An All-in-One 'Living Drug' CAR T Cell Platform for Safe and Effective Targeting of Metastatic Castration-Resistant Prostate Cancer

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Background

Metastatic castration-resistant prostate cancer (mCRPC) is a lethal and treatment-refractory stage of disease, often associated with bone metastases and poor prognosis. Although chimeric antigen receptor (CAR) T cell therapy holds promise for mCRPC, clinical translation has been limited by toxicities associated with validated targets such as PSMA. In first-in-human trials of PSMA-targeted CAR T cells armored with a dominant-negative TGF-β receptor II (dnTGFβRII) (NCT03089203, NCT04227275), and the ongoing REGN5678 αPSMA×CD28 bispecific trial (NCT03972657), encouraging responses were observed but accompanied by grade ≥3 cytokine release syndrome (CRS), hemophagocytic lymphohistiocytosis (HLH), and immune effector cell-associated neurotoxicity syndrome (ICANS). In addition to toxicity, antigen heterogeneity—particularly the emergence of antigen-negative escape variants—remains a key barrier to durable responses.

Methods

To address these challenges, we developed a next-generation, all-in-one "living drug" CAR T cell platform with triple-armoring and dual-targeting capabilities. The construct includes: (1) a STEAP2-directed CAR to reduce on-target/off-tumor toxicity; (2) localized secretion of αPSMA×CD28 bispecific antibodies to activate bystander T cells against STEAP2-negative/PSMA-positive escape variants while minimizing systemic effects; (3) rationally tuned CD28 affinity in the bispecific to balance efficacy with reduced CRS risk; and (4) dnTGFβRII to confer resistance to immunosuppressive signaling in the tumor microenvironment (TME). Bispecific secretion is confined to NFAT-activated CAR T cells upon STEAP2 engagement, thereby restricting CD28 co-stimulation to the tumor site and expanding the therapeutic window.

We evaluated this platform *in vitro* using a panel of human prostate cancer cell lines with variable STEAP2 and PSMA expression and high TGF-β secretion, to delineate STEAP2-CAR versus αPSMA×CD28-mediated cytotoxicity. For *in vivo* assessment, we utilized subcutaneous, intravenous, and intraosseous xenograft models to simulate primary tumors, systemic dissemination, and bone metastases, respectively. A novel huPSMA knock-in (KI) mouse model was also developed to assess safety by recapitulating physiological PSMA expression.

Results

We successfully engineered multiple αPSMA×CD28 constructs within a dnTGFβRII-armored STEAP2-CAR T cell framework. Bispecific secretion was confirmed by ELISA and dual-binding assays to PSMA+ tumor cells and CD28+ T cells. Engineered T cells exhibited potent *in vitro* cytotoxicity against heterogeneous prostate cancer lines. *In vivo*, dual-function CAR T cells demonstrated robust tumor control in LNCaP (STEAP2+/PSMA+) models through both STEAP2-CAR–mediated killing and bispecific-enhanced bystander

activity. Notably, bispecific secretion alone elicited strong antitumor effects in PC3-PSMA+/STEAP2– tumors.

Conclusions

This all-in-one CAR T cell platform integrates tumor-specific targeting, dual antigen recognition, localized immune activation, and resistance to TME suppression. Our findings support its potential as a safe and effective therapeutic strategy for mCRPC and other solid tumors characterized by antigen heterogeneity and immunosuppressive barriers.

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Conflicts of Interest Disclosure Statement

The authors declare no conflicts of interest related to this abstract.