**Preclinical Discovery and Characterization of Allogeneic Anti-PSMA γδ CAR-T Therapy for Prostate Cancer**

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### BACKGROUND

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein overexpressed in up to 90% of prostate cancers. PSMA expression can be increased in advanced stages of the disease, making it an attractive therapeutic target. Clinically, allogeneic anti-PSMA γδ CAR T cells have shown initial efficacy coupled with significant CR/PR in extirpating localized and locally advanced or metastatic prostate cancers. Additionally, γδ T cells demonstrate enhanced tumoricidal activity and reduced production of cytokines profiles that may decrease toxicity associated with γδ CAR T cells.

### METHODS

We used phase-painting to identify novel anti-PSMA binders with varying affinities. Anti-PSMA CAR expressing Jurkat cells were co-cultured with γδ T cells following adoptive transfer in a 72-hour co-culture period, using JSRV1 and JCRL62 cells. Results were taken as a representative of two technical replicates and are representative of triplicate biological replicates.

### Phage-painting was used to identify novel anti-PSMA binders with varying affinities.

**Figure 1.** (A) Overview of the process to identify and test PSMA binders. (B) Phenotypical characteristics of γδ T cells were characterized by flow cytometry analysis. (C) Confirmatory γδ T cells expressing PSMA CAR validated PSMA binding by flow cytometry. (D) Evaluation of γδ T cells targeting PSMA by ELISpot assay revealed increases in cytotoxicity with increasing antibody concentration. (E) Evaluation of γδ T cells targeting PSMA by Cytotoxicity Index assay showed increased cytotoxicity with increasing antibody concentration.

### Anti-PSMA CAR V01 T cells expand robustly and demonstrate potent cytotoxicity and proliferation against prostate cancer cell lines.

**Figure 2.** (A) Evaluation of proliferation of PSMA V01 T cells in the presence of JSRV1 and JCRL62 cells. (B) Viability analysis of JSRV1 and JCRL62 cells in the presence of PSMA V01 T cells. (C) Viability analysis of JSRV1 and JCRL62 cells in the presence of PSMA V01 T cells, 24 hours after addition of anti-PSMA CAR V01 T cells. (D) Viability analysis of JSRV1 and JCRL62 cells in the presence of PSMA V01 T cells, 72 hours after addition of anti-PSMA CAR V01 T cells.

### "Bolt-on" engineered anti-PSMA CAR V01 T cells express dnTGFβRII with corresponding reduction in pSMAD2/3 activity

**Figure 3.** (A) In vitro cytotoxicity assay showing a significant increase in cytotoxicity of PSMA V01 T cells in the presence of JSRV1 and JCRL62 cells. (B) ELISpot assay showing increased cytokine production in the presence of PSMA V01 T cells. (C) Flow cytometry analysis showing increased expression of DNAM-1 in the presence of PSMA V01 T cells. (D) Flow cytometry analysis showing increased expression of NKG2D in the presence of PSMA V01 T cells.

### "Bolt-on" expressing anti-PSMA CAR V01 T cells demonstrate potent in vitro cytotoxicity against prostate cancer cell lines and retain enhanced cytotoxic potential after rechallenge

**Figure 4.** (A) Evaluation of cytotoxicity of PSMA V01 T cells in the presence of PSMA 11 and PSMA 12 mAbs, showing increased cytotoxicity in the presence of PSMA V01 T cells. (B) ELISpot assay showing increased cytokine production in the presence of PSMA V01 T cells. (C) Flow cytometry analysis showing increased expression of DNAM-1 in the presence of PSMA V01 T cells. (D) Flow cytometry analysis showing increased expression of NKG2D in the presence of PSMA V01 T cells.

### Transcriptional and proteomic profiles of anti-PSMA CAR V01 T cells support functional enhancement provided by "bolt-on"

**Figure 5.** (A) Schematic representation of PSMA V01 T cells with decreased expression TGFβRII. (B) "Bolt-on" engineered anti-PSMA CAR V01 T cells with decreased expression TGFβRII. (C) "Bolt-on" engineered anti-PSMA CAR V01 T cells with decreased expression TGFβRII and increased expression of DNAM-1 and NKG2D.

### "Bolt-on" expressing anti-PSMA CAR V01 T cells demonstrate potent in vitro cytotoxicity against prostate cancer cell lines and retain enhanced cytotoxic potential after rechallenge

**Figure 6.** (A) Evaluation of proliferation of PSMA V01 T cells in the presence of JSRV1 and JCRL62 cells. (B) Viability analysis of JSRV1 and JCRL62 cells in the presence of PSMA V01 T cells. (C) Viability analysis of JSRV1 and JCRL62 cells in the presence of PSMA V01 T cells, 24 hours after addition of anti-PSMA CAR V01 T cells. (D) Viability analysis of JSRV1 and JCRL62 cells in the presence of PSMA V01 T cells, 72 hours after addition of anti-PSMA CAR V01 T cells.

### SUMMARY & CONCLUSIONS

V01 T cells modified to express de novo PSMA CARs were successfully generated and characterized. The resulting V01 CAR T cells expressed a predominant naïve/memory phenotype and were associated with potent in vitro cytotoxicity, production of proinflammatory cytokines, and proliferation against prostate tumor cell lines. Potential tumor growth inhibition was observed in heterogeneous and androgen PSMA tumor xenograft models. Functional heterogeneity of "hakari"-sensitive CARps was demonstrated for the anti-PSMA CAR V01 T cells both in vitro and in vivo. These data support further development of an armed allogeneic γδ CAR T cell therapy for prostate cancer.

### References