

AWARD NUMBER: W81XWH-20-1-0525

TITLE: CD8 T-cell Infiltration as a Predictor of Renal Cancer Progression After Surgery

PRINCIPAL INVESTIGATOR: Haydn Kissick

CONTRACTING ORGANIZATION: Emory University, Atlanta, GA

REPORT DATE: October 2022

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGE

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14. ABSTRACT Kidney cancer patients with AJCC/TNM Stage III disease represent a significant challenge in selecting the care that should be received after surgery. According to 2019 NCCN guidelines, the decision is whether these patients should either receive sunitinib, enter a clinical trial, or be placed on surveillance. Potentially, this may represent overtreatment or oversurveillance for some patients who are cured, and undertreatment for others. Currently, there are not good indicators to guide which path patients should be follow, thus, appropriate biomarkers are needed to improve prognostication for patients with this stage of disease.						
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The work in this proposal aims to determine how the level of CD8 T-cell infiltration into kidney tumors can be used to predict which patients will survive after surgery to remove their tumors. In addition, this project aims to study the basic mechanisms in kidney tumors that regulate the level of these important cells.

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

Kidney Cancer, T cells

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.

Below are the major tasks proposed for this grant, and the subtasks assigned to **year 1** and **year 2**:

Major Task 1 Validate Immunofluorescence quantification of CD8 infiltration as a biomarker in RCC patients with T3/M0/N0 disease

Subtask 1: Regulatory review of human subjects research by HRPO - **Complete**

Subtask 2: Collection of FFPE slides of 150 of T3N0M0 patients Emory University Hospital n=120, and the Atlanta VA hospital=30. **Complete (Awaiting follow up time for some patients analysis)**

Subtask 3: Immunofluorescence staining and image processing of 150 cases - **Complete**

Subtask 4: Obtain FFPE slides from Univ Wisconsin collaborator (Dr. Abel) - **Complete**

Subtask 5: Immunofluorescence staining of 100 U Wisc - **ongoing, 25% complete**

Major Task 2 Investigate the predictive power of the CD8 infiltration in response to checkpoint therapies:

Subtask 1: Analysis of FFPE slides of 125 patients who have progressed from initial surgery in Aim 1a

Major Task 3: Determine the role of de-novo generated fibroblastic reticular cells (FRCs) in the maintenance of a supportive antigen-presenting niche:

Subtask 1: Functional capacity of FRCs to recruit dendritic cells and stem-like T-cells using in vitro assays. Fibroblasts and immune cells are sorted from banked human tumor samples and incubated in transwell plates. Ability of cells to migrate is assessed after either 4 or 24 hours. - **ongoing**

Subtask 2: Single cell RNAseq to define FRC lineage and features of cancer cells from hot and cold tumors (30 patients). Cells are sorted from previously banked human tumor samples and processed by the Emory Genomics core - **Complete - analysis ongoing**

Major Task 4: Determine the role and development pathways of FRCs using mouse models

Subtask 1: Regulatory review and approval of animal studies. - **complete**

Subtask 2: Do additional FRCs in the tumor help establish the antigen presenting niche. - **ongoing**

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.

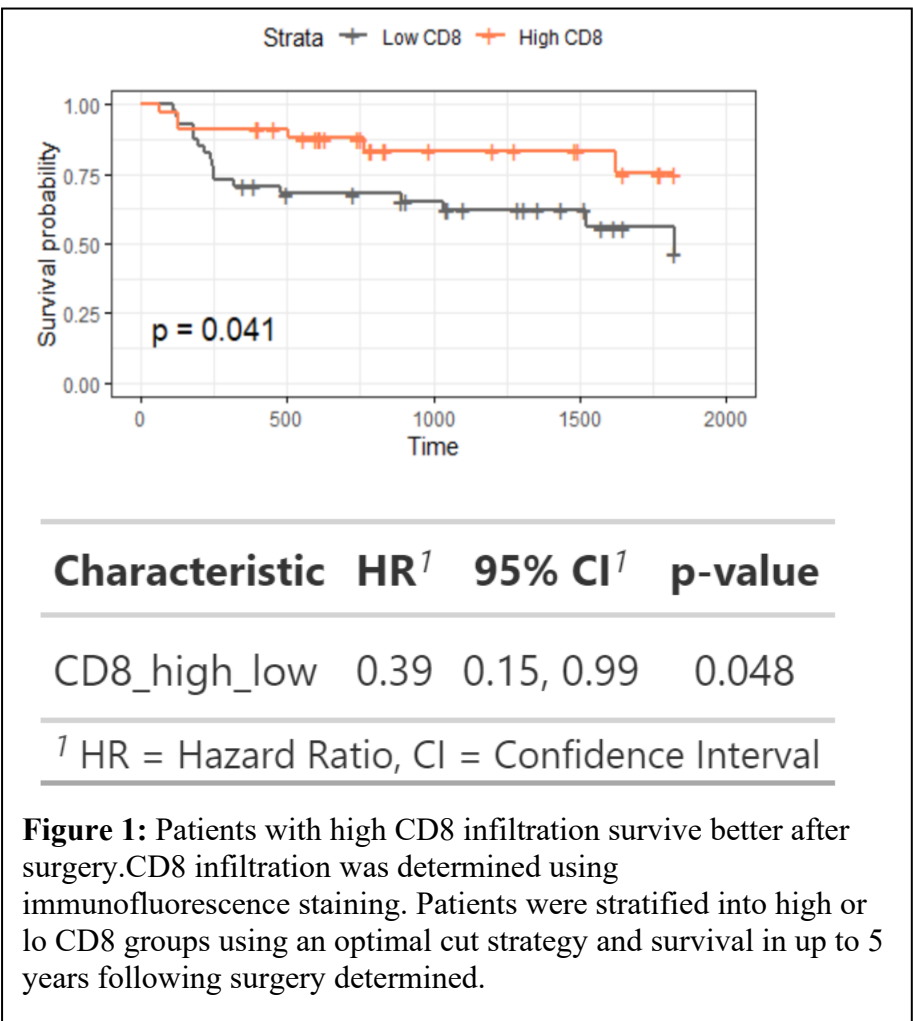
1. Major Activities

Major activities of this period were staining and analysis of all slides from the Emory RCC cohort, analysis of human fibroblast involvement in immune niche formation, and

2. Specific Objectives

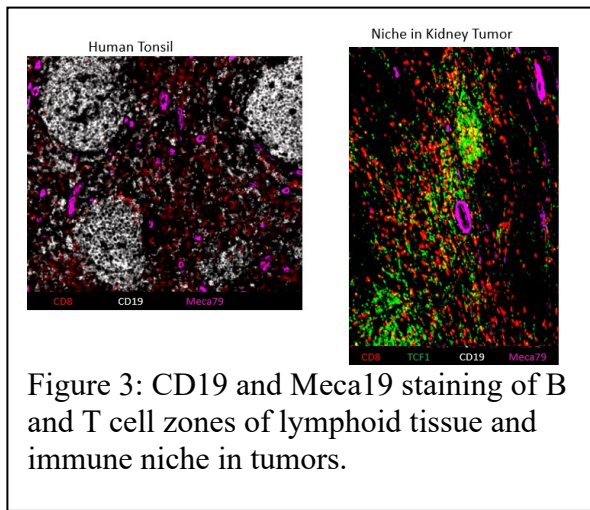
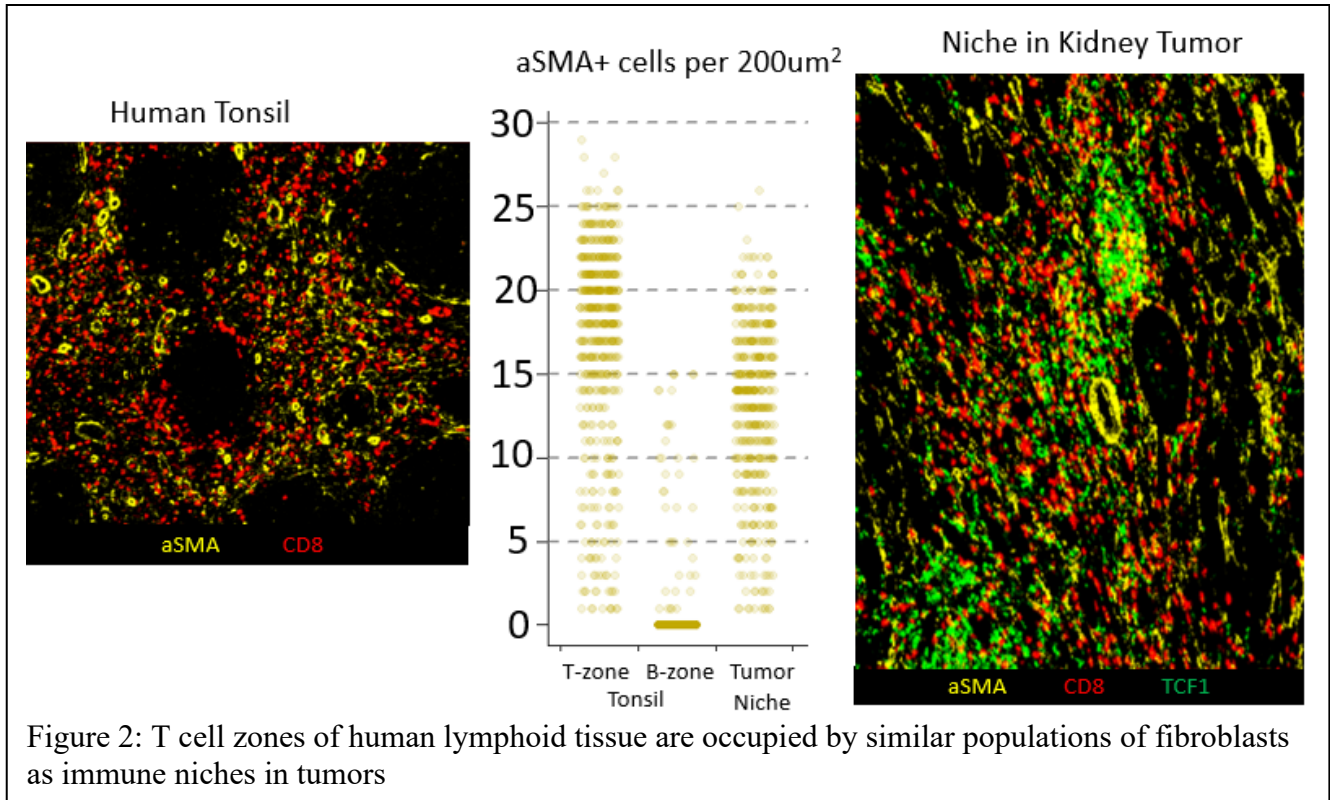
See above

3. Significant Results



i) Survival analysis of patients based on CD8 infiltration: Slides from patients with T3N0M0 disease were stained for 7 markers (CD8, CD4, TCF1, aSMA, Foxp3 and DAPI). Around 100 slides passed quality controls and 88 had at least 1 year follow up time from surgery. Patients were stratified into either CD8 high or CD8 low. This cutoff was 1.5% of total cells being CD8+, in close agreement with follow cytometry data on the same patients. This data is the first to confirm that CD8 infiltration into RCC patient tumors is a significant predictor of survival following surgery, and completion of a major goal of this project. Ongoing work is validating this in the Wisconsin validation cohort.

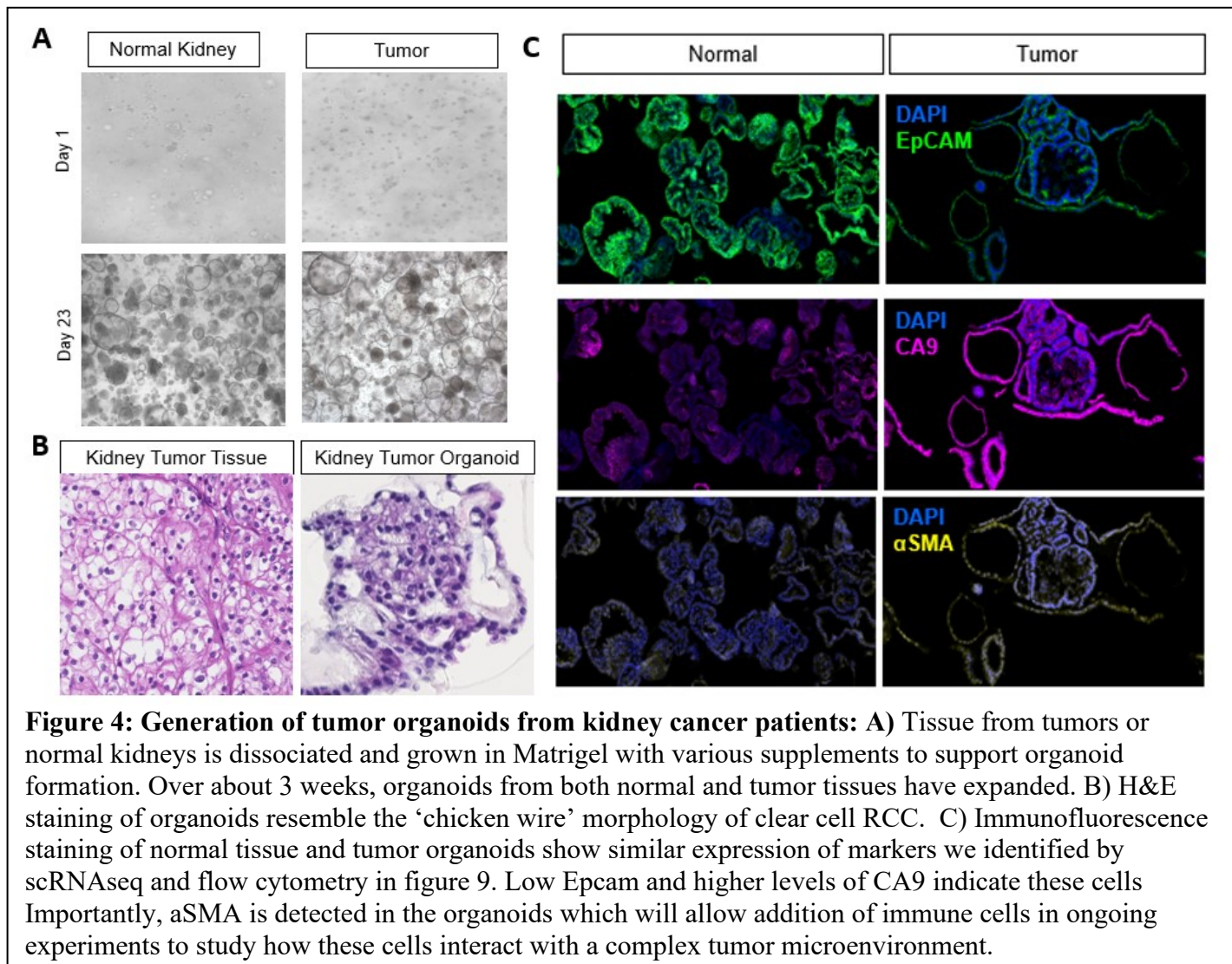
ii) Characterization of human fibroblasts in human tumors



A major task of this proposal is to characterize fibroblasts in the tumors of hot and cold tumors, and their association with immune niches. We have performed RNAseq of these cells in previous years and found they express several markers related to lymphoid tissue development. This year we performed immunofluorescence to better understand how fibroblasts and other cells associated with immune niches in tumors. We used human tonsil to compare the T cell zones of lymphoid tissue to immune niches in the tumor. We found similar levels of fibroblasts in these areas (Fig 2).

In another stain we found high endothelial venules associated with immune niches (Meca79+), also similar to T cell zones of lymphoid tissues (Fig 3). Several other markers related to the lymphoid structures have been identified, including high-endothelial venules, low numbers of B cells, presence of dendritic cells. These data are being used to complement RNA seq data of fibroblasts from immunologically hot and cold tumors.

iii) In vitro analysis of fibroblast interactions with immune cells



In prior experiments we isolated fibroblasts from tumors to perform functional experiments. We found these cells die very quickly in vitro and we weren't confident with the experimental setups. We have now developed an organoid culture system that reproduces the basic morphology of renal cell carcinoma (Fig4A &B). These organoids include cancer cells (CA9) and fibroblasts (aSMA). We have generated these organoids from patients with immune rich and poor tumors, and in the coming year we are studying how these different organoids recruit and activate human dendritic cells.

4. Other Achievements

No major deviations from the expected plan. We have had some issues constructing in vivo models of fibroblasts + Immune cells, but as shown above we have developed

organoid technologies that are allowing more complex analysis of cellular interactions in the tumor microenvironment which we anticipate using for the final year of this award.

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

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

Plans will continue as outlined in the statement of work

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

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

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

Data generated above has produced 2 key findings:

- i) CD8 infiltration in kidney cancer is a useful predictor of survival after surgery. This will be validated in the 2nd cohort of patients currently being analyzed.
- ii) Fibroblasts are specifically a part of immune niches in tumors and our ongoing research using organoid models will determine how these cells help organize immune cells in tumors.

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

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).*

Prokhnevskaya et al., CD8+ T cell activation in cancer requires initial activation in the lymph node and additional costimulation in the tumor, *Immunity*, 2022 – In Press.
This DOD award and other NIH grants were acknowledged in this publication

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.*

Cancer Research Institute, September, New York – Dr. Kissick presented work related to understanding the mechanisms that control immune niche formation

Society for the Immunotherapy of Cancer (SITC), November, Boston– Dr. Kissick was a session chair discussing tertiary lymphoid structures in cancer, and presented the work directly

- **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.)

Name: Haydn Kissick
Project Role: PI
Nearest person month worked: 2 months
Contribution to Project: Oversight of project
Funding support:

Name: Viraj Master
Project Role: PI
Nearest person month worked: 1
Contribution to Project: Oversight of project
Funding support:

Name: Baohan Vo
Project Role: Scientist
Nearest person month worked: 9
Contribution to Project: Optimized and performed all staining of slides in Aim 1.
Funding support: Additional funding support is provided by departmental funds.

Name: Yuan Liu
Project Role: Biostatistician
Nearest person month worked: 1
Contribution to Project: Experimental design
Funding support: NA

Name: Maria Cardenas
Project Role: Graduate Students
Nearest person month worked: 12
Contribution to Project: Performed ongoing mouse experiments

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.*