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TITLE: Mechanisms of Immune Checkpoint Resistance Mediated by LKB1 Tumor Suppressor in Lung Cancer

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Durham NC 27708-4677

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U.S. Army Medical Research and Development Command
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**14. ABSTRACT**

LKB1 is one of the most commonly lost tumor suppressors in lung cancer, with 30% loss in lung adenocarcinomas. It has recently been demonstrated that NSCLC patients with LKB1 loss have significantly worse outcomes to immune based treatment strategies, with lower response rates and decreased progression free and overall survival. Determining the mechanism for this immune refractory phenotype and a strategy to overcome it are urgent and unmet clinical needs.

This report will demonstrate progress characterizing LKB1-add back isogenic derivatives of immune resistant LKB1 mutant cell lines as model systems for study of LKB1-deficient immune resistance, the use of high throughput functional genomics approaches to identify candidate genes and pathways that may confer resistance, and preliminary validation of candidate mechanisms resulting from these experiments.

**15. SUBJECT TERMS:**

**Lung Cancer, Ferroptosis, CRISPR, Functional Genomics, KEAP1, LKB1, Immunotherapy, NRF2**

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## 1. INTRODUCTION:

This project aims to better understand immune evasion phenotypes driven the loss of the LKB1 tumor suppressor. The work combines three complementary approaches. First, characterization of immune response phenotypes using an in vitro model system with paired isogenic cell line derivatives – an immune resistant state with LKB1 mutation, and an immune sensitive state that occurs after re-expressing LKB1 WT. Second, high throughput functional genomics approaches are applied to this model system to identify candidate genes and pathways that are mechanistically important in conferring immune resistance. Third, analysis of patient specimens from a clinical trial of neoadjuvant pembrolizumab, as well as in silico analysis of other patient cohorts with molecular data allows us to assess the importance of candidate pathways in determining outcomes of lung cancer patients treated with immunotherapy.

## 2. KEYWORDS:

LKB1, KEAP1, NRF2, CRISPR, Functional Genomics, Immunotherapy, Ferroptosis, Lung Cancer

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

Major Task 1: Characterize IFN-γ response in genetically modified cell lines.
*Milestone(s) Achieved (12/2019): Identification of functional differences in IFN-γ response directly influenced by LKB1.*
*Percentage completion: 100%, with subsequent work evaluating interaction between LKB1 and KEAP1/NRF2 in regulating these phenotypes.*

Major Task 2: Candidate based approach to identify IFN mediators
*Milestone(s) Achieved (12/2019): Evaluated specified candidates on their effect on IFNG mediated growth suppression. The candidates tested did not appreciably affect IFNG response.*
*Percentage completion: 75%, with subsequent work testing novel candidates resulting from analysis of CRISPR-CAS9 screen in Major Task 3.*

Major Task 3: CRISPR-CAS9 screen to identifying IFN mediators
*Milestone(s) Achieved: Completed whole genome functional screen of modifiers of IFNG response in the A549 cell line and LKB1 add-back derivative (9/2019)*
*Percentage completion: 75%, with key primary screen completed and with ongoing work on subtask 2 to generate a targeted subgenomic target library and carry out further screens across other cell lines and conditions.*

Major Task 4: Pharmacologic screen to identify IFN mediators
*Based on preliminary analysis of the IFNG phenotype (Major Task 1) on which this screen was to be conducted, we concluded that the screen as originally designed had a low likelihood of success. Further pursuit of this aim is deferred for the time being.*
*Percentage completion: 20% - initial feasibility assessment completed, with plans not to pursue further experiments with a pharmacologic library.*

Major Task 5: Characterize gene expression and immune effectors in patients
*Milestone(s) Achieved: The clinical trial on which this aim is based has been completed. Sample identification and nucleotide extraction are being carried out (9/2020). DOD specific IRB is being prepared.*
*Percentage Completion: 75%.*

**What was accomplished under these goals?**

Major Task 1: Characterize IFN-γ response in genetically modified cell lines.
**Specific objectives**: To stably express wild-type LKB1 into parental LKB1-mutant cell lines and characterize the effects of LKB1 on interferon induced signaling and cellular phenotypes.
**Major activities:**

- Analyzed RNAseq mRNA expression data using pathway analysis to identify pathways differentially regulated by IFNG in the presence of LKB1.

- Stably expressed NY-ESO into target cell line H2023-Vector and H2023-LKB1 addback for purposes of replicating cytotoxicity assay
- Attempted to perform cytotoxicity assays - unsuccessful

**Significant results:**

1) Transcriptomic analysis: GSEA analysis identifies cholesterol biosynthesis/mevalonate pathway as the most differentially altered pathway by IFNG after LKB1 add-back (Fig 1A), in comparison to IFNG treated control (LKB1 mutant) cells, with FDR p-value 1e-17. Specific enzymes in the mevalonate pathway showing differential expression are highlighted in Fig 1B, representing the majority of the enzymes in this pathway, with mRNA expression data for A549 cell line shown in Fig 1C.

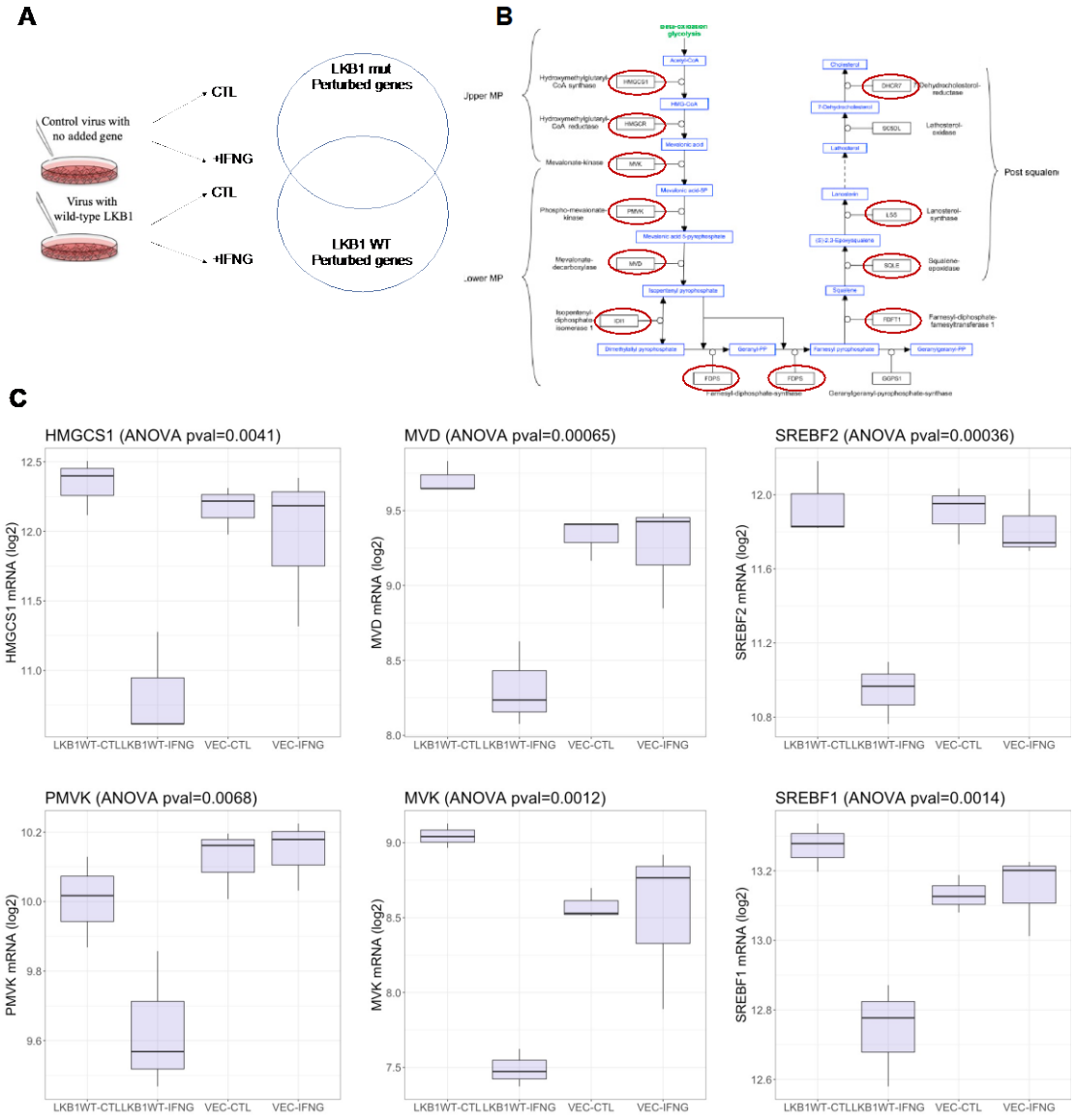



**Figure 1: Pathway analysis of RNAseq expression data from IFNG-treated cell lines identifies dysregulation of mevalonate pathway**

2) Analysis of TCGA patient data shows negative correlation between interferon stimulated gene signature (ISG) and mevalonate pathway in LKB1 WT tumors.
3) Three attempts were made to evaluate T-cell mediated cytotoxic response using T-cells specific to NY-ESO and cell line model (H2023-vector vs H2023-LKB1 add-back) with stable over-expression of NY-ESO antigen. Aliquots of antigen-specific T-cells in these experiments were purchased commercially (Cellero, Lowell MA), but in each case failed to proliferate and engage in cytotoxic response.

**Conclusions:** Our analysis of differential effects of IFNG after experimental LKB1 add-back highlights specific downstream metabolic effects suggesting crosstalk between IFNG signaling and mevalonate pathway

occurring in an LKB1-specific manner. Inhibition of the mevalonate pathway has previously been shown to enhance anti-tumor immunity in vaccine and anti-PD1 models (Xia et al, Cell 2018).

**Future directions:** Functional analysis of mevalonate pathway as target to enhance anti-tumor immunity in LKB1 deficient tumors is being further evaluated, including preliminary data highlighted in Major Task 2. We plan to evaluate inhibition of the mevalonate pathway using a T-cell co-culture model. We have also begun discussions with Dr. Michael Kelley, the national program director of Oncology at the VA network, regarding potential collaboration in which veteran patient data from CPRS could be used to evaluate whether statin use among LKB1 mutant lung cancer patients is associated with increased duration of response to anti-PD(L)1 inhibitors, as assessed by number of treatment cycles.

While attempts to use cytotoxic assay were unsuccessful, this appears to be due to technical issues with supply of T-cells. To address this further we have established collaboration with Carlotta Costa (Novartis, Basel, Switzerland) through which we will be able to obtain quality T-cell products, as well as viral vectors needed to successfully replicate this assay.

Major Task 2: Candidate based approach to identify IFN mediators

**Specific objectives**: To stably express wild-type LKB1 into parental LKB1-mutant cell lines and characterize the effects of LKB1 on interferon induced signaling and cellular phenotypes.

**Major activities:**

- Generated inducible shRNA knockdown constructs for NRF2, GPX4, SLC7A11 (xCT), SREBP1, SREBP2, expressed in target lines A549, H1944, and H2023, with and without LKB1 add-back, and measured effects of these perturbations on IFNG cell growth phenotype.
- Tested effects of ferroptosis inducer RSL3 and inhibitor of ferroptosis liproxstatin on IFNG cell growth phenotype in A549, H1944, and H2023, with and without LKB1 add-back.
- Tested effects of simvastatin and inducible shRNA knockdown of metabolic transcription factors SREBP1 and SREBP2 on IFNG cell growth phenotype in A549, H1944, and H2023, with and without LKB1 add-back, alone or in combination with exogenous mevalonate rescue.
- Generated STING and cGAS shRNA knockdowns and CRISPR knockout derivatives of H2023, A549, and H1944 with or without LKB1.
- Tested IFNG sensitivity in STING/cGAS derivatives after LKB1 add-back, and tested the enhancement of IFNG effects with statin use in these lines.

**Significant results:**

**1)** We observed that restoration of LKB1 into LKB1 mutant cell lines enhanced sensitivity to ferroptosis.

**2)** NRF2, xCT, and GPX4 inducible knockdowns, despite leading to expected alterations in sensitivity to redox and ferroptosis modulators (e.g. RSL3), did not appreciably affect IFNG sensitivity in target cell lines A549, H2023, or H1944 with or without LKB1 add-back.

**3)** Experiments using pharmacologic inducer of ferroptosis (RSL3) appeared to synergistically enhance the antiproliferative effects of IFNG. However, this was not consistent across cell lines and experiments. Further, inhibition of ferroptosis using liproxstatin was not able to reverse IFNG sensitivity caused by LKB1 add-back.

**4)** On the other hand, evaluation of mevalonate pathway demonstrated that use of ‘statin’ HMGCoA inhibitors potentiated the effects of IFNG, enhancing sensitivity of LKB1 mutant cells to IFNG similar to the levels seen after LKB1 add-back. These effects could be reverse with the use of exogenous mevalonate to bypass the statin inhibition, but were not affected by liproxstatin.

**5)** IFNG sensitivity induced by LKB1 add-back and/or by inhibition of the mevalonate pathway was affected only marginally by STING/cGAS manipulation, demonstrating that these effects are largely STING independent.

**Conclusions**

Modulators of ferroptosis had been identified as candidate resistance pathway based on our CRISPR screen in Major Task 3. Our follow up experiments on ferroptosis have yielded inconclusive results to demonstrate a causal role in immunotherapy resistance. LKB1 mutation status does appear to cause resistance to ferroptosis consistent with our hypothesis, and since the last update this has been demonstrated with a mechanistic model involving SCD1 (Wohlhieter et al, Cell 2020). On the other hand, the mevalonate pathway, which was implicated by our RNAseq analysis from Major Task 1, appears to have consistent effects on IFNG response across multiple cell lines and will be the target of ongoing mechanistic and functional studies.

**Future Directions**

As described in Major Task 1 we have established two collaborations that should allow testing modulators of ferroptosis and the mevalonate pathway on T-cell cytotoxicity in a co-culture model. We have also collected samples of three target cell lines +/- LKB1 and treated with IFNG or control which are being subjected to global metabolic profiling to identify altered levels of metabolites representing products of the mevalonate pathway, as well as poly-unsaturated lipids and oxygenated lipids reflecting the effects of ferroptosis. If mevalonate pathway influences cytotoxicity in co-culture experiment this will be focus of mechanistic manuscript with target completion date of 12/1/2021.

Major Task 3: CRISPR-CAS9 screen to identifying IFN mediators

**Specific objectives**: To carry out a whole genome functional screen to identify modifiers of IFNG anti-proliferative response in LKB1 deficient cell lines.

**Major activities:**

- CRISPR-CAS9 whole genome screen for modifiers of IFNG response was completed in initial portion of grant in 2019.
- Further screening is deferred until cytotoxicity co-culture model is established.

**Future Directions**

Whole genome screen using H2023 cell line with LKB1 add-back is planned to identify modifiers of T-cell cytotoxicity in co-culture model.

Major Task 4: Pharmacologic screen to identify IFN mediators

*Based on preliminary analysis of the IFNG phenotype (Major Tasks 1 and 2) on which this screen was to be conducted, we concluded that the screen as originally designed had a low likelihood of success due to technical reasons. Further pursuit of this aim is deferred for the time being.*

*Percentage completion: 20%*

Major Task 5: Characterize gene expression and immune effectors in patients

*Milestone(s) Achieved: The clinical trial on which this aim is based has been completed. Sample identification and nucleotide extraction are being carried out (9/2020).*

*Percentage Completion: 75%.*

**Major activities:**

- Continued analysis of neoadjuvant pembrolizumab patients (TOP1501; NCT02818920) has proceeded as part of a collaborative effort to characterize immune responses in responders and non-responders as indicated in the initial proposal, with project funded by Merck.
- IRB and HRPO approval was obtained, analysis of TOP1502 untreated control specimens was not able to be completed within the study timeframe. Allocated funds for this analysis will be refunded as stipulated.
- Continued genomic analysis of TCGA and other large patient datasets has revealed unique profile of immune infiltrating cells in LKB1 deficient tumors, including a novel phenotype of strong plasma cell infiltration.
- Participated in bioinformatics challenge (DREAM challenge, Sage Biosystems) using genomic features of LKB1 loss, NRF2 activation, and neuroendocrine differentiation to predict patient outcomes in the Checkmate 026 study. The submitted model was found to be a ‘top performer’ in the challenge, allowing more detailed analyses and inclusion in manuscript in coming months.

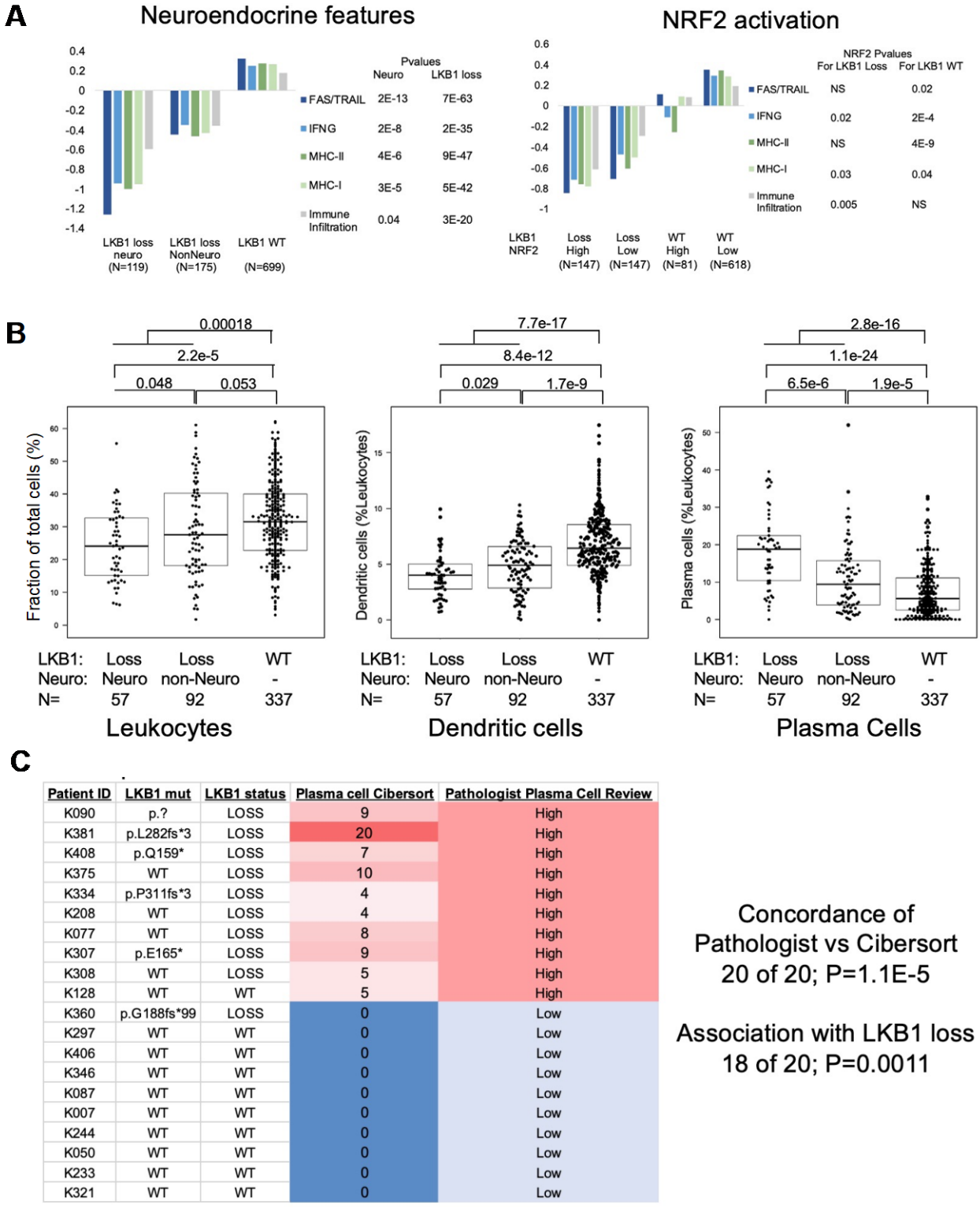
**Significant results:**

Both NRF2 activation and neuroendocrine features appear to modulate immune profiles associated with LKB1 loss (Fig 2). In particular the neuroendocrine subset appears to have especially decreased markers of immune activation and novel increase in plasma cell infiltration (Fig 2). A predictive model evaluating LKB1 loss and neuroendocrine phenotype was submitted to the ‘DREAM PD1 Challenge’, an open source competition for prediction of outcomes in the Checkmate 026 study, a randomized phase 3 study comparing first line nivolumab to chemotherapy, and was found to be a top predictor of immune outcomes, and will be included in the resulting manuscript.

We observe that this neuroendocrine subset is strongly enriched in 1) co-occurrence of LKB1 loss with ATM mutation 2) co-occurrence of LKB1 loss with focal amplification of 14q13 (containing lineage specific transcription factors FOXA1, TTF1, and PAX9). These pathways are likely important mechanistically for giving rise to their unique features.

We have established a collaboration with Dr. Doug Cress at Moffitt cancer center to evaluate immune infiltrating cell components and specifically evaluate plasma cell infiltration in these tumors. Thus far we have

validated our genomic findings, demonstrating that LKB1 deficient tumors have high plasma cell infiltration by pathology review.




C

| Patient ID | LKB1 mut | LKB1 status | Plasma cell Cibersort | Pathologist Plasma Cell Review |
|---|---|---|---|---|
| K090 | p.? | LOSS | 9 | High |
| K381 | p.L282fs*3 | LOSS | 20 | High |
| K408 | p.Q159* | LOSS | 7 | High |
| K375 | WT | LOSS | 10 | High |
| K334 | p.P311fs*3 | LOSS | 4 | High |
| K208 | WT | LOSS | 4 | High |
| K077 | WT | LOSS | 8 | High |
| K307 | p.E165* | LOSS | 9 | High |
| K308 | WT | LOSS | 5 | High |
| K128 | WT | WT | 5 | High |
| K360 | p.G188fs*99 | LOSS | 0 | Low |
| K297 | WT | WT | 0 | Low |
| K406 | WT | WT | 0 | Low |
| K346 | WT | WT | 0 | Low |
| K087 | WT | WT | 0 | Low |
| K007 | WT | WT | 0 | Low |
| K244 | WT | WT | 0 | Low |
| K050 | WT | WT | 0 | Low |
| K233 | WT | WT | 0 | Low |
| K321 | WT | WT | 0 | Low |

Concordance of Pathologist vs Cibersort
20 of 20; P=1.1E-5

Association with LKB1 loss
18 of 20; P=0.0011

**Figure 2. Neuroendocrine features are associated with potentiation of LKB1 immune exclusion phenotype.** A. Features of Neuroendocrine differentiation and NRF2 activity result in lower average expression of immune associated gene expression profiles. B. Neuroendocrine differentiation is associated with lower average immune infiltration and dendritic cells, and higher plasma cell infiltration. C. Plasma cell abundance estimated by Cibersort is concordant with blinded pathology assessment and strongly associated with LKB1 loss.

**Future directions:**

Continued collaboration with Moffitt as well as work continuing at Ohio State University will use immunofluorescence to evaluate plasma cell, T cell, B-cell and macrophage abundance across a panel of lung adenocarcinoma patients with known LKB1 status to further characterize the immune microenvironment and plasma cell abundance. Following this a manuscript will be prepared focused on heterogeneity of the NRF2 and neuroendocrine phenotypes within LKB1 loss and how it influences immune phenotypes – both interferon gamma signaling, as well as immune infiltrating phenotypes in these tumors. The clinical implication of the neuroendocrine subset as a predictive biomarker refining the immune refractory status of LKB1 deficient tumors will be evaluated through the DREAM challenge in both the Checkmate 026 and Checkmate 227 studies. This is a focus of ongoing translational work at OSU with evaluation of institutional immunotherapy outcome cohorts, and evaluation as a biomarker for immune response in clinical trials. These observations also form the basis of proposed preclinical and mechanistic studies including in vivo models, with grants thus far submitted to the International Lung Cancer Foundation, and OSU intramural Pelotonia Junior Investigator Award, and with plan for this to be the focus of upcoming K08 proposal.

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

- One on one work with mentor Kris Wood, PhD, to gain expertise in the application and analysis of high throughput functional genomics approaches such as CRISPR screens to understand oncologic phenotypes of interest.
- One on one work with mentor Neal Ready, MD, PhD, who is PI on the TOP1501 clinical trial, to gain clinical expertise in the treatment of NSCLC patients, design and execution of clinical trials in NSCLC, as well as understanding advances in translational approaches to immunotherapy.
- One on one work with mentor Scott Antonia, MD, PhD, providing guidance on translational research in immunotherapy. If staying on at Duke University for faculty, Dr. Antonia has agreed to take on a primary faculty mentoring role with me, providing further in-depth training and faculty development.
- Attended SITC annual meeting in November 2020.
- Presented findings at Duke Heme-Onc Grand Rounds
- Presenting findings as poster at SITC annual meeting 2021.
- After completing oncology fellowship at Duke, I have joined faculty as an assistant professor at the Ohio State University James Comprehensive Cancer Center, where I continue to work on projects directly stemming from these findings.

**How were the results disseminated to communities of interest?**

- Results were presented at IASLC Targeted Therapeutics in Lung Cancer 2020 in Santa Monica, CA at oral symposium under section ‘best fellow abstracts.’ Abstract entitled “Interferon Gamma Resistance in Setting of LKB1 Loss: Phenotypic Characterization and Investigation of Mechanism”
- Results are presented at SITC annual meeting 2021 as poster titled “Differentiation subgroups within LKB1-deficient lung cancer influence both the immune exclusion phenotype and cellular composition of the immune microenvironment.”
- Abstract was also submitted to ASCO annual meeting 2020 and was published electronically “Interferon gamma resistance in setting of LKB1 loss: Phenotypic characterization and investigation of mechanism.”
- Work was disseminated to our local oncology and immunotherapy colleagues at Duke at an oral presentation of Duke University Center for Cancer Immunotherapy seminar in September 2020.
- Work was presented to Duke oncology community at the Duke Oncology Fellowship Annual Research retreat at oral presentation January 2019 and January 2020.
- Work was presented to Duke Pharmacology and Cancer Biology graduate research program by poster at PCB annual retreat August 2019.

***4.*** **IMPACT:**

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

Patients with NSCLC who have loss of the LKB1 tumor suppressor have been shown to respond poorly to ‘Immune Checkpoint Inhibitors’ which are used for the treatment of most lung cancer patients. Understanding the various mechanisms of resistance that are caused by LKB1 loss will be crucial in order to develop treatment strategies to overcome this.

We have identified candidates that may play a role in causing resistance to immune attack in lung cancers with loss of LKB1. Specifically, we are evaluating a pathway known as 'ferroptosis' which represents an organized method of cell death that enhances immune recognition. LKB1 deficient cancers may be resistant to ferroptosis, which may be a key factor in avoiding effective anti-tumor immunity. Treatment approaches using drugs that sensitize these cancers to undergo ferroptosis may thus be a useful strategy which could be combined with immunotherapy. We are validating the effects of this pathway further and have obtained additional funding to perform more detailed analysis which we plan to include *in vivo* testing in mouse models.

**What was the impact on other disciplines?**
Results of this work, once fully completed, are likely to inform the understanding of how tumor cells evade surveillance and elimination by the immune system, with mechanisms that may be applicable to other tumor types beyond NSCLC and may be applicable to other genetic contexts beyond LKB1 and KEAP1 loss.

**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:

**Changes in approach and reasons for change**
Changes listed below were submitted in revised SOW 2020

- Included analysis of T-cell co-culture assays as a complementary approach to our proposed analysis of IFNG sensitivity phenotypes. This represents an improved surrogate measure of immune resistance phenotypes.
- Addition of ferroptosis and Hippo/YAP experiments to candidate pathways in Major Task 2. This is a clear extension to validate preliminary candidates from functional genomics screen in Major Task 3.
- Anticipated change to perform additional whole genome CRISPR screen using T-cell co-culture assay phenotype, rather than sub-genomic targeted library CRISPR screen on IFNG phenotype as described in Major Task 3.
- Elimination of pharmacologic inhibitor library (Major Task 4) to evaluate alterations to IFNG sensitivity suggested that experimental variability in the IFNG phenotype would lead to high likelihood of false positive and false negative results that would be difficult to resolve. We plan to instead conduct further functional genomics screens using complementary immune associated phenotypes, in particular immune co-culture.
- Inclusion of both treated and untreated clinical trial specimens in Major Task 5.

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

- Delay of eight weeks of lost laboratory time due to Covid institutional shut down, from March to May 2020. Restrictions on laboratory use due to social distancing remain in place, allowing productive advances.
- Submission of IRB proposal for analysis of clinical trial specimens (Major Task 5) was delayed with coordination of analysis across multiple collaborators and securing complementary funding to allow in depth characterization of these tumors (funding finalized July 2020 from Merck Investigator Studies Program). We are in final process of revisions for IRB submission which we anticipate will be submitted by October 9, 2020.

**Changes that had a significant impact on expenditures**

- Clinical trial specimens (TOP1501) initially proposed for analysis were instead analyzed through different funds through Merck Investigator Studies Program. Unused funds were returned to DOD at the end of the award period.

**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 or care of vertebrate animals**

Nothing to report.

**Significant changes in use of biohazards and/or select agents**

Nothing to report.

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **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**.
  - Kaufman, J. "Interferon Gamma Resistance in Setting of LKB1 Loss: Phenotypic Characterization and Investigation of Mechanism" IASLC Targeted Therapeutics in Lung Cancer, Santa Monica CA, 2020. Oral symposium. National Meeting.
  - Kaufman, J. "Interferon gamma resistance in setting of LKB1 loss: Phenotypic characterization and investigation of mechanism." ASCO 2020 Annual Meeting. E-abstract e21015. National Meeting
  - Kaufman, J. "Differentiation subgroups within LKB1-deficient lung cancer influence both the immune exclusion phenotype and cellular composition of the immune microenvironment" SITC 2021 Annual Meeting.

- **Website(s) or other Internet site(s)** Nothing to report

- **Technologies or techniques**
  - We developed a novel approach that we term 'Essentiality Enrichment Analysis' that leverages publicly available CRISPR dependencies from the Broad Institute Dependency Map (DEPMAP) to identify phenotypic clusters of genes that are statistically enriched in our phenotypic screen. Functionally related sets of genes, or 'phenotypic clusters' are identified based on statistical similarity in their CRISPR dependency phenotypes in DEPMAP, and these clusters are then cross-referenced with our candidate gene lists to identify clusters that are enriched – statistically over-represented – among our results. A representation of enriched phenotypic clusters is shown (Fig 7). Description of this technique and code to allow its application to other projects will be included in future publication.

- **Inventions, patent applications, and/or licenses** Nothing to report.

- **Other Products**
  Sequencing data with barcode abundances from analysis genomics screens will be made publicly available at time of publication.
  RNAseq expression data from diverse experiments will be made publicly available at time of publication.

**7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

**What individuals have worked on the project?**

*Name:* Jacob Kaufman
*Project Role:* PI
*Researcher Identifier (e.g. ORCID ID):* 0000-0001-9779-7228

*Nearest person month worked:* 12

*Contribution to Project:* Designed and carried out all experimental approaches and analyses described herein.

*Funding Support:* Duke House Staff
Allin Family Fellowship
DOD LCRP CA
SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship

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

SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship awarded $100,000 from July 2020 to June 2021 at 75% effort.

After completion of fellowship, Dr. Kaufman has joined faculty as an assistant professor at the OSU James Cancer Center in the thoracic oncology group. Salary is currently supported with 25% clinical effort and 75% research effort, and research efforts are supported with faculty startup package of $200,000 annually for the first five years of his position.

**What other organizations were involved as partners?**
National Cancer Institute, Bethesda MD
Collaborator Nicholas Restifo and post-doc Rigel Kishton collaborated to carry out pilot project of T-cell coculture assay shown in figure 3, and gave gift of plasmids for viral NY-ESO expression constructs as well as T-cell receptor specific for NY-ESO.

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** *Not Applicable.*

**QUAD CHARTS:** *Not applicable*

## 9. APPENDICES: *Jacob Kaufman Curriculum Vitae is attached*

# Jacob M. Kaufman, MD, PhD

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Jacob.kaufman@osumc.edu

## EDUCATION

**Duke University**, Durham, NC **2017-2021**
* Fellow, Division of Hematology and Oncology

**Duke University**, Durham, NC **2015-2017**
* Internal Medicine Residency Program

**Vanderbilt University School of Medicine**, Nashville, TN **2004-2015**
* M.D., Medical Scientist Training Program

**Vanderbilt University School of Medicine**, Nashville, TN **2007-2013**
* Ph.D., Cancer Biology

**Vanderbilt University**, Nashville, TN **2000-2003**
* B.S., Chemistry

## PERSONAL STATEMENT

I am committed to a career focused on basic and translational research to improve the treatment of lung cancer. Specifically, I have applied integrated multi-omics analyses and various experimental approaches to characterize gene expression profiles, signaling pathways, and drug sensitivity patterns exhibited by lung adenocarcinomas that have lost the LKB1 tumor suppressor. This gene, a serine-threonine kinase also known as STK11, is lost in approximately 30% of lung adenocarcinomas and has recently been shown to confer clinical resistance to immunotherapy. My current work applies high throughput functional genomics approaches to model systems of LKB1 loss with the goal of 1) identifying novel clinical targets for LKB1 deficient lung cancer, and 2) discover mechanisms of resistance to immune checkpoint inhibition in this subset of tumors.

I completed my Ph.D. in Cancer Biology in 2013 under the mentorship of David Carbone, and after finishing my medical training at Vanderbilt, I completed residency and fellowship at Duke University Medical Center through the ABIM research track pathway (PSTP). During the three research years of my fellowship I gained expertise in functional genomics approaches to inform translational and mechanistic questions in cancer biology.

I am excited now to have joined faculty at the James Cancer Center at Ohio State University as an assistant professor. Here I will again work closely with Dr. Carbone, who will serve as my primary clinical and research mentor, and I expect to gain tremendously from collaborations with leaders in their fields across our institution.

## POSITIONS AND HONORS

Positions

| | |
|---|---|
| 2021-current | Assistant Professor, OSU CCC James Cancer Center, Thoracic Oncology |
| 2017-2021 | Oncology Fellow, Duke Hematology and Oncology |
| 2015-2017 | Resident Physician, Duke Internal Medicine |

Honors

| | |
|---|---|
| 2019 | North Carolina Oncology Association; Outstanding Fellow |
| 2019 | Best Poster, co-Recipient; Duke Cancer Biology Research Retreat |
| 2019 | Best Presentation by Second Year Fellow; Duke Fellows Research Retreat |
| 2018-2021 | Recipient of Allin Family Fellowship; providing salary support for years 2-3 to a selected Oncology fellow nominated for his or her exceptional research potential. |
| 2016-2018 | Lefkowitz Society Research Award – For outstanding contributions to research and commitment to career as physician scientist received funding to hire research technician and supplies to pursue translational research project while in clinical training in residency and fellowship. |
| 2015 | Rudolph Kampmeier Prize in Medicine – For outstanding contributions in research as a medical student. |
| 2020 | IASLC Targeted Therapeutics in Lung Cancer Annual Meeting – Best Abstract, co-Recipient |
| 2020 | SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship |

## SCIENTIFIC CONTRIBUTIONS

### Peer Reviewed Publications

1. Koenig MJ, Agana B, **Kaufman JM**, Sharpnack M, Amann JM, Wysocki V, Oakes C, Carbone DP. LKB1 loss leads to global hypomethylation and altered FOXA binding in lung adenocarcinoma. *Cancer Research* (Submitted).
2. **Kaufman JM**, and Stinchcombe TE. Treatment of KRAS-Mutant Non-Small Cell Lung Cancer: The End of the Beginning for Targeted Therapies. *JAMA* 2017; **317**, 1835-1837. Review.
3. **Kaufman JM**, Yamada T, Park K, et al. A Transcriptional Signature Identifies LKB1 Functional Status as a Novel Determinant of MEK Sensitivity in Lung Adenocarcinoma. *Cancer Res* 2017; **77**, 153-163
4. Whang YM, Park SI, Trenary IA, Egnatchik RA, Lee C, **Kaufman JM**, Carbone DP, Young JD. LKB1 deficiency enhances sensitivity to energetic stress induced by erlotinib treatment in non-small cell lung cancer (NSCLC) cells. *Oncogene* 2016; **35**, 856-66.
5. **Kaufman JM**, Amann JM, Park K, Arasada RR, Li H, Shyr Y, Carbone DP. LKB1 Loss induces characteristic patterns of gene expression in human tumors associated with NRF2 activation and attenuation of PI3K-AKT. *J Thorac Oncol* 2014; **9**, 794-804.
6. **Kaufman JM**, Iams W, Puzanov I. The inhibition of PD1 and PD-L1 immune checkpoints in the treatment of malignant melanoma. *J Targeted Therapies Cancer* 2014. Review
7. Kikuchi T, Hassanein M, Amann JM, Liu Q, Slebos RJ, Rahman SM, **Kaufman JM**, Zhang X, Hoeksema MD, Harris BK, Li M, Shyr Y, Gonzalez AL, Zimmerman LJ, Liebler DC, Massion PP, Carbone DP. In-depth proteomic analysis of nonsmall cell lung cancer to discover molecular targets and candidate biomarkers. *Mol Cell Proteomics* 2012; **11**, 916-32.
8. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; **489**, 519-25.
9. Samanta D, **Kaufman JM**, Carbone DP, Datta PK. Long-term smoking mediated down-regulation of Smad3 induces resistance to carboplatin in non-small cell lung cancer. *Neoplasia* 2012; **14**, 644-55.

### Book Chapters

1. **Kaufman JM**, Horn L, Carbone D. Molecular Biology of Lung Cancer. Cancer: Principles and Practice of Oncology, 9e. DeVita, ed. (Lippincott, Williams, & Wilkins; USA), 2011, 789-798.
2. **Kaufman JM**, Carbone D. Molecular Profiling for Early Detection and Prediction of Response in Lung Cancer. Lung Cancer 3e. Roth, ed. (Wiley-Blackwell; USA), 2008.

### Selected Presentations

1. **Kaufman JM**, Ready NE, Wood KC. Interferon gamma resistance in setting of LKB1 loss: phenotypic characterization and investigation of mechanism. IASLC Targeted Therapeutics in Lung Cancer Meeting, 2020. Oral mini-symposium.
2. **Kaufman JM**, Lowe Cindy, Harris B, Boyd K, Amann JA, Eisenberg R, Carbone DP, Massion PP. Dysregulation of lung developmental pathways associated with LKB1 loss in lung cancer. Annual meeting of the Am Assoc Cancer Res, 2016. Poster.
3. **Kaufman JM**, Amann JM, Park K, Li H, Shyr Y, Carbone DP. LKB1 loss leads to activation of the CREB transcription factor and sensitivity to MEK inhibition in human lung cancer. Annual meeting of the Am Assoc Cancer Res, 2013. Oral mini-symposium.
4. **Kaufman JM**, Amann JM, Park K, Li H, Carbone DP. Loss of LKB1 induces a characteristic gene expression pattern and causes sensitivity to MEK inhibition in human tumors. TCGA lung adenocarcinoma meeting, 2012. Oral presentation.
5. **Kaufman JM**, Taguchi F, Kikuchi T, Girard L, Shyr Y, Wistuba I, Minna J, Carbone D. Prediction of Response to Chemotherapy in Non-Small Cell Lung Cancer. Annual meeting of the Am Assoc Cancer Res, 2008. Oral mini-symposium.

## RESEARCH SUPPORT

**Completed:**

2020/07/01-2021/06/30: SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship "Strategies to overcome immunotherapy resistance in LKB1 and KEAP/NRF2 mutated NSCLC: modulating ferroptosis to restore immune sensitivity."
Jacob Kaufman (PI).
This project will evaluate the resistance to ferroptotic cell death as a mechanism for intrinsic resistance to anti-tumor immunity, and explore modifiers of ferroptosis as potential means to restore sensitivity.

2020/02/01-2021/02/01: Correlative Science Analysis Merck MISP study 52567/TOP1501 (NCT02818920): "Immunophenotyping of Blood and Tumor, and Tumor Genomic Profiles to Understand Mechanisms of Primary Resistance for Early Stage Non-small Cell Lung Cancer Treated with Neoadjuvant Pembrolizumab"
Neal Ready (PI)
This project will perform correlative analysis on NSCLC patients treated with neoadjuvant pembrolizumab prior to surgical resection. Planned analyses include immunephenotyping with high dimensional flow cytometry, single cell RNAseq analysis, and genomic characterization of gene expression profiles and somatic mutations. Dr. Kaufman will plan and coordinate the genomic characterization and analysis, and integration of these data with immune and ssRNAseq results.

2019/08/15-2020/08/14: Department of Defense Lung Cancer Research Program Concept Award LC180633, "Mechanisms of Immune Checkpoint Resistance Mediated by LKB1 Tumor Suppressor in Lung Cancer"
Jacob Kaufman (PI).
This project aims to understand how LKB1 loss in lung cancer alters the response to and interactions with the immune system to induce immune evasion and resistance to immune checkpoint inhibition. Dr. Kaufman is responsible for all study design, laboratory experimentation, and interpretation of results. This work is being carried out in the physical laboratory of Kris Wood, who serves as Dr. Kaufman's research mentor.

2018/07/01-2021/06/30: Allin Family Fellowship Award.
"Effect of liver kinase B1 (LKB1, or STK11) tumor suppressor loss on pathway dependence and drug sensitivity phenotypes in non-small cell lung cancer."
The goal of the project is to understand the molecular drivers of LKB1 deficient lung cancer in order to identify novel treatment approaches. Dr. Kaufman serves as PI of projects detailed below, with the Allin Family Fellowship providing 100% salary support to allow Dr. Kaufman to pursue his research aims throughout fellowship.

2016/07/01-2018/05/30: Duke University Medical Center Lefkowitz Society Research Award.
"Effect of liver kinase B1 (LKB1, or STK11) tumor suppressor loss on pathway activation and differentiation phenotypes in non-small cell lung cancer."
Dr. Kaufman (Co-PI); Kris Wood (Co-PI)
Goal of this project was to identify mechanisms by which LKB1 loss influenced gut-like and neuroendocrine differentiation phenotypes within lung cancer and how these effects lead to specific targetable pathway dependencies. Dr. Kaufman and Dr. Wood served as co-PIs of this study. Dr. Kaufman was primarily responsible for study design and interpretation of results and met with research technician on a weekly to every other week basis.

2018/07/01-2019/06/30: North Carolina Lung Cancer Initiative Research Fellows Grant,
"Systematic characterization of pathway dependencies in LKB1 deficient lung cancer."
Jacob Kaufman (PI).
The goal of the project is to understand the molecular drivers of LKB1 deficient lung cancer in order to identify novel treatment approaches. Dr. Kaufman is responsible for all study design, laboratory experimentation, and interpretation of results. This work is being carried out in the physical laboratory of Kris Wood, who serves as Dr. Kaufman's research mentor.