantigen Discovery in mice

Milestone(s)	*Target Date*	*Completion Date*
Local animal ethics approval	01-Oct-20	05-Oct-20
ACURO review and approval	01-Jan-21	22-Oct-20
Complex-epitope mapping AB1 and AE17	01-Jul-22	15% complete
ID natural tumor induced neoantigen response	01-Jul-22	25% complete
ID vaccine induced neoantigen response	01-Jan-23	25% complete

Aim 3.2: Assessing tumor rejection following neo-antigen vaccination

Milestone	*Target Date*	*Completion Date*
Define protective neo-antigens	01-Apr-23	10% complete

Aim 3.3: Assessing tumor rejection following combined neo-antigen vaccination plus chemotherapy or neo-antigen vaccination plus ICPB

Milestone	*Target Date*	*Completion Date*
Define optimal peptide type (simple versus complex versus proteomically defined) and number to synergize with chemotherapy.	01-Jul-23	10% complete

Aim 3.4: Assessing tumor rejection following combined neo-antigen vaccination plus chemotherapy plus ICPB

Milestone	*Target Date*	*Completion Date*
Define optimal peptide type (simple versus complex versus) and number to synergize with ICPB.	01-Jul-23	0% Complete

Task 4:

Dissemination of Results	01-Jul-23	0% Complete

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.

Identify neoantigens in mesothelioma patients

To date, we have collected clinical samples and performed whole genome sequencing on tumour from 24 people with mesothelioma. RNA sequencing on matched samples was successful for 21 of these cases. Bioinformatic analysis of sequencing results is ongoing, results from 16 samples show that there are approximately 35 high confidence protein altering DNA mutations per sample (Figure 1). Bioinformatic prediction of candidate neoantigens has been performed for six cases; and peptides purchased and tested in four. Figure 2 shows the results for one of the mesothelioma patients. This patient was diagnosed with epithelioid mesothelioma when he was 59 years old. Nine months after diagnosis he received five cycles of combination platinum and pemetrexed chemotherapy which resulted in tumour shrinkage as seen radiologically and as a reduction in blood mesothelin levels. Whole genome sequencing of the tumour revealed there were 27 simple mutations and two complex structural mutations predicted to result in an altered protein product. Using bioinformatic prediction pipelines, developed in house, candidate neoantigens were predicted. We then performed functional screens of 82 synthetic peptides corresponding to the top candidate neoantigens from 23 mutational events. We have identified two neoantigens that are robustly recognised by T cells present in the blood prior to chemotherapy of this patient. We are currently examining post treatment blood from this patient and are continuing our studies to identify neoantigens from the remaining patients.

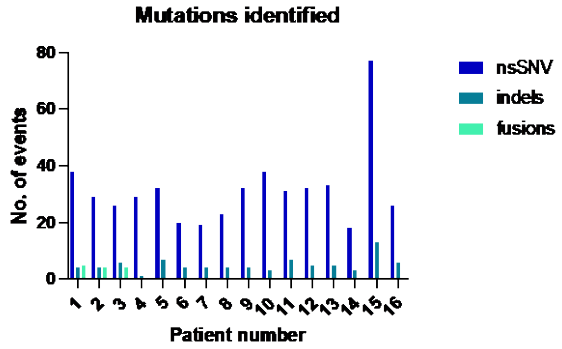

Figure 1 (Left): The number of protein altering mutations identified in 16 mesothelioma patients.
nsSNV – nonsynonymous single nucleotide mutation
indel – insertion/deletion mutation
fusion - NOTE: fusions have only been identified to date in 4 cases. Analysis is ongoing.

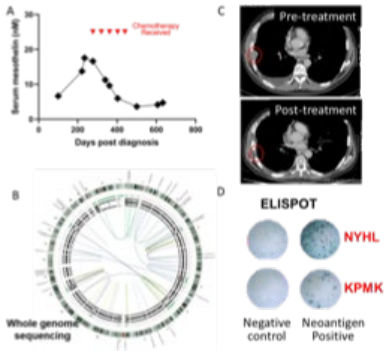

Figure 2 (Left): Results from one patient. (A) Serum mesothelin levels over time with the dates of treatment indicated. (B) Chest CT scans pretreatment (top) and after treatment (bottom) showing a reduction in tumour mass highlighted by red circle. (C) Representation of mutations in the tumour. The outer circle indicates chromosome location. Simple mutations are annotated and structural variations displayed by connecting lines within the circle. (D) Indicative wells from ELISPOT assay, each blue spot indicates a cell producing interferon-gamma in response to stimulation with either an irrelevant peptide (negative control; left column) or the two identified neoantigens (right column).

Define clonality of epitope specific T cells. We have stared our T cell receptor (TCR) diversity study. Shown in Figure 3 is the TCR usage of neoantigen positive T cells in the pleural effusion of a patient with mesothelioma. Tetramer: peptide, CFDPPLTRM (Gene KAT6A), restriction element, HLA-C*04:01. Shown are the common TCR beta chain sequence (red boxes) found as the dominant clone in the PD-1^{low} T cells (left) and as a subdominant clone in PD-1^{mid} (right) T cell population. Interestingly the PD1^{mid} T cells had a considerably more diverse TCR usage than PD1^{low} T cells. PD1^{mid} are stem like T cells, which we have demonstrated links to prolong patient survival (manuscript in preparation) in mesothelioma. PD1^{low} T cells may represent a recently recruited population of T cells.

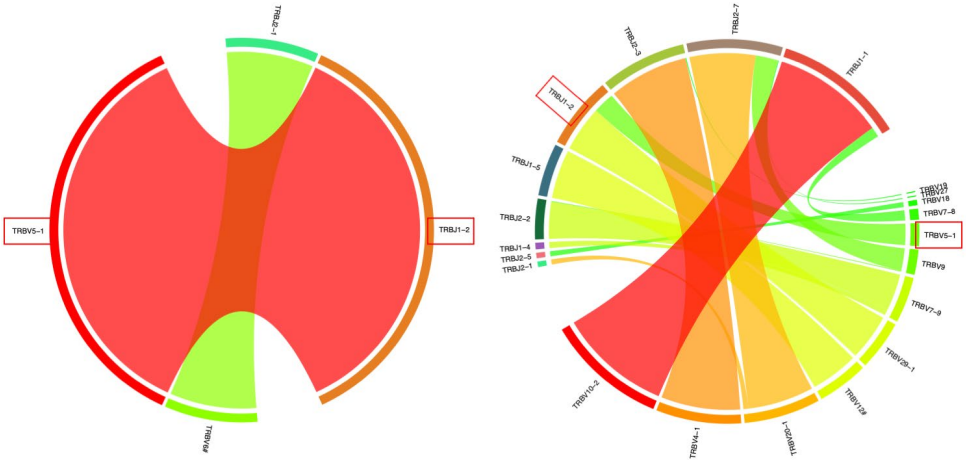

Figure 3, left. Circos plot of TCR usage directed toward the neoantigen CFDPPLTRM. Common TCR sequence CASSPPDHNYGYTF was found in 82% (left) and 4.6% (right) of T cell clones from PD1^{low} and PD1^{mid} T cell populations respectively (red boxes).

Pre-clinical evaluation of neo-antigen vaccination

Neoantigen discovery in mice

We have optimized our methods for screening of immunological responses to predicted neoantigens. Using this new methodology, we see significantly higher responses to known neoantigens than with our previous method (Figure 4). Using this novel approach, we have screened over 280 synthetic peptides corresponding to predicted neoantigens encoded by simple DNA mutations, interestingly however, only an additional 2 neoantigens been *robustly* demonstrated in mice. Similar studies are now underway in the AE17 model, albeit with less neo-antigen candidates (AE17 is a low mutation burden tumor). Thus far our data indicate (for simple SNV) mutations that spontaneous T cell responses can be reliably detected to four SNV neoantigens, of 218 genes tested (1.8%). This number is consistent with the literature and suggests that T cells are only capable of responding to a restrict set of neoantigens (this finding is presently being prepared as a manuscript for submission) and that this may be due to a hole in the T cell repertoire or due to issues with antigen processing and presentation.

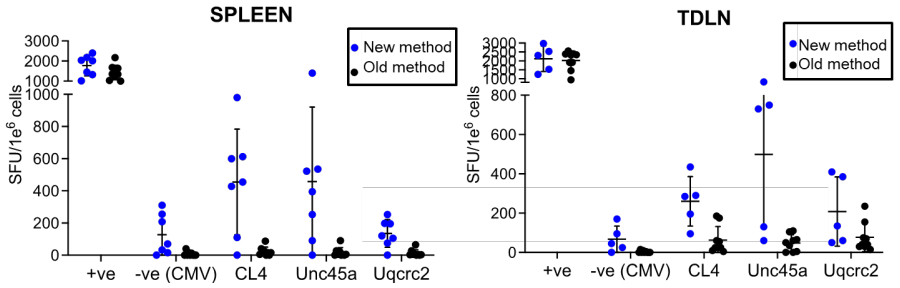

Figure 4: T cell responses to synthetic peptides encoding neoantigens and controls. Interferon-gamma responses presented as spot forming units (SFU) per million cells from (left) spleen and (right) tumor draining lymph node (TDLN) cells using the newly developed assay system relative to the old system. +ve control - mouse T-activator CD3/CD28 beads; -ve control – irrelevant peptide from cytomegalovirus; CL4 – class I epitope from the influenza haemagglutin gene; Unc45a neoantigen described in Creaney et al 2015 (PMID: 26140232); Uqcrc2 neoantigen described in Ma et al 2019 (PMID: 32002299)

Identifying vaccine induced neo-antigens. In parallel experiments we have increased the number of SNV mutations tested in the AB1-HA model to determine if poor T cell responses is due to a hole in the T cell repertoire, similar to that shown previously approx. 50% of SNV derived neo-antigens are capable of inducing T cell responses post vaccination. This also is consistent with the literature and indicates that low responses to neoantigens is NOT due to a hole in the T cell repertoire.

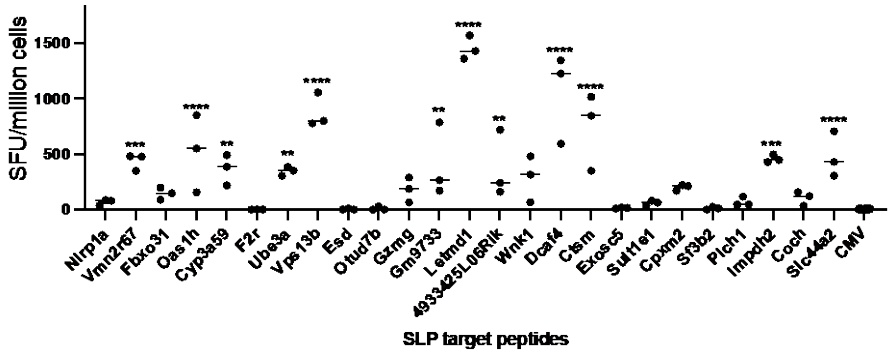

Figure 5. Vaccine induced immunogenicity testing. BALB/c mice were immunized with synthetic long peptides (SLP, mutated gene names are given) corresponding to SNV mutations in 25 different genes. T cell responses were detected to 12/25 (48%) of potential neoantigens. Data are compared to control peptide (CMV) via one way ANOVA with post hoc Dunnett's multiple comparison test.

We are experiencing difficulties with the grant-based focus of the work of identifying complex neoantigens in the mouse models from the mouse whole genome sequencing data. Our team in Queensland had previously generated extensive annotations and a database that facilitates the identification of structural variants from whole genome sequence in humans, however there has been unexpected difficulties in replicating such a database for the mouse genome. Whilst we continue to investigate solutions for this issue, we have been begun screening mice for immunological responses to neoantigen peptides identified from proteomic studies. In preliminary studies however, no immunological responses have been seen to 66 candidate proteomically identified neoantigens.

We will continue to work to determine whether we can improve our identification of complex neoantigens in the mouse. To this end we are investigating the use of long read RNA which we anticipate will facilitate these studies

Assessing tumor rejection following neo-antigen vaccination +/- immune checkpoint blockade therapy (ICPB). Thus far for SNV we have shown that, at least for anti-CTLA4, that CPB does not improve vaccine efficacy (data shown in previous reports). Full studies await elucidation of complex neoantigens.

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

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 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:*

The COVID19 pandemic has significantly inhibited progress on this project, which has been exacerbated by the collaborative nature of the study. The study, whilst coordinated in Perth, Western Australia, involves research groups in the states of Queensland and Victoria. The federal nature of Australian government has meant that each state, WA, Qld and Vic, have all had different stages and degrees of state government-imposed lockdowns. This has meant that even when research could progress in one State, it was delayed by halts imposed by State governments at another site. We anticipate that COVID-19 imposed delays are now behind us but it is highly likely that we will need to request an extension to this study.

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.

We have had technical problems that have been compounded by the COVID-19 induced delays discussed above. Specifically, the calling of structural variants (complex neoantigens) has been more technically demanding than anticipated. This has now been resolved for the human component of the work but remains a problem for the mouse model studies. We since changed protocol to allow long read RNA sequencing that we anticipate will resolve this issue.

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.

COVID-19 has significantly delayed spending, we anticipate this will now increase in line with the lifting of COVID-19 restrictions across all states in Australia

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

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."*

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.*

Local Presentations

Jessica BOULTER "Understanding the role of neoantigens in effective immunogenic therapy" 28-29 October, 2021, Perth Immunology Group Meeting, Rottnest Island, Western Australia

Alec REDWOOD "Towards personalised cancer vaccines" 16-17 September, 2021, NCARD Annual Scientific Meeting, Perth, Western Australia

Jessica BOULTER "Vaccines that flag your Nags" 16-17 September, 2021, NCARD Annual Scientific Meeting, Perth, Western Australia

Linda YE "Getting personal with cancer" 16-17 September, 2021, NCARD Annual Scientific Meeting, Perth, Western Australia

- **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. 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.

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;*
- *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.)*

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.

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:
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: *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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) 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.*