5-2024

Exploring the Role of IL-1β/IL-1R in the Pathogenesis of K-ras Mutant Lung Cancer

Avantika Krishna

Exploring the Role of IL-1β/IL-1R in the Pathogenesis of K-ras Mutant Lung Cancer

By

Avantika Krishna, B.S.

APPROVED:

DocuSigned by:
Seyed Javad Moghaddam
E79DAA932BA8421...

Seyed Javad Moghaddam, M.D.
Advisory Professor

DocuSigned by:
Jyotika Sharma
2B4D9515D3E24B4...

Jyotika Sharma, M.Phil., Ph.D.

DocuSigned by:
Humam Kadara
D1ED4441391940A...

Humam Kadara, Ph.D.

DocuSigned by:
Farrah Kheradmand
D9D8FE9F647E42D...

Farrah Kheradmand, M.D.

DocuSigned by:
Jianjun Zhang
2B9DC00A4A34425...

Jianjun Zhang, M.D., Ph.D.

APPROVED:

Dean, The University of Texas
MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences

Exploring the Role of IL-1β/IL-1R in the Pathogenesis of K-ras Mutant Lung Cancer

A Thesis

Presented to the Faculty of

The University of Texas MD Anderson Cancer Center

UTHealth Houston Graduate School of Biomedical Sciences

in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

by

Avantika Krishna, B.S.

Houston, Texas

May 2024

Acknowledgements

To begin I would like to express my sincerest appreciation to my advisor Dr. Moghaddam. His knowledge, kindness and guidance were invaluable throughout this journey. His insight and patience during challenging times have been immensely appreciated. Moreover, I can't thank him enough for his constant support in pursuing my future goals as well as always pushing me to grow as a scientist.

I would also like to extend my heartfelt thanks to my advisory committee members, Dr. Sharma, Dr. Kadara, Dr. Kheradmand and Dr. Zhang, for their invaluable feedback and time spent aiding in my success as a researcher as well as my project.

To my fellow, current as well as past lab members and mentees; Mikey, Maria, Kate, Nastaran, Sunu, Majo, Jocelynn, Vivian, and Arnav. Working alongside you all has truly been a source of joy, I still can't believe I was lucky enough to encounter. Meeting all of you provided me with a family I cannot imagine living without.

Special thanks go to my roommate Anna, one of the kindest and most comforting souls, I have been blessed to meet.

Additionally, I wish to express my appreciation towards my dearest friends, Fre, Mickey, Lauryn, Selam, Ashley, Ogechi, and Emma. Although miles apart your unwavering support and conversation never failed to uplift me during challenging times.

Most importantly, I owe an immeasurable debt of gratitude towards my parents, Priya and Ksheerabdhi and my siblings Aditya and Vedika. Your support means the world to me, and I am endlessly thankful for your love and encouragement.

Lastly, to everyone who has been part of this journey thank you for everything, this opportunity has provided me with invaluable knowledge but also unforgettable relationships I will cherish forever!

Exploring the Role of IL-1β/IL-1R in the Pathogenesis of K-ras Mutant Lung Cancer

Avantika Krishna, B.S.

Advisory Professor: Seyed Javad Moghaddam, M.D.

As the leading cause of cancer-related deaths worldwide, the development of targeted therapeutics to treat lung cancer remains crucial. Non-small cell lung cancer (NSCLC), the most common histological subtype predominantly comprises lung adenocarcinoma with driver mutations in the K-ras oncogene (KM-LUAD). KM-LUAD progression partly occurs through activation of the NF-κB pathway initiating an inflammatory response and creating a pro-tumor microenvironment. Notably, the pro-inflammatory cytokine IL-1β a potent activator and product of the NF-κB pathway is elevated in the lungs and sera of KM-LUAD patients. We have shown that IL-1β blockade promotes an anti-tumor immune phenotype in a mouse model of KM-LUAD driven by lung epithelial cell-specific expression of K-ras^{G12D} (CCSP^{Cre}/LSL-K-ras^{G12D}, CC-LR mouse), suggesting that IL-1β mediates tumor-promoting inflammation. Yet, cell-specific mechanisms that underlie this effect are still poorly understood. Thus, we sought to elucidate the role of IL-1β signaling via its ability to bind to its receptor, IL-1R, by conditionally knocking out IL-1R in K-ras-mutant lung epithelial cells in CC-LR mice (LR/IL-1R$^{\Delta/\Delta}$). Tumor development as well as immune microenvironment in 14 and 18-week-old LR/IL-1R$^{\Delta/\Delta}$ mice in comparison to control CC-LR littermates were studied. Notably a 30% reduction in tumor burden in LR/IL-1R$^{\Delta/\Delta}$ mice was evident at both time points tested when compared to their CC-LR counterpart. Reduced tumorigenesis was shown to be driven by decreased angiogenesis and an overall age-dependent effect on tumor-promoting inflammation was seen. Tumor reduction in 14-week-old LR/IL-1R$^{\Delta/\Delta}$ mice was associated with an abundance of myeloid cell subsets as well as a shift in dendritic cell phenotype suggesting an increase in T-cell priming. This differed in 18-week-old LR/IL-1R$^{\Delta/\Delta}$ mice where a stronger response to epithelial IL-1R targeting with a significant reduction in T-cell associated markers as well as NF-κB activation was observed. Overall, these findings provide insight into cell-specific mechanisms underlying the tumor-promoting effects of IL-1β signaling and support the role of tumor cell-intrinsic factors in this process via shaping the tumor microenvironment.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1 Lung Cancer Incidence and Relevance of KRAS Oncogene in NSCLC

Responsible for 20.8% of cancer-related deaths, lung cancer remains the leading cause of global cancer mortality.[1-3] Lung cancer can be classified into two common forms, the most predominant being non-small cell lung cancer (NSCLC) accounting for 85% of lung cancer cases, with 60-70% of those cases being diagnosed at their advanced stages.

NSCLC is known to comprise a heterogeneous class of tumors. The subtype lung adenocarcinoma (LUAD) represents 40% of all lung cancer cases.[4] With a five-year survival rate of less than 20%, the development of targeted therapeutics has been underway yet still met with little success due to rapidly acquired resistance.[5] Resistance to chemotherapeutic agents has been attributed to genetic and epigenetic factors, with genetic mutations causing activation of downstream signaling pathways. Mutations such as these are known as oncogenic driver mutations resulting in tumor metastasis and formation. Of interest the Kirsten rat sarcoma viral oncogene (KRAS) accounts for approximately 25% of all oncogenic mutations in NSCLC.[6] As part of the small guanosine triphosphate (GTP)ase family and modulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins, the KRAS protein switches between the "inactive", GDP-bound and the "active" GTP-bound forms.[7] The activated GTP-bound K-Ras triggers multiple downstream signaling pathways promoting cell survival, proliferation and division.[8] Gain-of-function mutations in the K-ras gene can result in the constitutive activation of the KRAS protein, thus preventing the hydrolysis of GTP and return to the "inactive" GDP-bound state. The continuous activation of its downstream signaling pathways causes potent oncogenic activities.[9] Due to its presence as well as role in NSCLC progression, the development of targeted therapies for this protein has been of high interest. Recently, small molecular weight inhibitors of mutant KRAS against advanced K-ras mutant LUAD (KM-LUAD) have been developed but have shown limited success due to acquired resistance.[10-12] Thus, new, or improved strategies are

urgently needed for clinical management of KM-LUAD, although, advancement in this area is hindered by our poor understanding of early events in the development and progression of this disease.

1.2 NF-κB pathway Activation Elicits Tumor-Associated Inflammation

The tumor microenvironment (TME) is comprised of an intricate network of stromal cells, inflammatory immune cells, and cancer cells which all play a pivotal role when it comes to tumor development and progression.[12] Inflammation heavily impacts the composition of the TME, where immune cells elicit the targeted destruction of cancer cells thus causing the immune system to act in an anti-tumorigenic manner. Opposingly it can advance tumorigenesis by exerting tumor-promoting signals onto epithelial and cancer cells.[13,14] Cancer-associated inflammation can develop extrinsically through insults such as smoking, the primary cause of lung cancer, as well as intrinsically, via cancer-initiating mutations. These mutations then cause subsequent recruitment and activation of inflammatory cells, such as macrophages and myeloid-derived suppressor cells (MDSCs), that promote metastasis, angiogenesis, and tumor growth.[15] Tumor cell-mediated inflammation is largely dependent on the activation of the NF-κB pathway. NF-κB is involved in inducing the expression of various mediators including cell survival, adhesion, differentiation, and growth.[16,17] Additionally, NF-κB signaling mediates cytokine/chemokine secretion and synthesis from immune cells, promoting their recruitment and capability to maintain a pro-tumor microenvironment.[18, 19] In the context of KM-LUAD, studies have indicated elevated activation of NF-κB in tumor tissue that is associated with unfavorable prognosis in patients.[20-23]

1.3 Interleukin-1β Associated Inflammation and Targeting in NSCLC

The development of NF-κB-associated inflammation can lead to production of cytokines such as IL-6, IL-17, and IL-22, which we have already shown to causally promote lung tumorigenesis.[24-26] However, research regarding the role of IL-1β as a potent activator of the NF-κB pathway in the context of KM-LUAD has yet to be addressed. Located upstream to the NF-κB pathway and associated with multiple inflammatory, metabolic, and infectious diseases, IL-1β plays an important role in the context of cancer metastatis.[27] Elevated levels of IL-1β has been attributed to tumor-associated inflammation,

leading to increased cell proliferation and angiogenesis. These high levels have been correlated with tumor progression in multiple cancer models.[28] Additionally, a prospective study (CANTOS) that evaluated the treatment of neutralizing IL-1β antibody (Canakinumab) in patients with atherosclerosis, indicated a reduction in lung cancer incidence and associated mortality.[29] This supported the interest in targeting IL-1β as an effective therapeutic target, especially for lung cancer patients who have elevated levels of IL-1β in their serum.[30] Additionally, a downstream effector of IL-1β induced signaling as well as a marker for chronic inflammation, the inflammatory protein CRP, was increased in patients with metastatic NSCLC and was associated with poor survival in NSCLC patients undergoing resection.[31] More importantly, our lab has previously found that antibody-based neutralization of IL-1β suppressed the development of KM-LUAD in human-relevant mouse models of the disease. IL-1β associated immunosuppression of the TME, which occurs through the recruitment of myeloid-derived suppressor cells (MDSCs), and neutrophil accumulation, with subsequent inhibition of cytotoxic T-lymphocytes (CD8+ T cells) was significantly reversed upon anti IL-1β antibody delivery transitioning from a pro-tumor immune phenotype to that of an anti-tumor immune phenotype.[32]

This study as well as many others led to the interest in wanting to target the IL-1 pathway as a means of treatment and due to the CANTOS trial, much research has gone towards targeting IL-1β in NSCLC. Three large-scale randomized phase III clinical trials (CANOPY-1, CANOPY-2, and CANOPY-A) as well as one phase II clinical trial (CANOPY-N) had gone into effect in the past few years, using Canakinumab in combination with other drugs.[33-36] Unfortunately, the outcome of these trials, were either halted due to low enrollment or terminated due to lack of efficacy.[37] This, in turn, led to questioning the validity of the drug but more importantly prompted the interest in wanting to further investigate the mechanistic effects of IL-1β inhibition in the context of NSCLC.

1.4 Interleukin-1 Inflammatory Pathway Activation, Signaling, and Targeting

There are four main IL-1 family members; IL-1α, IL-1β, IL-33, and IL-1 receptor antagonist (IL-1RA), all closely linked to inflammation in the context of tumor progression. Within this family, there are two pro-inflammatory cytokines IL-1α and IL-1β. Both cytokines are functionally distinct,

evident through differences in their expression and activation. IL-1α acts as a dual-function cytokine; in the nucleus as a transcription factor and in the cytoplasm as an 'alarmin'.[38] Interestingly research has shown that IL-1α production could be controlled by IL-1β.[39] Of the two cytokines, IL-1β, has been more commonly known to be a target in cancer treatment due to its ability to promote immunosuppression, induce tumor development and angiogenesis as well as promote cancer cell migration and invasion.

Beginning its synthesis as a pre-form protein, pro-IL-1β must be cleaved and converted to IL-1β, its active form. This process is highly controlled, requiring a 'priming' step which involves the transcription of the IL-1β gene, initiated by lipopolysaccharides (LPS), toll-like-receptors (TLRs), tumor necrosis factor (TNF), or even by itself as a positive feedback mechanism. Once pro-IL-1β has been released into the extracellular space, it must be cleaved by Caspase-1 to mature into IL-1β. Caspase-1 activation occurs via inflammasomes, specifically nucleotide-binding domain-like receptor protein 3 (NLRP3) with the aid of apoptosis-associated speck-like protein containing a CARD domain (ASC). Interestingly like IL-1β the NLRP3 inflammasome is controlled by activation of the NF-κB pathway, thus IL-1β as well as the NF-κB pathway heavily rely on one another to allow for continuous pathway activation and IL-1β production.[40, 41] Once in its active form IL-1β is generally released by immune cells such as monocytes, macrophages, and dendritic cells via pyroptosis. Pyroptosis is an NLRP3-regulated form of cell death, that involves Caspase-1 mediated cleavage of the effector molecule Gasdermin-D (GSDMD), leading to the formation of large pores in the cell membrane causing cell death. However, this mechanism has been proven to not be the only means of IL-1β secretion, with literature providing evidence of GSDMD-independent secretion as well as additional pathway involvement.[42]

IL-1β's ability to impact the immune microenvironment is only possible once it has bound to its respective membrane-bound receptor IL-1 receptor (IL-1R). The IL-1 family of receptors is comprised of 10 members, of which most form a trimeric signaling complex, that requires a co-receptor. IL-1β forms a trimeric complex comprised of both IL-1 Receptor 1 (IL-1R1) or IL-1 Receptor 2 (IL-1R2) and their associated accessory protein IL-1 Receptor 3 (IL-1R3).[43] When IL-1β binds to its receptor IL-1R1, a structural change occurs allowing IL-1R3 to bind. This complex elicits a MyD88-triggered signaling cascade of kinases initiating a pro-inflammatory response and activation of the NF-κB pathway. This is not the case when IL-1β binds to IL-1R2, for it acts as a decoy receptor, leading to the neutralization of IL-1β activity.[44] Differences in pathway activation or lack thereof, once the cytokine is bound can be seen in Figure 1. Since IL-1R1 is the sole receptor of IL-1β that initiates a pro-inflammatory response via the NF-κB pathway, it led us to our interest and my main hypothesis that; **IL-1β binding to the IL-1 receptor leads to a tumor intrinsic inflammatory response that promotes KM-LUAD.**

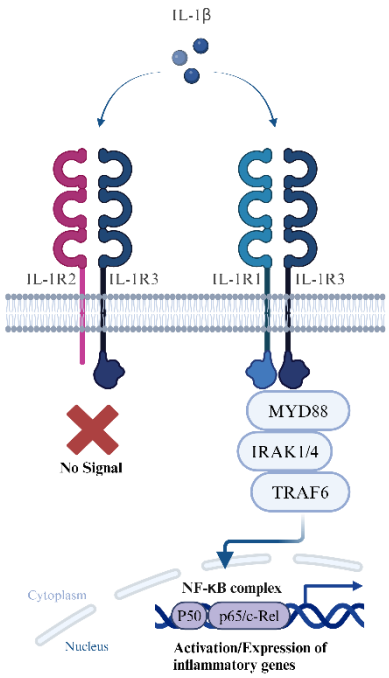


Figure 1. Binding of IL-1β to IL-1R2/IL-1R3 attenuates signal cascade, as opposed to IL-1R1/IL-1R3 (IL-1R), initiating NF-κB pathway activation. Created in Biorender.

In summation, IL-1β's ability to affect the immune cell compartment and induce tumor-promoting inflammation has yet to be fully understood. Hence, gaining greater insight into the mechanisms involved, as well as the importance of the interaction between IL-1β and its receptor, IL-1R, is necessary. These studies will allow for the refinement of targeting IL-1β as a prevention or treatment modality when used in the clinic.

Chapter 2: Results

2.1 Lung Epithelial Specific Conditional Deletion of IL-1R Reduces Tumor Burden at Both Early and Late-Stage K-ras Induced Lung Tumorigenesis

Previously we have shown that IL-1β inhibition significantly reduces tumor burden via transformation of the TME towards that of an anti-tumor immune phenotype.[32] We believe that occurs through the interaction between IL-1β and its receptor IL-1R, eliciting tumor promoting inflammation through immune and tumor cell communication. Thus, we investigated the role of tumor epithelial IL-1R in the context of K-ras mediated lung tumor progression. We accomplished this by our previously established K-ras^{G12D} driven LUAD mouse model (CC-LR). CC-LR mice have both the CCSP^{Cre} recombinase, incorporating the insertion of Cre into the Club cell secretory locus, as well as a Lox-Stop-Lox-Kras^{G12D} mutation. This in turn allows for lung epithelial specific constitutive expression of the Kras^{G12D} mutation and when crossed with our IL-1R conditional knockout mouse (IL-1R$^{f/f}$), leads to the simultaneous conditional deletion of the receptor within the lung epithelium (LR/IL-1R$^{\Delta/\Delta}$) (Figure 2A&B). We tested epithelial IL-1R deletion prior to and post adenocarcinoma formation, in 14- and 18-week-old mice. These two groups were termed the early and late-stage tumor development timepoints. To confirm conditional deletion of the receptor, immunohistochemical staining of IL-1R1 was performed on 14-week-old mice. A significant reduction in IL-1R1 presence was observed and quantified (Figure 2C).

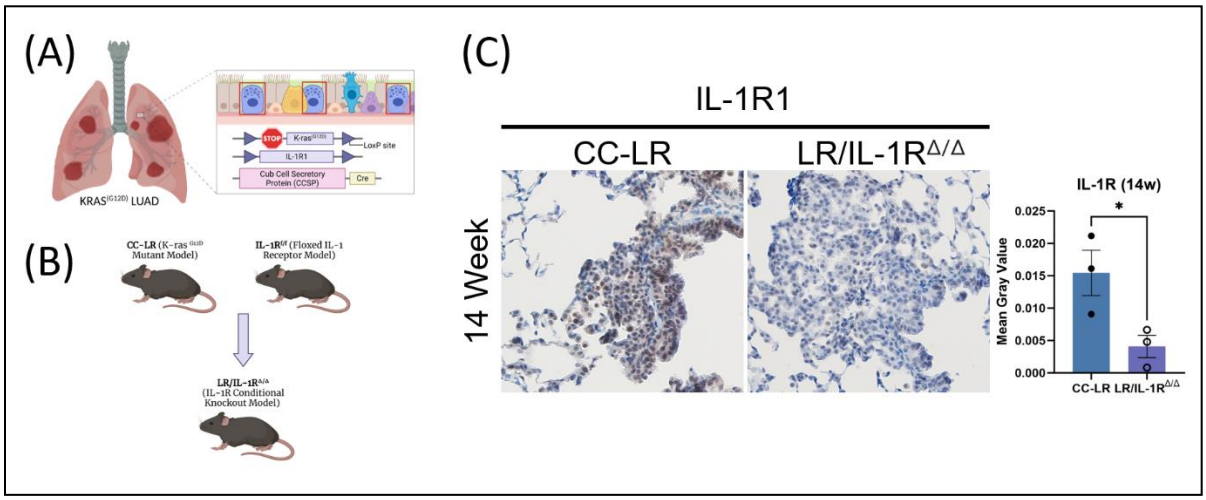
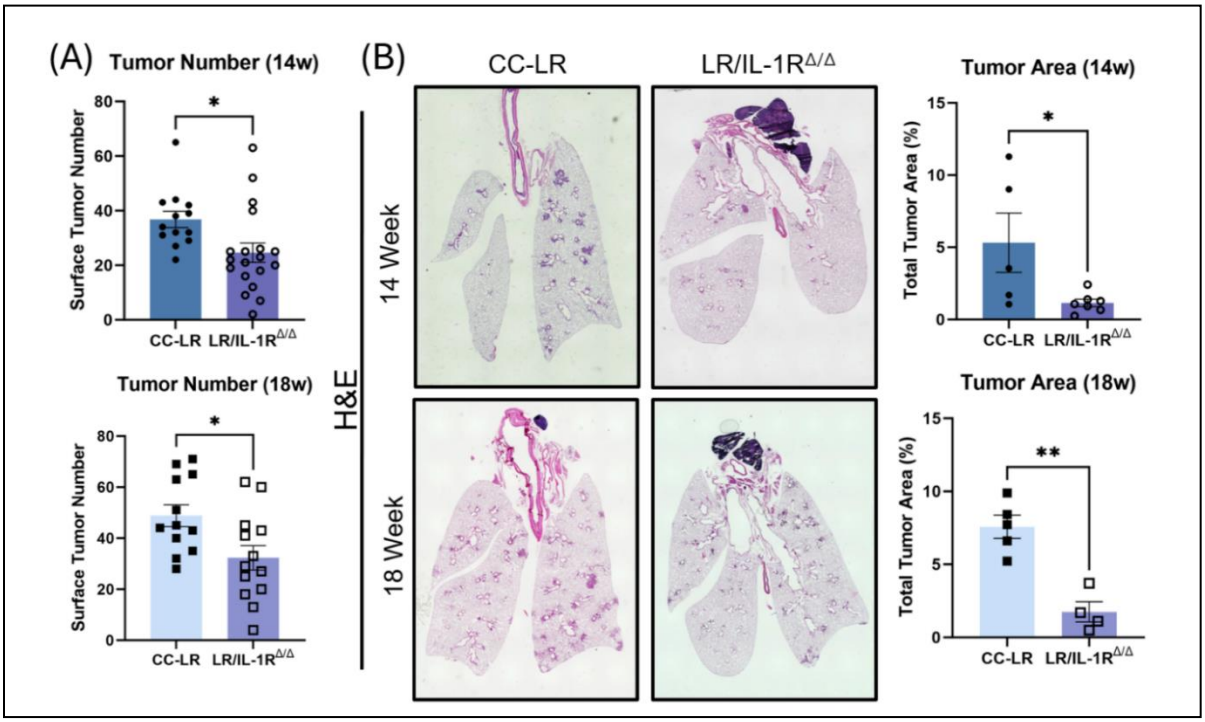


Figure 2: Conditional Knockout of IL-1R is Specific to the Lung Epithelium (A) Lung Epithelial IL-1R targeting is specific to the Club cell, which simultaneously possess the Kras^{G12D} mutation. (B) Representative mating pair involved in development of LR/IL-1R$^{\Delta/\Delta}$ mouse model. (C) Photomicrographs of IL-1R stained lung sections obtained from 14-week-old mice. Quantification presented as Mean Grey Value of IL-1R+ staining. Data represent mean ± SEM. *P < 0.05 by unpaired t test.

Upon conditional targeting of the receptor an average 30% reduction in surface tumor number was evident at both timepoints tested when compared to their CC-LR counterpart (Figure 3A). This was similarly seen via histopathological analysis which indicated a significant reduction in tumor area as well as lesion formation and size. Furthermore, a potential shift towards a more hyperplastic lesion phenotype as opposed to the expected adenoma/adenocarcinoma in late-stage LR/IL-1R$^{\Delta/\Delta}$ was visually seen (Figure 3B). These results led us to investigate the impact of tumor epithelial IL-1R deletion in the context of cell proliferation and angiogenesis previously known to be elevated in our CC-LR mice and inhibited upon IL-1β blockade.[32]



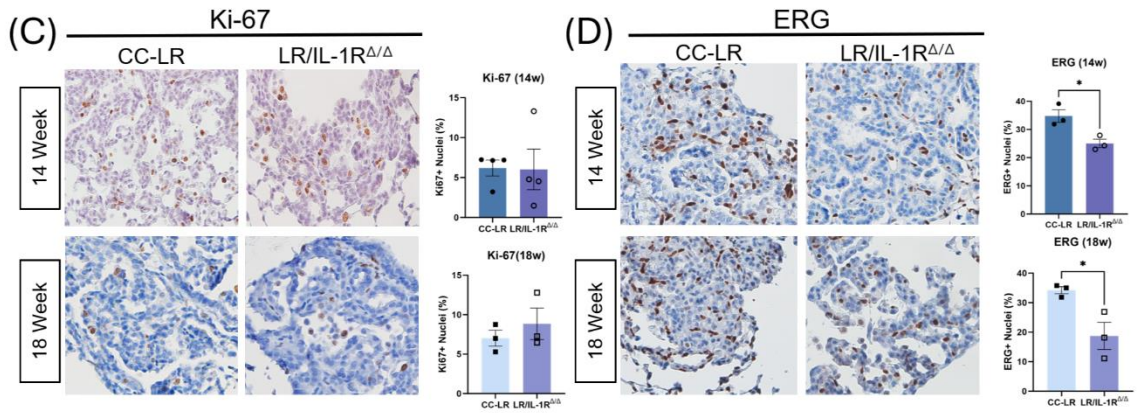


Figure 3: Conditional Knockout of Lung Epithelial-Specific IL-1R Reduces Tumor Burden and Angiogenesis but Not Cell Proliferation at Both Early and Late Stages. (A) Surface tumor number of 14- and 18-week-old mice. (B) Photomicrographs of H&E-stained lung, including representative calculated tumor areas measured using ImageScope Software. (C&D) Photomicrographs of Ki-67 and ERG-stained lung sections. Quantification presented as % of Ki-67 and ERG positive cells respectively. Data represent mean ± SEM. **P < 0.05, *P < 0.05 by unpaired t test.

Immunohistochemical staining of Ki-67 and ERG was performed respectively. Interestingly at both timepoints tested, no change in Ki-67+ cells were seen in LR/IL-1R$^{\Delta/\Delta}$ mice when compared to CC-LR's as opposed to the significant reduction in ERG+ cells found at both early and late stage timepoints (Figure 3C&D).

These results suggest that tumor epithelial IL-1R plays an important role in K-ras^{G12D} associated tumor progression potentially mediated via angiogenesis at both early and late-stage tumor development.

2.2 Lung Epithelial IL-1R Driven Angiogenesis Promotes K-ras Mediated Tumor Growth

Having seen a marked reduction in ERG upon lung epithelial IL-1R deletion, we wished to further assess the involvement of angiogenesis and its role in tumor progression. Thus, we performed immunohistochemical staining of vascular differentiation marker CD31 on tissue sections from both early and late-stage lesions. A distinct difference in CD31 staining was evident, amongst LR/IL-1R$^{\Delta/\Delta}$

mice at the late stage timepoint, indicating a decrease in vascularization that could be involved in the reduced tumor burden seen previously in LR/IL-1R$^{\Delta/\Delta}$ mice (Figure 4A).

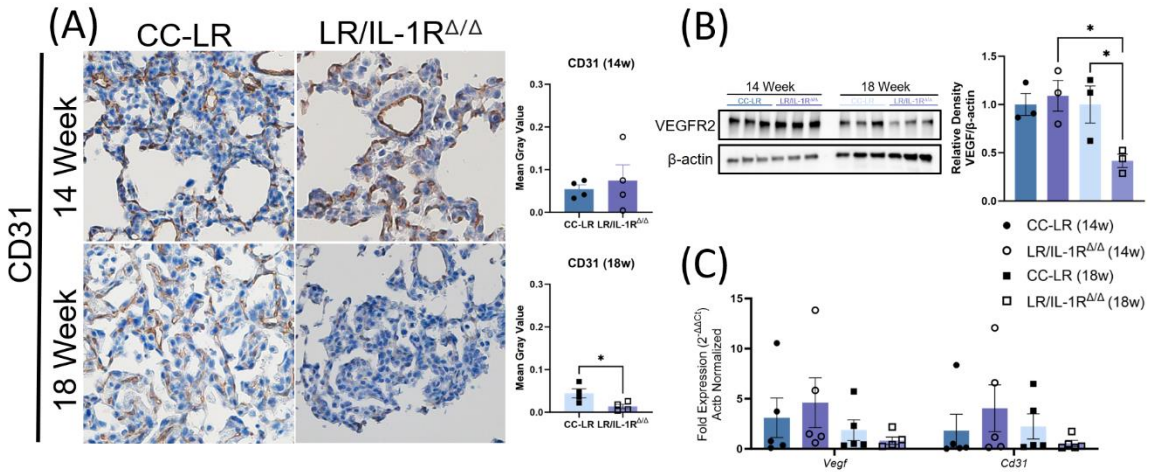
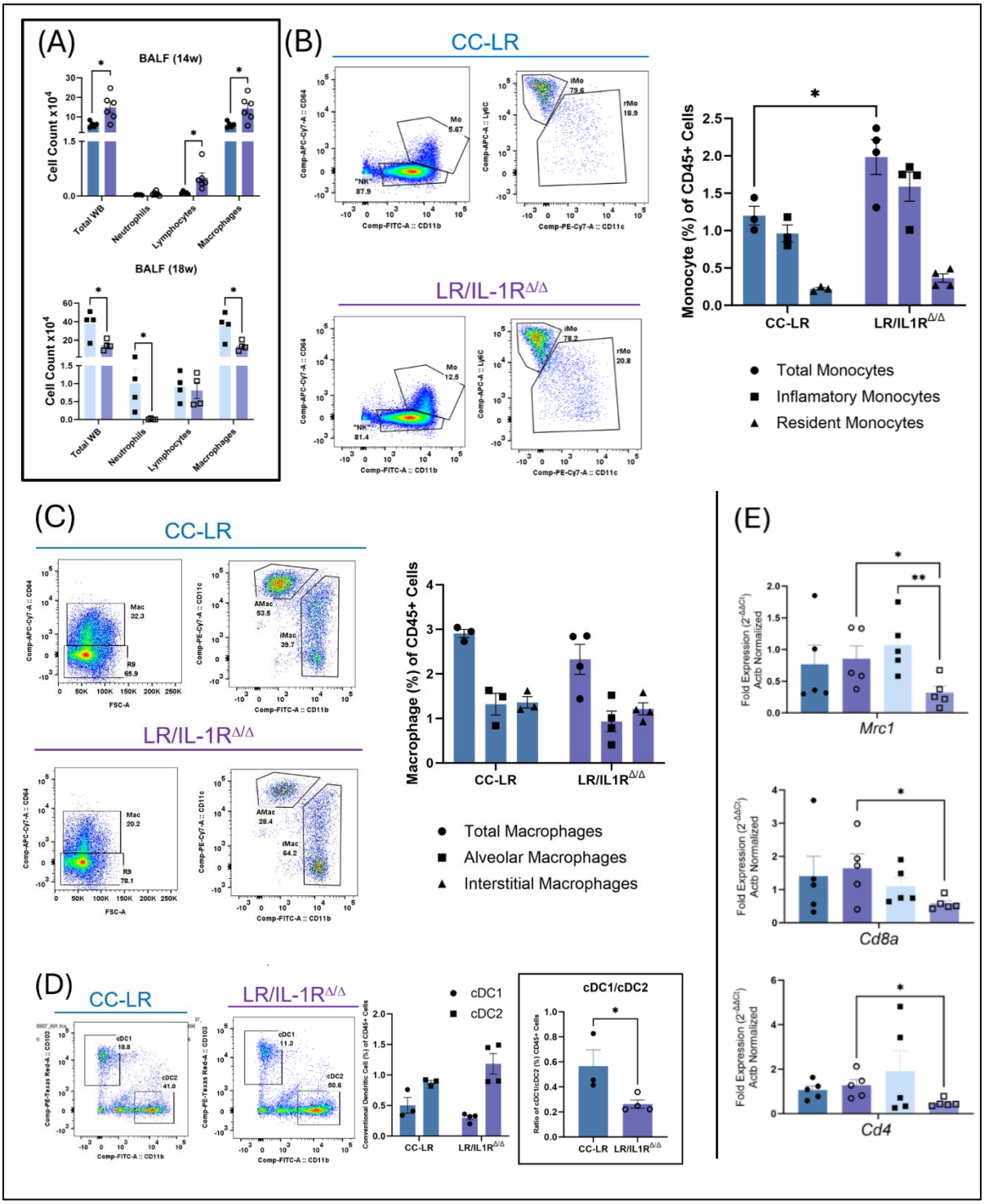


Figure 4: Lung Epithelial IL-1R Driven Angiogenesis Promotes K-ras Mediated Tumor Growth. (A) Photomicrographs of CD31 stained lung sections obtained from 14- and 18-week-old mice. Quantification presented as Mean Grey Value of CD31+ staining. (B) Representative WB images and analysis of VEGFR2 and β-actin protein levels in whole-lung tissue. (C) Relative mRNA expression of VEGF and CD31 markers in whole lung normalized to Actb. Data represent mean ± SEM. *P < 0.05 by unpaired t test.

Furthermore, we were interested in measuring VEGFR2 expression, a receptor for the major angiogenic regulator VEGF and shown to be regulated by the NF-κB.[45] Western blot analysis revealed a significant decrease in VEGFR2 expression in LR/IL-1R$^{\Delta/\Delta}$ at the late stage timepoint when compared not only to its CC-LR counterpart but additionally to early stage LR/IL-1R$^{\Delta/\Delta}$ mice (Figure 4B). qPCR analysis of VEGF and CD31 markers, further confirmed these findings, with a trend for a decrease in these markers primarily seen in 18-week-old LR/IL-1R$^{\Delta/\Delta}$ mice (Figure 4C). These findings provided evidence for the role of IL-1β/IL-1R induced angiogenesis in tumor progression and indicated its primary involvement at the late stage timepoint.

2.3 Early and Late-Stage Changes in Lung TME Upon IL-1R Targeting

IL-1β is produced and secreted by multiple cell types, primarily myeloid cells which are involved in the induction of early lung cancer metastasis.[46] Thus, we were interested in evaluating the role of immune cells in the context of tumor inhibition seen upon lung epithelial IL-1R deletion.



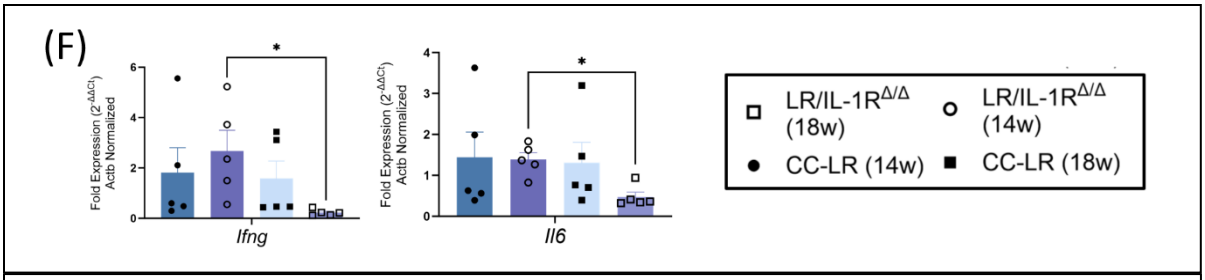


Figure 5. Early and Late-Stage Contexture of Immune Cells Upon IL-1R Targeting Tumor Differs (A) Inflammatory cell and lineage-specific white blood cell numbers from BALF's of 14- and 18-week-old mice. (B-D) Representative flow cytometry analysis performed on 14-week-old mice. Quantification of monocytes (CD64+CD11b+), macrophages (CD64+), dendritic cells (CD103+). (E) Relative mRNA expression Mrc1, Cd8a, Cd4, Ifnγ, and Il6 markers normalized to Actb or Cd45 expression. Data represent mean ± SEM. *P < 0.05 by unpaired t test.

Evaluation of Wright-Giemsa stained bronchoalveolar lavage fluid (BALF) from early stage LR/IL-1R$^{\Delta/\Delta}$ indicated an overall increase in inflammation compared to age matched CC-LR's. This was evident through a significant increase in total white blood cells, primarily comprised of lymphocytes and macrophages. Conversely a trend for decrease in total white blood cells, lymphocytes and macrophages was prominent in LR/IL-1R$^{\Delta/\Delta}$ mice at the late stage timepoint (Figure 5A). This difference in inflammation or lack thereof at later stages of tumor development indicates a time dependent role that immune cells have regarding tumor promoting IL-1β /IL-1R crosstalk.

To evaluate these findings, flow cytometry analysis was performed on whole lung tissue obtained from early stage LR/IL-1R$^{\Delta/\Delta}$ and CC-LR mice. Overall, a significant increase in total monocytes was seen, however, when evaluating their inflammatory and resident subsets no significant differences in their abundances were observed. There was however an increased trend in inflammatory monocytes found in LR/IL-1R$^{\Delta/\Delta}$ mice compared to their control (Figure 5B). Further analysis of macrophages and dendritic cells (DCs) was performed, as they are known to differentiate from myeloid progenitor cells. Overall, no significant difference in amount of macrophage and dendritic cell subsets was seen, yet an interesting shift towards the conventional dendritic cell 2 subset (cDC2) was noticed (Figure 5C&D) These results not only highlight the role of monocytes in early-stage tumor prevention,

however, additionally allude to the recruitment or involvement of T-cells in combatting tumor progression and metastasis.

Quantitative real time-PCR (rt-qPCR) was performed on whole lung tissue to better characterize the immune cells involved. A significant reduction in expression of M2 macrophage associated gene Mrc1 was evident in late-stage LR/IL-1R$^{\Delta/\Delta}$ mice compared to both age matched CC-LR and early-stage LR/IL-1R$^{\Delta/\Delta}$ mice. Unexpectedly a significant decrease in Cd8a, Cd4 and IFN-γ was evident only in late stage LR/IL-1R$^{\Delta/\Delta}$ mice, differing from increased T-cell associated cytotoxic activity seen upon IL-1β inhibition (Figure 5E&F).[32] When comparing both early and late-stage time-points, a decrease in pro-inflammatory cytokine known to be play a role in the activation of the NF-κB pathway; IL-6, was seen (Figure 5F). These results suggest the involvement of macrophages regarding IL-1β driven tumor growth via the induction of a tumor promoting M2 associated phenotype. Additionally, a decrease in overall immune cell associated markers in late stage LR/IL-1R$^{\Delta/\Delta}$ suggests their differing involvement regarding tumor progression. Early-stage tumor epithelial IL-1R deletion in combating tumor growth may be driven primarily by immune cell associated signaling as opposed to late-stage deletion which relies more on tumor cell associated signaling.

2.4 IL-1β/IL-1R Medicated NF-κB Pathway Activation is Mainly Associated with Late-Stage Tumor Phenotype

K-ras mediated tumor progression is associated with inflammation, arising from the activation of multiple pro-inflammatory pathways. This activation can be elicited by tumor-immune cell crosstalk but additionally through the release of cytokines such as IL-1β. Having seen a differing role of immune cell subsets as well as inflammation at the timepoints tested we wished to evaluate the activation of pro-inflammatory pathway NF-κB.

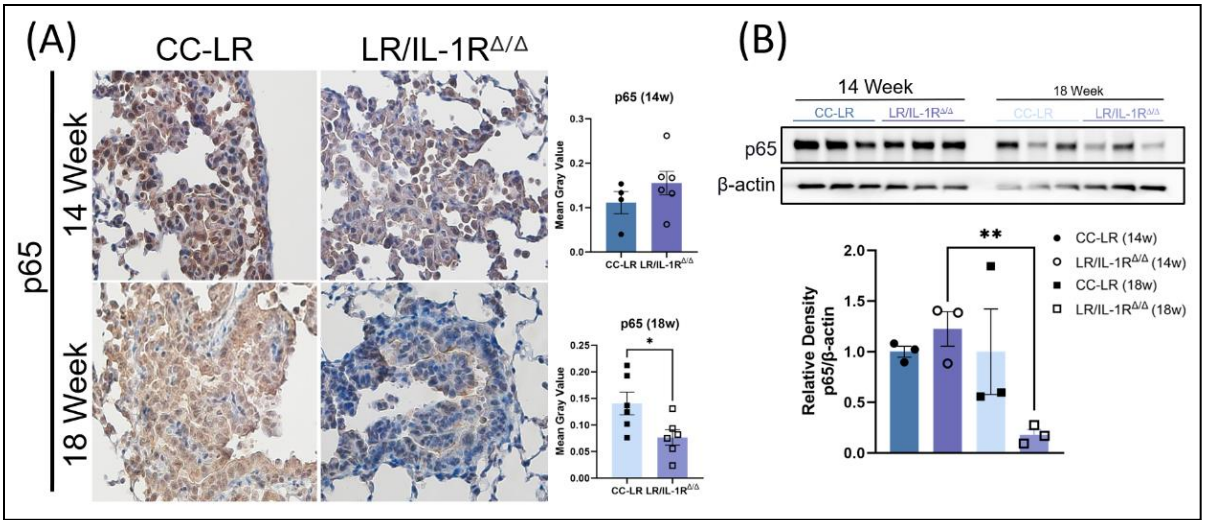


Figure 6. IL-1β/IL-1R Mediated NF-κB pathway Activation is Mainly Associated with Late-Stage Tumor Phenotype. (A) Photomicrographs of p65 stained lung sections obtained from 14- and 18-week-old mice. Quantification presented as Mean Grey Value of p65+ staining. (B) Representative WB images and analysis of p65 and β-actin protein levels in whole-lung tissue. Data represent mean ± SEM. **P < 0.001 *P < 0.05 by unpaired t test.

Immunohistochemical analysis of p65, a subunit of NF-κB that is predominantly involved in its activation, did not show a significant decrease in early-stage LR/IL-1R$^{\Delta/\Delta}$ mice compared to their CC-LR control, however a lack of p65 expression was evident in late-stage LR/IL-1R$^{\Delta/\Delta}$ mice (Figure 6A). These results were confirmed via Western blot analysis, where expression of p65 greatly differed amongst 14- and 18-week-old LR/IL-1R$^{\Delta/\Delta}$ mice (Figure 6B). Lack in p65 expression suggests involvement of NF-κB pathway activation in tumor progression in later-stage of tumorigenesis as well as the role of tumor epithelial IL-1R in prompting its activation. No difference in p65 expression was seen in early-stage LR/IL-1R$^{\Delta/\Delta}$ mice, supporting the more dominant involvement of immune cells, such as myeloid cells known to respond to IL-1β regulated NF-κB activation in the context of tumor progression.

2.5 Tumor/Epithelial-Specific-IL-1R Conditional Targeting Has No Affect KM-LUAD Associated Stem-Like Properties

Research has shown involvement of IL-1β in regulation and function of stem-like properties,

which are upregulated in our mouse model and mediated via NF-κB associated cytokine IL-22.[26, 47] Thus, we wished to evaluate the effects that tumor/epithelial IL-1R targeting would have on stemness.

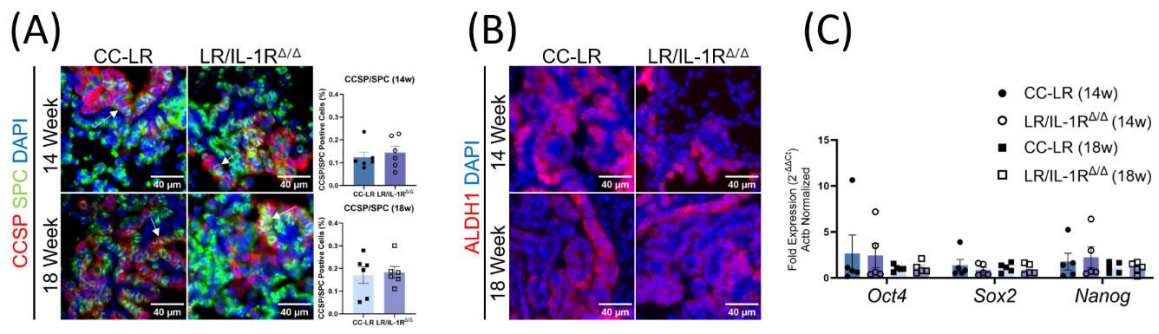


Figure 7. Tumor/Epithelial-Specific-IL-1R Conditional Targeting Has No Affect KM-LUAD Associated Stem-Like Properties. Photomicrographs of immunofluorescent stained lung sections obtained from 14 and 18-week-old mice. (A) Quantification presented double positive CCSP/SPC cells. DAPI (Blue), CCSP (Red), and SPC (Green) cells. (B) ALDH1 (Red) expression. (C) Relative mRNA expression of Sox2, Nanog and Oct4 markers in whole lung normalized to Actb. Data represent mean ± SEM. *P < 0.05 by unpaired t test.

Immunofluorescence (IF) imaging of known subset of cancer stem cells (CSC's); Club Cell Secretory Protein (CCSP) and Surfactant Protein C (SPC) double positive cells was performed. CCSP and SPC were used as they are primarily found in the bronchoalveolar duct junction where most stem-like cells are in the context of KM-LUAD. No significant difference in number of these double positive cells was seen at both early and late-stage LR/IL-1R$^{\Delta/\Delta}$ mice when compared to their controls (Figure 7A). These results were supported when measuring the CSC marker ALDH1, which visually showed no difference in expression (Figure 7B). Furthermore, PCR analysis of core stem cell genes, Sox2, Nanog and Oct 4, indicated no change in expression at both time-points tested (Figure 7C) further confirming our IF findings. Overall, these results indicate lack of involvement of IL-1β signaling in the induction of stemness properties of double positive CSCs in the context of KM-LUAD progression.

Chapter 3: Discussion and Future Direction

Taken together, our results indicate a time dependent effect for tumor/epithelial cell specific IL-1R in KM-LUAD progression (Figure 8). Epithelial IL-1R targeting resulted in a significant tumor reduction at both early and late-stage time points. Of interest, a difference in lesion phenotypes was observed between 14-week and 18-week-old mice, a time point primarily associated with adenoma/adenocarcinoma development. 18-week-old mice had a greater number of hyperplastic lesions which may have resulted from a decrease in IL-1R presence on tumor cells. This in turn could halt the ability of the receptor to interact with pro-inflammatory IL-1β causing the inhibition of tumor growth.

The tumor growth elicited by IL-1β/IL-1R tumor epithelial interaction was demonstrated to be primarily induced by angiogenesis. This confirmed our previous findings which indicated the involvement of IL-1β in promoting angiogenesis[32] and endothelial cell specific IL-1R inflammatory pathway involvement in prompting VEGF production.[48] The response mentioned above, however, was seen more significantly in our LR/IL-1R$^{\Delta/\Delta}$ late-stage mice, as opposed to early-stage. These results indicate an age dependent sensitivity, with greater involvement of tumor cell specific production of VEGF in eliciting tumor growth at the late stage timepoint. Further analysis measuring the proangiogenic cytokines COX-2 and HIF-1α induced by IL-1β which are shown to mediate angiogenesis, would be of interest.[49, 50] The observed alterations in histological progression at both timepoints which could potentially be mediated by angiogenic factors brought our attention to incorporate the use of a p53 mouse model to further evaluate the effects of IL-1R conditional targeting in the context of metastasis.

Along with cancer cells other immune cell subsets secret IL-1β. Therefore, we expected that inhibiting IL-1R on the tumor epithelium could play a role in dampening the pro-inflammatory immune cell-cancer cell crosstalk. The mentioned results were expected to be seen more drastically in myeloid cells, the primary producers of IL-1β, shown to have tumor promoting properties in the TME of the

lung. Furthermore, we had previously confirmed these findings, where targeting IL-1β in CC-LR mice led to the depletion of neutrophils and polymorphonuclear (PMN) myeloid-derived suppressor cells.[32] Interestingly, an increase in inflammation was apparent upon tumor epithelial IL-1R deletion at the early-stage timepoint. Further analysis indicated the involvement of macrophages, as well as cDC2 cell subsets playing a role in tumor development in the early stage timepoint. This observation suggests that a compensatory mechanism may be at play, where lack of IL-1R in the tumor could potentially lead to an increase in cytokine secretion. This influx of certain cytokines in the TME may increase activation of other immune cell subsets such as T-cells, whose involvement is of interest due to changes seen in the cDC2 cell compartment.

cDC2's are less well known within the context of cancer and are believed to elicit a tumor promoting Th2 response. Recently, however, literature has shown these cells possess a dual role in tumor regulation. In humans, cDC2's has been evidenced to be involved in priming CD8+ T cell responses under inflammatory conditions.[51] Additionally, evidence regarding cDC2 association with better prognosis in lung cancer patients has come to light.[52] Thus, our results along with previous findings by others support our interest in better understanding T-cell priming involvement in the context of IL-1β/IL-1R mediated tumorigenesis.

The above-mentioned observation was less conspicuous when studying tumor epithelial IL-1R inhibition in late-stage lesions, where a noticeable decline in inflammation was evident. Further evaluation of immune associated markers indicated a decrease in CD4+ and CD8+ T cells. This could potentially be due to the role of IL-1β in the context of T-cell activation and dendritic cell priming. Dendritic cell release of IL-1β can promote CD4+ T cell differentiation and activation.[53] Furthermore, it has been shown that the NLRP3 inflammasome-IL-1R axis can initiate CD8+ T cell activity via pyroptosis.[54] Thus upon early-stage IL-1R targeting, the initial influx of immune cells may be present due to elevated levels of IL-1β or lack of response from the tumor upon IL-1β release. However, as time progresses within this model, the decrease in IL-1β production by the tumor due to inhibition of its IL-1β auto stimulatory loop could perhaps lead to a decrease in DC priming and an overall anti-

inflammatory response. Furthermore, failure to initiate a cytotoxic T-cell response via DC priming of dying tumor cells in the absence of IL-1R, as well as exogenous IL-1β has been demonstrated.[55] These conclusions, however, must be confirmed through further analysis of the lymphoid cell subset. Experiments, such as Single-Cell RNA sequencing, could additionally be used to exacerbate the exact T-cell subtypes involved in tumor regression in these contexts.

These studies also signify the importance of IL-1β signaling and its relation to the myeloid cell compartment. Hence, targeting the IL-1R in the myeloid cell compartment using a bone marrow transplant approach should be considered.

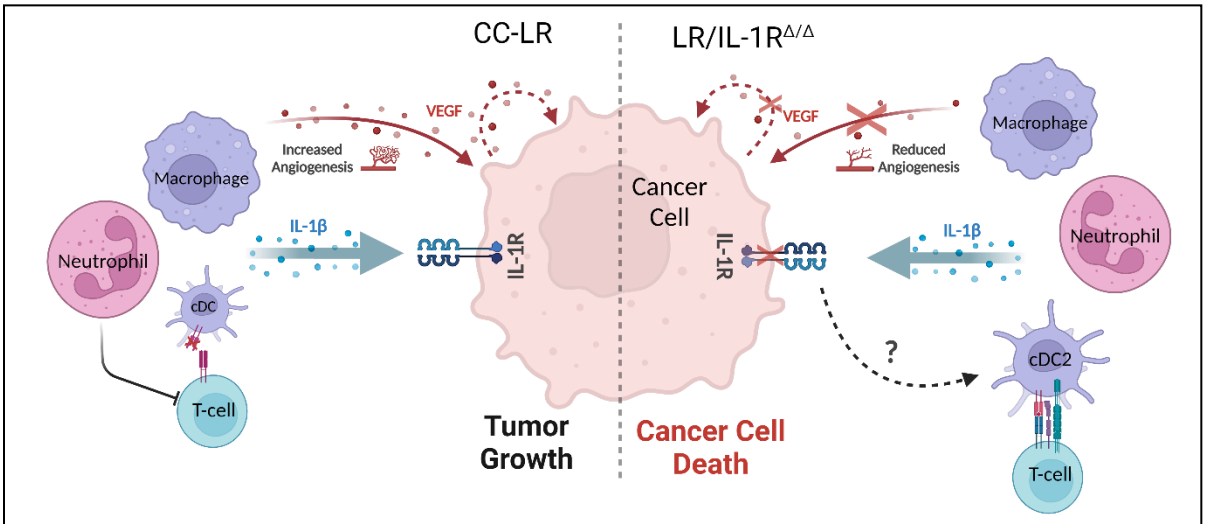


Figure 8. IL-β/IL-1R signaling in the tumor/epithelial cell is involved in tumor promotion via changes in the immune microenvironment in early and late tumor stages. (Left) IL-1R/IL-1β signaling is intact, prompting tumor associated inflammation characterized by increased angiogenic factors, production of PMN-MDSCs, and neutrophils involved in tumor cell dissemination/invasion. Furthermore, inhibition of cytotoxic T-cell function and infiltration occurs via the release of neutrophil elastase. (Right) IL-1R/IL-1β signaling is partially inhibited due to conditional deletion of IL-1R on the tumor epithelium, leading to cancer cell death. This causes a reduction in tumor and immune cell mediated angiogenesis, lack of response to IL-1β producing immune cells, and potential involvement of T-cell activity which must be further evaluated. Created in Biorender.

Following these findings, we studied the involvement of the NF-κB pathway, known to be heavily activated by IL-1β and downregulated upon its inhibition. Once again, a time dependent response was evident, where NF-κB activity was more affected in the late-stage time point. These results may confirm the involvement of tumor cell intrinsic mechanisms mainly participating in tumor progression at the late-stage timepoint, through inhibition of the NF-κB pathway. Additionally, it could suggest the role of alternative inflammatory pathways being involved in early-stage IL-1R/IL-1β associated tumor development. It would be essential to assess the STAT3 and MAPK/ERK pathway activities which has been shown that could be activated by NF-κB regulated cytokines.[24,26] Furthermore, we can evaluate the p38-MAPK pathway associated with promoting cell migration via IL-1R/IL-1β endothelial cell interaction.[56]

Lastly, having previously shown the involvement of NF-κB regulated cytokines in promoting cancer stemness properties and evidence indicating the role of IL-1β in facilitating stemness associated tumor growth, we were interested in evaluating the effects of cancer stemness in our LR/IL-1R$^{\Delta/\Delta}$ mice.[26,47] Interestingly no change in markers associated with cancer stemness was evident, potentially indicating a lack of involvement of IL-1β in promoting KM-LUAD related stem-like properties. On the other hand, our previous studies indicate that tumor of advanced stages in the lung, express more of an alveolar type II cell phenotype (AT2).[57] These AT2 cells could be derived from Club cells due to the activated K-ras oncogene and the observed phenotypic shift may be involved in prompting aggressive tumor growth. AT2 cells can generate AT1 cells, to maintain lung homeostasis in response to injury. This differentiation is mediated by IL-1β associated signaling.[58] It has been shown that upon differentiation towards an AT1 cell phenotype, AT2 cells first transit into alveolar intermediate cells (AICs). AIC's have been characterized to be Krt8+, which expand upon KM-LUAD associated pathogenesis.[59] Thus, these findings prompt the intent to study Krt8+ presence and activity when knocking out IL-1R on the tumor epithelium.

Our studies support the ability of IL-1β to elicit an inflammatory response in the context of KM-LUAD development. Hence, our intent to inhibit IL-1β to treat other lung associated diseases with

elevated levels of this cytokine would be of interest. Chronic Obstructive Pulmonary Disease (COPD), caused by exposure to inhaled pollutants, can increase the risk of lung cancer (3 to 10-fold) and 50-70% of most lung cancer individuals have COPD at the time of diagnosis.[60] COPD is caused by neutrophil and pro-inflammatory cytokine mediated airway inflammation.[61] These neutrophils show increased NF-κB signaling, which in turn regulates and is activated by alveolar macrophages.[62] Furthermore, there has been evidence linking the NLRP3 inflammasome and its subsequent inflammatory cytokines to the inflammation observed in COPD patients. Of note, one of these cytokines being IL-1β, commonly elevated in the lungs of patients with COPD.[63] Additionally, we have shown that COPD-like airway inflammation increases lung carcinogenesis through the induction of a pro-inflammatory microenvironment.[57] Thus in the future we plan to test effects of targeting IL-1β as a means of inhibiting COPD-associated lung tumorigenesis.

Regarding potential caveats to this study, it is important to note that the experiments completed were performed using whole lung lysates, thus the combination of both tumor epithelial and immune cells was present during analysis. Therefore, for future studies the goal of isolating epithelial cells from immune cells to allow for better characterization and precise analysis of what occurs upon tumor epithelial IL-1R targeting must be accomplished. Furthermore, we should not forget that targeting IL-1R, not only inhibits IL-1β signaling but additionally IL-1α signaling. Thus, potential effects of tumor depletion upon IL-1R targeting could be attributed to IL-1α depletion as well, however its role in the context of tumor progression especially within KM-LUAD is still not well understood.[64]

Altogether, these findings support the time dependent effects of IL-1β/ IL-1R tumor epithelial signaling in promoting tumor growth. Due to the drastic differences evident within the immune cell compartment at both timepoints, evaluating the effects of IL-1R targeting in a time-dependent manner would be of interest in the future. This could be done using a tamoxifen induced mouse model, where IL-1R deletion is controlled as opposed to conditionally knocked out since birth. This in turn would aid in the assessment of targeting the receptor as a potential means of treatment for KM-LUAD within the clinic.

In conclusion, our study provides new perspectives regarding the mechanisms involved in the immune associated tumor promoting effects of IL-1β signaling in the context of KM-LUAD. Furthermore, increased understanding of IL-1β's time dependent roles along with the involvement of lymphoid cell recruitment and activation could pave way for the development of novel context-specific targets for the prevention and treatment of KM-LUAD.

Chapter 4: Methods

4.1 Mice

Our lab has developed a K-ras Driven Mouse Lung Adenocarcinoma Model, known as the CC-LR mouse as previously described[53]. This model is created by mating mice carrying a Cre recombinase inserted into Club Cell Secretory Protein locus ($CCSP^{Cre}$) to mice harboring the LSL-K-ras^{G12D} to allow for lung epithelial specific constitutive expression of the mutant K-ras^{G12D} and the development of KM-LUAD. LR/IL-$1R^{\Delta/\Delta}$ mice were created by crossing CC-LR mice with mice kindly provided by Tylor Jacks harboring conditional allele of IL-1R (IL-$1R^{\Delta/\Delta}$). 14 and 18 weeks of age were chosen as early and late stage timepoints, with adenocarcinoma formation primarily associated with 14- to 18-week-old mice. Mice were housed under pathogenic free conditions and handled according to MD Anderson IACUC Guidelines.

4.2 Assessment of Lung Tumor Burden and Inflammation

Prior to dissection, mice were anesthetized via intraperitoneal (IP) injection of avertin (Sigma), and their tracheas were cannulated and sutured into place. Upon dissection, visible surface tumors were counted. To collect tissue for RNA and Protein, mice first underwent BALF collection, encompassing the administration and collection of two 1 mL aliquots of PBS through a cannula. Blood collection via the posterior vena cava was then performed. Subsequently lungs were perfused with PBS and separated and snap frozen for RNA or protein analysis. For tissue histology, following perfusion, lungs were inflated with 10% buffered formalin (Sigma) for the duration of 10 minutes, then collected and held in formalin then PBS for two consecutive days prior to paraffin embedding for further experimentation.

4.3 Immunohistochemistry (IHC) / Immunofluorescent (IF) Staining

IHC staining for proliferation marker Ki-67 (1:200; Cat ab16667, Abcam), angiogenesis marker ERG (1:200; Cat ab92513, Abcam), vascular differentiation marker CD31 (1:200; Cat ab77699T, Cell Signaling), NF-kB pathway activation marker p65 (1:2000; Cat ab32536, Abcam) and the IL-1 receptor marker (1:50; Cat sc-393998, Santa Cruz), were completed as previously established,

including optimization prior to experimentation. Stained slides were then imaged using an upright microscope. Ratio of positive staining to total tumor cells obtained from 20x field images slides where then quantified via ImageJ (NIH).

4.4 Isolation of Lung Inflammatory Cells by Flow Cytometry

Lung homogenate obtained from PBS perfused lungs post dissection for 14-week-old mice were obtained following protocol as previously described.[32] Cells obtained were primarily stained with surface markers prior to permeabilization using transcription factor-staining kit (Invitrogen) for intracellular cytokine detection. Antibodies used are listed in Table 1. Data was obtained using the LSRFortessa X-20 (BD) and analyzed using FlowJo software and an established gating strategy previously used by our lab.

Table 1. Myeloid Panel

Antibody	Clone	Fluorescence	Dilution Factor
Ghost Dye	--	Violet 510	1:100
CD45.2	104	redFluor 710	1:100
CD11b	MI/70	FITC	1:100
CD11c	N418	PE-Cy7	1:100
Ly6C	HK1.4	APC	1:100
Ly6G	1A8	PE	1:100
CD206	C068C2	BV421	1:100
I-A/I-E	M5/114.15.2	BV711	1:100

4.5 Quantitative RT-PCR Analysis (qRT-PCR)

RNA was obtained from lung homogenate, extracted using the Qiagen extraction kit following the manufacturer's provided protocol. qRT-PCR experiments were performed using qScript cDNA SuperMix (Quanta Biosciences) and SYBR Green FastMix (Quante Biosciences). The CFX96 Touch

Real-Time PCR Detection System (Bio-Rad) was used and fold changes for each group measured was obtained via the $2^{-\Delta\Delta Ct}$ method. Primers used can be found in Table 2.

Table 2

Gene	Forward	Reverse
IL-6	5'CTGATGCTGGTGACAACCAC3'	5'CAGACTTGCCATTGCACAAC3'
Mrc1	5'TTGGACGGATATGGAGGG3'	5'CCAGGCAGTTGAGGAGGTTC3'
CD4	5'GAAGATTCTGGGGCAGCATGGCAAAG3'	5'TTTGGAATCAAAACGATCAA3'
CD8	5'CTGCGTGGCCCTTCTGCTGTCCT3'	5'GGGACATTTGCAAACACGCT3'
IFNg	5'TTTTTCCAGCAGACCAGCTT3'	5'AGAGATTATCGGAGCGCCTT3'
VEGF	5' CTGCTGTAACGATGAAGCCCTG3'	5'GCTGTAGGAAGCTCATCTCTCC3'
CD31	5' CCAAAGCCAGTAGCATCATGGTC3'	5'GGATGGTGAAGTTGGCTACAGG3'

List of primers used in real-time qPCR.

4.6 Western Blot Analysis (WB)

Protein extracts were prepared from lung homogenate using a solution comprised of RIPA buffer (Sigma) and protease inhibitor cocktail (Thermo Fisher Scientific). Protein extraction and WB experimentation were performed as previously described. Concentration of protein was evaluated using a bicinchoninic acid assay. Primary antibodies used are as follows; VEGF (1:1000; Cat 9698s Cell Signaling), IL-1R (1:1000; Cat sc-393998, Santa Cruz), and p65 (1:1000; Cat ab32536, Abcam) and β-actin (1:1000; Cat 4970s, Cell Signaling).

4.7 Statistical Analysis

Data analysis was performed using two-tailed unpaired t-tests, where a P-value <0.05 was considered significant. Data was presented as a mean ±SEM and graphed using GraphPad Prism.

Bibliography

1. National Cancer Institute. (2018). *Cancer of the Lung and Bronchus - Cancer Stat Facts*. National Cancer Institute. https://seer.cancer.gov/statfacts/html/lungb.html
2. Siegel, R. L., Giaquinto, A. N., & Jemal, A. (2024). Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians*, *74*(1), 12-49.
3. Global Burden of Disease Cancer Collaboration, Fitzmaurice, C., Abate, D., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdel-Rahman, O., Abdelalim, A., Abdoli, A., Abdollahpour, I., Abdulle, A. S. M., Abebe, N. D., Abraha, H. N., Abu-Raddad, L. J., Abualhasan, A., Adedeji, I. A., Advani, S. M., Afarideh, M., Afshari, M., Aghaali, M., Murray, C. J. L. (2019). Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study. *JAMA oncology*, *5*(12), 1749–1768.
4. Siegel, R., Naishadham, D., & Jemal, A. (2012). Cancer statistics, 2012. *CA: a cancer journal for clinicians*, *62*(1), 10–29.
5. Gironés, R., López, P., Chulvi, R., Cañabate, M. & Dolores, T. others Ten years of lung cancer in a single center: gender, histology, stage and survival. *J. Cancer Metastasis Treat.* 1, 201–207 (2015)
6. Friedlaender, A., Drilon, A., Weiss, G. J., Banna, G. L., & Addeo, A. (2020). KRAS as a druggable target in NSCLC: Rising like a phoenix after decades of development failures. *Cancer treatment reviews*, *85*, 101978.
7. Zeissig, M. N., Ashwood, L. M., Kondrashova, O., & Sutherland, K. D. (2023). Next batter up! Targeting cancers with KRAS-G12D mutations. *Trends in cancer*, *9*(11), 955–967.
8. Myers, D. J. & Wallen, J. M. (2019, January 11). Cancer, Lung Adenocarcinoma. Nih.gov; Stat Pearls Publishing.

9. Cox, A. D., Fesik, S. W., Kimmelman, A. C., Luo, J., & Der, C. J. (2014). Drugging the undruggable RAS: Mission possible?. *Nature reviews. Drug discovery*, *13*(11), 828–851.
10. Román, M., Baraibar, I., López, I., Nadal, E., Rolfo, C., Vicent, S., & Gil-Bazo, I. (2018). KRAS oncogene in non-small cell lung cancer: clinical perspectives on the treatment of an old target. *Molecular cancer*, *17*(1),
11. Román, M., Baraibar, I., López, I.. KRAS oncogene in non-small cell lung cancer: clinical perspectives on the treatment of an old target. *Mol Cancer* 17, 33 (2018)
12. Huang, X., Ding, L., Liu, X., Tong, R., Ding, J., Qian, Z., Cai, L., Zhang, P., & Li, D. (2021). Regulation of tumor microenvironment for pancreatic cancer therapy. *Biomaterials*, *270*, 120680.
13. Greten, F. R., & Grivennikov, S. I. (2019). Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity*, *51*(1), 27–41.
14. Koebel, C., Vermi, W., Swann, Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* 450, 903–907 (2007).
15. Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y., & Li, Y. (2021). Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal transduction and targeted therapy*, *6*(1), 263
16. Chen, W., Li, Z., Bai, L., & Lin, Y. (2011). NF-kappaB in lung cancer, a carcinogenesis mediator and a prevention and therapy target. *Frontiers in bioscience (Landmark edition)*, *16*(3), 1172–1185.
17. Raghuwanshi, S. K., Nasser, M. W., Chen, X., Strieter, R. M., & Richardson, R. M. (2008). Depletion of beta-arrestin-2 promotes tumor growth and angiogenesis in a murine model of lung cancer. *Journal of immunology (Baltimore, Md. : 1950)*, *180*(8), 5699–5706.
18. Pereira, F., Ferreira, A., Reis, C. A., Sousa, M. J., Oliveira, M. J., & Preto, A. (2022). KRAS as a Modulator of the Inflammatory Tumor Microenvironment: Therapeutic Implications. *Cells*, *11*(3), 398.

19. Wong, K. K., Jacks, T., & Dranoff, G. (2010). NF-kappaB fans the flames of lung carcinogenesis. *Cancer prevention research (Philadelphia, Pa.)*, *3*(4), 403–405

20. Nair, V. S., Gevaert, O., Davidzon, G., Plevritis, S. K., & West, R. (2014). NF-κB protein expression associates with (18)F-FDG PET tumor uptake in non-small cell lung cancer: a radiogenomics validation study to understand tumor metabolism. *Lung cancer (Amsterdam, Netherlands)*, *83*(2), 189–196.

21. Qin, H., Zhou, J., Zhou, P., Xu, J., Tang, Z., Ma, H., & Guo, F. (2016). Prognostic significance of RelB overexpression in non-small cell lung cancer patients. *Thoracic cancer*, *7*(4), 415–421.

22. Gu, L., Wang, Z., Zuo, J., Li, H., & Zha, L. (2018). Prognostic significance of NF-κB expression in non-small cell lung cancer: A meta-analysis. *PloS one*, *13*(5), e0198223.

23. Barbie, D. A., Tamayo, P., Boehm, J. S., Kim, S. Y., Moody, S. E., Dunn, I. F., Schinzel, A. C., Sandy, P., Meylan, E., Scholl, C., Fröhling, S., Chan, E. M., Sos, M. L., Michel, K., Mermel, C., Silver, S. J., Weir, B. A., Reiling, J. H., Sheng, Q., Gupta, P. B., … Hahn, W. C. (2009). Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*, *462*(7269), 108–112.

24. Caetano, M. S., Zhang, H., Cumpian, A. M., Gong, L., Unver, N., Ostrin, E. J., Daliri, S., Chang, S. H., Ochoa, C. E., Hanash, S., Behrens, C., Wistuba, I. I., Sternberg, C., Kadara, H., Ferreira, C. G., Watowich, S. S., & Moghaddam, S. J. (2016). IL6 Blockade Reprograms the Lung Tumor Microenvironment to Limit the Development and Progression of K-ras-Mutant Lung Cancer. *Cancer research*, *76*(11), 3189–3199.

25. Chang, S. H., Mirabolfathinejad, S. G., Katta, H., Cumpian, A. M., Gong, L., Caetano, M. S., Moghaddam, S. J., & Dong, C. (2014). T helper 17 cells play a critical pathogenic role in lung cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(15), 5664–5669.

26. Khosravi, N., Caetano, M. S., Cumpian, A. M., Unver, N., De la Garza Ramos, C., Noble, O., Daliri, S., Hernandez, B. J., Gutierrez, B. A., Evans, S. E., Hanash, S., Alekseev, A. M., Yang,

Y., Chang, S. H., Nurieva, R., Kadara, H., Chen, J., Ostrin, E. J., & Moghaddam, S. J. (2018). IL22 Promotes *Kras*-Mutant Lung Cancer by Induction of a Protumor Immune Response and Protection of Stemness Properties. *Cancer immunology research*, *6*(7), 788–797.

27. Dinarello C. A. (2011). A clinical perspective of IL-1β as the gatekeeper of inflammation. *European journal of immunology*, *41*(5), 1203–1217
28. Karki, R., & Kanneganti, T. D. (2019). Diverging inflammasome signals in tumorigenesis and potential targeting. *Nature reviews. Cancer*, *19*(4), 197–214.
29. Ridker, P. M., Thuren, T., Zalewski, A., & Libby, P. (2011). Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *American heart journal*, *162*(4), 597–605.
30. Kolb, M., Margetts, P. J., Anthony, D. C., Pitossi, F., & Gauldie, J. (2001). Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *The Journal of clinical investigation*, *107*(12), 1529–1536.
31. Kanoh, Y., Abe, T., Masuda, N., & Akahoshi, T. (2013). Progression of non-small cell lung cancer: diagnostic and prognostic utility of matrix metalloproteinase-2, C-reactive protein and serum amyloid A. *Oncology reports*, *29*(2), 469–473.
32. Yuan, B., Clowers, M. J., Velasco, W. V., Peng, S., Peng, Q., Shi, Y., Ramos-Castaneda, M., Zarghooni, M., Yang, S., Babcock, R. L., Chang, S. H., Heymach, J. V., Zhang, J., Ostrin, E. J., Watowich, S. S., Kadara, H., & Moghaddam, S. J. (2022). Targeting IL-1β as an immunopreventive and therapeutic modality for K-ras-mutant lung cancer. *JCI insight*, *7*(11), e157788.
33. Study of Efficacy and Safety of Pembrolizumab Plus Platinum-based Doublet Chemotherapy With or Without Canakinumab in Previously Untreated Locally Advanced or Metastatic Non-squamous and Squamous NSCLC Subjects (CANOPY-1). ClinicalTrials.gov Identifier: NCT03631199.

34. Phase III Study Evaluating Efficacy and Safety of Canakinumab in Combination With Docetaxel in Adult Subjects With Non-small Cell Lung Cancers as a Second or Third Line Therapy (CANOPY-2). ClinicalTrials.gov Identifier: NCT03626545.
35. This Study Will Evaluate the Effect of Canakinumab or Pembrolizumab Given as Monotherapy or in Combination as Neo-adjuvant Treatment for Subjects With Early Stages NSCLC. (CANOPY-N). ClinicalTrials.gov Identifier: NCT03968419
36. Study of Efficacy and Safety of Canakinumab as Adjuvant Therapy in Adult Subjects With Stages AJCC/UICC v. 8 II-IIIA and IIIB (T>5cm N2) Completely Resected Non-small Cell Lung Cancer Acronym: CANOPY-A (Canopy-A). ClinicalTrials.gov Identifier: NCT03447769.
37. Lythgoe, M.P., Prasad, V. Repositioning canakinumab for non-small cell lung cancer—important lessons for drug repurposing in oncology. *Br J Cancer* 127, 785–787 (2022).
38. Kaneko, N., Kurata, M., Yamamoto, T., The role of interleukin-1 in general pathology. *Inflamm Regener* 39, 12 (2019).
39. Horai, R., Asano, M., Sudo, K., Kanuka, H., Suzuki, M., Nishihara, M., Takahashi, M., & Iwakura, Y. (1998). Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *The Journal of experimental medicine*, *187*(9), 1463–1475.
40. Dinarello C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological reviews*, *281*(1), 8–27.
41. Monteleone, M., Stanley, A. C., Chen, K. W., Brown, D. L., Bezbradica, J. S., von Pein, J. B., Holley, C. L., Boucher, D., Shakespear, M. R., Kapetanovic, R., Rolfes, V., Sweet, M. J., Stow, J. L., & Schroder, K. (2018). Interleukin-1β Maturation Triggers Its Relocation to the Plasma Membrane for Gasdermin-D-Dependent and -Independent Secretion. *Cell reports*, *24*(6), 1425–1433.

42. Garlanda and Mantovani “Interleukin-1 in Tumor Progression, Therapy, and Prevention.” *Cancer Cell* 39,8 (2019)
43. Rébé, C., & Ghiringhelli, F. (2020). Interleukin-1β and Cancer. *Cancers*, *12*(7), 1791.
44. Chevallier, M., Borgeaud, M., Addeo, A., & Friedlaender, A. (2021). Oncogenic driver mutations in non-small cell lung cancer: Past, present, and future. *World journal of clinical oncology*, *12*(4), 217–237
45. Tu J, Fang Y, Han D, Activation of nuclear factor-κB in the angiogenesis of glioma: Insights into the associated molecular mechanisms and targeted therapies. *Cell Prolif*. 2021; 54:e12929
46. Sangaletti, S., Ferrara, R., Tripodo, C., Garassino, M. C., & Colombo, M. P. (2021). Myeloid cell heterogeneity in lung cancer: implication for immunotherapy. *Cancer immunology, immunotherapy : CII*, *70*(9), 2429–2438.
47. Lu, L., Wang, P., Zou, Y., Zha, Z., Huang, H., Guan, M., Wu, Y., & Liu, G. (2020). IL-1β Promotes Stemness of Tumor Cells by Activating Smad/ID1 Signaling Pathway. *International journal of medical sciences*, *17*(9), 1257–1268.
48. Kiriakidis S, Andreakos E, Monaco C, Foxwell B, Feldmann M, Paleolog E. VEGF expression in human macrophages is NF-kappaB-dependent: studies using adenoviruses expressing the endogenous NF-kappaB inhibitor IkappaBalpha and a kinase-defective form of the IkappaB kinase 2. *J Cell Sci*. (2003) 116:665–74. 10.1242/jcs.00286
49. Jung, Y. J., Isaacs, J. S., Lee, S., Trepel, J., & Neckers, L. (2003). IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *17*(14), 2115–2117.
50. Nakao, S., Kuwano, T., Tsutsumi-Miyahara, C., Ueda, S., Kimura, Y. N., Hamano, S., Sonoda, K. H., Saijo, Y., Nukiwa, T., Strieter, R. M., Ishibashi, T., Kuwano, M., & Ono, M. (2005). Infiltration of COX-2-expressing macrophages is a prerequisite for IL-1 beta-induced

neovascularization and tumor growth. *The Journal of clinical investigation*, *115*(11), 2979–2991.

51. Si, Y., Wang, Y., Tian, Q., Wang, Q., Pollard, J. M., Srivastava, P. K., Esser-Kahn, A. P., Collier, J. H., Sperling, A. I., & Chong, A. S. (2023). Lung cDC1 and cDC2 dendritic cells priming naive $CD8^+$ T cells in situ prior to migration to draining lymph nodes. *Cell reports*, *42*(10), 113299.
52. Zilionis, R., Engblom, C., Pfirschke, C., Savova, V., Zemmour, D., Saatcioglu, H. D., Krishnan, I., Maroni, G., Meyerovitz, C. V., Kerwin, C. M., Choi, S., Richards, W. G., De Rienzo, A., Tenen, D. G., Bueno, R., Levantini, E., Pittet, M. J., & Klein, A. M. (2019). Single-Cell Transcriptomics of Human and Mouse Lung Cancers Reveals Conserved Myeloid Populations across Individuals and Species. *Immunity*, *50*(5), 1317–1334.e10.
53. Van Den Eeckhout, B., Tavernier, J., & Gerlo, S. (2021). Interleukin-1 as Innate Mediator of T Cell Immunity. *Frontiers in immunology*, *11*, 621931.
54. Hatscher, L., Amon, L., Heger, L., & Dudziak, D. (2021). Inflammasomes in dendritic cells: Friend or foe?. *Immunology letters*, *234*, 16–32.
55. Ghiringhelli, F., Apetoh, L., Tesniere, A., Aymeric, L., Ma, Y., Ortiz, C., Vermaelen, K., Panaretakis, T., Mignot, G., Ullrich, E., Perfettini, J. L., Schlemmer, F., Tasdemir, E., Uhl, M., Génin, P., Civas, A., Ryffel, B., Kanellopoulos, J., Tschopp, J., André, F., Zitvogel, L. (2009). Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nature medicine*, *15*(10), 1170–1178.
56. Jagielska, J., Kapopara, P. R., Salguero, G., Scherr, M., Schütt, H., Grote, K., Schieffer, B., & Bavendiek, U. (2012). Interleukin-1 assembles a proangiogenic signaling module consisting of caveolin-1, tumor necrosis factor receptor-associated factor 6, p38-mitogen-activated protein kinase (MAPK), and MAPK-activated protein kinase 2 in endothelial cells. *Arteriosclerosis, thrombosis, and vascular biology*, *32*(5), 1280–1288.

57. Moghaddam, S. J., Li, H., Cho, S. N., Dishop, M. K., Wistuba, I. I., Ji, L., Kurie, J. M., Dickey, B. F., & Demayo, F. J. (2009). Promotion of lung carcinogenesis by chronic obstructive pulmonary disease-like airway inflammation in a K-ras-induced mouse model. *American journal of respiratory cell and molecular biology*, *40*(4), 443–453.

58. Choi, J., Park, J. E., Tsagkogeorga, G., Yanagita, M., Koo, B. K., Han, N., & Lee, J. H. (2020). Inflammatory Signals Induce AT2 Cell-Derived Damage-Associated Transient Progenitors that Mediate Alveolar Regeneration. *Cell stem cell*, *27*(3), 366–382.e7.

59. Han, G., Sinjab, A., Rahal, Z., Lynch, A. M., Treekitkarnmongkol, W., Liu, Y., Serrano, A. G., Feng, J., Liang, K., Khan, K., Lu, W., Hernandez, S. D., Liu, Y., Cao, X., Dai, E., Pei, G., Hu, J., Abaya, C., Gomez-Bolanos, L. I., Peng, F., Kadara, H. (2024). An atlas of epithelial cell states and plasticity in lung adenocarcinoma. *Nature*, *627*(8004), 656–663.

60. Syamlal, G., Kurth, L. M., Dodd, K. E., Blackley, D. J., Hall, N. B., & Mazurek, J. M. (2022). Chronic Obstructive Pulmonary Disease Mortality by Industry and Occupation - United States, 2020. *MMWR. Morbidity and mortality weekly report*, *71*(49), 1550–1554.

61. Jasper, A. E., McIver, W. J., Sapey, E., & Walton, G. M. (2019). Understanding the role of neutrophils in chronic inflammatory airway disease. *F1000Research*, *8*, F1000 Faculty Rev-557.

62. Schuliga M. (2015). NF-kappaB Signaling in Chronic Inflammatory Airway Disease. *Biomolecules*, *5*(3), 1266–1283

63. Birrell, M. A., & Eltom, S. (2011). The role of the NLRP3 inflammasome in the pathogenesis of airway disease. *Pharmacology & therapeutics*, *130*(3), 364–370.

64. Chiu, J. W., Binte Hanafi, Z., Chew, L. C. Y., Mei, Y., & Liu, H. (2021). IL-1α Processing, Signaling and Its Role in Cancer Progression. *Cells*, *10*(1), 92

Vita

Avantika Krishna is from Austin, Texas, where she was born as the eldest of her twin brother and sister. In 2008, her family relocated to Aix-En-Provence, France, where she spent her formative years. Avantika attended the Centre International Pour L'Education et la Culture (CIPEC) for her fifth year of elementary school before advancing to the International Bilingual School of Provence, where she pursued her education until her sophomore year of high school. Here, she studied the Cambridge International General Certificate of Secondary Education curriculum (IGCSE). In 2015, Avantika and her family settled in Ellicott City, Maryland, USA. Completing her high school education at Mount Hebron in May 2017, Avantika immersed herself in diverse experiences that kindled her passion for scientific research. Notably she seized the opportunity to intern at the prestigious National Institute of Health (NIH) during the summer of 2016, solidifying her dedication to scientific inquiry. These experiences paved the way for Avantika to embark on her academic journey with the support of a STEM Build scholarship funded by the NIH. This scholarship funded her undergraduate studies at the University of Maryland Baltimore County, where she pursued a Bachelor of Science in Biochemistry and Molecular Biology, graduating in May 2021. Continuing her pursuit of knowledge, Avantika was admitted to the Master of Science in Biomedical Sciences program at The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences. Specializing in Immunology, she is set to graduate in May 2024, equipped with a wealth of expertise and a master's degree that signifies her commitment to advancing scientific understanding and medical breakthrough.