Engineering Next-Generation STEAP1 CAR T Cells: Development and Optimization for Enhanced Anti-Tumor Efficacy in Prostate Cancer

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Background:

CAR T-cell therapy has achieved success in treating hematologic malignancies and is now being explored for solid tumors such as prostate cancer (PCa). Current targets, such as PSCA and PSMA, show variable expression in metastatic castration-resistant prostate cancer (mCRPC), highlighting the need for new antigens. STEAP1, overexpressed in over 80% of mCRPC cases and linked to disease progression, is a promising target. STEAP1-targeted CAR-T cells (STEAP1-BBζ) have shown strong antitumor activity and safety in preclinical models, though STEAP1 loss could pose a major resistance issues.

Methods:

To predict in-vitro binding and activation of various PSMA or edited-STEAP1 CAR-T cells, we employed co-culture assays and assessed T cell activation using Enzyme-Linked Immunosorbent Assay (ELISA) for IFNγ. In-vivo efficacy was evaluated using C42B subcutaneous and 22RV1 metastatic tumor models. Genome-wide CRISPR screens were conducted to identify genetic knockouts that might enhance STEAP1 CAR-T cell killing efficacy.

Results:

Key challenges to STEAP1-BBζ CAR-T cell therapy for mCRPC include antigen escape and CAR-T cell exhaustion, driven by an immunosuppressive tumor microenvironment and prolonged antigen exposure. To address antigen escape, we are developing dual-targeting CAR-T cells against both PSMA and STEAP1. We optimized PSMA CAR-T cells by testing various scFvs and found NB37-scFv (a proximal PSMA binder) with an IgG4 spacer superior to J591 scFv (a distal PSMA binder). In preclinical models, NB37-PSMA CAR T cells showed improved tumor-killing efficacy and persistence. We are now developing and characterizing STEAP1-PSMA dual CAR-T cells to maximize anti-tumor effects while minimizing toxicity.

To tackle STEAP1 CAR T cell exhaustion and improve metabolic fitness, we created an in vitro model where STEAP1 CAR T cells were repeatedly exposed to 22RV1 and C42B tumor cells, inducing functional exhaustion. A genome-wide CRISPR knockout screen was performed, followed by rechallenge with tumor cells in varying CD4:CD8 ratios. Top knockout candidates were evaluated for their impact on CD4 and CD8 activity. Pathway analysis of the enriched knockouts highlighted disruptions in anabolic and catabolic metabolism, providing insights into genetic modifications that could enhance the metabolic fitness of STEAP1 CAR-T cells. We are currently validating and prioritizing the top 200 hits from the genome-wide screen to identify essential genetic knockouts that could improve STEAP1 CAR-T cell fitness and anti-tumor efficacy.

Conclusions:

Our research advances STEAP1-BBζ CAR-T cell therapy by addressing challenges such as antigen escape and functional exhaustion. Optimized PSMA CAR T cells exhibit enhanced anti-tumor efficacy, supporting the development of dual-targeting PSMA-STEAP1 CAR T cells. Additionally, our in vitro model and CRISPR screen identified genetic modifications that enhance metabolic fitness and CAR T cell activity. Ongoing validation will refine these strategies, aiming to improve the effectiveness and persistence of STEAP1 CAR T cell therapy.

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