Single-cell RNA sequencing reveals priming professional antigen-presenting macrophages and chemokine expressing T cells in tumor microenvironment by AXL inhibitor, SKI-G-801

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Sponsored by Oscotec Inc.

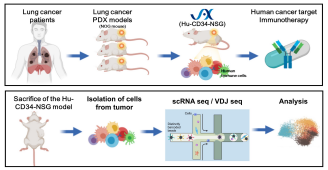
Abstract

The mechanistic investigations have defined that AXL inhibition reprograms the immunological microenvironment leading to increased T cells and antigen-presenting cells. However, previous reports did not clearly demonstrate the linchpin mechanism of anti-tumor effects with AXL inhibitors. It may be possible to elucidate the immunological mechanism of AXL inhibitor based on a comprehensive analysis of cell type-specific transcriptomic changes using scRNA-seq. The human CD34-NSG mice were engrafted LUSC tumor. These mice were divided into four dose groups: Vehicle, SKI-G-801, pembrolizumab, and combination groups. The tumor-associated cells were collected for flow cytometry and scRNA-seq analysis, and FFPE sample of tumor tissue was analyzed by a multispectral imager. The combination group demonstrated the superior anti-cancer efficacy to vehicle, pembrolizumab, and SKI-G-801 groups (all $p<0.05$). The scRNA-seq was performed with Seurat 4.0.

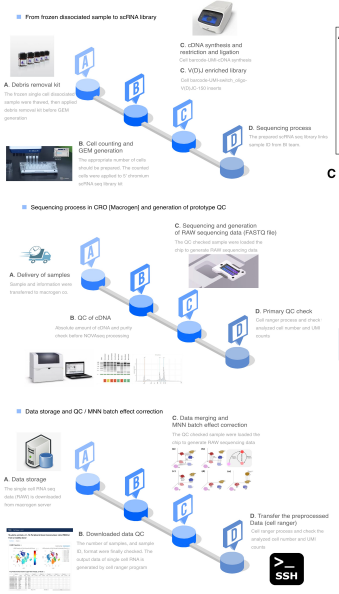
The cells were separated into total 9 clusters. Following SKI-G-801 treatment, the T cells showed higher expressions of CD2, CCL5, CCL4, GZMA, and GZMB to both pembrolizumab and vehicle groups (both p<0.05). The gene expression of CD2 and GZMA were higher than pembrolizumab and vehicle groups (both $p<0.05$). The CDR3 length differences of TCRs and diversity of T cell clones were higher increased in SKI-G-801, pembrolizumab and combination groups than vehicle group ($p<0.05$). In the macrophage cluster, the HLA-A, HLA-DRA, HLA-DRB1, and IL-1B genes were commonly expressed in SKI-G-801 and combination group ($p<0.05$). Mostly, the professional antigen-presenting macrophages (MHC class 2 protein complex high, $p<0.05$) were increased in the combination group. The combination effects between pembrolizumab and SKI-G-801 can be addressed by enhanced antigen presenting machinery, T cell activation and unique T cell clones. These transcriptomic results were highly correlated with the results of multispectral imaging and flow cytometry. Tumor infiltrations of helper T cells and cytotoxic T cells significantly increased in combination and SKI-G-801 groups ($p < 0.01$, $p < 0.001$ respectively). In multiplex IHC analysis, the proportion of $CD4^+$ and $CD8^+$ T cells were significantly increased in the tumor nest (both $p<0.05$), but the Tregs were not changed in the tumor nest which observations were validated by further flow cytometry analysis. A novel AXL inhibitor, SKI-G-801 drives priming of professional antigen-presenting cells and tumor-infiltrating T cells, leading to immunological synapsis for tumor killing, which is significantly enhanced by the combination with pembrolizumab. These results suggest the inhibition of AXL signal pathway by SKI-G-801 could confer a solid rationale for clinical investigation of lung cancer cells.

Methods

scRNA seq / VDJ seq of humanized mouse tumor



Processes of scRNA seq and analysis



Results

Figure 1 Drug efficacy test with pembrolizumab and SKI-G-801

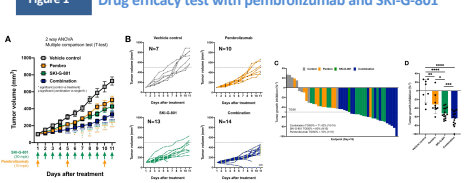


Figure 2 Classification of immune cell and cancer cells in cluster by canonical gene markers

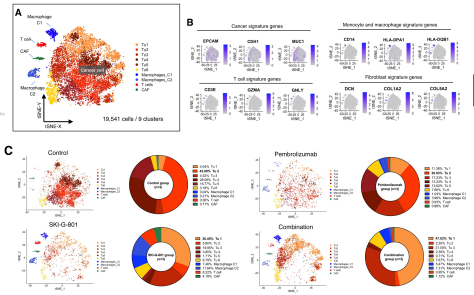


Figure 3 Transcriptional changes of T cells after treatment of SKI-G-801 and Pembrolizumab

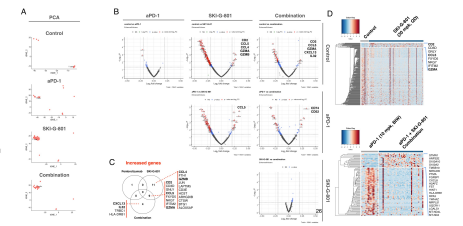


Figure 4 TCR repertoire analysis between treatment groups (T cells)

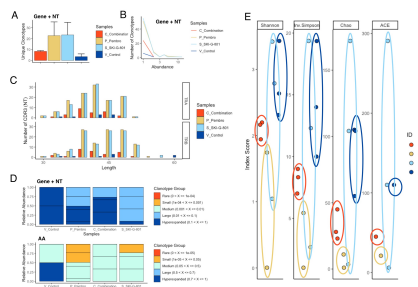


Figure 5 Transcriptional changes of macrophages after treatment of SKI-G-801 and Pembrolizumab

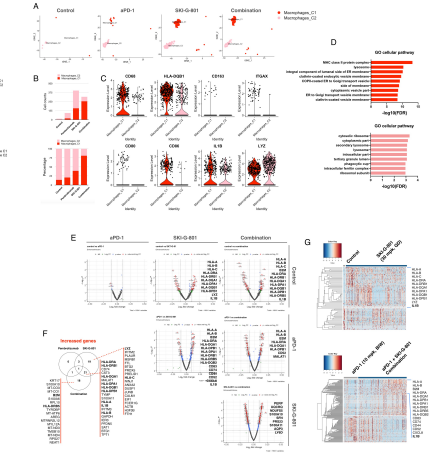
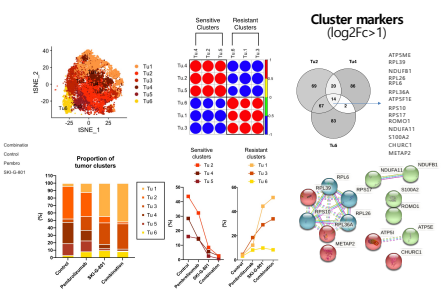
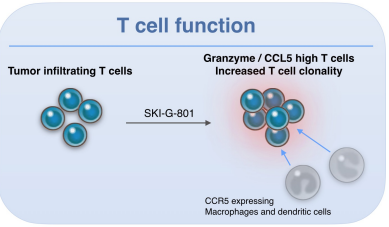


Figure 6 Biomarkers of SKI-G-801 sensitive tumor

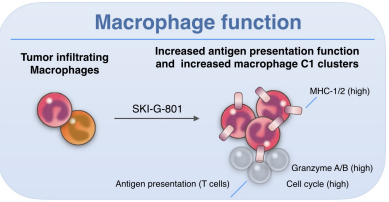


Summary

T cell function



Macrophage function



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