PSMA-specific CAR T-Stem Cell Memory Therapy Shows Potent anti-Tumor Effects in Solid Tumor Prostate Cancer Models

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Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein overexpressed on the surface >80% of primary and metastatic prostate cancer. PSMA is a promising therapeutic target, but early first generation chimeric antigen receptor (CAR) T-cell therapies have lacked clinical efficacy. Here we developed a novel CAR T-cell product (P-PSMA-101) via non-viral piggyBac™ transposition of a tri-cistronic transgene encoding a safety switch, a PSMA-specific Centyrin-based CAR (CARTyrin), and a selection gene- features that may improve safety and therapeutic efficacy.

We identified a lead anti-PSMA CARTyrin (P-PSMA-101) that displayed exquisite specificity for PSMA isoform 1 in a safety screen testing >5,600 surface proteins. Our production methodology led to >95% CAR+ T-cells after selection and expansion, as well as >60% T-stem cell memory (Tscm) cells. Early memory cells are long-lived, self-renewing and multi-potent, with recent evidence suggesting a correlation with complete responses in CD19 CAR and TCR T-cell clinical trials. *In vitro*, P-PSMA-101 specifically proliferated, lysed, and secreted IFN-γ and IL-2 against PSMA+ LNCaP or PSMA-engineered K562s. P-PSMA-101 cells produced from PBMCs obtained from stage IV prostate cancer patients showed comparable functionality. Lastly, no evidence of tonic signaling or exhaustion was detected.

In an *in vivo* dose titration study, P-PSMA-101 eliminated established LNCaP subcutaneous tumor in 100% of immune-compromised mice for the duration of the study at a 12e6 dose (42 days post-treatment), while 2/3 of the low-dose (5e6) animals remained tumor-free. In a second study, we tested the previously clinically-applied anti-PSMA J591 scFv-based CAR for comparability. P-PSMA-101 demonstrated enhanced anti-tumor efficacy and survival (>70 days, end of study) in comparison to J591 CAR T-cell-treated mice (41 days) at a "stress test" dose of 4e6 T-cells against subcutaneous LNCaP solid tumors in NSG mice. The >60% Tscm P-PSMA-101 expanded *in vivo* and gave rise to differentiated effector CAR+ T-cells that were detected in the peripheral blood concomitant with a decrease in tumor burden below detectable caliper and BLI imaging limits. P-PSMA-101 then contracted yet persisted in the peripheral blood with >70% of T-cells retaining a Tscm phenotype. Additional *in vivo* studies utilizing a PSMA-engineered PC3 bone metastasis prostate cancer model demonstrated potent P-PSMA-101 anti-tumor efficacy at a 4e6 dose. In comparison, the J591 CAR showed significant anti-tumor activity at a 12e6, but not at 4e6.

P-PSMA-101 is a first-in-class Centyrin-based CAR T-cell therapeutic that exhibits a persistently high frequency of Tscm and mediates durable anti-solid tumor efficacy that surpasses previously established anti-PSMA CAR T-cell therapy in these *in vivo* models. Current efforts will continue towards clinical application in patients with metastatic castrate resistance prostate cancer.

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