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TITLE: Cancer Cell Autonomous Regulation of Immunogenicity: Revisiting the Immunoediting Hypothesis

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14. ABSTRACT

The project aims to identify specific tumor genotypes that naturally impact the process of immune surveillance and the efficacy of immune checkpoint therapy. Our project uses genetically engineered mouse models that feature a fully competent immune system that is required to study gene-environment interactions. Identifying particular genotypes that actively suppress immune surveillance and those that are particularly sensitive to immune surveillance will reveal therapeutic approaches to enhance the durability of ICT. Moreover, defining the impact made by cancer-driving cell-autonomous mutations on immune surveillance and ICT will shift our mechanistic understanding of the immunoediting hypothesis and the etiology of human lung cancer.

15. SUBJECT TERMS
None listed.

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1. Introduction

Our overarching goal is to implement a novel *in vivo* screening platform to determine the extent to which inactivation of specific tumor suppressor genes changes the immunogenicity threshold of a tumor to affect cancer cell immune surveillance and the efficacy of ICT. Our approach combines the power of genetically-engineered mouse models of lung cancer, CRISPR-based somatic cell gene perturbation, genetically-barcoded neoantigen expression, and a DNA sequencing-based method to quantitatively assess the in vivo growth of tens to hundreds of genetically diverse tumors in a multiplex fashion.

Specific Aim 1: Determine the impact that inactivation of each of the top 10 most commonly mutated tumor suppressor genes in lung adenocarcinoma has on neoantigen-induced tumor immune surveillance.

Specific Aim 2: Determine the impact of tumor suppressor gene mutations on the potency of immune checkpoint therapy.

2. Keywords

Mouse model, Kras, lentivirus, immunogenicity

3. Accomplishments:

(Text from the SOW is included for context. Accomplishments are boxed in blue.)

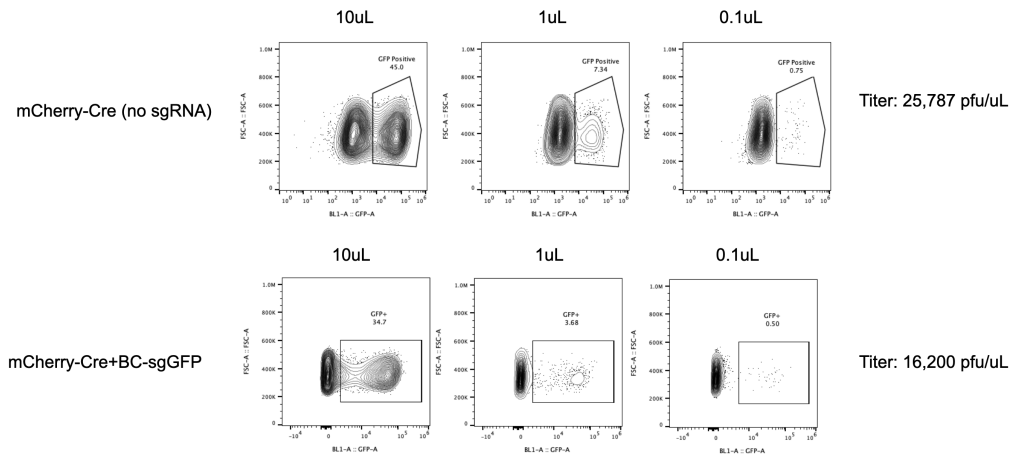
Specific Aim 1: Determine the impact that inactivation of each of the top 10 most commonly mutated tumor suppressor genes in lung adenocarcinoma has on neoantigen- induced tumor immune surveillance.

Subtask 0.2 – Submit documentation to DoD ACURO and receive approval required for the use animals (*Kras*$^{LSL\text{-}G12D/+}$;*p53*$^{flox/flox}$; *Rosa26*$^{LSL\text{-}Cas9/+}$ mice)

This was completed and approved on 12/01/2021

Subtask 1 – Establish virus stock for each unique sgRNA-expressing lentivirus. HEK293T cells will be used to generate lentivirus stocks. Generation 3 lentivirus production methods will be used. Each stock will be functionally titered using a Cre-dependent reporter line (GreenGo) to obtain tumor initiating particles per uL. Virus stocks will be pooled such that each is equally represented and the final number of functional particles equals $1.0x10^5$ in a single 50uL dose (one mouse equivalent).

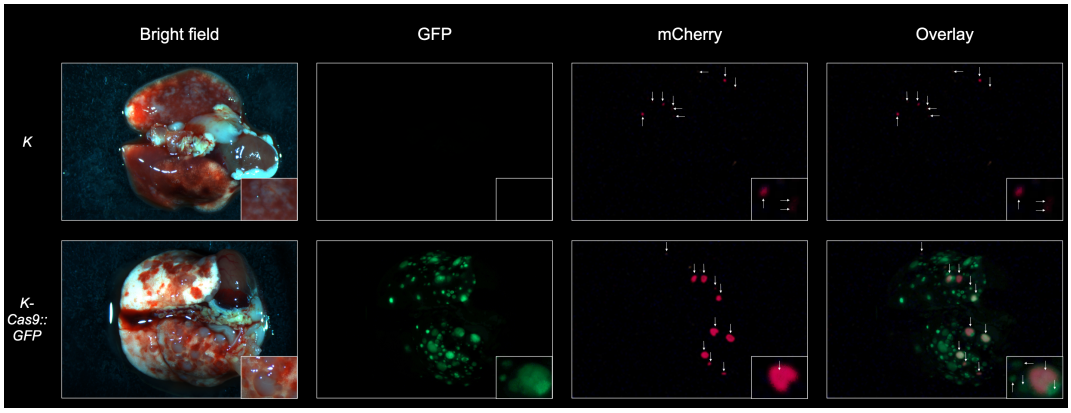
Representative flow plots demonstrating effective lentiviral titers using a Cre-inducible GFP cell line. Viral preps were diluted across 10, 1, and 0.1 uL of virus to determine the pfu/uL. These values were used to determine the amount of lentivirus to transduce each mouse.



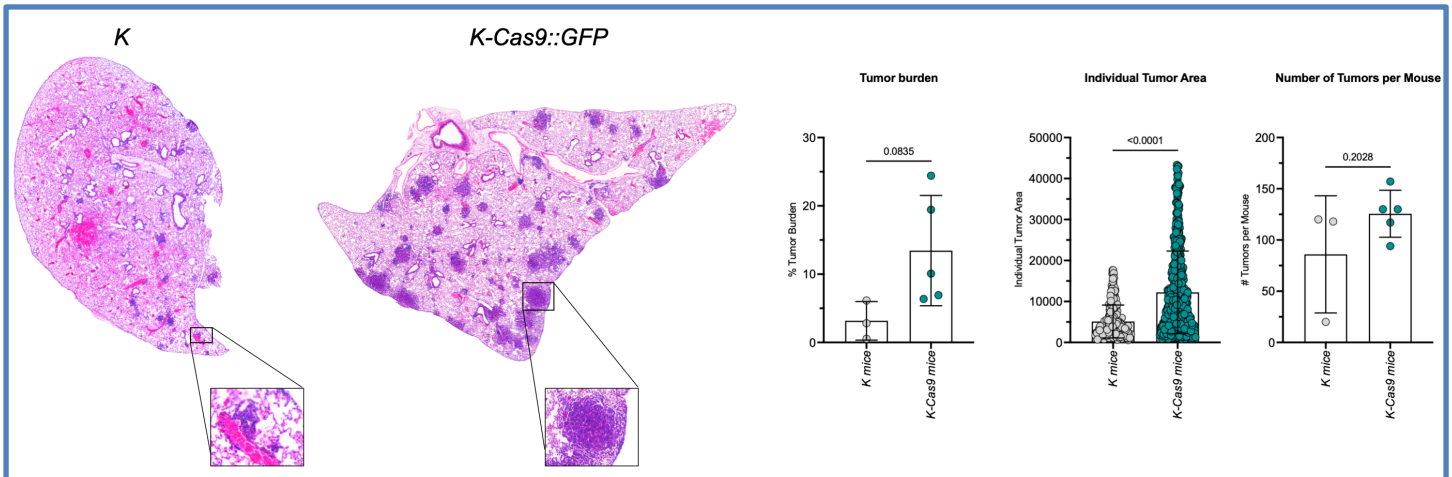
Subtask 2- Transduce cohorts of *Kras*$^{LSL\text{-}G12D/+}$;*p53*$^{flox/flox}$; *Rosa26*$^{LSL\text{-}Cas9/+}$ mice with pooled Lenti-CRISPR-SIIN (antigen expressing) or Lenti-CRISPR-X (antigen absent). We will use 12 mice per experimental arm (24 total).

Completed. No physical data to show.

Subtask 3- Harvest mouse lungs, prepare libraries, and sequence.

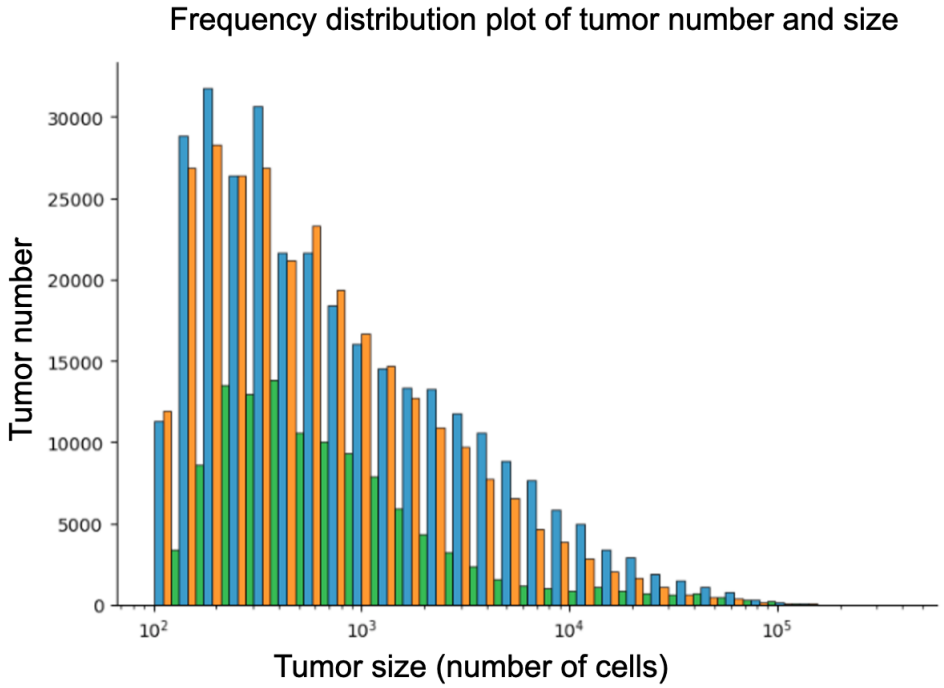


Representative lungs from transduced mice of the indicated genotype (left) visualized via fluorescence microscopy to detect GFP expressed from the Cas9::GFP transgene, mCherry expressed from the lentivector pool, and teh overlay. Note larger tumors in the Cas9::GFP lungs demonstrating the effect of Cas9-mediated tumor suppressor gene inactivation.



Histological analysis from lungs after pooled lentiviral transduction. Note larger tumors in the Cas9::GFP lungs demonstrating the effect of Cas9-mediated tumor suppressor gene inactivation.

Subtask 4- Data analysis: Enumerate tumor number and size from each tumor clone. Log- rank tumor clones in percentile format and assess changes between genotypes for tumors at discrete percentile rankings.

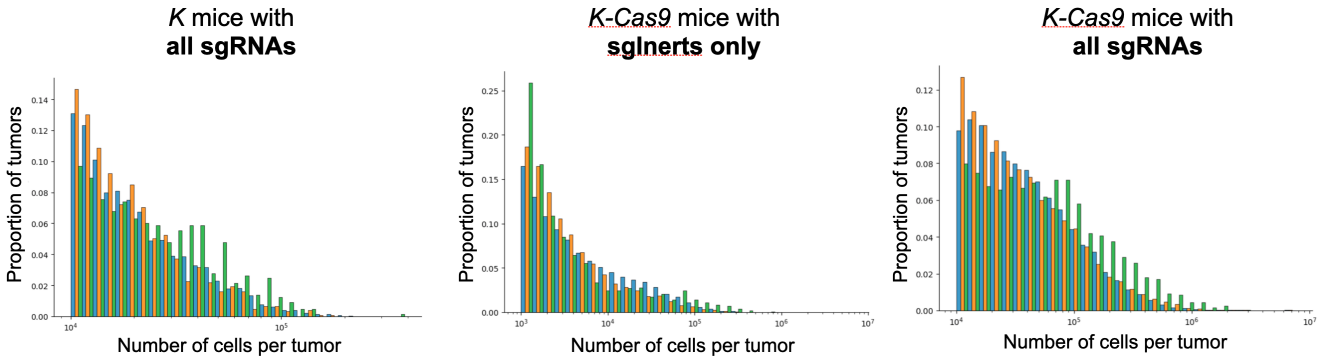


NI=Non-immunogenic (Cre only)

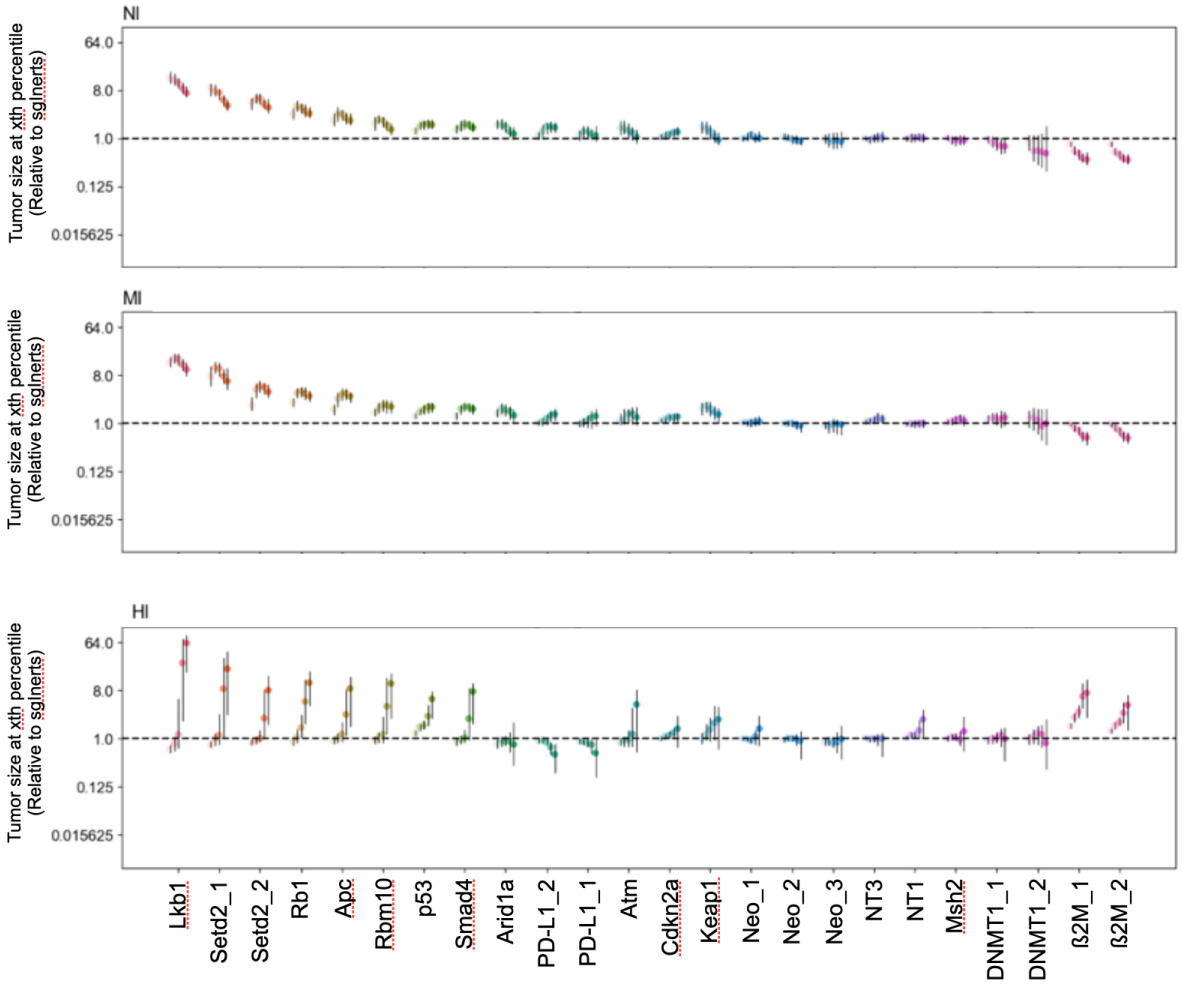
MI=Mildly immunogenic (mCherry-Cre)

HI=highly immunogenic (mCherry-SIINFEKL-Cre)

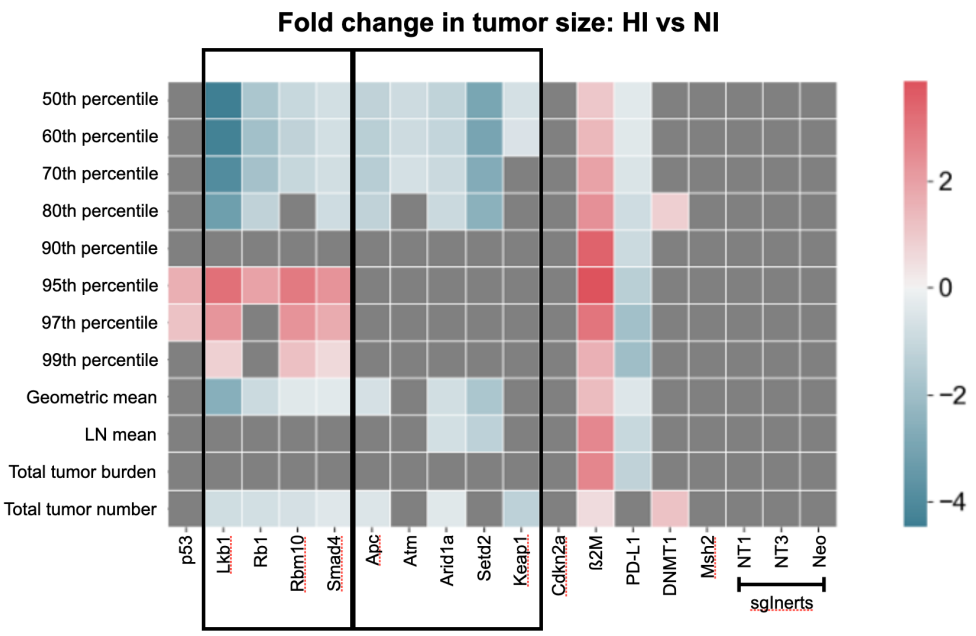
SIINFEKL expression greatly reduces the number and size of tumors initiated. There is a much lower frequency of tumors initiated with the HI vector compared to NI or MI. The average tumor initiated by the HI vector is smaller than the average tumor initiated by either the NI or MI vectors. Together, this suggests that SIINFEKL expression promotes an immune response that limits tumors from entering the “escape” phase of immunoediting



SIINFEKL expression also enriches for a small subset of very large tumors. High levels of immunoediting during early tumor development lead to the outgrowth of very large (and presumably immune evasive) tumors. This is a general phenomenon that occurs independently of tumor genotype.



SIINFEKL expression induces different levels of tumor immunoediting depending on the genotype of each tumor. NI= non Immunogenic UBC-Cre (Top), MI= Mildly Immunogenic UBCmCHerry (Middle), and HI= Highly immunogenic UBCmCherry-SIIN (Bottom). Shown are tumor size ratios relating percentiles of tumor size (50%, 75%, 90%, 95%, and 99% indicating by increasing pigment enrichment for each dot associated with the sgRNA indicated at the bottom) with sizes of tumors expressing sgInert for each percentile.



Inactivation of specific tumor suppressor genes modifies the immunogenicity threshold. Inactivation of the majority of tumor suppressor genes lowers the immunogenicity threshold. Comparison of Non-immunogenic tumors to Highly-immunogenic tumors presented as a fold change in tumor size across relative tumor size percentiles in each genotype. Generally, tumors grow smaller in the HI condition. Inactivation of a subset of these tumor suppressor genes promotes immunoediting but also facilitates immune escape:
A small subset of tumors belonging to these genotypes are better able to enter the “escape” phase of immunoediting. These tumors grow larger in the HI condition at the top percentiles of tumor size

Specific Aim 2: Determine the impact of tumor suppressor gene mutations on the potency of immune checkpoint therapy.

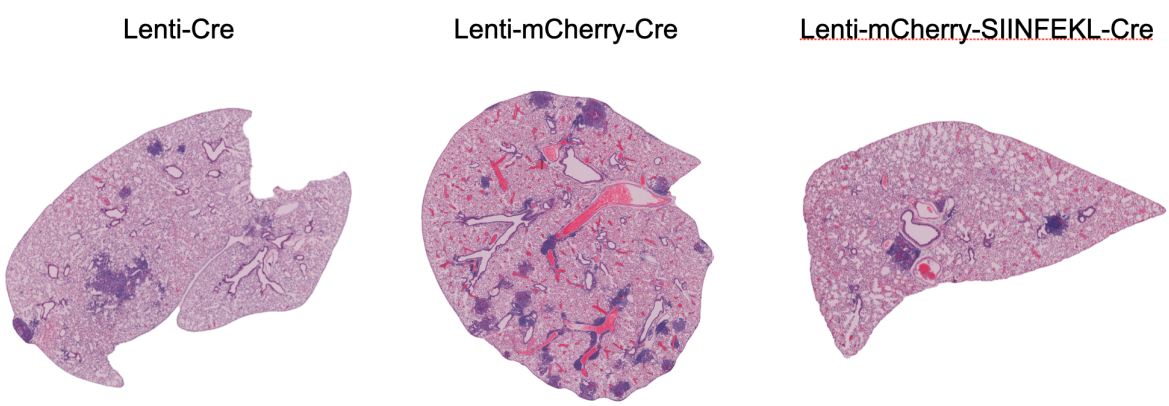
Subtask 1- Transduce cohorts of KrasLSL-G12D/+;p53flox/flox; Rosa26LSL-Cas9/+ mice with pooled Lenti-CRISPR-SIIN (antigen expressing) or Lenti-CRISPR-X (antigen absent). We will use 12 mice per experimental arm (24 total).

This was completed. No physical data to show.

Subtask 2- Initiate treatments with control and anti-PD1 antibodies.

This was completed. No Physical data to show.

Subtask 4- Data analysis: Enumerate tumor number and size from each tumor clone. Log- rank tumor clones in percentile format and assess changes between genotypes for tumors at discrete percentile rankings.



Tumors were harvested and processed for analysis. Representative histology from samples associated with the experiment to demonstrate acquire material for analysis and success of the experiment. Analysis has not been completed but is ongoing.

4. Impact

This project has minimally identified one critical determinant of lung cancer immunosurveillance. This has the potential to segment the lung cancer population into those amenable to ICT and potentially explain the large hetrogeneity of antigenic peptides present in human lung cancers. Our work made possible by this pilot funding mechanism is likely to fuel the acquisition of new larger scale grants to uncover the molecular mechanisms of the immunogenicity threshold.

5. Changes/Problems

During the coarse of the experimental set up we realized that the mCherry vector had a small but significant degree on immunogenicity. We used this as an opportunity to expand our vector repertoire by using an additional vector that expresses neither mCherry nor SIINFEKL. We therefore performed each of our screens with the following 3 vectors to create an allelic series of immunogenicity: Lenti:Cre, Lenti-mCherry/Cre and Lenti:mCherry-SIINFEKL/Cre.

6. Products

None

7. Participants & Other Collaborating Organizations

None

8. Special Reporting Requirements

None

9. Appendices

None