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14. ABSTRACT

Our proposed studies have profound implications in improving our understanding of cachexia development in lung cancer patients, which affects about 60% of the lung cancer patients and contributes to morbidity and mortality from the disease1,2. The pathophysiology of the cachexia syndrome is still poorly understood; in particular, there is little understanding of how specific oncogenic mutations might drive this problem. Investigating oncogene based models of cancer cachexia that, mimic human disease will improve our knowledge about potential mechanisms and factors responsible for disease development and aid in discovery novel targets for reducing the severity of cachexia.

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Introduction

Adenocarcinoma is the most common subtype of non-small cell lung cancer in the U.S. Among active military personnel and U.S. Veterans, lung cancer is the leading cause of cancer related deaths. Exposure to dust, fumes, and other toxic substances/ carcinogens from burn pits and other environmental pollutants puts them at a higher risk for lung cancer development as compared to the civilians.

Cachexia is a devastating complication of cancer affecting about 60% of the lung cancer patients. It takes a heavy toll on quality of life in cancer patients due to involuntary weight loss, reductions in muscle strength and adipose tissue mass as well as elevated toxicity of chemotherapy and other postoperative treatments.

We proposed to study the possible role of certain oncogenic mutations in promoting the development of lung adenocarcinoma and simultaneously inducing systemic inflammation, adipose tissue and muscle loss using a mouse model.

Body

Our specific aim was to determine if loss of Keap1 function in mutant Kras^{G12D} driven lung tumors reprograms cellular redox balance, glucose metabolism and immune response to promote rapid tumor growth, adipose tissue loss and tumor induced cachexia. Our preliminary studies revealed that tumors in mice harboring oncogenic Kras^{G12D} mutation that are also deficient in Keap1 show rapid tumor growth and dramatically reduced survival (Fig. 1). Correlation between tumor burden and weight loss or tumor burden and overall survival is lacking in mice harboring Keap1 deficient

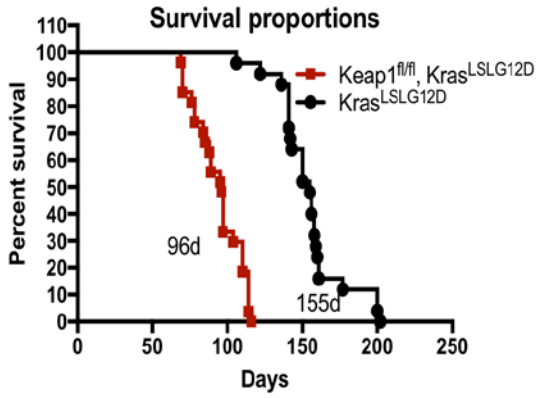

Figure 1*: Kaplan Meier survival curve showing the overall survival* ***of*** *Keap1fl/fl;KrasLSL* and *K-rasLSL infected with adenovirus CMV-cre recombinase intratracheally.*

mutant Kras tumors. On the contrary, mice carrying Kras tumors (wild type for Keap1) show positive correlation between tumor burden and survival and do not exhibit rapid weight. Our results will help determine whether identifying co-existing Keap1 and Kras mutations can serve as a biomarker for predicting cachexia development in patients with lung adenocarcinoma.

Major Task 1: Breeding, tumor initiation and Imaging to monitor tumor burden, body fat and lean content, measure heart function.

Subtask 1: Expand mouse colony of *Keap1* $^{fl/fl}$ *Kras* $^{LSL\text{-}G12;}$ and *Kras*$^{LSL\text{-}G12D}$ mice. Genotype the mice and initiate lung tumors by intratracheal administration of adenoviral cre recombinase	**Progress:** We have successfully expanded the genetically-engineered mouse colony, with genotype confirmation. Tumors have been initiated by administration of cre recombinase. **Problems/ Challenges/ Solutions:** Multiplicity of tumors was lower than expected in our initial experiments. Using a different lot of viral cre recombinase in new series of animals did increase multiplicity, but not to the same level as in our preliminary experiments. Experiments are ongoing.
Subtask 2: SPECT-CT imaging to monitor lung tumor burden. Eco-MRI to measure body composition and determine body water, fat and lean content. Echocardiography to measure heart function in tumor bearing mice. Analyze muscle function by grip test. Isolate and record the weight of gastrocnemius and quadriceps muscles. N=8 mice/ group- 3 time point (total 24). Genotypes: *Keap1* $^{fl/fl}$ *Kras* $^{LSL\text{-}G12;}$ and *Kras*$^{LSL\text{-}G12D}$ mice N=8-10 mice/ group- (total 16-20). 2 Genotypes.	**Progress:** Imaging studies were initiated as proposed. **Problems/ Challenges/ Solutions:** Equipment failure (core facility) resulted in a need to change instruments used to monitor lung tumor burden and body water, fat, and lean content. As a result, data from our initial multi-month experiment was not interpretable. Using funds from our no-cost extension, we initiated another series of experiments, but the Core facility for these measurements closed in March, 2020 due to COVID-19 restrictions.

Major Task 2: Analysis of pulmonary inflammation and systemic inflammation.

Subtask 1: Analyze pulmonary inflammation by flow cytometry	**Progress:** We have measured inflammatory cells in airways as proposed in a subset of animals.

	Problems/ Challenges/ Solutions: Because multiplicity of tumors was lower than expected in initial experiments, (resulting in use of a different lot of viral cre recombinase), we planned to repeat these measurements in a new series of animals. These experiments were terminated before completion, due to COVID19-related restrictions.
Subtask 2: Analyze inflammatory gene expression by real time R-PCR. Measure inflammatory cytokine levels in BAL fluid (from lung) and serum by ELISA.	**Progress:** We also initiated a repeat of these experiments due to lower than expected multiplicity of tumors in initial experiments. However, these repeat experiments were terminated before completion, due to COVID19-related restrictions. **Solutions:** We plan to repeat these measurements in a new series of animals.
Subtask 3: Targeted metabolite analysis in the adipose tissue and muscles.	**Progress:** These studies have not yet been conducted, due to lower than expected multiplicity of tumors in initial experiments and premature termination of repeat experiments. **Solutions:** We plan to repeat these measurements in a new series of animals.

Major Task 3: IFNγ neutralization to block IFNγ signaling and reduce inflammation and adipose tissue loss.

Subtask 1**:** IFNγ neutralization studies first in tumor bearing *Keap1* $^{fl/fl}$ *Kras* $^{LSL\text{-}G12}$ mice.	**Progress:** These studies have not yet been conducted, due to lower than expected multiplicity of tumors in initial experiments and premature termination of repeat experiments. **Solutions:** We plan to repeat these measurements in a new series of animals.
Subtask 2: Imaging by CT, Eco-MRI, muscle function test	**Progress:** These studies have not yet been conducted, due to lower than expected multiplicity of tumors in initial experiments and premature termination of repeat experiments.

	Solutions: We plan to repeat these measurements in a new series of animals.
Subtask 3*: Analysis of pulmonary inflammation, Immunohistochemistry*	**Progress:** These studies have not yet been conducted, due to lower than expected multiplicity of tumors in initial experiments and premature termination of repeat experiments. **Solutions:** We plan to repeat these measurements in a new series of animals.

Conclusions

We have faced significant logistical challenges in this project. First, it took over 2 months to resolve all administrative hurdles for using a mouse model. Subsequently, tumor growth/burden was significantly lower than in prior experiments which forced us to repeat our experiments using new virus stock and fresh animals. We also had to deal with equipment failure in our core facility that resulted in switching to different methodology to measure body fat. Since there is some normal variation in tumor development in our mouse model the animals were kept alive for a longer period of time than originally planned. During this extended period we did not see acceleration of tumor growth, and therefore we decided to repeat this series of experiments and not to analyze the collected data from initial experiments. Unfortunately, due to COVID19-related restrictions on the use of animal care facilities, our repeat experiments were terminated just before our scheduled completion. We have conserved funds, and with our extension, we plan to repeat the experiments.