**T cell receptor engineering targeting FOXM1 for the treatment of lung cancer**

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**Abstract**

Background: Despite its success, checkpoint blockade immunotherapy has proven challenging in selected lung cancer patient populations. This is in part due to the extensive intratumor heterogeneity at play and infiltration of bystander T cells which recognize non-tumor antigens. Recent clinical trials have demonstrated some efficacy for adoptive cell therapy using bulk unenriched tumor infiltrating lymphocytes, but success remained limited. Accordingly, novel tumor antigens are needed to further improve upon this success of cellular immunotherapy in lung cancer. Forkhead box M1 (FOXM1) is a transcription factor expressed in 90% of lung cancers and lacks expression in brain tissue, making it an appealing target for T cell receptor (TCR) engineering. Interestingly, up-regulation of FOXM1 is associated with drug resistance to tyrosine-kinase inhibitors (TKIs), highlighting another potential therapeutic application for this target. Here, we assessed the immunogenicity of FOXM1 and its potential as a cellular therapy target in non-small cell lung cancer.

Methods: Antigen-specific T cells were isolated and then expanded by peptide stimulation of HLA-matched healthy donor PBMCs. Antigen-specific T cells were then isolated by tetramer sorting and underwent single-cell TCR sequencing to identify full length alpha and beta chains of the TCR. TCRs were retrovirally-engineered into healthy donor PBMCs and function was assessed via chromium release cytotoxicity, ELISpot (IFN-γ secretion) and ELISA (MIP-1β secretion).

Results: An epitope of FOXM1 (FLVPRQDPF) was immunogenic when presented on HLA-A*02:01 (42% of United States population). This epitope was confirmed to be naturally-processed and presented using H1975 cells. Assessment of cytotoxicity revealed that 51% of H1975 cells were lysed by TCR-engineered PBMCs, compared to only 10% for H1975 parental cells devoid of FOXM1 expression (p<0.0001). Cytokine assessment via ELISpot demonstrated a significant increase in IFN-γ spots (p<0.05) and MIP-1β secretion by ELISA (p=0.05).

Conclusion: Our findings confirm the immunogenicity of FOXM1 when presented on the most prevalent HLA allele in the United States and support the feasibility of TCR-engineered targeting FOXM1 for the treatment of lung cancer.

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**T cell generation workflow**

**FOX1 T cell reactivity**

**Conclusions**

- FOXM1-derived epitopes are immunogenic when presented on HLA-A*02:01;
- FOXM1-derived epitopes are naturally endogenously-processed and presented on HLA-A*02:01;
- T cell receptor engineering confers FOXM1-specificity to healthy donor PBMCs.

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