

COUNTERACTING TCR-T CELL DYSFUNCTION IN SOLID TUMORS THROUGH COMBINATION OF FAS-BASED SWITCH RECEPTORS AND CD8-CORECEPTOR

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Background The immunosuppressive tumor microenvironment (TME) impedes solid tumor treatment by adoptive T cell therapies. Tumor-resident cells express multiple inhibitory ligands that contribute to T cell exhaustion, including FAS ligand (FASL), a transmembrane protein that binds to FAS receptor on T cells, triggering apoptosis. Moreover, cancer cells lack costimulatory ligands mediating optimal T cell activation upon TCR engagement, which is critical for T cell engraftment and persistence.

We envisaged that expression of switch receptors (SwR) composed of extracellular FAS-domain and intracellular costimulatory domains will overcome the inhibitory effects of FASL and provide co-stimulation to improve T cell functionality and persistence. Furthermore, coordinated CD4+ and CD8 + TCR-T cell response broadens and deepens clinical responses, necessitating additional incorporation of a CD8 coreceptor, thus raising payload and expression challenges in TCR-T construct design.

Here, we screened a library of FAS-based SwRs in combination with an HLA-A*02:01-restricted MAGE-A1 TCR and a CD8 co-receptor and determined lead candidates enabling TCR-T cell function and persistence in FASL-expressing milieus.

Methods Nineteen different SwR constructs in combination with a MAGE-A1 targeting TCR currently under clinical development (NCT05430555) were retrovirally delivered to CD8+ T cells. SwR candidates that were efficiently expressed, as determined by flow cytometry, were further analyzed in CD4+ and CD8+ T cells by assessing the T cell phenotype, metabolic fitness, and functional activity against FASL-expressing cancer cell lines, under chronic or repeated antigen stimulation.

Results Expression of ten out of 19 SwR constructs was confirmed by increased surface expression of FAS. The constructs were further evaluated in long-term killing assays against FASL expressing cancer cells at 1:1 effector to target ratio. The four SwR candidates comprising intracellular CD40, CD30, OX40 and CD27 domains enabled CD8+ TCR-T cells to effectively eliminate FASL-expressing cancer cells in a dose dependent manner, whereas TCR-only T cells showed limited cytotoxicity due to FASL-induced apoptosis. When selected SwR candidates were co-expressed with MAGE-A1 TCR and CD8 co-receptor in CD4+ and CD8+ T cells, they triggered augmented eradication of target cells and higher proliferation rate upon antigen stimulation, while maintaining a less differentiated T cell phenotype and higher metabolic fitness.

Conclusions Armoring TCR-T cells with FAS-based SwR and a CD8 co-receptor mediated stronger cytotoxic activity upon sustained or repeated antigen stimulation, and enhanced T cell fitness. Our data support this dual approach as a promising strategy to overcome tumor resistance mechanisms and further improve clinical outcomes.

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