


AUTHOR'S VIEW

CAR T-cell intrinsic PD-1 checkpoint blockade: A two-in-one approach for solid tumor immunotherapy

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ABSTRACT

PD-L1/2 expression in solid tumors inhibits chimeric antigen receptor (CAR) T-cell efficacy. A PD-1 dominant negative receptor expressed in CAR T cells provides cell-intrinsic checkpoint blockade and augments antitumor efficacy. A combinatorial immunotherapeutic strategy of combining CAR T cells with checkpoint blockade is a promising treatment approach for solid tumors.

Abbreviations: CAR, chimeric antigen receptor; CRISPR, clustered regularly interspaced short palindromic repeats; LAG3, lymphocyte-activation gene 3; MDSC, myeloid-derived suppressor cell; MSLN, mesothelin; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PSCA, prostate-specific cell antigen; TAM, tumor-associated macrophage; TIM-3, T-cell membrane protein 3; Treg, regulatory T cells

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Chimeric antigen receptors (CARs) are synthetic receptors that redirect T cells to cancer cell surface antigens to eliminate the targeted cancer cells. The components of CARs are an extracellular single chain variable fragment (scFv), a transmembrane domain, and an intracellular signaling domain. First-generation CARs linked scFv with the CD3 ζ intracellular signaling domain to induce antigen-specific T-cell activation. Subsequent generations of CARs incorporated additional co-stimulatory signals (e.g., domains of CD28, 4-1BB, and OX40) alone (second generation) or in combination with (third generation) to further augment and sustain T-cell effector functions.

Application of CAR T-cell therapy to solid tumors faces a series of challenges, including efficient trafficking and infiltration to tumor, and overcoming tumor-mediated immunosuppression. We have demonstrated augmented efficacy following regional administration of mesothelin (MSLN)-specific CAR T cells in an orthotopic model of pleural mesothelioma; a single, low dose of CAR T cells was able to suppress tumor growth and establish long-term immunity.¹ In this clinically-relevant model, we investigated the effect of repeated antigen stress on CAR T cells that results in CAR T-cell exhaustion and further explored strategies to enhance CAR T-cell functional persistence.² We used three CARs that incorporated an anti-human, MSLN-specific scFv and CD3 ζ , CD28/ CD3 ζ , or 4-1BB/ CD3 ζ signaling domains (Mz, M28z, and MBBz). We purposefully administered a very low dose of MSLN-targeted CAR T cells (Effector to Target, E:T ratio = 1:7,500). Although second generation CAR T cells (M28z and MBBz) exhibited

superior antitumor efficacy compared with first-generation CAR T cells (Mz), mice treated with M28z eventually died from tumor relapse, whereas a relatively higher proportion of MBBz-treated mice remained tumor free. Upon *ex vivo* stimulation, tumor-harvested M28z cells had decreased cytotoxicity and Th1 cytokine production, which is a phenotype associated with T-cell exhaustion. Notably, although MBBz cells better retained effector functions compared with M28z cells, overall cytotoxicity and cytokine production also decreased with repeated antigen stimulation, thus indicating that MBBz cells are also sensitive to exhaustion, albeit at a relatively lower magnitude. These observations underscore the need to establish CAR T-cell *functional persistence* within the immunosuppressive solid tumor microenvironment for long-term antitumor efficacy.

Exhausted CAR T cells overexpressed inhibitory receptors, including programmed death-1 (PD-1), with corresponding upregulation of PD-1 ligands (PD-L1 and PD-L2) on the tumor cells. In line with the beneficial effects of PD-1 and PD-L1 blocking antibodies in the clinical treatment of solid tumors,³ treatment with the PD-1 blocking antibody was able to partially rescue effector functions of M28z both *in vitro* and *in vivo*, and reduce tumor burden significantly. However, tumor relapse was observed following cessation of antibody administration. While long-term, repeated antibody treatment was able to control tumor progression it did not achieve tumor eradication. Taken together, our observation suggests that *adaptive resistance* plays an important role in CAR T-cell exhaustion. The PD-1 blocking antibody was able to rescue M28z function; its

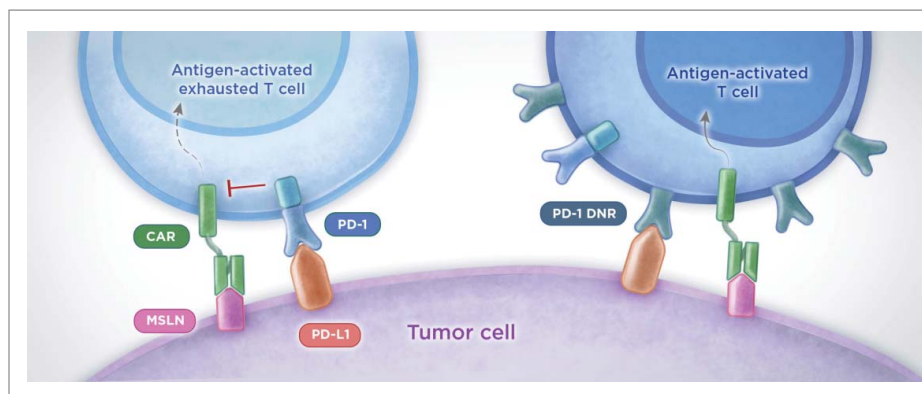


Figure 1. PD-1 cell-intrinsic strategy counteracts PD-L1-mediated immunosuppression and enhances efficacy of CAR T-cell therapy. To overcome PD-L1 mediated immunosuppression, MSLN-specific CAR T cells were genetically engineered to overexpress a PD-1 dominant negative receptor (PD-1 DNR) that lacks the intracellular inhibitory signaling domain. PD-1 DNR competes with the endogenous PD-1 and saturates the PD-L1 ligand expressed on tumor cells, thereby limiting activation of PD-1 inhibitory signaling. PD-1 DNR CAR T cells rescue effector functions *in vitro* and enhance control of tumor burden *in vivo*. PD, programmed-death; MSLN, mesothelin; CAR, chimeric antigen receptor; DNR, dominant negative receptor.

effect is short-lived and repeated antibody administration is required to suppress tumor progression.

To license CAR T cells with checkpoint blockade without being reliant on repeated antibody administration, we genetically engineered CAR T cells to overexpress a PD-1 dominant negative receptor (PD-1 DNR) that lacks the PD-1 transmembrane and intracellular signaling domains (see Fig. 1). We hypothesized that PD-1 DNR expressed on the CAR T-cell surface can act as a “decoy receptor” to bind and block the PD-L1/2 inhibitory signal. Indeed, M28z CAR T cells co-transduced with PD-1 DNR cells had increased proliferation, enhanced cytotoxicity, and augmented cytokine secretion upon repeated antigen stimulation compared with M28z cells. Mice injected with M28z PD-1 DNR cells better controlled tumor burden and prolonged median survival. Other strategies used to intrinsically block the PD-1 inhibitory signal in CAR T cells include co-expression of a switch receptor that contains the PD-1 extracellular domain and CD28 transmembrane, and intracellular domains (PD-1 CD28) in anti-MSLN or anti-prostate-specific cell antigen (PSCA) CAR T cells expressing the 4-1BB co-stimulatory signal,⁴ as well as the generation of PD-1 deficient anti-CD19 CAR T cells using CRISPR/Cas9 gene editing technology.⁵

In our study, PD-1 blockade by both anti-PD-1 antibody and PD-1 DNR augmented CAR T-cell efficacy. Compared with combined CAR T-cell and antibody treatment, there are several advantages of using genetically engineered cells. First, blockade of the PD-1 signal through genetic engineering provides a sustainable effect. Second, genetically engineered CAR T cells provide tumor-limited PD-1 blockade. Anti-PD-1 antibody efficacy can be limited by inefficient tumor penetration, short half-life, and non-specific toxicity. Nevertheless, MSLN-targeted CARs, in combination with checkpoint blockade either by antibody administration or by PD-1 DNR, provides opportunity to treat a wide array of solid tumors.⁶ We are planning to initiate a combination immunotherapy clinical trial for patients with metastatic lung cancer, breast cancer, and mesothelioma at Memorial Sloan Kettering Cancer Center.

Some mice treated with a single dose of M28z PD-1 DNR CAR T cells demonstrated long-term tumor relapse, which suggests that there are redundant mechanisms of immunoinhibition that must be overcome within the tumor microenvironment. In addition to PD-1, multiple co-inhibitory receptors (e.g., TIM-3, LAG3, and TIGIT) are also expressed on exhausted T cells.⁷ Simultaneously targeting multiple inhibitory pathways may further enhance CAR T-cell potency. A better understanding of inhibitory mechanisms influencing tumor-infiltrating T cells, both endogenous and CAR T cells can help design next-generation immunotherapy. Adoptive T-cell therapy by T-cell engineering provides opportunities to design rational combinatory strategies to improve CAR T-cell therapy that may lead to successful treatment of solid tumors.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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