**Phase 1 Clinical Trial of PSMA-directed/TGFβ-insensitive CAR-T cells in Metastatic Castration-Resistant Prostate Cancer: Safety, Correlative Studies, and Future Directions following Preliminary Dose Escalation**

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**Background:**
Adoptive immunotherapy with CAR-T cells has transformative potential for the treatment of cancer. However, a primary challenge to the success of these therapies in prostate cancer is the immunosuppressive microenvironment, including high levels of TGFβ, encountered by re-directed T cells upon tumor infiltration. Importantly, these immunosuppressive functions of TGFβ can be inhibited in T cells using a dominant negative TGFβ receptor (TGFβRdn), thereby enhancing antitumor immunity. In *in vivo* disseminated tumor models, co-expression of TGFβRdn on PSMA-directed CAR-T cells led to increased T cell proliferation, enhanced cytokine secretion, long-term persistence, and greater induction of tumor eradication. Mechanisms of adaptive tumor resistance are unknown.

**Methods:**
We initiated a first-in-human phase 1 clinical trial to evaluate the safety and preliminary efficacy of lentivirally-transduced PSMA-directed/TGFβ-insensitive CAR-T cells (CART-PSMA-TGFβRdn) in men with treatment-refractory metastatic CRPC (NCT03089203). In preliminary dose-escalation cohorts, patients received a single dose of 1-3 x $10^7/m^2$ (Cohort 1) or 1-3 x $10^8/m^2$ (Cohort 2) CART-PSMA-TGFβRdn cells without lymphodepleting chemotherapy in a 3+3 design. All treated patients underwent metastatic tumor biopsies at baseline, as well as on day +10 following the CAR-T cell infusion. Quantitative PCR of CART-PSMA-TGFβRdn DNA was performed at serial timepoints to evaluate for CAR-T expansion and persistence in peripheral blood and trafficking to target tissues. Bioactivity of CART-PSMA-TGFβRdn cells in peripheral blood was evaluated through Luminex analyses of immune and inflammatory factors.

**Results:**
Six patients have received CART-PSMA-TGFβRdn cell infusions at the specified dose levels (Cohort 1, N=3; Cohort 2, N=3). All CART-PSMA-TGFβRdn infusion products have met target transduction efficiency. Evaluation of CAR-T cellular kinetics via qPCR of CART-PSMA-TGFβRdn DNA has demonstrated peripheral blood T cell expansion **(Figure 1)**, as well as tumor tissue trafficking in post-treatment tumor biopsies **(Table 1)**. In Cohort 2, two patients developed anticipated Grade 3 cytokine release syndrome (CRS), and one patient developed Grade 3 CAR-T neurotoxicity requiring corticosteroids. Marked increases in inflammatory cytokines (IL-6, IL-15, IL-2, IFNgamma) and ferritin correlated with all Grade 3 CRS events **(Figure 2)**. All CRS events rapidly resolved with tocilizumab (anti-IL6R) rescue. No dose-limiting toxicities have occurred.

**Conclusions:**
Cellular therapy with CART-PSMA-TGFβRdn is safe and feasible in men with metastatic CRPC. CAR-T cell expansion and tumor tissue trafficking is observed. We observed clinically significant CRS, which is a critical marker of biologic activity with CAR-T therapy. In order to further

enhance CART-PSMA-TGFβRdn expansion, persistence, and antitumor efficacy, ongoing cohorts are evaluating co-administration with lymphodepleting chemotherapy, as well as serial CART-PSMA-TGFβRdn re-infusions. Critical planned analyses will examine mechanisms of adaptive resistance via comprehensive phenotyping of tumor-infiltrating T cells and the prostate cancer microenvironment, as well as circulating tumor material and T cell subtypes.

**Conflict of Interest:** None

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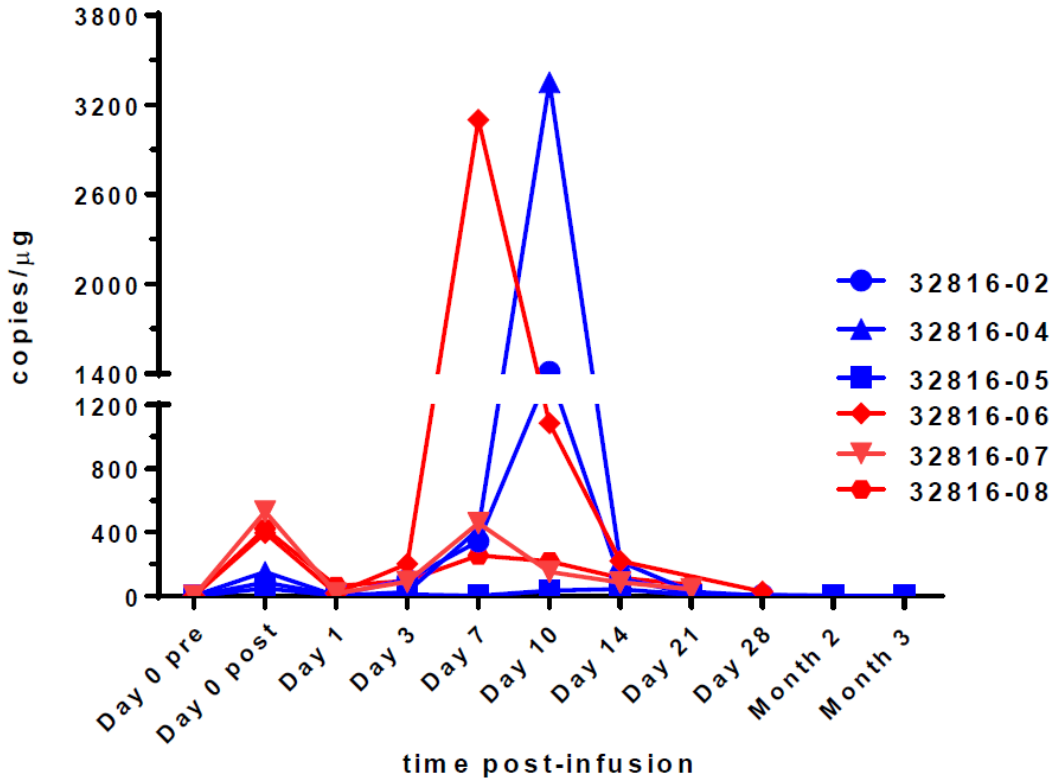


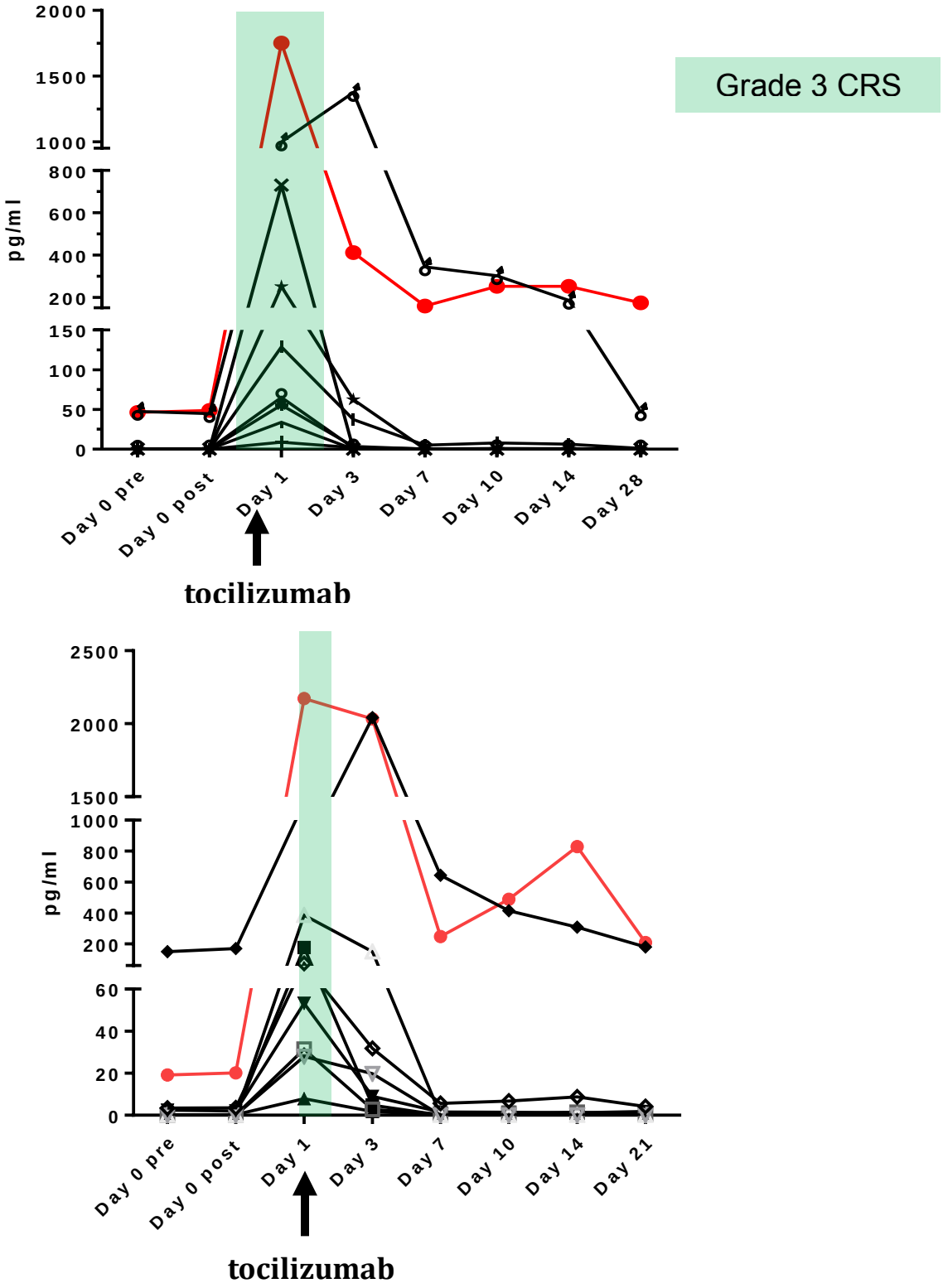

**Figure 1. Detection of PSMA CAR by qPCR in the peripheral blood of prostate cancer subjects.** Blue lines represent Cohort 1 subjects; red lines represent Cohort 2 subjects.

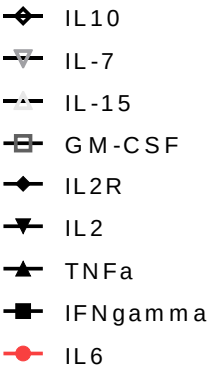
**Table 1. CART-PSMA-TGFβRDN cell trafficking: qPCR detection in tissue biopsy samples in infused subjects**

| Subject ID | Cohort | Time point | Sample Type | Results* |
|---|---|---|---|---|
| 32816-02 | 1 | Day 10 | Bladder (FFPE tissue curls) | 122.32 |
| | | | Bladder (FFPE tissue curls) | 57.99 |
| | | Day 21 | Bone marrow biopsy core | ND |
| | | | Bone marrow | 27.12 |
| | | Month 2 | Bone marrow biopsy core | ND |
| 32816-04 | 1 | Day 10 | Bone (FFPE tissue curls) | 133.36 |
| | | | Bone (FFPE tissue curls) | 211.16 |
| 32816-05 | 1 | Day 10 | Lymph node (FFPE tissue) | 758.51 |
| 32816-06 | 2 | Day 10 | Lymph node (FFPE tissue) | 98.24 |
| 32816-07 | 2 | Day 10 | Bone (FFPE tissue curls) | ND |
| 32816-08 | 2 | data analysis pending | | |

*copies/µg gDNA
Abbreviations
FFPE – formalin-fixed, paraffin embedded
ND – not detected

**Figure 2. Inflammatory Cytokines in Subjects with Cytokine Release Syndrome (Top: Subject 6; Bottom: Subject 7)**




IL10
IL-7
IL-15
GM-CSF
IL2R
IL2
TNFa
IFNgamma
IL6