

Preclinical development of AB-3028, a programmable circuit CAR T cell product intended for the treatment of metastatic castration-resistant prostate cancer (mCRPC)

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Background:

Long-term survival for metastatic castration-resistant prostate cancer (mCRPC) patients remains low. New modalities show promise, but have yet to demonstrate durable responses. While multiple CAR T products have been approved in hematological malignancies, their activity in solid tumors has thus far been limited by off-tumor toxicity, poor persistence, inflammatory toxicity, and tumor microenvironment (TME) suppression. To overcome these challenges, we developed AB-3028, an autologous logic-gated CAR T cell product for potential mCRPC treatment. Non-viral T cell engineering is used to integrate the AB-3028 DNA cassette, which includes three functional modules: a sequential "AND" logic gate for dual antigen recognition to limit off-tumor toxicity; an improved shRNA-miR module targeting FAS and TGFBR2 to resist TME suppression; and a synthetic pathway activator (SPA) to potentiate T cell functional persistence via activation of JAK/STAT signaling.

Methods:

We evaluated tumor antigen expression using IHC on mCRPC patient tumor samples and tumor cell lines. We assessed AB-3028's dual-antigen specificity *in vitro* using single and dual antigen-expressing target cells, and *in vivo* using single and dual antigen-positive tumors established on contralateral flanks. We performed an *in vitro* repetitive tumor challenge assay with dual antigen-positive tumor cells to assess the functional contributions of the SPA and TGFBR2 shRNA modules. The SPA module was additionally assessed in an *in vitro* repetitive stimulation CAR T assay, coupled with single cell RNA-seq to assess gene expression. We also assessed AB-3028's anti-tumor activity and its capability to overcome TME-mediated suppression *in vivo* using a PC3 mCRPC xenograft model. We used a constitutive CAR incorporating a dominant-negative TGF- β receptor (dnTGF β R2) as a control.

Results:

In both *in vitro* co-cultures and the dual-flank *in vivo* xenograft model, AB-3028 showed selective anti-tumor activity against cells co-expressing the target antigens. AB-3028 maintained long-term killing during an *in vitro* repetitive stimulation assay, and this functional persistence depended on the SPA and TGFBR2 shRNA modules. Analysis of cytokine secretion from AB-3028 compared to controls revealed that AB-3028 secretes lower amounts of inflammatory cytokines, and that this functional difference is dependent on the SPA module. Single cell RNA-seq characterization revealed that the SPA module prevented the induction of TOX and other hallmarks of T cell exhaustion. *In vivo*, AB-3028 exhibited anti-tumor efficacy in the PC3 mCRPC xenograft model and provided a survival benefit at doses 4-fold lower than a constitutive CAR including a dominant-negative TGF- β receptor (dnTGF β R2). Additionally, AB-3028 showed rapid tumor killing in a PC3 intratibial tumor model.

Conclusions:

Preclinical data demonstrate AB-3028's selectivity for mCRPC tumor targeting, and its modular features that enhance T cell fate and function in the tumor microenvironment. These results support clinical evaluation of AB-3028 for mCRPC treatment in a phase I/II clinical trial being initiated in early 2026.