IL-15 signaling regulates the protective NK cell-cDC1 axis in melanoma.

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Background

- Type 1 conventional dendritic cells (cDC1s) play a critical role in cancer by presenting antigens from tumor cells to T cells within the tumor microenvironment (TME) or in tumor-draining lymph nodes, thus regulating T cell proliferation, survival, and effector functions.[1]
- Despite their scarcity, cDC1s are associated with better patient survival across multiple cancers and are predictive of responsiveness to anti-PD-1 immunotherapy in melanoma patients.[1]
- The cytokine FLT3LG in the TME influences the levels of cDC1s and is produced by Natural Killer (NK) cells.[2]

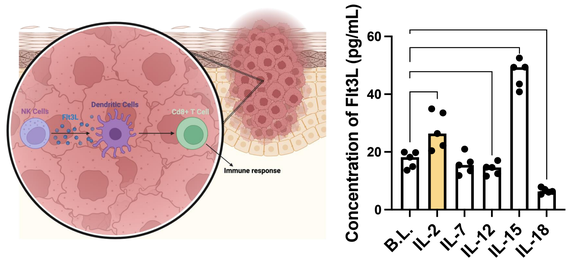


Figure 1: Amount of Flt3L produced by *in vitro* stimulated NK cells quantified by ELISA.

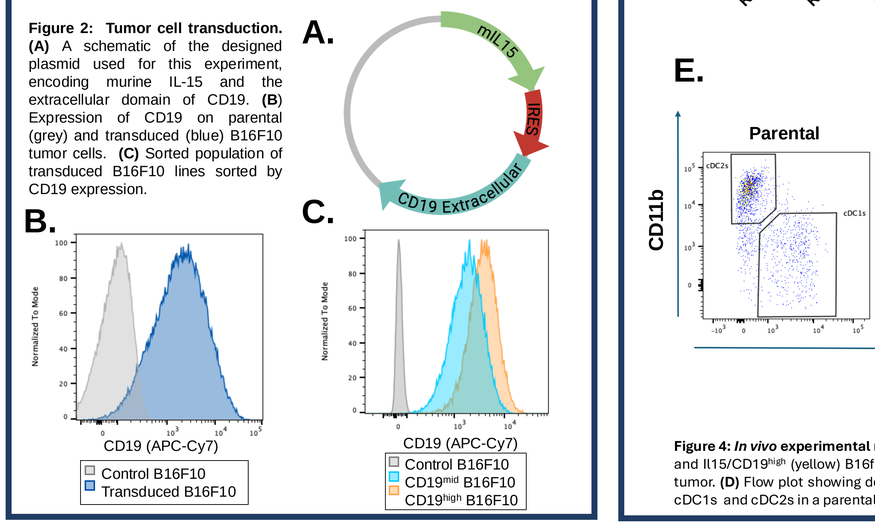
- Our lab has shown that NK cells produce the most Flt3L when exposed to cytokine IL-15.
- Our goal: To investigate the impact of elevated IL-15 levels in the tumor microenvironment on *Flt3l* expression and its possible effect on the amount of cDC1s.

References

1. Böttcher JP, Reis e Sousa C. The Role of Type 1 Conventional Dendritic Cells in Cancer Immunity. Trends Cancer. 2018 Nov;4(11):784-792.
2. Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, Nelson AE, Loo K, Kumar R, Rosenblum MD, Alvarado MD, Wolf DM, Bogunovic D, Bhardwaj N, Daud AI, Ha PK, Ryan WR, Pollack JL, Samad B, Asthana S, Chan V, Krummel MF. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nat Med. 2018 Aug;24(8):1178-1191.

Transduction and Sorting

Figure 2: Tumor cell transduction. (A) A schematic of the designed plasmid used for this experiment, encoding murine IL-15 and the extracellular domain of CD19. **(B)** Expression of CD19 on parental (grey) and transduced (blue) B16F10 tumor cells. **(C)** Sorted population of transduced B16F10 lines sorted by CD19 expression.



Characterization of the Sorted Tumor Lines

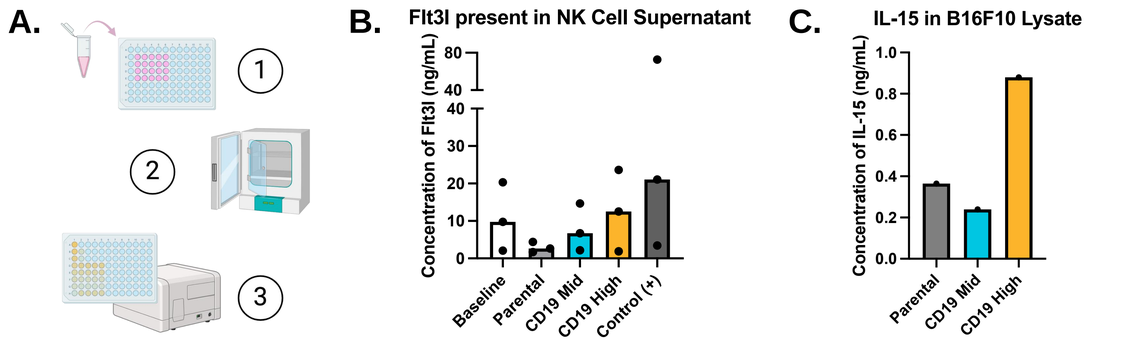


Figure 3: Characterization of B16F10 tumor lines. (A) Procedure followed for ELISA assays. **(B)** Flt3L production by NK cells when exposed to B16F10 tumor cell line supernatant quantified by ELISA. **(C)** Quantification of IL-15 concentration in supernatants of different B16F10 tumor cell lines, measured by ELISA .

In Vivo Analysis of Tumor Models

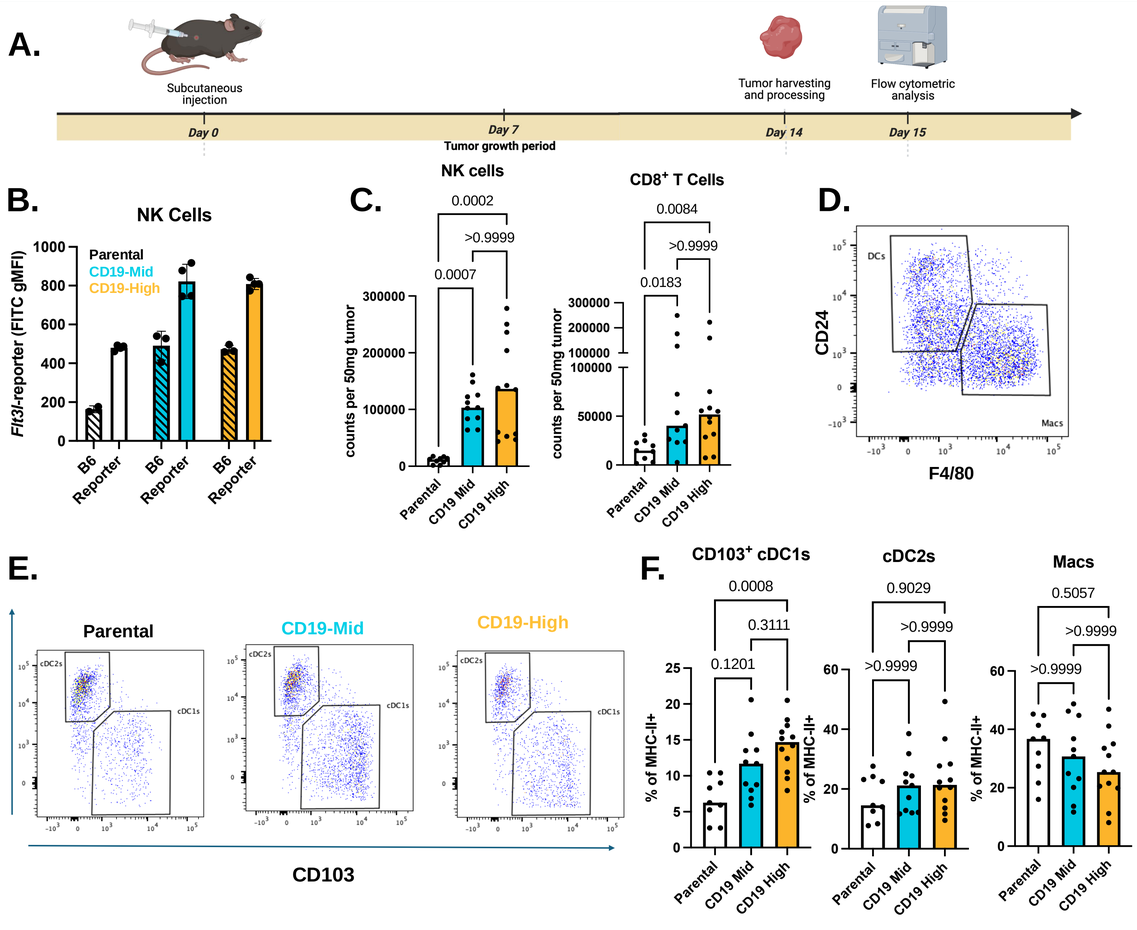


Figure 4: *In vivo* experimental results. (A) Experimental design for *in vivo* mouse experiments. **(B)** Production of *Flt3l* by total NK cells in parental (black), Il15/CD19^{mid} (blue), and Il15/CD19^{high} (yellow) B16f10 tumors, measured using a transcriptional *Flt3l*-reporter mouse strain. **(C)** Counts of NK cells, CD4^{+} T cells, and CD8^{+} T cells in 50 mg of tumor. **(D)** Flow plot showing dendritic cell population and macrophages in a parental tumor. Gating scheme includes CD45^{+}, lineage^{-}, and MHC-II^{+}. **(E)** Flow plot showing cDC1s and cDC2s in a parental, il15/CD19^{mid}, and il15/CD19^{high} B16F10 tumor. **(F)** cDC1s, cDC2s, and macrophages per tumor line as a percent of MHC-II^{+} cells present.

Spatial analysis of NK-cDC1 axis

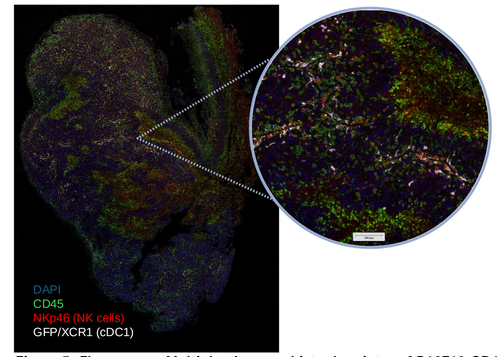


Figure 5: Fluorescent Multiplex Immunohistochemistry of B16F10-CD19-Mid tumor. XCR1-venus mice were transplanted with tumor and stained for DAPI (blue), CD45 (green), NKp46 (red), and GFP/XCR1 (white).

Conclusion

- Tumor lines were successfully transduced, sorted, and confirmed to be producing IL-15.
- High and mid Il15/CD19 expression levels were associated with increased quantities of NK cells, CD8 T cells, and cDC1s, with the most significant rise being a threefold increase in cdc1s
- These results demonstrate an enhanced immune response, characterized by increased NK cell, cDC1, and CD8^{+} T cells, when tumors are treated with IL-15. This finding holds promise as a potential treatment for tumor control in humans and suggest IL-15 regulates the NK cell-cDC1 axis *in vivo*.

Next steps

- Identify the suppressive factor(s) in B16F10 supernatants.
- Replicate the experiment in a mouse model with NK cell knockout or using NK cell-depleting antibodies to determine if the observed effects are NK cell-dependent.
- Combine this experiment with checkpoint blockade to evaluate potential tumor control.

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