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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

LKB1	Functional Genomics	KEAP1	Lung Cancer
Immunotherapy	NRF2	CRISPR	Ferroptosis

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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: 50%, with ongoing 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: 50%, with ongoing 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: 50%, 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: 25%.

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:

- Stably expressed LKB1 into panel of cell lines (A549, H2023, H1944, H2122)

- Evaluated effect of LKB1 on IFNG-induced gene expression by RNAseq (A549, H1944, H2023, in parental and LKB1 add-back state)
- Evaluated effect of LKB1 on IFNG growth inhibition (A549, H1944, H2023, H2122 in parental and LKB1 add-back state)
- Evaluated effect of LKB1 on T-cell mediated cytotoxicity (H2023)
- Evaluated effect of LKB1 and IFNG on key immune signaling components

Significant results:

1) Transcriptomic analysis: Over-expression of wild-type LKB1 leads to induction of a key immune associated genes in the baseline state (Fig 1A) and alters the expression patterns of key immune associated genes after IFNG treatment (Fig 1B). Additional data beyond figure 1 include baseline transcriptomic data for H2122, A549, H1944, and H2023 for control vs LKB1 add-back, and RNAseq transcriptomic data for A549, H1944, and H2023 after treatment with exogenous IFNG.

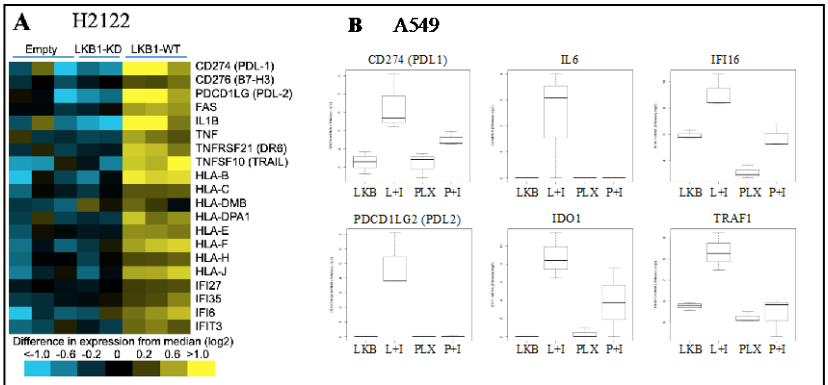

Figure 1: Evaluation of effect of LKB1 add-back on expression of key immune associated genes. A) Selected gene expression in H2122 cell line with vector control or stable transduction of either kinase-dead LKB1 or wild-type LKB1. B) Selected gene expression in A549 cell line with vector control or stable transduction of wild-type LKB1 expression, with or without exogenous IFNG.

2) Effect of LKB1 on IFNG growth inhibition: Over-expression of wild-type LKB1 potentiates antiproliferative effects of IFNG (Fig 2). In four cell lines, IFNG had relatively little effect on cell growth in empty vector and LKB1 KD control cells, but significantly reduced cell growth after LKB1 add-back.

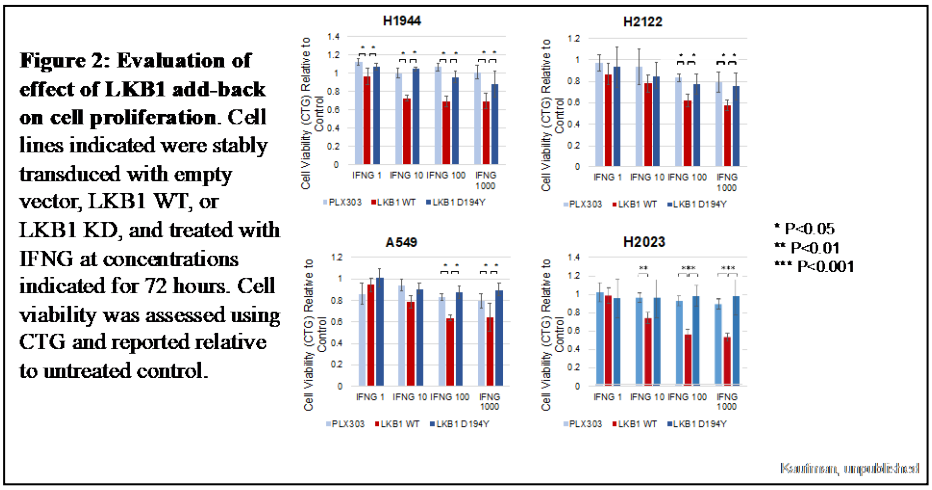

Figure 2: Evaluation of effect of LKB1 add-back on cell proliferation. Cell lines indicated were stably transduced with empty vector, LKB1 WT, or LKB1 KD, and treated with IFNG at concentrations indicated for 72 hours. Cell viability was assessed using CTG and reported relative to untreated control.

3) Effect of LKB1 on T-cell mediated cytotoxicity: LKB1 derivatives of H2023 cell line (HLA-A2 haplotype) were sent to the lab of collaborator Nicholas Restifo at NCI, where they were further transduced to express the NY-ESO antigen and co-cultured with NY-ESO specific CD8 T-cells derived from three different anonymous donors. In each of three experiments using three different donor CD8 T-cells LKB1 was observed to significantly potentiate anti-tumor cytotoxicity as measured by flow cytometry. While measurement of T-cell responses suggested induction of an activated phenotype to similar extent across the three cell line derivatives (data not shown), the anti-tumor cell cytotoxicity was significantly enhanced by LKB1 expression in each case (Fig 3).

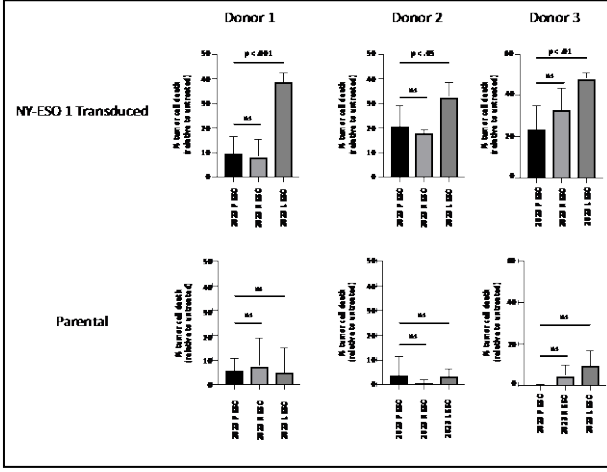

Figure 3: LKB1 re-expression in H2023 cell line enhances T-cell mediated anti-tumor immunity. Stable LKB1 add-back derivatives were transduced with NY-ESO-1 antigen; parental or NY-ESO expressing cells were then co-cultured for 12 hours with CD8 T-cells from three donors transduced with anti-NY-ESO specific TCR. Cell death measured by flow cytometry using Live/Dead marker (Thermo). (In collaboration with R. Kishton and N. Restifo; NCI)

4) Effect of LKB1 and IFNG on key immune signaling components: LKB1 derivatives of cell lines A549, H1944, and H2122 were treated with IFNG and assayed for expression of key immune signaling components. We observed increased expression of STING and PDL1 in each cell line tested, consistent with previously published work (Barbie); PDL1 expression assessed by flow cytometry is shown (Fig 4). H1944 cell line was further characterized by western blot and showed significant induction of FAS, TRAIL, and NLRP3.

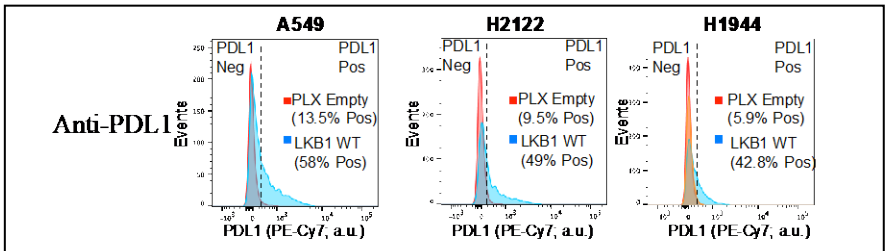

Figure 4: LKB1 re-expression in induces PDL1. Stable LKB1 add-back derivatives were assayed for PDL1 expression by flow cytometry using PE-Cy7 conjugated anti-PDL1 antibody. Percent positive cells are indicated.

Conclusions: Taken together, these results demonstrate that our model system recapitulates key features associated with LKB1 loss in patient tumors: with 1) decreased expression of a variety of genes/proteins essential for promoting effective anti-tumor immunity including immune effector proteins, innate immune 'pattern recognition' machinery, etc 2) reversion of these phenotypes downstream of wild-type LKB1 expression, 3) enhanced anti-tumor effects of IFNG, and 4) enhanced cytotoxic anti-tumor immunity. Our RNAseq experiments represent a significant achievement and demonstrate that a core set of interferon driven genes are induced by IFNG regardless of LKB1 status, while there are a range of context specific IFNG-induced genes that are significantly expressed only the context of wild-type LKB1. Further efforts are to integrate these findings with genomic analysis of LKB1 and immune associated genes e.g. from TCGA, and to integrate these findings with results from genome wide functional screens to identify candidates that show differential IFNG induction in the setting of LKB1 and may be mechanistically important in conferring immune sensitivity or resistance.

Future directions: Work thus far has centered on experiments re-expressing LKB1 in cell lines. Ongoing work will explore the role of KEAP1/NRF2 in modulating these phenotypes. Effects of NRF2 are implicated by the results of the whole genome screen conducted in Major Task 3, namely a role of detoxification of ROS and lipid peroxidation products in promoting resistance to IFNG effects. The cell lines used thus far exhibit constitutive activation of NRF2, but experiments to decrease NRF2 activity are confounded by cellular dependence on this pathway. We are pursuing an alternative approach of activating NRF2 constitutively in KEAP1 wild-type cells and evaluating for an induction of IFNG and immune resistance. A key change for future experimentation in these aims will be the inclusion of T-cell co-culture assays, as shown in figure 3, which will complement our findings pertaining to IFNG and represent a better in vitro model that captures more of the complexities of anti-tumor immunity relevant to clinical outcomes. Overall, we estimate this aim to be 50% completed.

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:

- Evaluated change in IFNG sensitivity phenotypes across LKB1 add-backs and controls of three cell lines: A549, H1944, and H2122
- We tested effects of phenformin, rapamycin, trametinib, paclitaxel, and MLN0128. Cells were cultured in the combination of IFNG and each of the listed drugs at multiple concentrations and effect on cell viability was determined using CTG assay.
- Based on results from functional screen in Major Task 3, we identified additional candidates pertaining to ferroptosis and the Hippo/LATS pathway and are testing drugs and genetic perturbations to these pathways using approaches analogous to the experiments proposed for the initial panel of drugs.

Significant results:

1) None of the tested candidates that had been prespecified in the project narrative significantly improved IFNG sensitivity in the LKB1 deficient cells tested. Possible explanations include 1) context specificity – that other cell lines, for instance KEAP1 wild-type lines without NRF2 activation – may exhibit sensitization to IFNG in the setting of specific pathway inhibition, 2) the inherent effect size of altered sensitivity may be small relative to the noise in the assay, 3) the pathways and inhibitors specified to be of interest may have no effect on IFNG signaling.

Overall 50% of the initial experiments proposed have been completed with analysis of the effects of drugs on IFNG sensitivity carried out in the LKB1 deficient and LKB1 add-back setting, but not yet with follow up experiments in which KEAP1 is manipulated.

2) Rather than continue evaluation of the initial pathways specified a priori, we plan to focus further candidate based testing on the evaluation of additional pathways of interest, namely ferroptosis and the Hippo/YAP pathway, which were implicated in the regulation of tumor immune response on the basis of our functional genomics screen conducted in Major Task 3. LATS1/2 has previously been shown to confer immune resistance (Moroishi et al, “The Hippo Pathway Kinases LATS1/2 Suppress Cancer Immunity”, Cell 2016), but has not been shown to be dysregulated in LKB1 deficient tumors. Ferroptosis has similarly been shown to be functionally important in determining immune response (Lang et al, “Radiotherapy and Immunotherapy Promote Tumoral Lipid Oxidation and Ferroptosis via Synergistic Repression of SLC7A11,” Cancer Discovery 2019; and Wang et al, “CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy”, Nature 2019).

3) Evaluated effect of LKB1 and IFNG on ferroptosis/lipid peroxidation: based on results of whole genome CRISPR-CAS9 screen implicating a role of ferroptosis in regulating sensitivity to IFNG, we performed initial validation experiments showing that LKB1 add-back enhances sensitivity to the ferroptosis inducer RSL-3 (Fig 5a). Treatment of LKB1-expressing cells with IFNG significantly induced ferroptosis, with increased lipid peroxidation as measured by flow cytometry using the BODIPY™ 581/591 C11 dye assay (Fig 5b). Further, in H810, H2286, and A549 cells (but not H1755 or H1395), treatment with RSL3 and IFNG together demonstrated synergistic effects on cell growth (Fig 5c).

We have also conducted initial experiments with shRNA and CRISPR mediated knock-out of key pathway members NRF2, SLC7A11, and GPX4 – however, these experiments were complicated by significant reduction in cell viability with both shRNA and CRISPR targeting of these genes, which prevented our evaluation of IFNG effects as planned.

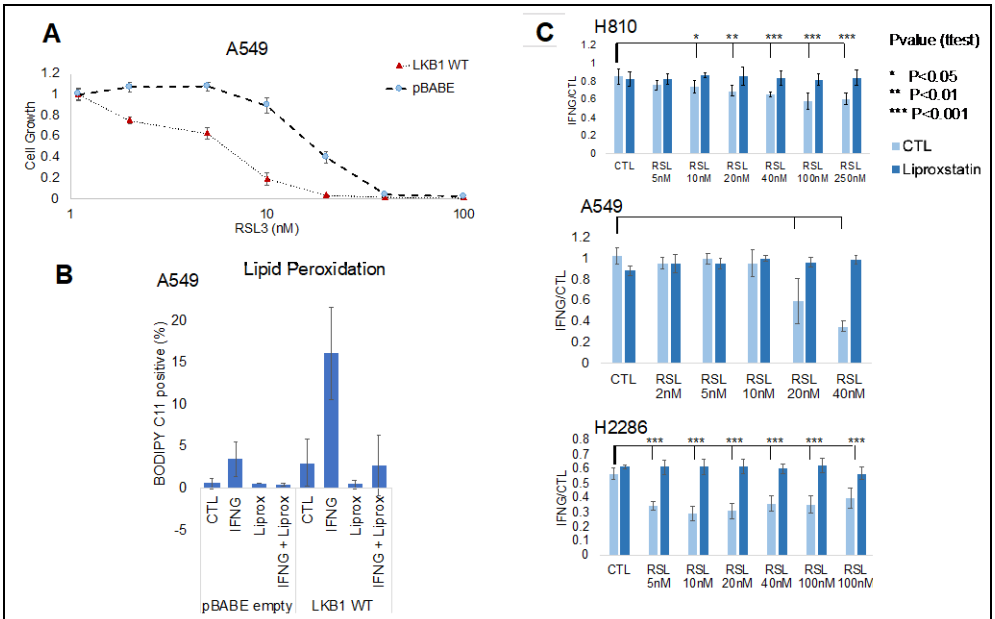

Figure 5: LKB1 and IFNG jointly influence susceptibility to ferroptosis. A) Sensitivity to ferroptosis inducer RSL3 was tested in control and LKB1 add-back derivative of A549 using CTG assay. B) Lipid peroxidation induced by 48h treatment with IFNG was assessed by flow cytometry using the BODIPY™ 581/591 C11 assay. C) Parental LKB1 mutant lines H810, A549, and H2286 were treated with IFNG 10U/ml and RSL3 at concentrations listed in the absence or presence of ferroptosis inhibitor liproxstatin (200 nM). Cell proliferation is determined using CTG assay and reported as fraction of IFNG treated vs control.

4) We have also conducted preliminary experiments testing the effects of the Hippo/LATS pathway on IFNG sensitivity. We have used shRNA targeting NF2 (Merlin) and LATS1/2, and small molecule inhibitors of the LATS1/2 kinases, as well as CRISPR-mediated knockout of the NF2 gene, and tested effects on IFNG response using CTG assay to assess proliferation, and RNAseq to assess IFNG-driven changes in mRNA expression. Initial experiments were conflicting, with modest induction of IFNG sensitivity observed using small molecule LATS1/2 inhibitors, as well as with NF2 shRNA constructs, but without reproducibility across multiple cell lines (data not shown).

Conclusions

The initial pathway inhibitors that we specified to be of interest, phenformin (mitochondrial electron transport chain), rapamycin (mTORC1), trametinib (MEK), paclitaxel (microtubules; cytotoxic), and MLN0128 (mTORC1/2), were found to have no significant effect on resistance to IFNG in the setting of LKB1 loss. However, evaluation of candidates identified in Major Task 3 appear more promising. In particular, we observe that 1) restoring LKB1 in the A549 cell line leads to sensitization of this cell line to the effects of ferroptosis (Fig 5a), 2) IFNG significantly induces ferroptosis in the LKB1 add-back setting, with much less effect seen in LKB1-loss control, and 3) IFNG has synergistic anti-proliferative effects when combined with the ferroptosis inducer RSL3.

Future Directions

Given the initial negative results from testing the panel of inhibitors, we do not plan to evaluate these further at this time. Rather, we revise this aim to perform further candidate-based evaluation on the effects of LKB1 and NRF2 in regulating ferroptosis and thereby influencing susceptibility to immune attack. To support more detailed evaluation, a proposal based on this concept was submitted to the SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship, which was awarded funding for 2020-2021. Further work will include evaluation of ferroptosis in different cellular contexts including manipulation of both LKB1 and KEAP1/NRF2 pathways, and evaluation of the interplay of ferroptosis with immune sensitivity using immune co-culture assays (as in Fig 3) and in vivo work funded through the SITC fellowship.

<u>Major Task 3: CRISPR-CAS9 screen to identifying IFN mediators</u>

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:

- Conducted a whole genome CRISPR-CAS9 screen in duplicate using the A549 LKB1 mutant cell line and isogenic derivative with LKB1 add-back in the presence of absence of IFNG (10 U/mL).
- Processed screen with nested PCR to amplify 'bar code' regions containing CRISPR guide target sequences.
- Employed high throughput sequencing approaches to quantify the bar-coded regions.
- Applied standard statistical approaches to deconvolute results and determine ranked candidate lists on basis of effect size.
- Developed novel statistical analysis to identify enriched pathways within these candidate lists by integrating results with large scale publicly available CRISPR phenotypic screens (Broad Institute DEPMAP; Ghandi et al, "Next-generation characterization of the Cancer Cell Line Encyclopedia." Nature 2019).
- Obtained funding for follow up study on ferroptosis mechanism identified through results of CRISPR screen
- Presented results at oral presentation of national meeting "Translational Therapeutics in Lung Cancer" of the IASLC in Santa Monica, CA, 2020; selected as one of five "best fellows' abstracts."

Significant results:

We carried out the CRISPR-CAS9 screen as proposed, using the A549 vector control and LKB1 add-back control lines (Fig 6). This was used instead of the H1944 cell line because of rapid growth rates and facile transduction with the CRISPR gDNA library. The TKOv3 library was used (Hart et al, "Evaluation and Design of Genome-Wide CRISPR/SpCas9 Knockout Screens" G3 (Bethesda), 2017), which targets ~19,000 genes with ~77,000 gDNA probes, typically targeting each gene with four independent probes. Cells were maintained at population sizes corresponding to >500X library coverage throughout the experiment. Four pellets were processed for each cell derivative to comprise one experimental replicate: these pellets were from T_0 (two days after puromycin), T_1 (six days after puromycin, at time of addition of IFNG), and $T_{final\text{-}CTL}$ and $T_{final\text{-}IFNG}$ representing cumulative effects of proliferation for control cells and IFNG-treated cells after 8-10 cell doublings. Two replicates were carried out for both the A549 vector control and the LKB1 add-back derivative, for a total of 16 samples sent for sequencing and analysis. Samples were analyzed using two sequencing lanes on Illumina NextSeq 500 High-Output instrument (75bp SR). Runs performed within specifications and the total library coverage for all specimens was >200X.

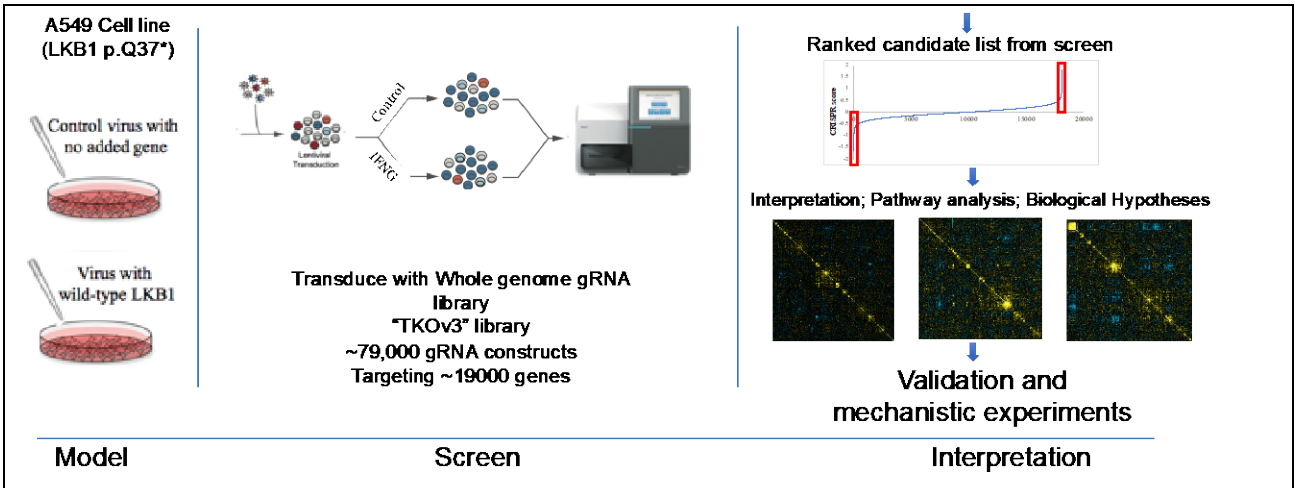

Figure 6: Schema representing whole genome functional screen for modifiers of IFNG response

To identify candidate genes that may affect IFNG response, the normalized barcode abundance was compared between the $T_{final\text{-}CTL}$ and $T_{final\text{-}IFNG}$ samples for each cell line derivative and replicate. In the LKB1 deficient state, we focused on genes and pathways that were implicated in sensitizing the to the effects of IFNG. Conversely, for LKB1 add-back we ranked genes according to their ability to either sensitize to IFNG, or to induce IFNG resistance. For purposes of pathway analysis, we evaluated the top 400 to 500 ranked genes for each of these analyses. In addition to standard pathway analysis approaches, 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).

We focus our initial attention on candidate genes that result in the sensitization of the IFNG resistant LKB1 mutant state, as these candidates may include therapeutic targets of interest to overcome immune resistance associated with LKB1 loss. Essentiality Enrichment Analysis divulged three sets of targets are statistically enriched among our results: 1) Genes comprising the Hippo/LATS pathway (Fig 8a), 2) a cluster of genes associated with cellular metabolism including several members of purine biosynthetic pathway, and 3) genes regulating ROS and lipid peroxide detoxification (Fig 8b). The detoxification of lipid peroxides prevents ferroptotic cell death and has clear links to the KEAP1/NRF2 ROS detoxification pathway. Genes in this pathway that scored as candidates in our screen include the key ferroptosis inhibitor GPX4, both members of the xCT cysteine-glutamate transporter – SLC7A11 and SLC34A2 – and four enzymes involved in the

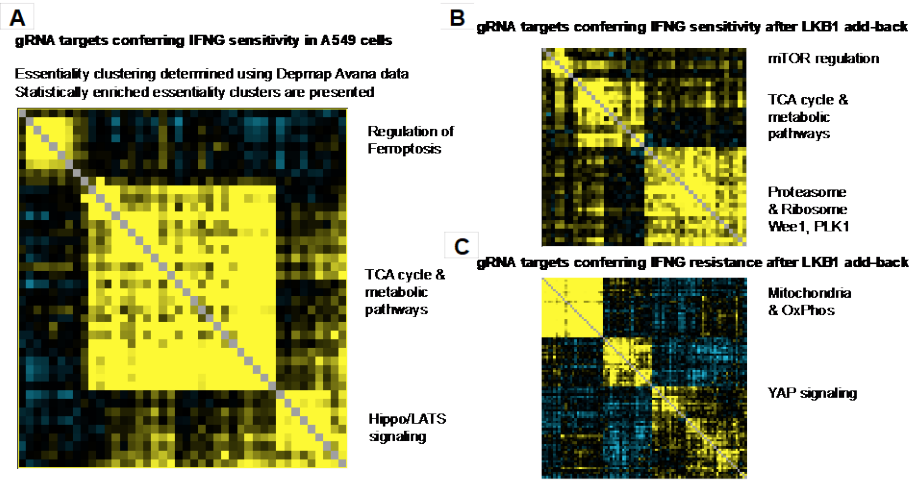

Figure 7: Essentiality Enrichment Analysis for screen candidate genes implicated in modifying IFNG response in A549 cell line. The top 400-500 candidate genes were ranked according to standard approaches. Essentiality Enrichment Analysis was then applied to each list of candidate. A) Functionally enriched gene clusters in which gRNA targets were depleted in LKB1 deficient A549 cells after IFNG treatment, suggesting a sensitization to IFNG. B) Functionally enriched gene clusters in which gRNA targets were depleted in A549 LKB1 add-back cells after IFNG treatment, suggesting a sensitization to IFNG. C) Functionally enriched gene clusters in which gRNA targets were enriched in A549 LKB1 add-back cells after IFNG treatment, suggesting induction of IFNG resistance. Each row and column represents a single gene, with matrix colored to show the degree of statistical similarity in DEPMAP between gene pairs, thus representing log10 transformed p-values after linear regression of dependency data.

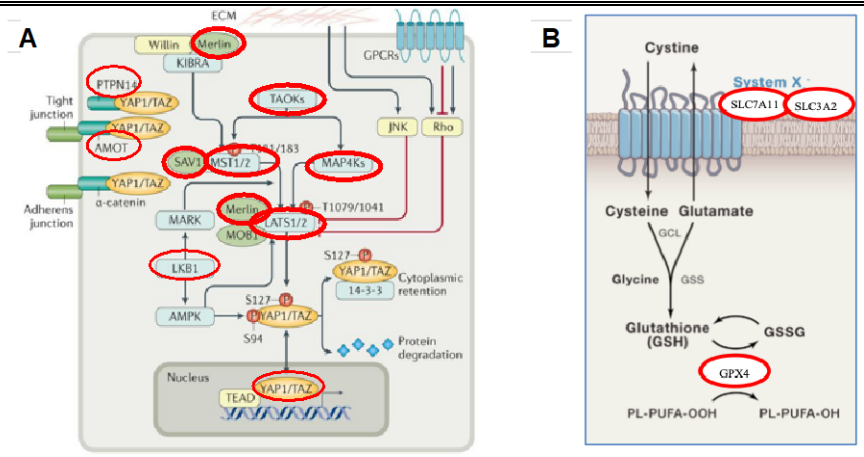

Figure 8: Schema representing functionally enriched candidate pathways representing potential IFNG resistance pathways implicated by functional genome wide screen of IFNG modifiers. A) The Hippo/LATS/YAP pathway shows functional enrichment among the screen with significant hits from the screen highlighted in red. B) GPX4 detoxification of lipid peroxidation products is also implicated, with significant hits including the xCT transporter, GPX4, and proteins involved in selenium processing and maturation of the GPX4 protein.

processing of the essential selenocysteine moiety essential for GPX4 function – SECISBP2, EEFSEC, PSTK, and SEPSECS. Ferroptosis has recently been shown to represent an immunogenic form of cell death and

modifiers of ferroptosis had significant effects on anti-tumor immunity in murine models (Lang et al, "Radiotherapy and Immunotherapy Promote Tumoral Lipid Oxidation and Ferroptosis via Synergistic Repression of SLC7A11," Cancer Discovery 2019; and Wang et al, "CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy", Nature 2019). Thus, we chose to focus on GPX4 mediated resistance to ferroptosis as a mechanistic hypothesis that may contribute to immune resistance in LKB1 and KEAP1 deficient tumors. Preliminary validation of this candidate pathway was shown in figure 5, and to further support more detailed evaluation we submitted a proposal entitled "Strategies to overcome immunotherapy resistance in LKB1 and KEAP/NRF2 mutated NSCLC: modulating ferroptosis to restore immune sensitivity" to SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship, which was awarded funding for 2020-2021.

Conclusions

We have successfully conducted our initial whole genome phenotypic screen for modifiers of IFNG response using paired isogenic derivatives of the A549 cell line in which the LKB1 mutant state exhibits IFNG resistance and the LKB1-add-back state exhibits increased IFNG sensitivity. We have developed novel techniques for interpretation of these data that leverage the phenotypes from large scale publicly available CRISPR phenotypes across several hundred cell lines, revealing functional clusters of genes that are enriched among our results and form the basis for further validation experiments. We have specifically focused on GPX4 mediated inhibition of ferroptosis as a key hypothesis and are evaluating this further as a candidate pathway added to Major Task 2, and represents a focus of further detailed evaluation supported by additional funding directly made possible by preliminary results supported by this DOD LCRP CA. We had initially proposed a next step of conducting further sub-genomic screens using a smaller targeted library representing candidate genes resulting from this screen. However, we have made advances in our experimental methodology (Fig 3) and now propose to modify this goal and instead conduct further genome wide screens with two phenotypes that will further inform our understanding of LKB1/KEAP1 mediated immune resistance with complementary approaches, as described below.

Future Directions

We will continue mechanistic work to evaluate the ferroptosis pathway as a key mediator of immune resistance in LKB1/KEAP1 deficient lung cancer. This is anticipated to be the main focus of a manuscript resulting from the preliminary results described above in Major Tasks 1, 2, and 3. We will evaluate genetic and pharmacologic approaches to modify this pathway across a range of cellular contexts including multiple cell lines and derivatives in which LKB1, KEAP1, GPX4, and other key pathway members are genetically perturbed with over-expression, knockdown and knock-out approaches. We will evaluate the effects of these various perturbations on hallmarks of ferroptosis, IFNG sensitivity, T-cell mediated anti-tumor cytotoxicity using a T-cell co-culture model, and *in vivo* experiments. Much of this work goes beyond the scope of the DOD LCRP CA award and is now supported by separate funding through a SITC fellowship that was awarded as a direct result of the successes in our preliminary results described herein.

Rather than generating a sub-genomic library of candidates identified in the initial screen, we propose carrying out an additional whole genome CRISPR screen that will test two immune associated phenotypes that are highly relevant to understanding LKB1-mediated immune resistance. First, we plan to conduct whole genome screen using TKOv3 library to identify tumor intrinsic modifiers of T-cell mediated anti-tumor immunity with using a T-cell co-culture assay (Figure 3). We have obtained vector constructs from collaborator Nicholas Restifo that allow over-expression of NY-ESO antigen in target cell lines, as well as a separate construct that over-expresses both NY-ESO and HLA-A2, so that any cell line can be compatible with CD8 T-cells modified to express HLA-A2 NY-ESO specific T-cell receptor, for which we also have viral constructs. Second, we will perform a flow cytometry-based screen for modifiers of STING expression, using anti-STING fluorescent antibody. STING is a key modifier of anti-tumor immunity, and acts to integrate and amplify signals from the innate and adaptive immune response. LKB1 deficient cells typically exhibit absent or low STING expression, which is induced after LKB1 add-back (Kitajima et al). We see similar phenotypes in our model cell lines, with characteristics of near absent STING expression in our deficient lines and strong induction after LKB1 add-back that will likely yield a technically successful screen with significant power to identify phenotypic modifiers. Both of these phenotypes can be assessed in a single experiment, with five pellets processed for each cell line derivative representing T_0, STING+, STING-, $T_{final\text{-}Tcell+}$, and $T_{final\text{-}no\text{-}Tcells}$. To conduct the screen with two biologic replicates for the LKB1 mutant and LKB1-add-back derivatives of a cell line will comprise 20 pellets, similar in scope to our previous screen. We propose to carry this out for the A549 line, with phenotypes that can be directly compared to the results from our previous screen, as well as an additional cell line, either H1755 or H1944 to evaluate these phenotypes in a different context allowing evaluation of generalizability. We propose to carry out this screen in lieu of generating a subgenomic CRISPR library and carrying out further screens on diverse cell types using the IFNG phenotype.

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%

After obtaining initial results as above, specifically, after review of the initial candidate inhibitors in Major Task 2, we are concerned that the typical magnitude of difference observed for modifiers of the IFNG phenotype may not be sufficient to assess the pharmacologic library envisioned in Major Task 4. We anticipate that carrying out the approach as initially proposed would have a high risk for both false positive and false negative results and would be difficult to interpret. Therefore, we propose to defer testing of the collection of small molecule inhibitors, and rather apply the resources that had been envisioned for conducting this screen conduct additional whole genome screens described in the Future Directions section of Major Task 3, which we estimate to have a high likelihood of success and will be complementary to the already informative screen we have conducted thus far.

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: 25%.

Major activities:

- The clinical trial (NCT02818920) of neoadjuvant pembrolizumab has been completed enrollment and initial clinical data assessed.
- Additional funding has been obtained from Merck Investigator Studies Program to allow detailed analysis of samples.
- Conducted initial review of specimens available for molecular analysis.
- Revising IRB proposal, anticipated submission by October 9, 2020.
- Conducted meta-analysis of immune associated genomic features across three large datasets of lung adenocarcinoma to identify novel phenotypes within LKB1-deficient lung cancer that appear to modify immune resistance.

Significant results:

The multicenter phase 2 single arm study of neoadjuvant pembrolizumab (TOP1501; NCT02818920) completed enrollment and initial neoadjuvant and surgical management has been completed with last resection performed in March 2019 (Fig 9a). Initial review of surgical safety outcomes and pathologic assessment of tumor response after two cycles of pembrolizumab. 30 patients received at least one dose of pembrolizumab, of which 1 patient withdrew consent, four patients did not undergo surgery after identifying more advanced disease than appreciated at enrollment, and 25 patients underwent surgery (Fig 9b). Of these 25 evaluable patients, 7 (28%) had major pathologic responses with less than 10% viable tumor cells observed at time of resection, including two patients with complete response (Fig 9c).

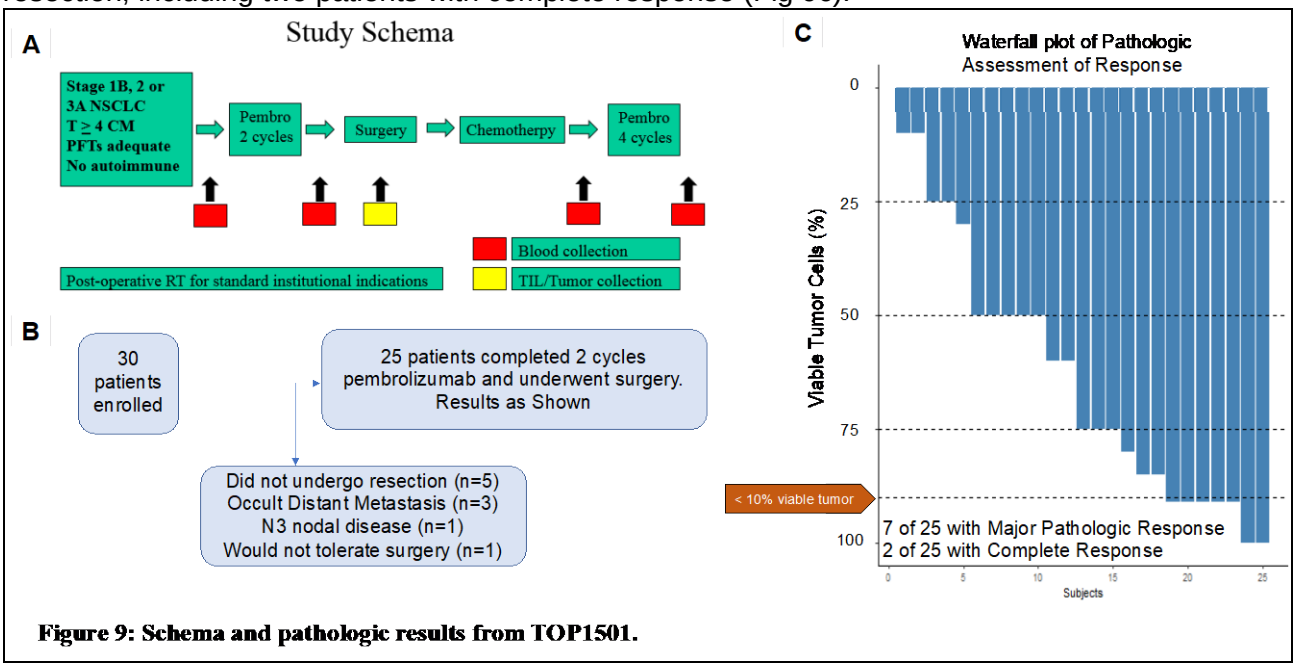

Figure 9: Schema and pathologic results from TOP1501.

We have conducted review of biospecimens available for analysis, including FFPE, OCT, fresh frozen specimens, and peripheral blood, and when possible, we have identified pre-treatment specimens that will allow pre- and post- treatment analyses of key biomarkers. Additional funding from Merck Investigator Studies Program will allow additional characterization of these tumors, including immune profiling of tumor infiltrating and peripheral lymphocytes using high dimensional 28 color flow cytometry, as well as in depth characterization of B-cell and plasma cell phenotypes using single cell RNAseq. This is a large collaborative effort within our institution. Analysis associated with the DOD LCRP CA will evaluate the LKB1 and NRF2 phenotypes of these tumors, and if remaining funds allow, may also expand the approach to include evaluation of the LKB1 and NRF2 phenotypes of untreated control specimens of a companion trial which conducted analogous biospecimen collection with standard-of-care surgical treatment (TOP1502; NCT02848872), and/or contribute to the single cell RNAseq analysis, with collaborator Simon Gregory. LKB1 and NRF2 phenotypes will be assessed using exome sequencing to determine somatic mutations, and RNAseq carried out to determine LKB1 and NRF2 transcriptional phenotypes and other immune-associated and tumor intrinsic transcriptional phenotypes of interest. Revisions to IRB proposal to include these changes are underway and we anticipate submission to IRB within 1-2 weeks (by October 9, 2020).

Further, we have conducted meta-analysis of transcriptional phenotypes associated with LKB1 loss over a set of >1000 lung adenocarcinomas, identifying heterogeneity within LKB1 deficient tumors related both to NRF2 activation and to expression of markers of neuroendocrine differentiation that significantly influence immune infiltration and activation phenotypes on the basis of gene expression profiles (Fig 10). Our genomic characterization of TOP1501 tumors will be integrated with the results of this meta-analytical approach to identify features most consistently associated with tumor response to immunotherapy. Additionally, we are evaluating other patient cohorts with known immunotherapy outcomes and genomic data through collaborations with Chad Pecot at UNC, and exploring options of academic/industry collaborations with BMS (Checkmate 026 study), and Caris Life Pharmaceuticals, for additional validation of our findings.

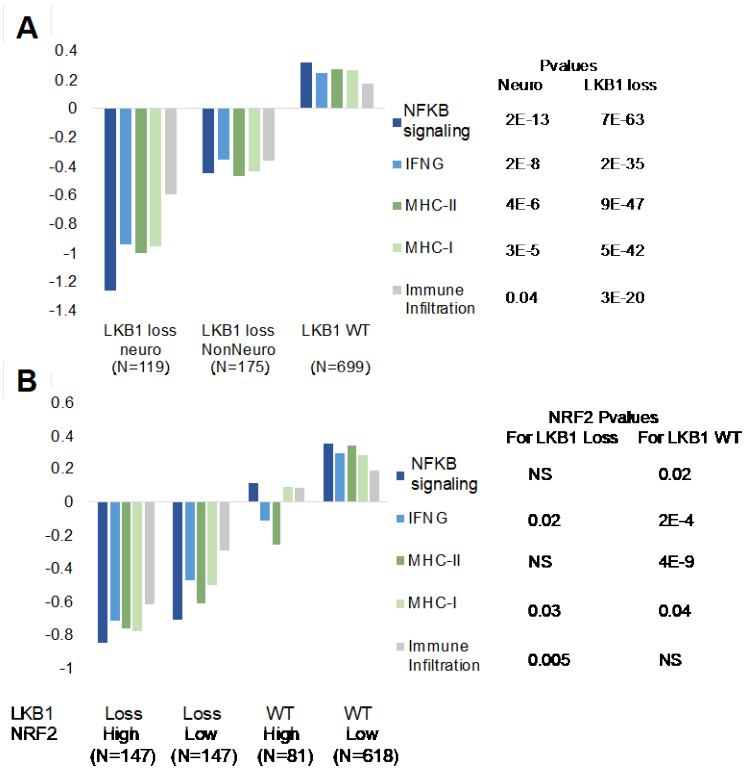

Figure 10: Heterogeneous phenotypes associated with LKB1 loss influence immune phenotypes. A) Immune associated gene expression profiles for NFKB signaling, IFNG activation, MHC-I and MHC-II antigen presentation, and immune infiltration, averaged across lung adenocarcinomas with the specified LKB1 loss and Neuroendocrine phenotypes. P-values represent results from bivariate linear model. B) Immune associated gene expression profiles, averaged across lung adenocarcinomas with the specified LKB1 loss and NRF2 activation phenotypes. P-values represent results from bivariate linear model.

Future directions:

The IRB and HRPO applications will be submitted by October 9, 2020. Internal IRB protocol will under exempt category as all information will be de-identified and all patients in TOP1501 trial have given informed consent for use of their biospecimens as described. Where available we will evaluate gene expression in both pre- and post-treatment specimens using RNAseq. Exome sequencing will be used to evaluate somatic mutations in LKB1, KEAP1, and NRF2. Patients will be classified as LKB1 loss or LKB1 WT on basis of combined use of somatic mutations for STK11 gene and expression of a previously validated 16-gene signature of LKB1 loss. Neuroendocrine differentiation is of additional interest based on preliminary results from our meta-analysis of combined expression data which suggests that this phenotype has particularly strong association with expression profiles suggestive of immune resistance (Fig 10b).

Our primary clinical question will be the comparison the fraction of patients with Major Pathologic Response (MPR: defined as <10% viable tumor cells remaining at time of resection), between LKB1 loss and LKB1 wild-type cohorts using Fisher test. We will accept a two-tailed P-value < 0.1 as being statistically significant. We will also be able to conduct comparable genomic testing of untreated control specimens from companion clinical trial TOP1502, a 'biospecimen only' trial of untreated NSCLC patients who had identical specimen processing to TOP1501. Further analyses will be largely descriptive, using the end analytical results from immunophenotyping and single cell RNAseq analysis, e.g. prevalence of particular T-cell, B-cell and other immune cell populations of interest, and comparing these data across patient cohorts defined by the molecular classes of interest above. This subgroup analysis is expected to be limited in statistical power, with plans to overcome this by leveraging the results of our meta-analysis as above, as well as collaborations with other groups who have access to genomic data from cohorts of NSCLC patients with known immunotherapy outcomes. Specifically, Chad Pecot, MD, at UNC has agreed to share data in collaboration from approximately 50 NSCLC patients with RNAseq data and treatment outcomes after immunotherapy.

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.
- Participated in the IASLC Targeted Therapeutics in Lung Cancer 2020 in Santa Monica, where I presented my work entitled "Interferon Gamma Resistance in Setting of LKB1 Loss: Phenotypic Characterization and Investigation of Mechanism" at oral symposium under section 'best fellow abstracts.'
- Participated in career development seminar at IASLC Targeted Therapeutics in Lung Cancer 2020
- Participated in Duke Cancer Biology Research Retreat and was co-recipient of award for 'best poster' presenting this work.
- Participated in Duke Fellows Research Retreat 2019 and was awarded 'best presentation by second year fellow.'
- Participated in Duke Fellows Research Retreat 2020 and was awarded the Silber award for best overall presentation.
- Submitted follow up grant proposal to ASCO Conquer Cancer Young Investigator Award to evaluate the role of the Hippo/YAP pathway in conferring immune resistance in LKB1 deficient lung cancer, which was not awarded.
- Submitted follow up grant proposal to SITC-AstraZeneca Immunotherapy in Lung Cancer Clinical Fellowship, to evaluate resistance to ferroptosis as mechanism of immune resistance in LKB1 deficient lung cancer. This proposal received funding for $100,000 one-year award.
- I have participated in Duke University Center for Cancer Immunotherapy seminars and have presented my work at a monthly meeting of this association.

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"
- 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
Changes listed above overall are cost neutral.

- Funds that had been planned to support the pharmacologic library screen will be used to support the development of the T-cell co-culture assay.
- Funds that had been planned to support the development of sub-genomic CRISPR libraries and analysis of different cell line derivatives, will be used instead to carry out whole genome screen of modifiers of T-cell anti-tumor immunity using T-cell co-culture assay.
- Final number of clinical trial participants in TOP1501 was 25 rather than the 32 samples proposed in initial project narrative. Of these 25, a few do not have adequate biospecimens for genomic analysis (e.g. two patients with complete response without residual tumor cells amenable for expression analysis). This will allow analysis of untreated control specimens from TOP1502 and is expected to be close to neutral cost.
- Preliminary evaluation of ferroptosis and Hippo/YAP candidate pathways used funds that had been planned to support more in depth characterization of other candidate pathways in Major Task 2, which were not carried forward due to lack of phenotype observed in IFNG experiments.

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

- **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.

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

Duke University Medical Center ● DUMC #3841 ● Morris Building ● Durham, NC 27710 ●

EDUCATION

Duke University, Durham, NC **2017-present**
- 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. My work uses a variety of experimental and analytical approaches to define and characterize genetic subsets of lung cancer with the goal of identifying novel precision treatment approaches. Specifically, my previous work has characterized gene expression profiles, signaling pathways, and drug sensitivity patterns that define 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 significant 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 have pursued residency and fellowship at Duke University Medical Center through the ABIM research track pathway (PSTP). In June 2018 I completed my clinical training in both internal medicine and oncology and have since transitioned to the laboratory for the remainder of my fellowship to focus on basic and translational research interests as above. I have worked in the lab of Kris Wood, gaining expertise in functional genomics approaches in oncology research, and additional clinical and translational mentorship from Neal Ready throughout my fellowship.

POSITIONS AND HONORS

Positions

2017-current 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. *J Thoracic Oncol* (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

Current:

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.

Completed
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.