Understanding and **targeting** the association between **REST** activation, **neuroendocrine** pathway **suppression**, and an **inflamed tumor microenvironment** in **small cell lung cancer**

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Background

- Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine (NE) carcinoma associated with poor clinical outcomes and modest efficacy of immune checkpoint inhibition (ICI) despite high tumor mutation burden.
- A subset of SCLC tumors exhibit increased REST and NOTCH pathway activity and increased inflammation of the tumor microenvironment (TME). REST is a transcriptional repressor that is widely expressed except in neuronal and NE cells.
- Lysine-specific demethylase 1 inhibition (LSD1i) in SCLC has potent anti-tumor effects via upregulation of REST and NOTCH pathways and suppression of canonical NE genes such as ASCL1.
- We therefore sought to test the following two hypotheses:
  - Increased REST activity drives a transition to a more inflamed subtype of SCLC, and understanding of the mechanism by which this occurs could lead to new therapeutic strategies in SCLC.
  - LSD1i can pharmacologically recapitulate natural epigenetic plasticity to induce a REST- and NOTCH-high state that increases sensitivity to ICI in SCLC.

REST expression correlates with immune infiltration in SCLC transcriptomic analysis

We performed unsupervised hierarchal clustering of immune-related gene expression using published RNA-seq data from 81 SCLC primary tumors. We found that approximately one third of tumors exhibited increased inflammatory infiltrate as evidenced by immune cell specific transcripts. Surprisingly, inflamed tumors were associated with higher REST levels and suppression of REST target genes.

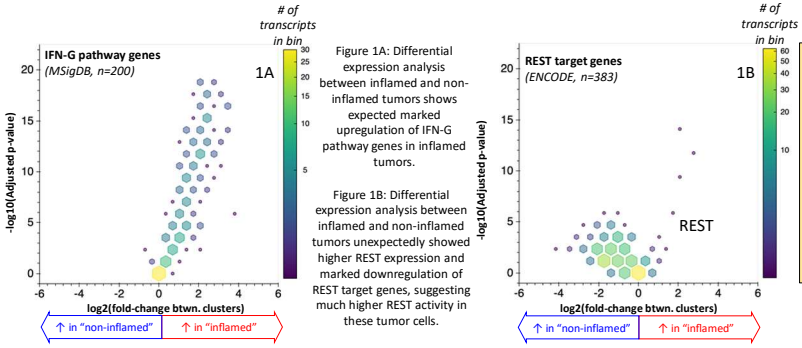

Figure 1A: Differential expression analysis between inflamed and non-inflamed tumors shows expected marked upregulation of IFN-G pathway genes in inflamed tumors.

Figure 1B: Differential expression analysis between inflamed and non-inflamed tumors unexpectedly showed higher REST expression and marked downregulation of REST target genes, suggesting much higher REST activity in these tumor cells.

Based on this finding, we hypothesized that REST activity leads to a pro-inflammatory tumor cell state via unknown mechanisms. We undertook multiple subsequent studies including generation of a GEM model of REST activation in SCLC.

Generation of REST-high SCLC GEMM to characterize the REST-dependent immune TME

To examine the effect of REST activation in an autochthonous system, we bred a Cre-inducible hREST allele into the standard murine SCLC GEM model. We sought to establish the first GEM model of SCLC with suppressed levels of the major NE transcription factors and to test whether REST activation and NE pathway suppression was sufficient to drive anti-tumor inflammation.

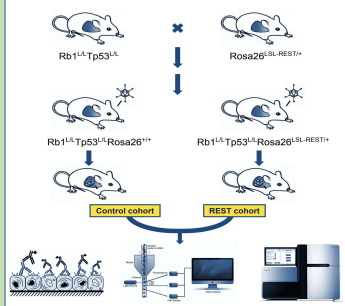

Figure 2A: An Cre inducible REST construct in the Rosa26 locus was bred into the standard SCLC GEMM. Recombination was induced via intratracheal administration of Cre-expression adenovirus and the cohorts were aged to facilitate multiple analyses.

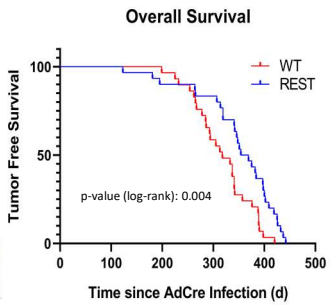

Figure 2B: Kaplan-meier survival curve showing percent freedom from lung tumors by Rosa26 allele status (WT: wild-type, REST: heterozygous for LSL-REST).

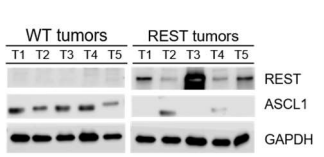

Figure 2C: Tumors resected from mice in the LSL-REST cohort exhibit considerably increased REST and decreased ASCL1 protein level.

We successfully established a SCLC GEMM with high REST and low ASCL1. An intriguing survival advantage was observed in the LSL-REST cohort. Ongoing analyses include histological and transcriptomic analyses of the resected tumors and cell lines that were established from them.

LSD1i & anti-PD1 combination therapy synergize and increase immune infiltration

LSD1 inhibition can upregulate REST and NOTCH pathways. We sought to test whether LSD1i synergized with ICI in an immunocompetent flank tumor model of SCLC. To model the high neoantigen burden present in human SCLC, we used $Rb1^{-/-}Tp53^{-/-}$ mSCLC cell lines that had been engineered to express potent MHC class I and class II antigens.

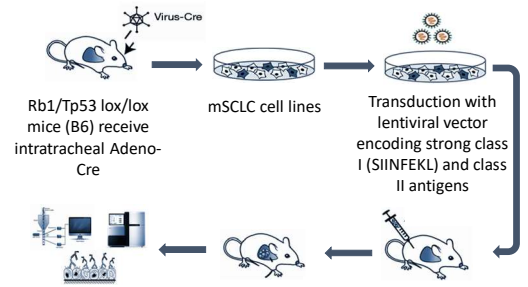

Figure 3A: Schematic of mSCLC cell line generation and engineering of MHC class I and class II antigen expression.

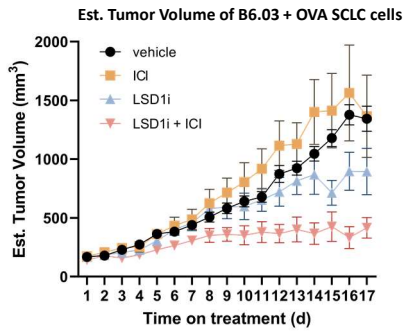

Figure 3B: mSCLC flank tumor growth was synergistically inhibited by LSD1i + ICI treatment. All treatment groups were significantly different from one another via two-way ANOVA with multiple testing correction.

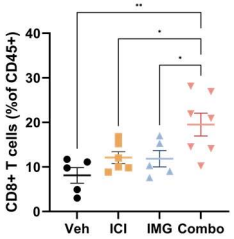

Figure 3C: Combination therapy with LSD1i and ICI led to a significant increase in $CD8^+$ T cell infiltration relative to monotherapy with either agent or vehicle.

Ongoing analyses include immunohistochemical and transcriptomic profiling of tumors to identify mechanisms of sensitization to combination therapy. We are planning a clinical trial of LSD1i in combination with maintenance ICI in newly diagnosed extensive stage SCLC.

REST chromatin occupancy

REST occupancy has not been previously interrogated in SCLC. We performed CUT&RUN in a REST-low mSCLC cell line with engineered REST overexpression to identify direct REST targets.

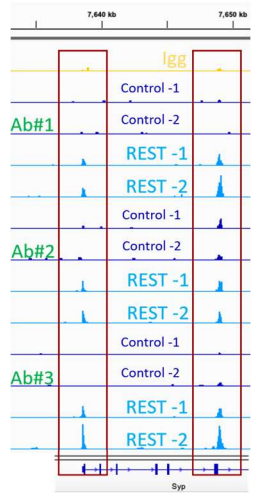

Figure 4: Inspection of high confidence REST target genes shows clear peak enrichment in the REST-high lines across all three antibodies.

Next Steps

- Complete characterization of REST overexpression GEMM samples (IHC, transcriptomes)
- Complete characterization of LSD1i/ICI combination treatment tumors (IHC, transcriptomes)
- Perform flank tumor growth studies of REST overexpression in neoantigen-expressing mSCLC cells
- Intersect chromatin occupancy data with transcriptomic analyses to identify pro-inflammatory pathways that are REST-dependent
- Implement clinical trial of LSD1i and maintenance ICI in newly diagnosed ES-SCLC

References

- Augert A, et al. Targeting NOTCH activation in small cell lung cancer through LSD1 inhibition. Sci Signal. 2019 Feb 5;12(567):eaau2922. doi: 10.1126/scisignal.aau2922. PMID: 30723171; PMCID: PMC6530478.
- Gay et al. Patterns of transcription factor programs and immune pathway activation define four major subtypes of SCLC with distinct therapeutic vulnerabilities. Cancer Cell. 2021 Mar 8;39(3):346-360.e7. doi: 10.1016/j.ccell.2020.12.014. Epub 2021 Jan 21. PMID: 33482121; PMCID: PMC8143037.
- Roper, N., Velez, M.J., Chiappori, A. *et al.* Notch signaling and efficacy of PD-1/PD-L1 blockade in relapsed small cell lung cancer. *Nat Commun* **12,** 3880 (2021). https://doi.org/10.1038/s41467-021-24164-y