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

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CONTRACTING ORGANIZATION: Emory University, Atlanta, GA

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6. AUTHOR(S)

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E-Mail:

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13. SUPPLEMENTARY NOTES

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.

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**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

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:

Kidney Cancer, T cells

3. ACCOMPLISHMENTS:

What were the major goals of the project?

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

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. ~60% complete (88 samples collected)
Subtask 3: Immunofluorescence staining and image processing of 150 cases 50% complete
Subtask 4: Obtain FFPE slides from Univ Wisconsin collaborator (Dr. Abel) - ongoing

Major Task 2 Investigate the predictive power of the CD8 infiltration in response to checkpoint therapies:
No sub tasks proposed for year 1.

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 – 50% complete

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?

1) Major activities
Major activities of this period were acquisition of kidney cancer slides from pathology and collaborators, RNAseq of fibroblasts from human tumors, beginning functional experiments using human fibroblasts.

2) Specific Objectives
See major goals section above for the specific objectives of this project.

3) Significant results
All aims of this project are in early phases and no final results from any experiments have been collected. Most of the first year has been optimizing protocols and performing initial experiments. Details are outlined below.

i) Optimization of tissue staining protocols
We spent the first 6 months optimizing a slightly new staining protocol that now allows us to stain up to 7 colors at a time to allow more detailed analysis. This procedure has been performed on 88 patients and we will continue to collect and stain the planned 150 patients from Emory and 100 from Wisconsin.

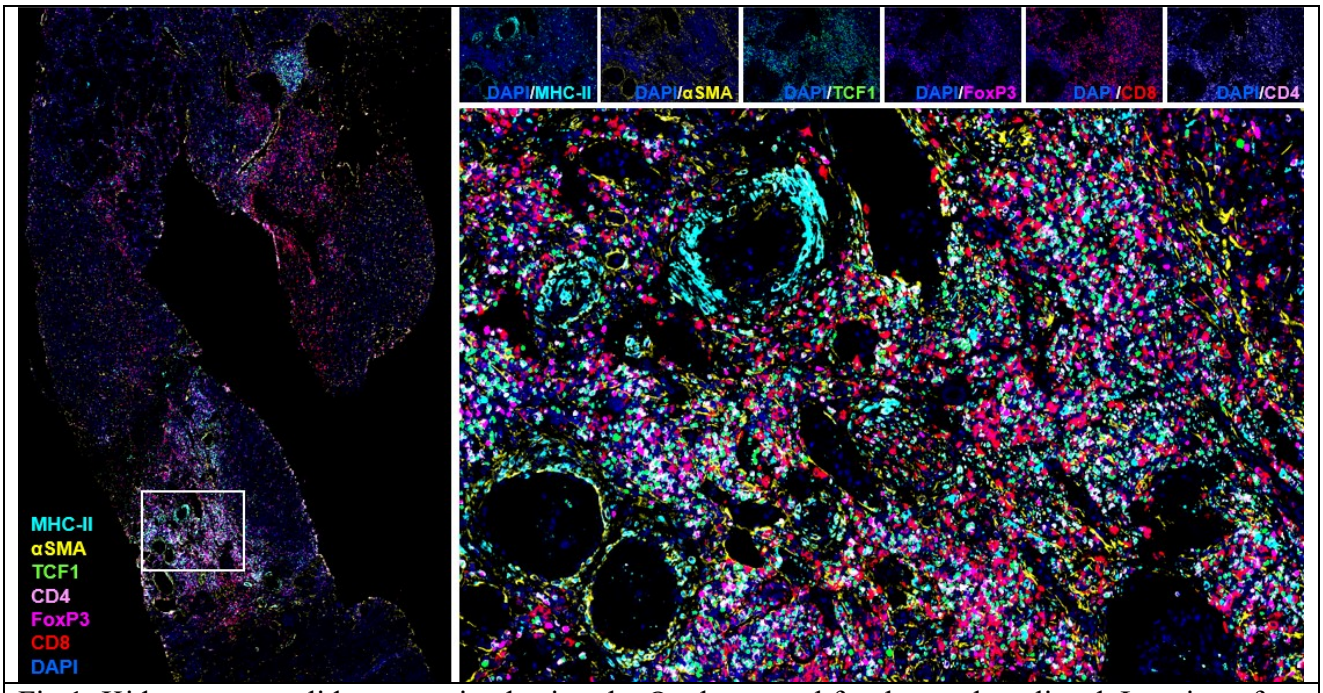

Fig 1: Kidney cancer slides are stained using the Opal protocol for the markers listed. Imaging of

entire slides are then taken using fluorescent slide scanner. This has been completed for 88 patients. Analysis for how various parameters correlate with patient survival is ongoing.

ii) Collection of RNAseq data from fibroblasts in human patients

Data has been collected from 19 patients. Data analysis is only preliminary to ensure it is of sufficient quality for future analysis. Full analysis will be completed once all the samples are collected.

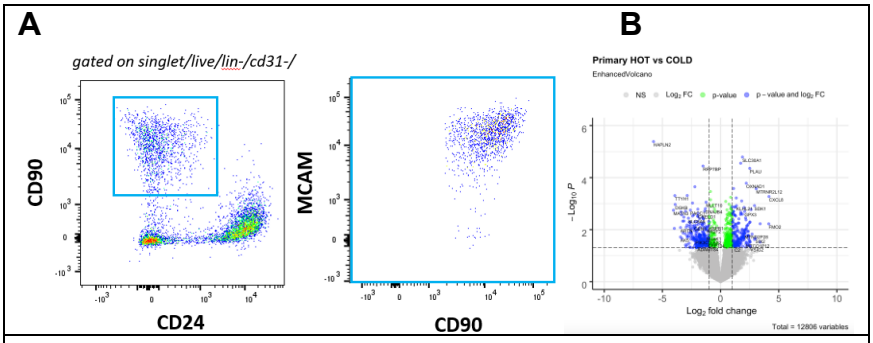

Fig 2 A) Fibroblasts isolation from kidney cancer are based on expression of CD90, MCAM and negative expression of lineage markers of other cell types. B) Intermediate analysis of RNA from these 19 samples begun, but fill analysis will wait for the full collection of patients.

iii) Begun in vitro functional assays using human fibroblasts

We have begun performing various functional experiments using human fibroblasts. The early part of this proposal was spent developing methods to effectively grow fibroblasts from human tumors in a manner that retained their phenotype. We found that an organoid 3d culture gave us the best results at expanding these cells. Ongoing experiments are optimizing the use of these fibroblasts to test their functionality to recruit different immune cell populations from the tumors. Data will be reported in the next progress report.

iv) Begun mouse experiments on fibroblasts

Experiments studying mouse fibroblasts are underway and we can detect these cells in mice. We are delaying functional experiments until better information is gathered from human RNAseq in the next ~6 months.

4) Other achievements

A staff scientist, Baohan Vo, was hired on this project and has made excellent progress completing the work outlined.

The only goal not met on time was collection of slides from our Wisconsin collaborator. This was delayed due to the MTA process taking longer than expected but is still well within the scope of the project.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Plans will continue as outlined in the statement of work. We do not foresee any deviations from the planned work

4. IMPACT:

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

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Slow MTA approval by Emory and Wisconsin legal offices. These have mostly been approved and slides are being selected at Wisconsin to send to Emory in the next 3 months.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

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:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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: RNAseq, optimization of fibroblast experiments, mouse experiments
Funding support: NA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: