IL-34 inhibition targeting macrophage-mediated immune suppression in murine SPCcre Kras lung tumor model

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Introduction

Lung adenocarcinoma remains a leading cause of cancer mortality. The tumor microenvironment (TME)—particularly myeloid cells and macrophages—plays a central role in tumor progression and immune suppression.

IL-34, a ligand for the colony stimulating factor-1 receptor (CSF1R), supports macrophage survival and differentiation. Increased IL-34 expression in tumors has been shown to correlate with enrichment of immunosuppressive macrophages and resistance to immune checkpoint therapy.

Prior studies from the Varner Lab identified PI3Kγ as a key myeloid signaling node integrating cytokine, TLR, and receptor tyrosine kinase inputs, promoting immune suppression and tumor growth [1-3]. Targeting PI3Kγ or upstream regulators such as IL-34 may reprogram macrophages toward a proinflammatory, T-cell–supportive phenotype [3].

This study examines whether IL-34 blockade or genetic deletion of IL-34 alters the immune landscape in SPCcre KrasG12D murine lung tumors.

Objectives

1. Quantify macrophage (F4/80+), total T-cell (CD3+), and cytotoxic T-cell (CD8+) infiltration in control, anti-IL-34-treated, and IL-34 knockout (IL-34$^{-/-}$) SPCcre Kras lung tumors.
2. Compare staining intensity and distribution across groups to assess shifts in immune composition.
3. Determine whether IL-34 suppression prevents potential macrophage-mediated immune suppression within the TME.

Methods

SPCcre Kras mice (6 weeks old) were treated with 200ug of anti-IL-34 antibody every 3 days for 2-3 weeks via IP injection. SPCcre Kras IL-34$^{-/-}$ mice were used as a comparison for the exogenous anti-IL-34 treatment. Mice were sacrificed and lungs resected at 8-9 weeks old.

Murine lung sections were fixed in formalin, stained with H&E, followed by IHC for: CD3, CD8, and F4/80 to detect T cells, CD8+ T cells, and macrophages, respectively.

QuPath software was used for automated cell detection and quantification of DAB-stained cells. Thresholds of cell detection were adjusted for slides with high background DAB or faint H&E staining after qualitative assessment of staining distribution and background signal. Cell density was compared between groups.

Images in Figures 1-4 were captured at 10x (Fig. 1-4, A&C) and 30x (Fig. 1-4, B&D) magnification on QuPath.

Figure 1. CD3+ quantification of control (Fig. 1A-1B) vs. anti-IL-34-treated (Fig. 1C-1D) SPCcre Kras lungs

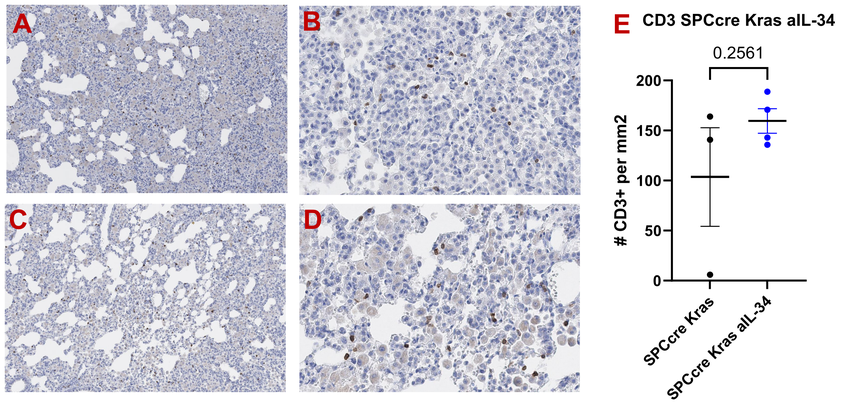


Figure 2. CD8+ quantification of control (Fig. 2A-2B) vs. anti-IL-34-treated (Fig. 2C-2D) SPCcre Kras lungs

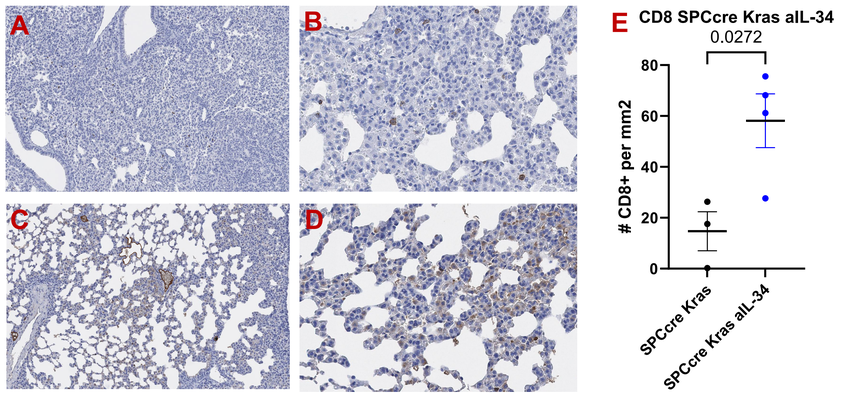


Figure 3. F4/80+ quantification of control (Fig. 3A-3Banti-IL-34-treated (Fig. 3C-3D) SPCcre Kras lungs

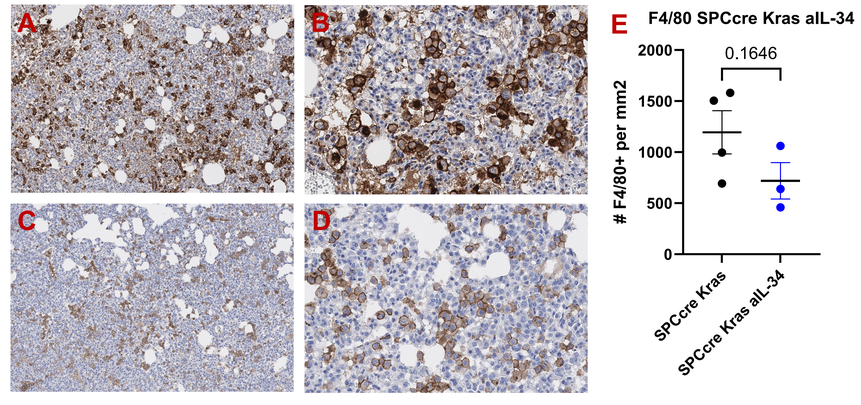
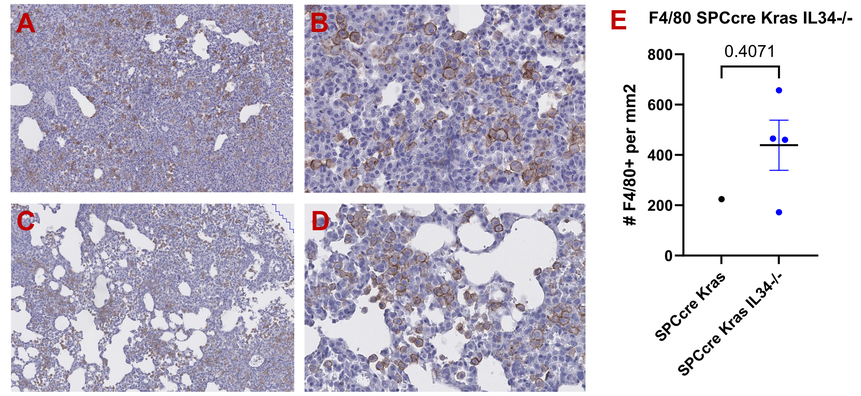


Figure 4. F4/80+ quantification of control SPCcre Kras (Fig. 4A-4B) vs. SPCcre Kras IL-34$^{-/-}$ (Fig. 4C-4D) lungs



Results

T-cell infiltration (CD3):

- Anti-IL-34-treated mice showed tumors with more consistent and higher CD3+ counts, indicating enhanced T-cell presence.

Cytotoxic T cells (CD8):

- Anti-IL-34-treated mice showed tumors with increased CD8+ cells, though some slides displayed DAB background signal.
- Suggests a trend toward a cytotoxic T-cell-enriched microenvironment after IL-34 inhibition.

Macrophage infiltration (F4/80):

- Anti-IL-34 tumors exhibited reduced F4/80 staining intensity and fewer macrophages. Decreased macrophage density suggests successful blockade of IL-34-mediated recruitment.
- IL-34$^{-/-}$ mice did not display significant difference in F4/80+ macrophage accumulation compared to the control group—suggests possible rerouting of macrophage-mediated immune suppression through an alternative signaling pathway from PI3Kγ.

Conclusions

Exogenous IL-34 blockade reduces macrophage infiltration in SPCcre Kras murine lung tumors, and increased CD3+ and CD8+ infiltration suggests enhanced adaptive immune engagement.

Findings support a model in which IL-34 promotes an immunosuppressive macrophage phenotype via pathways converging on PI3Kγ, and that IL-34 inhibition may promote the immunogenicity of the tumor microenvironment.

These data align with prior evidence that myeloid-targeted therapies (PI3Kγ inhibitors, CSF1R blockade) synergize with T-cell-directed immunotherapies to overcome tumor immune resistance [2,3].

References

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