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TITLE: Molecular and Cellular Determinants of Malignant Transformation in Lung Squamous Cell Carcinoma

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
During the first funding period we partially completed Major Tasks 1 and 2. The areas of interest were identified in 22 squamous cell lung cancer patient and isolated by LCM. Genomic DNA was isolated from these areas and whole exome sequencing was performed. The data is being analyzed for the progression-associated, premalignant- and malignant-specific mutations. The mutational data is being analyzed in the pathway context. Based on the mutational analysis, neoantigens are being identified as well as analysis of the lesion-infiltrating lymphocytes.

15. SUBJECT TERMS
Lung cancer, premalignancy, microenvironment, progression, driver mutations, neoantigens, whole exome sequencing (WES)

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1. INTRODUCTION:

Lung cancer is the leading cause of cancer death among US Veterans as well as the world's leading cause of cancer death. Squamous cell carcinoma (SQCC) accounts for 23% of non-small cell lung cancer cases in the US, however, to date no studies have focused on the mutational landscapes relevant to progression from premalignancy to invasive SQCC. One of the major driving forces of carcinogenesis is somatic mutagenesis. Over 75% of lung cancers bear driver mutations that are causally implicated in cancer development, while the remainder of lung cancers does not bear mutations in known oncogenes or tumor suppressors. Bronchial epithelium of many SQCC patients and subjects at risk for developing lung cancer often contain small proliferative premalignant lesions, such as squamous metaplasia (SQM), dysplasia (SQD), basal cell hyperplasia (BCH) and carcinoma *in situ* (SCIS). Current studies suggest that SQM and SQD may be precursors of SCIS and, subsequently, to invasive pulmonary SQCC. Factors that determine the fate of a premalignant lesion, i.e. whether it will progress to cancer or recede, remain enigmatic. Patients might be at increased risk for progression from these early premalignant lesions for years before developing clinically detectable lung cancer. Uncertainty about the clinical behavior of a premalignant lesion can lead to either inappropriate inaction or inappropriate aggressive treatment, either of which can result in harm to the patient. Unravelling the factors that determine whether premalignant lesions will progress to cancer or recede, including the modulation of cellular immunity, may change the way premalignant lesions are approached clinically. We anticipate that these studies could ultimately lead to the development of novel the approaches for lung cancer interception through immunoprevention and treatment of the very earliest phase of the disease.

2. KEYWORDS:

Lung cancer, premalignancy, microenvironment, progression, driver mutations, neoantigens, whole exome sequencing (WES)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1(specified in proposal)	Timeline	Site 1	Site 2	Status after Year 1
Major Task 1	Months			
Subtask 1: Review the slides to identify the areas of interest for LCM and IHC	1-3	Drs. Wallace and Dubinett	Drs. Huang and Dubinett	75%completed
Subtask 2: To isolate areas of interest by LCM	2-4	Dr. Krysan		75% completed
Subtask 3: To isolate genomic DNA and perform quality control	5	Dr. Krysan		75% completed
Milestone(s) Achieved:				75% completed
Local IRB/IACUC Approval: Active, IRB#17-000726-CR-00001		Dr. Dubinett	Dr. Dubinett	Completed
Major Task 2				
Subtask 1: To construct sequencing libraries and perform exome enrichment (30 cases)	6-8	Dr. Krysan		50% completed
Subtask 2: To perform next generation sequencing	9-11	Sequencing Core facility		50% completed
Subtask 3: To perform data analysis and identify progression-associated mutations	12-14	Drs. Krysan and Tran		50% completed
Milestone(s) Achieved: Specific Aim 2				50% completed
Major Task 3				
Subtask 1: To perform multi-color IHC, slide scanning and image analysis	15-20	TPCL, Dr. Wallace		5% completed
Subtask 2: To relate the expression of immune	21-24	Drs. Tran and		0% completed

regulators to the mutational landscapes of the tissues		Krysan		
Milestone(s) Achieved:				Ongoing

What was accomplished under these goals?

During the first funding period we partially completed Major Tasks 1 and 2. We performed LCM and isolated the regions of interest, including 3 BCH, 28 SQM, 22 SQD, 20 SCIS, BCH and 26 SQCC lesions and the adjacent normal bronchial epithelium from 22 lung cancer patients (**Table 1**). Sample collection is ongoing to achieve the projected number of 30 cases in the course of the second funding period. Sequencing libraries were constructed followed by exome enrichment and WES was conducted with at least 2 x 10^{10} bases sequenced per exome, which has been frequently achieved in published WES studies.

Patient ID	SQM	SQD	SCIS	BCH	SQCC
P01	2	2	0	0	1
P02	0	1	6	0	1
P03	0	2	1	0	1
P04	0	0	2	0	1
P05	1	0	0	0	1
P06	1	0	1	0	1
P07	1	1	0	0	1
P08	0	1	0	0	1
P09	3	0	3	0	1
P10	4	1	0	0	2
P11	0	1	0	0	1
P12	4	3	0	3	2
P13	3	3	0	0	1
P14	0	0	2	0	1
P15	0	0	4	0	1
P16	1	2	0	0	1
P17	1	0	0	0	1
P18	1	2	1	0	1
P19	2	0	0	0	1
P20	2	0	0	0	2
P21	1	2	0	0	2
P22	1	1	0	0	1

Table 1. Summary of regions sequenced in each patient.

Mutational and neoantigen analyses as well as the analysis of the lesion-infiltrating lymphocytes are ongoing. Our Vectra Polaris instrument (Perkin Elmer) has been recently upgraded to 10-colors detection capability and we are currently optimizing our protocols for the extended panels of markers. In our initial experiments, we immunostained the tissue sections for PD-L1, PD-1, CD8, CD4, CD11c, CD68, CD163, FOXP3 and granzyme B utilizing Opal kits (Perkin Elmer). The representative images are shown in **Figure 1**.

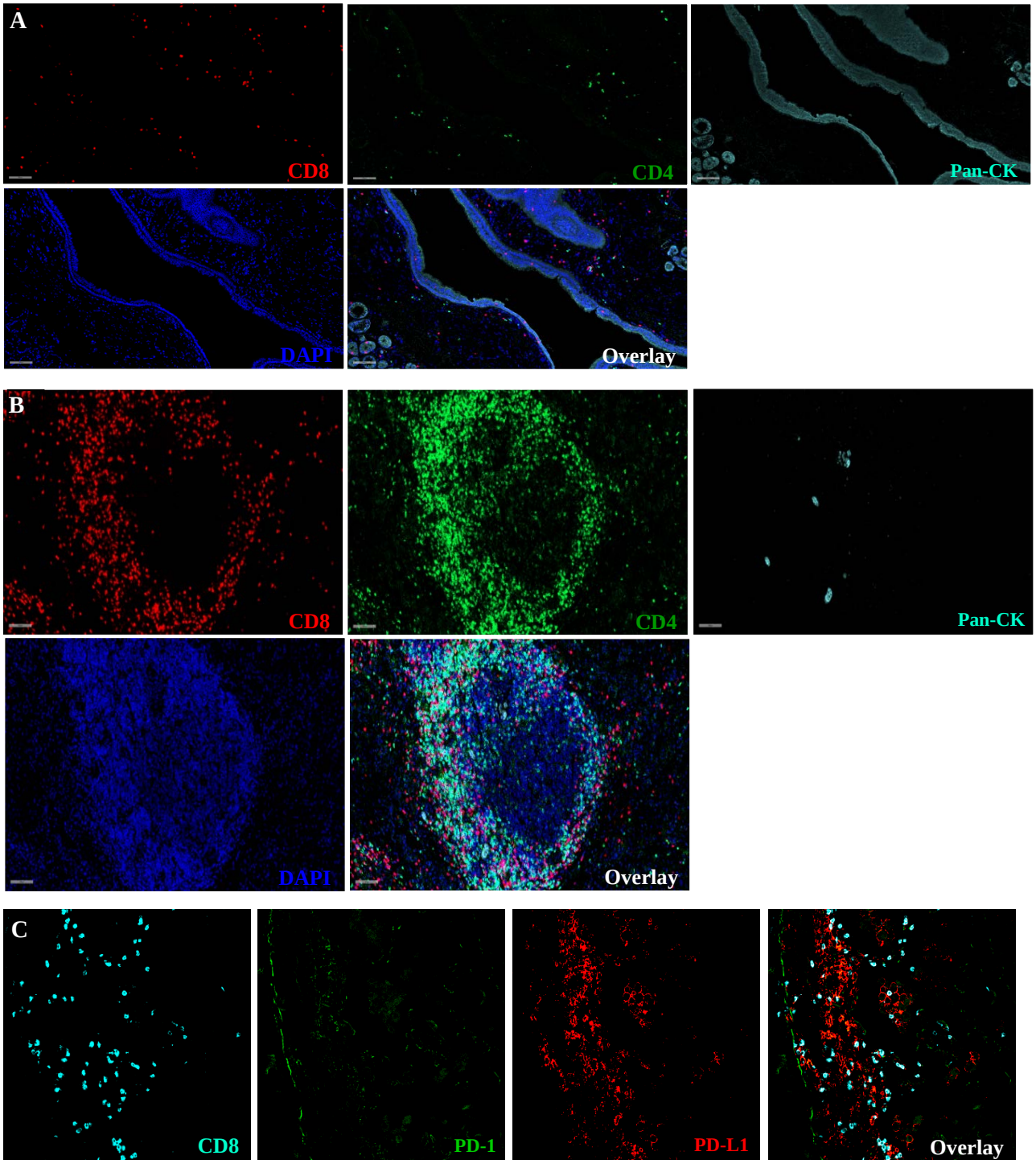

Figure 1. Representative MIF staining of: (A) SQM, (B) SQCC, (C) SCIS.

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

Kostyantyn Krysan and Linh Tran participated in conference calls related to pulmonary premalignancy. The conferences included investigators from other institutions involved in the MCL Pre-Cancer Atlas Program, as well as, the NCI Moonshot Pre-Cancer Atlas Program.

How were the results disseminated to communities of interest?
Nothing to report on how the results were disseminated to communities of interest.

We plan to continue to complete major tasks 1, 2 and 3.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
We anticipate that these results will add significant new insights into the pathogenesis of pulmonary squamous premalignancy. This turn will inform efforts related to the application of lung cancer interception.

What was the impact on other disciplines?
We anticipate that these studies will have an impact in lung cancer screening, pulmonary medicine and thoracic oncology.

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:

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

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
Nothing to report.

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:

- **Publications, conference papers, and presentations**
  Nothing to report.

  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?
Steven M. Dubinett - No change
Kostyantyn Krysan - No change
Linh M. Tran - No change
W. Dean Wallace - No change
Min Huang - 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?
SU2C and CIRM awards received

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES:

Nothing to report.