AWARD NUMBER: **W81XWH-16-1-0530**

TITLE: **Tissue-Engineered Cancer Metastasis to Improve the Abscopal Effect and Cancer Immunotherapy in Melanoma**

PRINCIPAL INVESTIGATOR: **Zhuang A. Wang, MD, Associate Professor**

CONTRACTING ORGANIZATION: **University of North Carolina at Chapel Hill**
Chapel Hill, NC 27599

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Zhuang A. Wang

E-Mail:

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University of North Carolina at Chapel Hill
101 manning drive
Chapel Hill, NC 27599

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Background: Our application aims to address the PRCRP topic area “melanoma and other skin cancers” and the Military Relevance Focus Area of “Gaps in cancer treatment that may affect the general population but have a particularly profound impact on the health and wellbeing of military members, Veterans, and their beneficiaries”. Cancer immunotherapy has been shown to be an exciting new treatment for melanoma. There has been high interest in the development of approaches that can further improve cancer immunotherapy. Radiotherapy (radiosurgery) has shown to be synergistic with immunotherapy and can lead to abscopal effect. However, not every patient is eligible for radiosurgery and the treatment can lead to significant side effects.
Objectives: The key objective of this application is to improve cancer immunotherapy for melanoma using novel tissue engineered cancer metastasis models and the abscopal effect. We hypothesize that we can induce a robust abscopal effect by engineering cancer metastasis ex vivo and administering lethal radiotherapy prior to in vivo administration.

Specific Aims: Our proposal has 3 specific aims.

Aim 1: To engineer 3D melanoma lung metastases using decellularized lung matrix and evaluate the engineered metastases in cancer immunotherapy

Aim 2: To engineer and evaluate 3D melanoma lung metastasis models with pro-inflammatory agents such as CpG, IL-12 and GM-CSF embedded in the matrix

Aim 3: To engineer 3D lung metastasis models using melanoma cells from patient surgical specimens or circulating tumor cells from blood samples

Progress: Our work mainly focused on B16F10 mouse model of melanoma and confirmed the data using TyrRAS Ink4a/Arf KO (TRIA) model. First, we were able to establish engineered lung metastasis using B16F10 cells. Using decellularized lung tissue, we identified conditions that enable 3D growth of melanoma cells. We also examined the use of a bioreactor model and showed melanoma cells can also be cultured in 3D using decellularized lung in bioreactor. Using these models, we studied whether these ex vivo cultured metastasis can be utilized to improve cancer immunotherapy. Using mouse model of B16F10 melanoma, we demonstrated that irradiated engineered metastasis can function as a cancer vaccine and reduce tumor growth as well as prevent lung metastasis. The immunotherapy effects were improved with the addition of STING agonists. Lastly, we studied whether we can culture patient circulating tumor cells using our metastasis models. This work is ongoing as circulating tumor cells are uncommon and the harvest of these cells remain challenging.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF: **Reports with the UNLIMITED distribution designation which will become accessible to the general public through the Defense Technical Information Center (DTIC) data repository**			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
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Unclassified	Unclassified	Unclassified			

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

The key objective of this application is to improve cancer immunotherapy for melanoma using novel tissue engineered cancer metastasis models and the abscopal effect. We hypothesize that we can induce a robust abscopal effect by engineering cancer metastasis ex vivo and administering lethal radiotherapy prior to in vivo administration.

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

Cancer immunotherapy, abscopal effect, ex vivo metastasis, engineered metastasis, cancer vaccine

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.

Major Task 1: Optimize 3D engineered metastases for vaccine administration

Subtask 1: IACUC and ACURO approval
Subtask 2: Develop in vitro 3D colonies of B16F10 and TRIA using lung biomatrix. 30 Rats will be sacrificed and their lungs will be harvested. Tumor cells will be plated on dishes coated with lung biomatrix (varying level) and we will identify the proper condition to generate 3D colonies. These colonies will be harvested in further immunotherapy experiments.
Subtask 3: Develop bioreactor 3D colonies of B16F10 and TRIA using lung biomatrix. 30 Rats will be sacrificed and their lungs will be harvested (new set of rats than previous task). The lungs will be incorporated into our established bioreactor and tumor cells will be introduced into the reactor
Subtask 3: Optimal timing for metastasis vaccine administration The metastases from the above experiments will be lethally irradiated with 100 Gy of radiotherapy. The optimal time to utilize the irradiated metastasis is when most of the tumor cells are undergoing radiation induced mitotic death and release their tumor antigens. To identify the optimal time, 3D metastases will be irradiated and examined at 12 hr, 24 hr, 36 hr, 48hr, 60h and 72h after irradiation. Mitotic death will be characterized using Tunnel assay and Annexin V staining. The time point when most of the tumors are undergoing mitotic death will be used in subsequent experiments.
Subtask 4: In vivo evaluation of metastasis vaccine with checkpoint inhibitors with B16F10 and TRIA model (10 mice per group) 1.Control: mice with tumors and no treatment
Subtask 5: Re-challenge long-term complete responding mice and correlative science experiments

Major Task 2: Evaluate mechanism of engineered metastases in immunotherapy

Subtask 1: Analysis of T-cell populations

Correlative science: if we are able to achieve a therapeutic signal with engineered metastasis, we will repeat the above in vivo experiments with the experimental arms and 2 of the control arms. We will characterize the CD4+ and CD8+ T cells as well as the T reg cells in the tumors as well as spleen. This will be done using

Specific Aim 2: To engineer and evaluate 3D melanoma lung metastasis models with pro-inflammatory agents

Major Task 1: Optimize pro-inflammatory conditions for vaccine administration

To engineered 3D *ex vivo* metastases with proinflammatory microenvironment using the bioreactor model, we will take two different approaches. In one method, the entire bioreactor will be given 100 Gy of irradiation. The proinflammatory molecules will then be injected into the bioreactor and allowed to be adsorbed by the biomatrix (250 µg/ml CpG, 10 ng/ml IL-12, 5 µg/ml GM-CSF, based on published reports). We will assess the level of these molecules in media to determine the level of adsorption. After 12 hours of incubation, we will harvest the tumor metastases for use in further studies. Since the bioreactor model allows the culturing and settlement of antigen-presenting cells (dendritic cells), we will also engineer metastases with dendritic cells. Dendritic cell culture will be generated using an established technique. Similar to the above experiment, we will first develop bioreactors that have been incubated with proinflammatory molecules. Following 12 hours of incubation, we will then inject dendritic cell cultures into the bioreactor and allow the culture to settle for 12 hours. We theorize that the dendritic cells can establish themselves in the biomatrix and become activated antigen presenting cells. The metastases will then be harvested and used for further experimentation.

Will need 30 rats for the lung bioreactors

Major Task 2: Determine effect of pro-inflammatory tumor microenviroment on immune activation

First, we plan to determine the optimal combination of proinflammatory molecules for cancer immunotherapy using the spontaneous 3D model. Similar to Aim 1, mice (groups of 10) bearing
0.5 cm flank tumors will be given checkpoint blockade agents (regimen determined from Aim 1). Metastases containing a single proinflammatory molecule (CpG, IL-12, GM-CSF) will be compared to each other, as well as combinations (two of CpG, IL-12, GM-CSF; and all three molecules). 3D models without proinflammatory molecules will be used as control.

Subtask 2: **Correlative science studies**

Analysis of T-cell populations using pro-inflammatory engineered metastasis

Specific Aim 3: Engineer 3D lung metastasis models using melanoma cells from patient surgical specimens or circulating tumor cells from blood samples

Major Task 1: Culture tumor cells from biopsies or CTCs on biomatrices

Subtask 1: Accrual of patients for CTC isolation Targeted Accrual: 10 patients

Subtask 2: Accrual of patients for surgical biopsy Targeted Accrual: 10 patients

Subtask 4: Culture of CTCs and biopsy specimens on matrices and bioreactor

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.

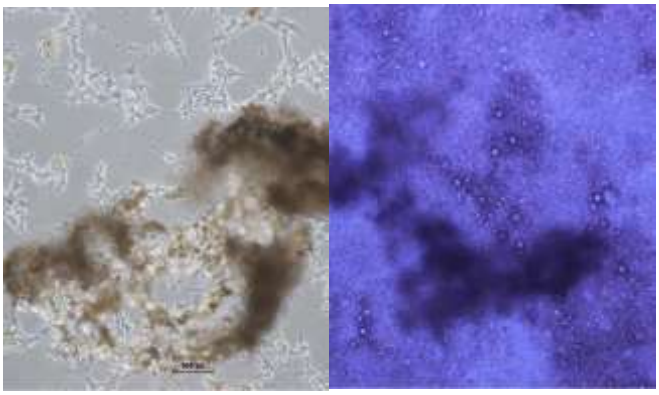
Major Task 1: Optimize 3D engineered metastases for vaccine administration

Subtask 1: IACUC and ACURO approval

Progress: we obtained IACUC and ACURO approval in 08/2016.

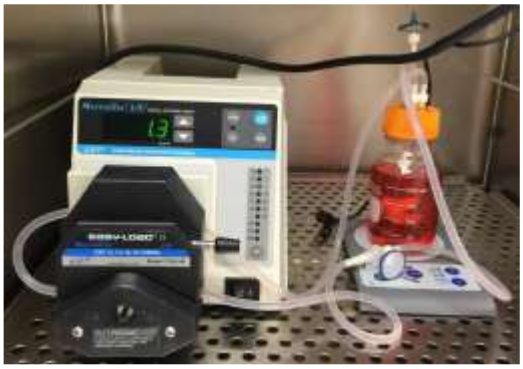
Subtask 2: Develop in vitro 3D colonies of B16F10 and TRIA using lung biomatrix.

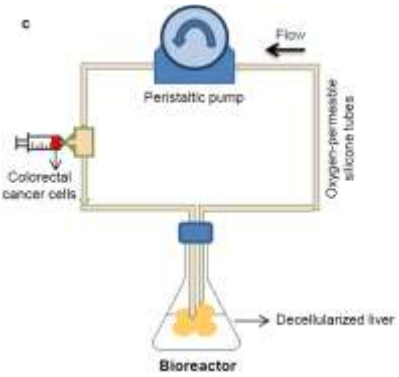
Progress: Using decellularized lung tissue from rats, we engineered 3D in vitro/ex vivo tumor colonies using B16F10 and TRIA cells. In this experiment, lung matrix is grounded using a freezer mill and plated onto plates. For each type of melanoma cells, we examined the different level of lung matrix that is required for 3D growth. We then identified the proper seeding density for 3D colonies. B16F10 cells are highly aggressive and required a lower cell seeding density. Representative images of the 3D colonies are shown below. First is B16F10 and second is TRIA with methylene blue staining.

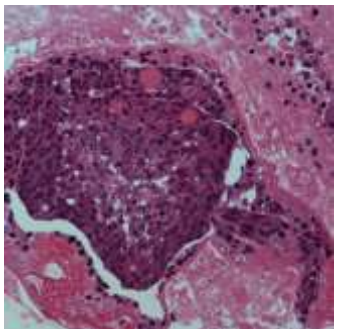


Subtask 3: Develop bioreactor 3D colonies of B16F10 and TRIA using lung biomatrix. 30 Rats will be sacrificed and their lungs will be harvested (new set of rats than previous task).

Progress: in this experiment, we kept the decellularized lung intact and perfused the organ in a bioreactor (see below figure). We then introduced tumor cells into the "circulation" showed that the cancer cells can also form 3D colonies in the decellularized organ (figure below).





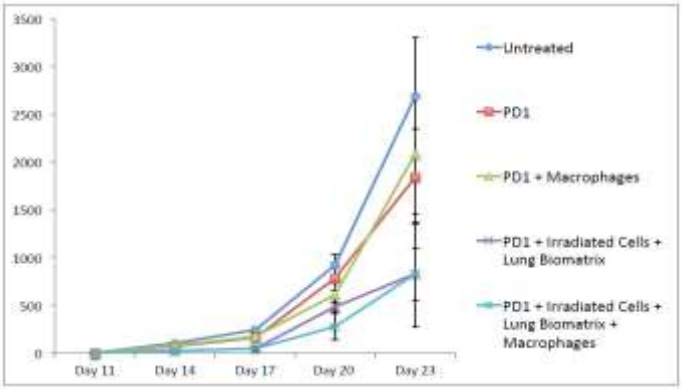


Subtask 3: Optimal timing for metastasis vaccine administration

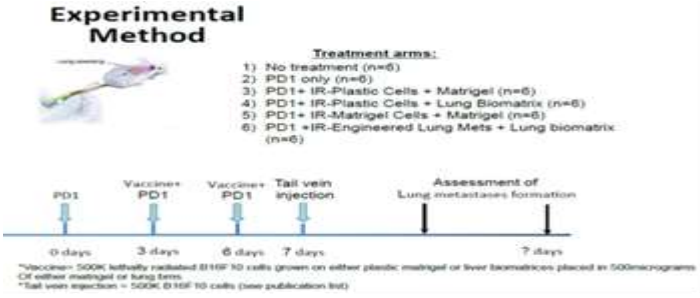
Progress: We examined different time points (post radiation) for irradiated 3D tumors (metastasis vaccine) administration, including 3 hours, 6 hours, 12 hours, 24 hours and 48 hours. We did not observe any difference in efficacy. (data is not shown because of lack of difference between the arms).

Subtask 4: In vivo evaluation of metastasis vaccine with checkpoint inhibitors with B16F10 and TRIA model (10 mice per group)

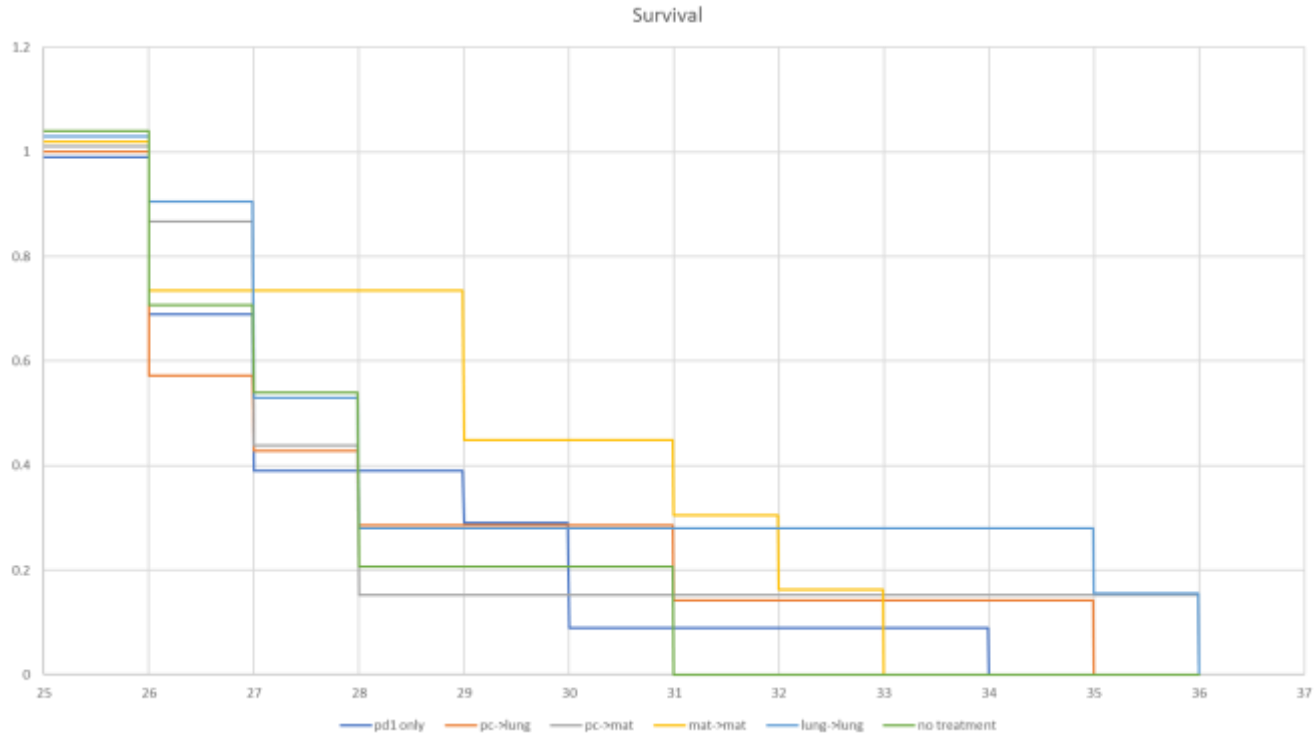
Progress: To examine the efficacy of engineered metastasis as vaccines, we first studied it using B16F10 model. In this experiment, we examined the vaccine as well as IL-2 expressing macrophages as an adjuvant. We demonstrated that the irradiated metastasis is effective and prolongs survival. Adjuvant did not improve efficacy. Figure below



We also studied whether the vaccine can prevent the development of lung metastases. Experimental protocol is shown below



We showed that irradiated lung metastasis vaccine can indeed prevent lung metastasis development and improve survival. (survival curves shown below)



Subtask 5: Re-challenge long-term complete responding mice and correlative science experiments
Progress: We were unable to achieve full cures and thus, we did not conduct re-challenge experiments.

Major Task 2: Evaluate mechanism of engineered metastases in immunotherapy

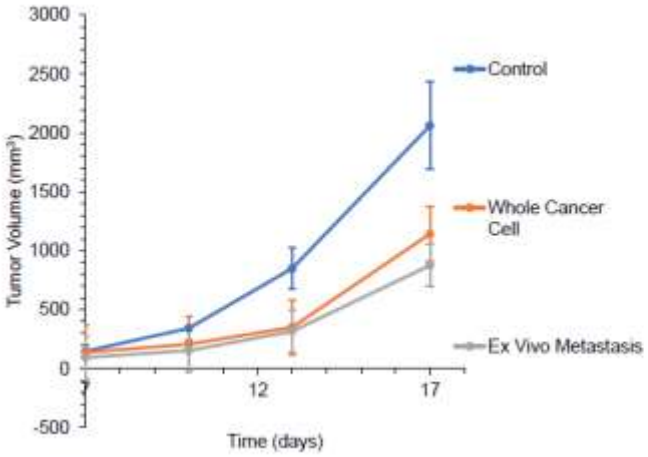
Subtask 1: Analysis of T-cell populations

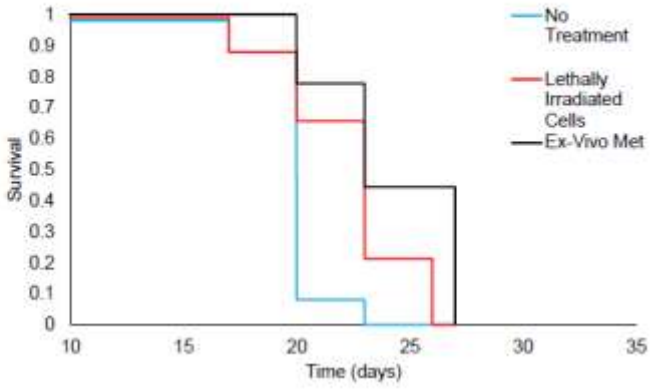
Progress: We examined the T cell population changes post metastasis vaccine administration. We found that CD8+ T cells increased while Treg population decreased, consistent with vaccination effect. No significant difference in CD4+ T cell population.

Specific Aim 2: To engineer and evaluate 3D melanoma lung metastasis models with pro-inflammatory agents

Major Task 1: Optimize pro-inflammatory conditions for vaccine administration

Progress:
We engineered ex vivo metastasis models using the bioreactors and integrated CpG into the matrix. We were unable to integrate IL-12 effectively, thus we resorted to macrophages as in Aim 1 (see data above). We were unable to integrate GM-CSF effectively into the tumor microenvironment. On the other hand, we were able to utilize these adjuvants to activate dendritic cells.
These bioreactor metastases were irradiated and examined in vivo. We should they can also improve immunotherapy (PD-1) efficacy and the adjuvants can improve efficacy even further. However, they did not further improve upon in vitro metastases vaccine from Aim 1. Bioreactor metastasis is more effective than irradiated cells is shown below





Major Task 2: Determine effect of pro-inflammatory tumor microenviroment on immune activation

Progress: Given the difficulty in integrating some of the immune adjuvants, we did not examine the different combinations. Our ongoing work is studying other types of adjuvants, including STING agonists, TLR agonists in conjunction with biomatrix.

Subtask 2: **Correlative science studies**
Progress: Similar to Aim 1, we showed that CD8+ T cells increased while Treg population decreased, consistent with vaccination effect.

Specific Aim 3: Engineer 3D lung metastasis models using melanoma cells from patient surgical specimens or circulating tumor cells from blood samples

Major Task 1: Culture tumor cells from biopsies or CTCs on biomatrices

Subtask 1: Accrual of patients for CTC isolation Targeted Accrual: 10 patients

Progress: we have accrued 6 patients on our melanoma CTC protocol thus far. We could have accrued more than 10 but due to the technical difficulties of culturing these cells, we decided to suspend accrual and identify optimal conditions for harvesting and culturing. One major difficulty in harvesting these cells is our device relies on EpCAM capture and it appears that many melanoma cells do not express the biomarker. Another difficulty is detachment of the cells from device once they are captured. Our current work involves modifying our device to include multiple biomarkers to enable better capture and adding chemistry to the bottom of capturing antibodies to allow easy detachment.

Subtask 2: Accrual of patients for surgical biopsy Targeted Accrual: 10 patients

Progress: we have accrued 10 patients on our melanoma surgical tissue protocol. We have shown that cells from surgical tissue can be successfully cultured for a period of 3 weeks on the lung biomatrix. We are studying conditions that will allow longer cultures.

Subtask 3: Culture of CTCs and biopsy specimens on matrices and bioreactor

Progress: Please see above for our culturing progress.

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.

This project provided training opportunities for 4 postdoctoral fellows (Drs. Roche, Tian, Sun and Mi) and one MD/PhD student (Hagan). Dr. Roche was involved in the research from 2016-2017. The translational nature of this work sparked his interest in medicine and he successful matriculated at Georgetown Medical School following his postdoctoral training. He is pursuing a career as a physician scientist. Dr. Tian was involved from 2016-2018 and she is working in industry. Drs. Sun and Mi are still conducting research related to this work. Charles Tilden Hagan is a MD/PhD student and he has been working on this project in 2018. This research has enriched his experience in cancer immunotherapy.

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.

Although we have not published any original research publication based on this work yet, we have published a review in Advanced Science discussing materials approaches to cancer immunotherapy.

Emerging nano/micro approaches for cancer immunotherapy
Journal: Advanced Science, in press

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

We plan to continue this line of investigation and identify optimal adjuvants for engineered metastasis as cancer vaccines

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).

When we finish our investigation and publish our work, we will provide a new strategy to improve cancer immunotherapy

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:*

Nothing to report

ber

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

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).*

Emerging nano/micro approaches for cancer immunotherapy Journal: Advanced Science, in press Original research publications under preparation

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. 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.)*

Kyle Roche

Project Role: *Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *6*

Contribution to Project: *Kyle conducted the in vivo experiments involving cancer immunotherapy.*
Funding Support: *DOD and NIH*

Xi Tian

Project Role: *Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *9*

Contribution to Project: *Xi generated ex vivo metastases and harvested lungs.*
Funding Support: *DOD and NIH*

Yu Mi

Project Role: *Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *3*

Contribution to Project: *Yu conducted the in vivo experiments involving cancer immunotherapy..*
Funding Support: *DOD and NIH*

Bo Sun

Project Role: *Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *4*

Contribution to Project: *Bo conducted the in vivo experiments involving cancer immunotherapy..*
Funding Support: *DOD and NIH*

Charles Tilden Hagan

Project Role: *graduate student*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *6*

Contribution to Project: *Tilden generated ex vivo metastases and harvested lungs.*
Funding Support: *DOD and NIH*

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*

No other institutions

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.*

Not applicable

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.*

none