PSMA-specific CAR-T Memory Stem Cell Therapy Eliminates Solid Tumors in Multiple Prostate Cancer Models

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ABSTRACT

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein overexpressed on the surface >80% of primary and metastatic prostate cancers. 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 the non-viral piggyBac™ DNA Modification System for 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.

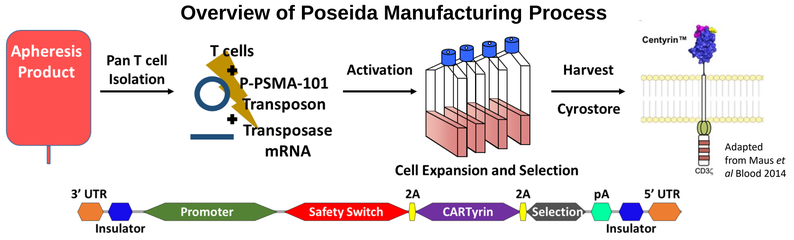
We identified a lead anti-PSMA CARTyrin (P-PSMA-101) that displayed exquisite specificity for PSMA isoform 1 in a safety screen testing >5,600 surface proteins. Using piggyBac we obtained >95% CAR+ T-cells after selection and expansion. Importantly, our unique production methodology leads to exceptionally high levels of T-stem cell memory (Tscm) cells, an early memory population that has recently been reported to correlate with clinical responses in several CAR 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 eliminated established solid tumors in two different *in vivo* prostate cancer models, one subcutaneous and the other intratibial to model bone metastasis. In the first 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 the 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.

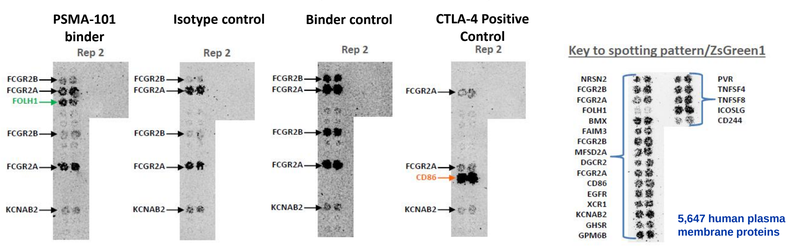
P-PSMA-101 is a first-in-class Centyrin-based CAR T-cell therapeutic that exhibits an exceptionally high, persistent frequency of Tscm and mediates durable anti-solid tumor efficacy that surpasses previously established anti-PSMA CAR T-cell therapy in these *in vivo* models. Current efforts will continue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

INTRODUCTION

Overview of Poseida Manufacturing Process



Confirmatory Screen of Centryin Binder



Phenotype of Poseida T cell Products

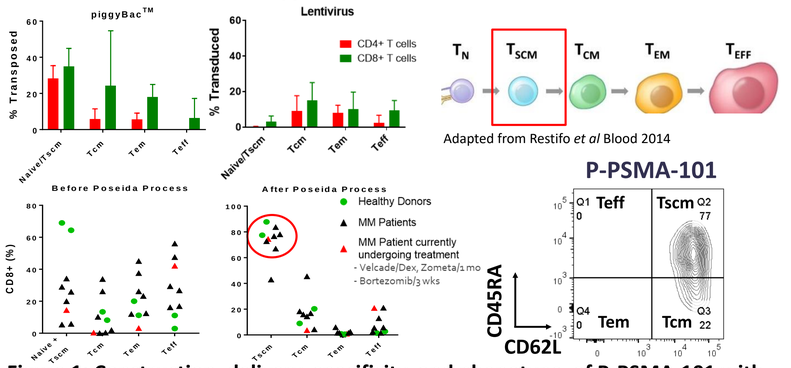


Figure 1: Construction, delivery, specificity, and phenotype of P-PSMA-101 with piggyBac transposition and Poseida manufacture process.

METHODS & RESULTS

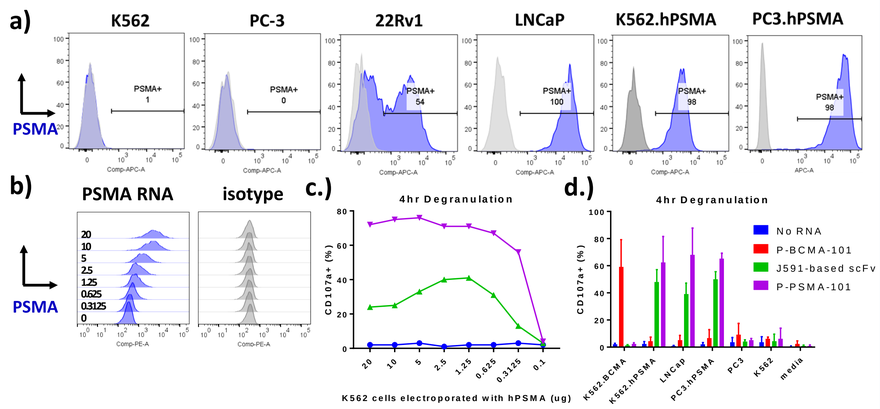


Figure 2: Initial screen of RNA-electroporated CARTyrin cells. (a) Surface expression of human PSMA (hPSMA) on tumor cells. K562.hPSMA and PC3.hPSMA were engineered to stably express hPSMA. (b) Surface expression of PSMA on RNA-electroporated K562 cells. PSMA RNA amounts per reaction (ug). (c/d) Degranulation of indicated RNA-electroporated CAR T cells against K562 cells transiently expressing varying levels of hPSMA (c) or tumor cells (d).

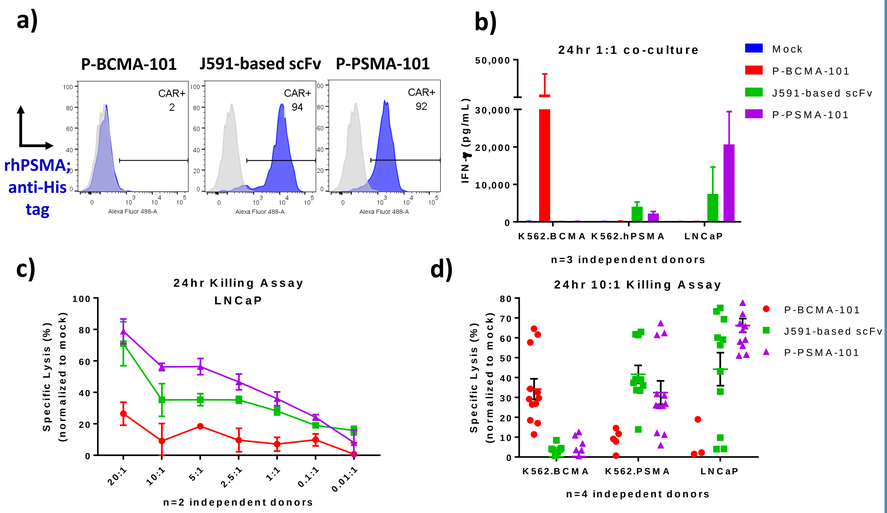
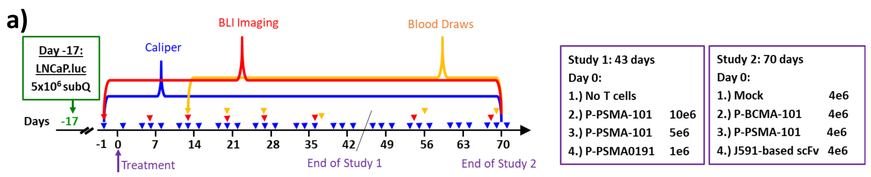


Figure 3: *In vitro* activity of piggyBac-engineered CARTyrin cells against PSMA+ tumor cells. (a) Binding of histidine (His)-tagged, recombinant hPSMA antigen (rhPSMA) with an anti-His tag antibody. Mock transposed T cells in gray. The CAR indicated above each histogram is shown in blue. PSMA-specific J591 antibody-based scFv (J591) transposed CAR T cells were used to test comparability. (b) IFN-γ secretion against indicated target tumor cells at 1:1 effector: target ratio. (c/d) Cytolytic activity against the indicated tumor cells in 24-hr assays.



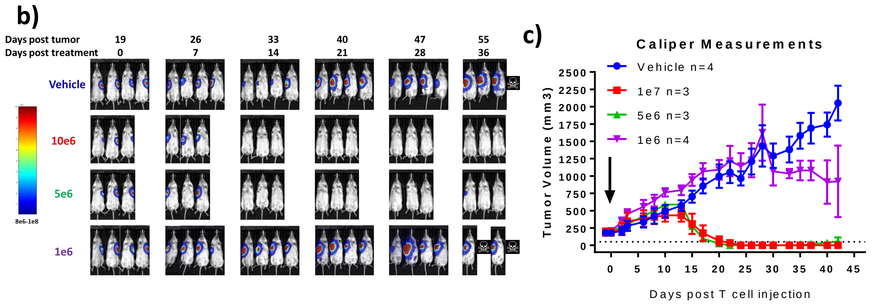


Figure 4: *In vivo* activity of piggyBac-engineered CARTyrin cells against established LNCaP tumor. (a) Experimental outline. Study 1: dose study at 1, 5, or 10e6 P-PSMA-101 cells. Study 2: efficacy study at 4e6 P-PSMA-101 cells, Mock-transposed and P-BCMA-101 cells as specificity controls, and J591 CAR-T cells. (b/c) Bioluminescence (BLI) imagining (b) and caliper measurement (c) of Study 1 over time.

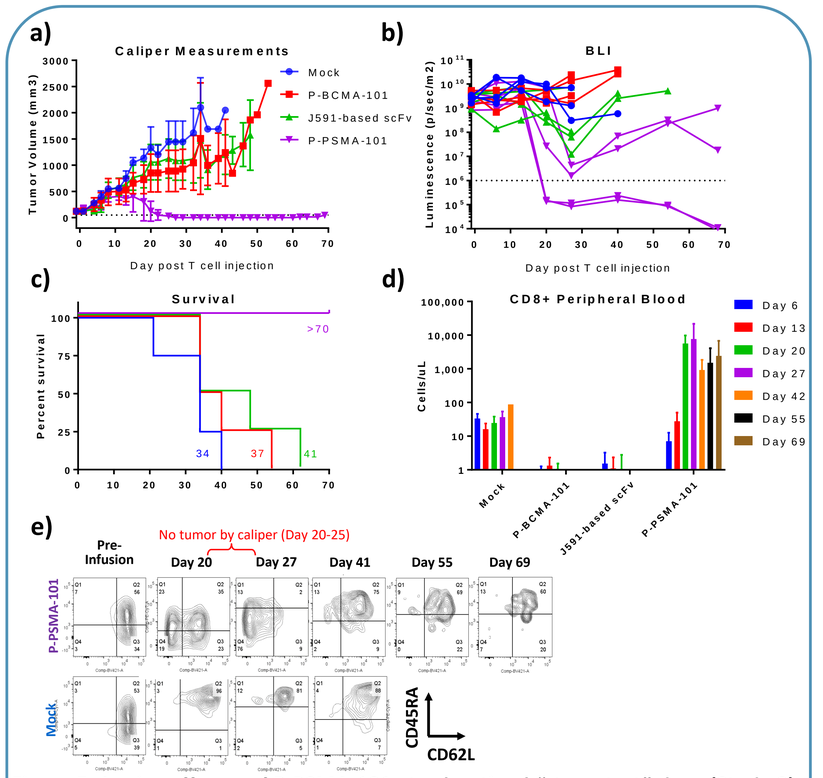


Figure 5: *In vivo* efficacy of P-PSMA-101 at suboptimal "stress test" dose (Study 2). (a/b) Caliper measurements (a) and quantification of BLI (b) of NSG mice bearing established LNCaP tumor. Mice were treated with 4e6 of the indicated CARTyrin cells at Day 0. (c) Survival curves of Study 2. Median survival in days indicated by number. (d) Quantification of CD8+ cells/µl of peripheral blood at the indicated time points. Cells were gated on size, human CD45+CD3+CD8+ and quantified with beads. (e) Flow cytometric analysis of CD45RA versus CD62L in a representative P-PSMA-101 CARTyrin-treated mouse (top) or Mock-transposed T cells (bottom) over time. Cells were gated on size, mouse CD45(-), human CD3+, CD8+ T cells.

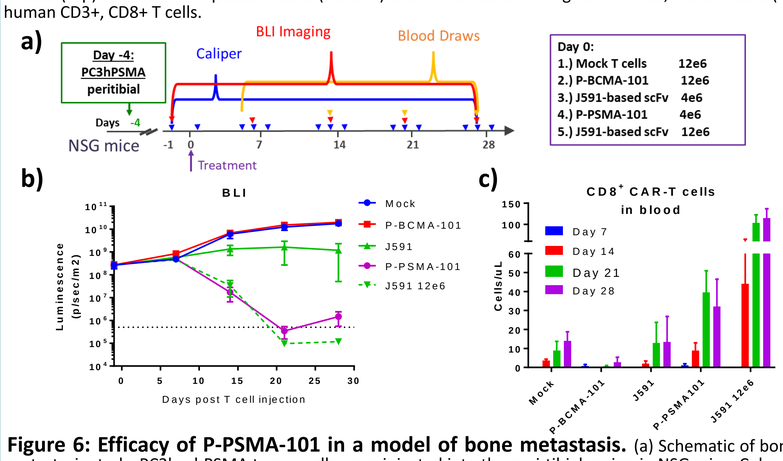


Figure 6: Efficacy of P-PSMA-101 in a model of bone metastasis. (a) Schematic of bone metastasis study. PC3luc.hPSMA tumor cells were injected into the peri-tibial region in NSG mice. Cohorts were treated 4 days post tumor implant with the indicated CAR and dose. (b) Quantification of bioluminescent imaging (c) Quantification of CD8+ cells/µl of peripheral blood at the indicated time points. Cells were gated on size, human CD45+CD3+CD8+ and quantified with beads.

CONCLUSIONS

- **P-PSMA-101 is Poseida's CAR-T memory stem cell product for mCRPC**
- **P-PSMA-101 eliminated solid tumors and significantly prolonged survival in an established subQ LNCaP model**
- **P-PSMA-101 gave rise to differentiated CAR-T populations, but following solid tumor elimination, persisted as CD45RA⁺CD62L⁺ memory CARTyrin⁺ cells**
- **P-PSMA-101 reduced tumor burden at 3x lower dose than a clinical PSMA binder in an peri-tibial bone metastasis model**

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