# Elucidating the Role of IL-18 in NSCLC: Integrated Analysis through Imaging Mass Cytometry, Mass Cytometry, and Single-Cell Sequencing

T1030-02-12

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## PURPOSE

Interleukin-18 (IL-18) plays a pivotal role in non-small cell lung cancer (NSCLC) progression, influencing both tumor growth and immune response dynamics. Recent insights into the heterogeneity and functional status of tumor-infiltrating T cells have underscored their critical impact on antitumor immunity and responses to immunotherapy, paralleling the observed complexity in IL-8 interactions within the tumor microenvironment. This study employs Imaging Mass Cytometry (IMC), Mass Cytometry (CyTOF), and Single-Cell RNA Sequencing (scRNA-seq) to map IL-18 expression, explore its impact on immune cell subsets, and identify its role in the tumor microenvironment.

## METHOD(S)

### Sample collection

Tumor samples collected at different stages of the disease were obtained from non-small cell lung cancer (NSCLC) patients ensuring a diverse representation of tumor phenotypes. Formalin-fixed paraffin-embedded (FFPE) tissues were used for Imaging Mass Cytometry (IMC), while fresh-frozen samples were analyzed via mass cytometry and single-cell RNA sequencing (scRNA-seq).

### Imaging Mass Cytometry (IMC)

Tissue sections were stained with metal-tagged antibodies for a panel of proteins including IL-8 protein, followed by UV laser ablation and ion detection using time-of-flight mass spectrometry (TOF-MS).

### Mass Cytometry (CyTOF)

CyTOF was performed on dissociated tumor samples stained with a 40-marker panel to systemically profile IL-8 and other immune markers. Data were analyzed using tSNE and FlowJo software to correlate IL-8 expression with immune cell infiltration and tumor characteristics.

### Single-Cell RNA Sequencing (scRNA-seq)

RNA was extracted from fresh-frozen NSCLC tumor samples, and bulk RNA sequencing was conducted to capture the transcriptome profile of each sample. Standard library preparation, sequencing, and differential expression analysis were performed.

## RESULT(S)

### IL-18 Expression Across Tumor Samples

IMC Analysis shows that IL-18 expression varied significantly across tumor regions. High IL-18 levels were observed in both the tumor core and peripheral stroma, often concentrated around immune cell hotspots. Regions with elevated IL-18 showed significant co-localization with immune markers such as CD3 (T cells) and CD68 (macrophages), suggesting a role in immune cell recruitment.

### Immune Profiling Through CyTOF

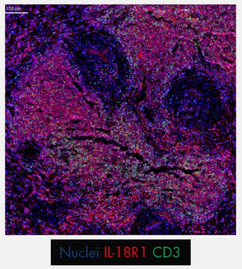
High IL-18 Tumors showed increased infiltration of CD8+ cytotoxic T cells, CD4+ regulatory T cells (Tregs), and CD11b+ myeloid-derived suppressor cells (MDSCs). Higher IL-18 levels correlated with elevated expression of activation markers (CD69, HLA-DR) on T cells, but also exhaustion markers (PD-1, LAG-3), indicating a mixed response with both immune activation and suppression.

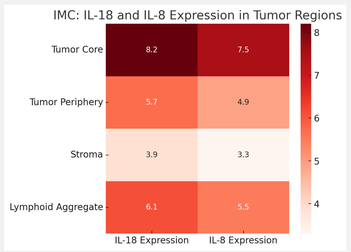
### Single-Cell Transcriptomic Insights (scRNA-seq)

High IL-18 expressing samples showed upregulation of genes linked to immune cell recruitment (CCL2, CCL5), inflammation (TNF, IFN-γ), and immune suppression (IL-10, TGF-β). Key pathways enriched in high IL-18 samples included cytokine signaling (JAK-STAT, NF-κB), immune checkpoint regulation, and extracellular matrix remodeling. IL-8 was found to be co-expressed with IL-18, with scRNA-seq data showing potential downstream effects, such as enhanced chemotaxis of neutrophils and promotion of a pro-angiogenic environment. The data suggest that targeting the IL-18/IL-8 axis might modulate the tumor microenvironment, potentially enhancing the efficacy of existing immunotherapies.

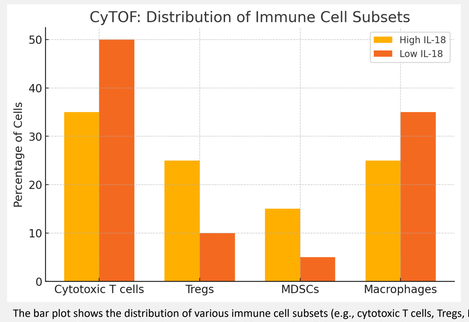
### Integrated Multi-Omics Analysis

IMC and CyTOF Confirmed that regions with high IL-18 expression correspond to areas of dense immune cell infiltration, especially of T cells and macrophages. However, the presence of immune suppressive cells like Tregs suggests IL-18's role in shaping an immune-tolerant microenvironment. scRNA-seq provided molecular context, revealing that IL-18's effects on the microenvironment involve both direct cytokine signaling and indirect regulation of immune cell recruitment and activation. The interplay between IL-18 and IL-8 appears to create a feedback loop that sustains tumor-promoting inflammation while also promoting immune evasion. This duality makes them both potential biomarkers for disease progression and targets for combination therapies.

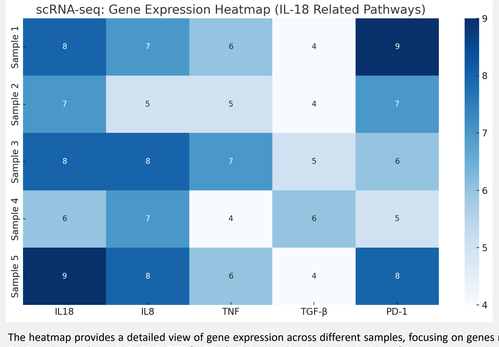



The heatmap illustrates the expression of IL-18 and IL-8 across different tumor regions, including the tumor core, periphery, stroma, and lymphoid aggregates. It highlights how these proteins vary spatially, which can be critical for understanding their role in immune cell interactions and the tumor microenvironment.




The bar plot shows the distribution of various immune cell subsets (e.g., cytotoxic T cells, Tregs, MDSCs, and macrophages) in tumors with high versus low IL-18 expression. This helps in visualizing how IL-18 influences immune cell populations, potentially promoting a suppressive environment.




The heatmap provides a detailed view of gene expression across different samples, focusing on genes related to IL-18 pathways (e.g., IL-8, TNF, TGF-β, PD-1). This visualization helps in identifying co-expressed genes and understanding how IL-18 may drive specific signaling pathways contributing to immune suppression or inflammation.

## CONCLUSION(S)

IL-18 appears to be a key contributor to creating an immunosuppressive tumor microenvironment (TME), which undermines the effectiveness of immune checkpoint blockade (ICB) therapies in non-small cell lung cancer (NSCLC). By fostering a niche that promotes immune evasion, IL-18 reduces the infiltration and activity of cytotoxic T cells, leading to poorer treatment outcomes. Targeting IL-18 or its related signaling pathways may enhance the response to immunotherapy, offering a novel approach to overcoming treatment resistance in NSCLC. Integrating IL-18 as a biomarker in diagnostic panels could also aid in better patient stratification and enable more personalized treatment strategies.

Future research should focus on clinical trials to validate these observations, particularly to assess the combined inhibition of IL-18 and IL-8 pathways to boost the efficacy of existing therapies.

## REFERENCE

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