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CONTRACTING ORGANIZATION: University of Washington

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<b>14. ABSTRACT</b> In Project 3, we have made substantial progress despite a slow-down of research activities due to the ongoing COVID-19 pandemic. We have found that immune cells in FLC are biased towards being in the stromal bands or peritumoral interface. We have found that PD-1 and IL-10 blockade each have some effects against FLC slice cultures in vitro. We are presently completing a manuscript detailing our findings from the first year of the project.						
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### 1. Introduction

In Project 3, we have been working to define the immune response to fibrolamellar carcinoma (FLC) and test hypotheses on improving anti-tumor immune responses. Despite challenges posed by the ongoing COVID-19 pandemic, we have made progress on the project as detailed below.

### 2. Keywords

CXCR – C-X-C chemokine receptor

CD – Cluster of differentiation

FLC – Fibrolamellar carcinoma

IL – Interleukin

mIHC – multiplex immunohistochemistry

scRNAseq – single-cell RNA sequencing

### 3. Accomplishments

We have made substantial progress in Project 3 over the past year in terms of the Major Tasks for each Specific Aim, as described below. We are nearing completion of a manuscript detailing our findings and anticipate submitting this for publication within the next 2 months.

#### Specific Aim 1

**Major Task 1:** Define spatial relationship of tumor immune cells

We performed mIHC for CK18, CD8, CD4, FoxP3, CD68, HLA-DR, and DAPI on tumors from 6 patients. This demonstrated that T cells were more densely located within the stromal bands and tumor interface than in the main tumor compartment.

**Major Task 2:** Single-cell RNAseq (scRNAseq) analyses of FLC

We are awaiting procurement of appropriate tumors to be able to perform this task. We have, however, collaborated with the University of California San Francisco in our current manuscript to include scRNAseq data from a single FLC tumor.

## **Specific Aim 2**

### **Major Task 1: Combining CXCR4 and PD1 inhibition**

We have performed in vitro treatment with combination CXCR4 and PD-1 inhibition, as well as IL-10 blockade, in slice cultures from two tumors. We have completed data analysis from the first tumor and are presently completing analysis of the second case. These data will be included in the manuscript in preparation.

### **Major Task 2: Live imaging of immune-mediated killing**

We performed live imaging of the slice culture experiments described above.

## **4. Impact**

Our findings suggest that immunotherapy for FLC has great potential. As we gather further data, we will be able to define which immune checkpoints have the best chance to provide clinical benefit.

## **5. Changes/Problems**

We have had a substantial slow-down in our laboratory work due to the COVID-19 pandemic. However, we have been able to continue to progress the project.

## **6. Products**

None

## **7. Participants & Other Collaborating Organizations**

N/A

## **8. Special Reporting Requirements**

N/A

## **9. Appendices**

N/A