Optimizing T Cell Activation Kinetics for TCR Engineering in Head and Neck Cancer

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Background

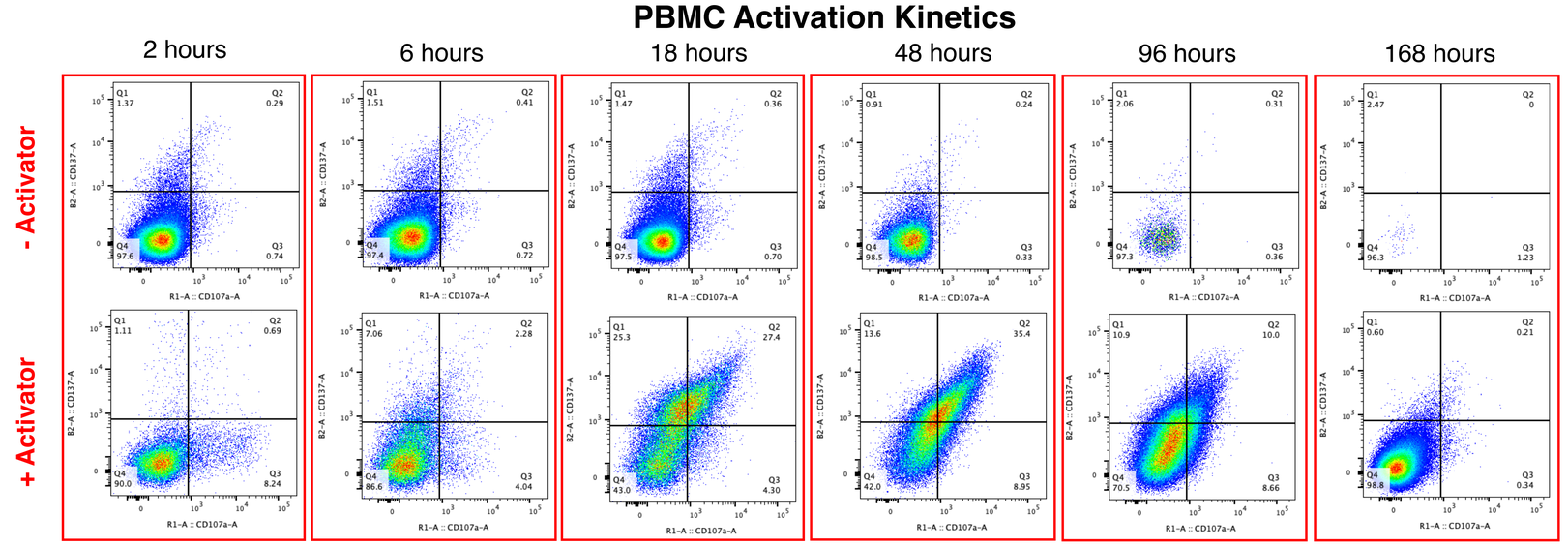
- Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy with limited options for precision immunotherapy.
- T cell receptor (TCR)-engineered T cell therapies offer a promising strategy, requiring reliable platforms to activate, modify, and assess T cells.
- Peripheral blood mononuclear cells (PBMCs) serve as scalable platforms for CRISPR-Cas9–mediated TCR engineering.
- In contrast, tumor-infiltrating lymphocytes (TILs) offer a window into naturally occurring tumor-specific TCRs.
- Effective TCR engineering requires precise control over T cell activation and reactivation to enable gene editing and downstream functional testing.
- We developed a CD137-based flow cytometry assay to define the optimal activation timeline in both PBMCs and TILs from HNSCC patients, establishing a foundational tool for future TCR discovery and engineering workflows.

Methods

1. TILs were isolated from tumor specimens and PBMCs were obtained from pretreatment peripheral blood draws.
2. Cryopreserved TILs and PBMCs were thawed, expanded in cytokine-supplemented media, cytokine-starved for 5 days to return to baseline, and CD8+ selected using CD8 MicroBeads (Miltenyi).
3. These were divided into activated (IL7/IL15 25 µl/million cells) and non-activated control arms.
4. T cell activation was assessed by double-staining for CD137 (Miltenyi) and CD107a (Miltenyi) at various timepoints.
5. Due to baseline CD137 positivity, CD137-negative populations were flow sorted (BD FACSAria III).
6. Analysis at all time points utilized MACSQuant 16, with additional staining for CD8, CD4, and 7-AAD.

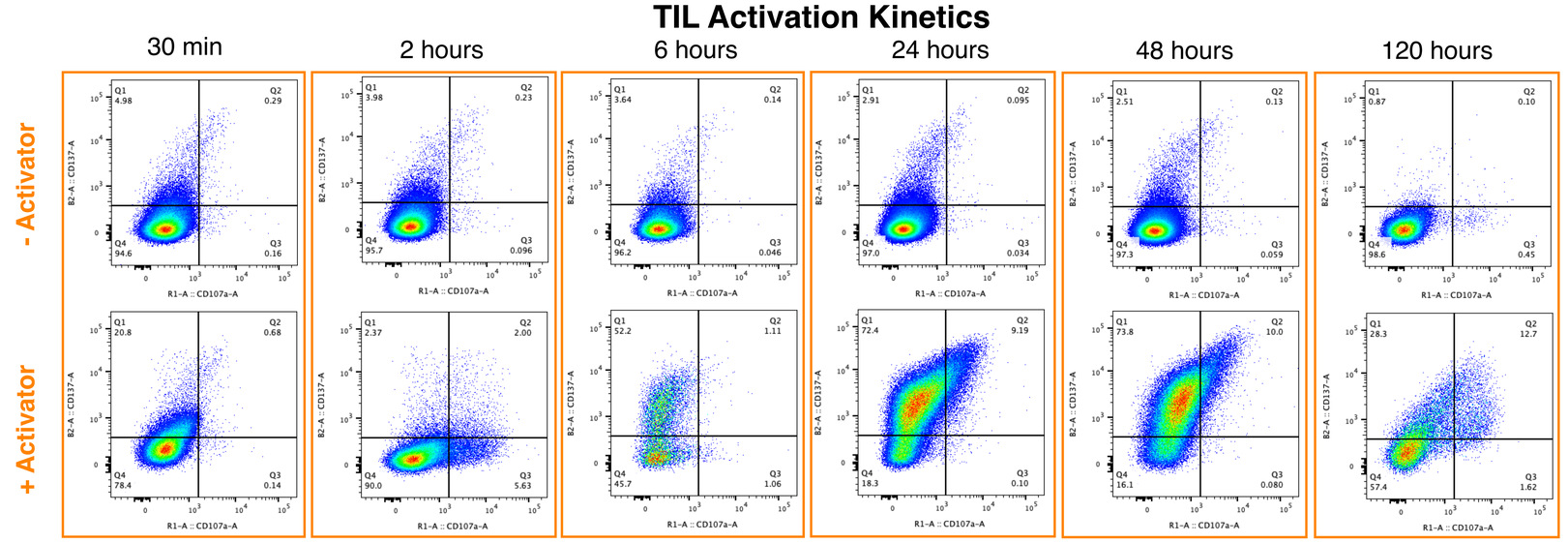
Results

PBMC Activation Kinetics



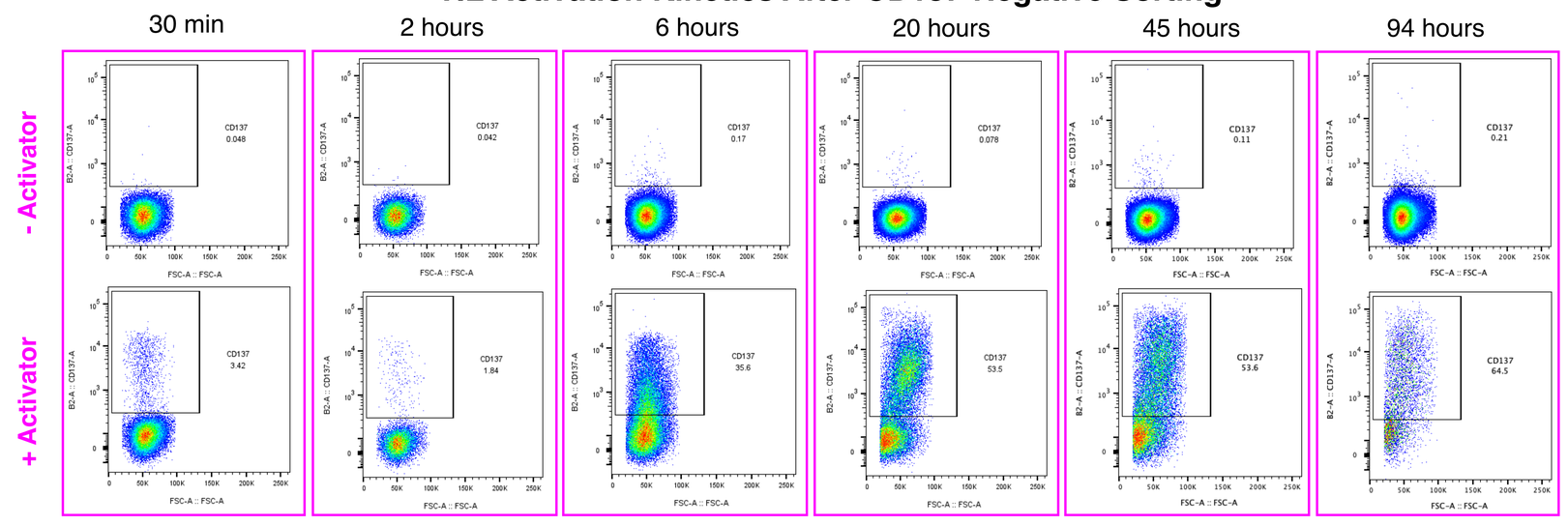
CD107a + CD137

TIL Activation Kinetics



CD107a + CD137

TIL Activation Kinetics After CD137-Negative Sorting



CD137

Discussion and Conclusion

- CD137 outperformed CD107a as an activation marker. This may be due to CD137's stable upregulation following TCR engagement, whereas CD107a is transiently expressed and requires active degranulation, which may not occur with cytokine-based stimulation alone.
- Peak activation for both PBMCs and TILs occurred at 24 hours at 50-60%, with no significant increase at later timepoints.
- Baseline CD137 positivity was observed in unstimulated cells, potentially confounding results. Pre-sorting for CD137-negative cells prior to stimulation eliminated background signal and improved assay precision.
- These findings establish a reproducible, optimized assay for evaluating T cell activation in HNSCC. The assay will serve as a foundational tool for future CRISPR-based TCR engineering workflows, enabling identification and validation of tumor-specific T cells.