Editorial

Commentary on "A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors"

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The field of adoptive immunotherapy has grown from the early days of a few academic groups administering cytotoxic lymphocytes (CTL) or tumor infiltrating lymphocytes (TIL), to the use of genetically engineered T cells being developed as drug products by multinational pharmaceutical companies. The first demonstration of the effective use of gene engineered T cells was reported in 2006 when T-cell receptor (TCR) engineered cells were shown to mediate cancer regression in 2 of 13 patients with malignant melanoma (1). In the subsequent years, several groups spearheaded the clinical developed an alternative antigen targeting technology based on chimeric antigen receptors (CAR), which mediate tumor antigen recognition via ligand binding domains (generally antibodies) and bypass the TCR complex by being fusion proteins with T cell signaling domains such as CD3zeta (2-4). CARs based on anti-CD19 mAbs have been shown to mediate durable cancer regression in leukemia and lymphoma in multiple clinical trials and it is these results that most stimulated the excitement of this therapy as a new treatment modality for cancer (5-7).

While there has been consistent success in the application of CAR therapy in CD19-expressing hematological malignancies, there has not been reproducible success in the application of CAR technology in solid cancers. There are most certainly multiple reasons for the lack of effective tumor treatment by CAR engineered T cells (CART) in solid malignancies, with the tumor microenvironment the most widely proposed, and the subject of the research reported in Liu *et al*. Just as tumors vary from type to type (e.g., breast *vs*. colon), there is no one universal tumor microenvironment. While tumor microenvironments vary greatly, there are some consistent themes that may permit researchers to focus efforts on the most prevalent inhibitory factors. The immune system has a series of checkpoints that dampen the immune response and restore balance post-antigen exposure. One of the most widely studied systems is the programmed death 1 (PD-1) receptor interaction with its' two major ligands PD-L1 and PD-L2. Antibodies targeting the PD-1/PD-L1 proteins have been studied in several clinical trials and have demonstrated to mediate responses in several solid tumor histologies (e.g., melanoma, non-small cell lung cancer) (8,9). T cell checkpoint blockade is the focus of the work by Liu *et al*. Genetic engineering has the potential to endow cells with novel functions using synthetic biology. Liu *et al*. apply the concept of synthetic biology in CART cells using what they describe as a "switch receptor complex".

The idea of switch receptors is to produce a chimeric molecule that combines a ligand binding domain with an alternative signaling domain and this approach has been reported for a number of different constructs (10,11). In the specific example investigated in Liu *et al*., the investigators fused the extracellular domains of PD-1 to the transmembrane and intracellular signaling domains of CD28. This approach was initially reported by others and expanded in this report using more detailed *in vivo* modeling to demonstrate increase efficacy when combined with CART cells (12-14), as shown in *Figure 1*, when PD-1 normally binds one of its ligands in signals through the

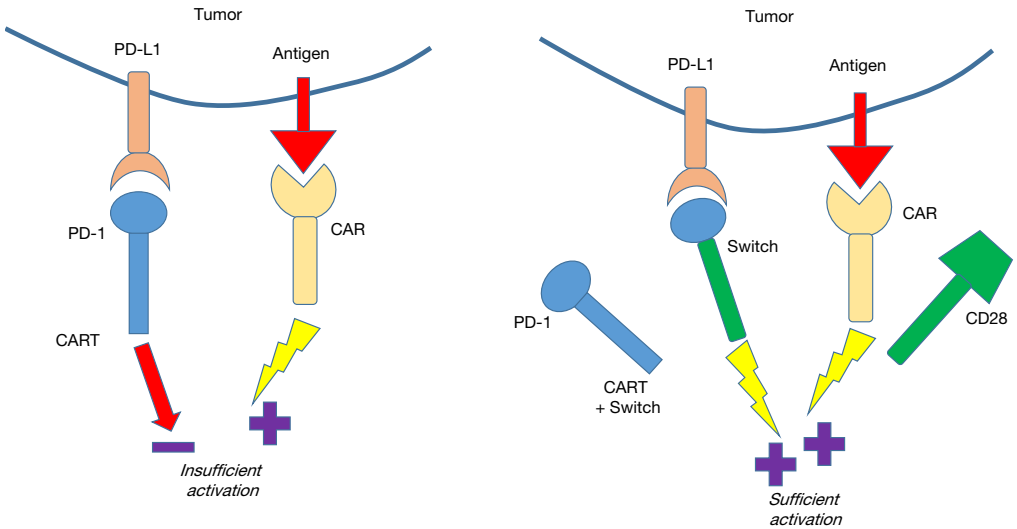

Figure 1 When a CAR T cell encounters a tumor cell expressing both the tumor antigen and the PD-L1 ligand, the CAR T cell can get mixed signals (A). T cells expressing the CAR and PD-1 will receive both the positive T cell activation signal from the CAR and the negative inhibitory signal from the PD-1/PD-L1 interaction, potentially leading to insufficient activation. If as shown on (B), the CAR T cells have been engineered to express the PD-1 flip receptor, the T cell can receive two positive signals (from the CAR and switch receptor) and this may lead to sufficient activation to mediate an anti-tumor response. CAR, chimeric antigen receptors; PD-1, programmed death 1.

Smad proteins to blunt T cell signaling. CD28, on the other hand, is one of the main TCR co-stimulator receptors and upon engagement of its ligands (e.g., CD80) boosts the T cell response through a number of positive signaling events resulting in enhanced proliferation and cytokine production. By using a PD-1/CD28 switch receptor fusion protein, it is possible to turn the negative signal normally propagated by the PD-1/PD-L1 interaction, into a positive signal that a T cell normally receives through CD28/CD80 co-stimulation.

Liu *et al*. use three gene transfer methods to test this approach, RNA electroporation, gamma-retroviral vector, and lentiviral vector mediated gene transfer and they combined these methods in a number of antigen targeting systems with CARs recognizing CD19, PSCA, and mesothelin. RNA electroporation can quickly introduce most any protein into a cell and the co-electroporation of a CD19 CAR and the switch receptor was performed to test the impact on T cell recognition of tumor cell lines expressing both CD19 and PD-L1. In the case where the CAR alone engineered T cells encounter PD-L1 expressing targets, they produced less cytokine and showed reduced tumor cell killing than when cultured with PD-L1-negative cell lines. In the case of the CAR-switch receptor co-expressing T cells, this reduction in effector T cell responses was not observed. While RNA electroporation can be used to rapidly evaluate synthetic biology approaches, the amount of gene expression afforded by the method is generally much higher than viral-vector mediated gene transfer methods. In the second and third figures in their report, Liu *et al*. use gamma-retroviral and lentiviral vectors (for anti-PSCA and anti-mesothelin CARs, respectively) and reproduce the findings of the CD19 CAR switch receptor RNA electroporation experiment by demonstrating the lack of blocking activity when CART cells encounter tumor cell lines expressing PD-L1. Moreover, when comparing the activity of switch receptor-engineered T cells to CAR alone T cells, data suggest that these cells are more active when encountering PD-L1 expressing target cells.

In a last series of experiments, Liu *et al*. explore the biology of CAR plus switch receptor engineered T cells in tumor xenograft models. These models used well established tumors (unlike previous reports) and are a typical assay used in the CART field to validate *in vitro* biology. In both PD-L1 expressing mesothelin and PSCA expressing tumor models significantly better anti-tumor activity was observed when mice were administered T cells engineered with both the CARs and the switch receptor. What may be of particular interest to the application of these approaches in cancer patients were the findings on cell

phenotype and persistence. PD-1 is not the only inhibitory receptor involved in control T cell activity, proteins Lag3 and Tim3 have also been associated with decreased T cell functions (15). When Liu *et al*. analyzed T cells from mice that received the CAR switch combination, there were fewer T cells co-expressing these additional exhaustion markers. Although the precise biology behind the co-expression of inhibitory T cell markers is still being worked out, it is clear that these markers can be co-expressed and cells expressing multiple markers are more impaired than cells expressing PD-1 alone. Finally these investigators also report that the number of TIL in mice receiving the CAR plus switch receptor engineered T cells were greater than TIL from animals that received only the CART. Whether this resulted from increased persistence, cell expansion, or tumor trafficking (or a combination of mechanisms) was not determined.

These results are encouraging and suggest a potential way in which anti-tumor T cells might be engineered to make them more active in tumors which express PD-L1/PD-L2. A note of caution in the over interpretation of these results should be taken from the experimental design. The tumor targets used had been engineered to over-express either the target antigen and/or the PD-L1 ligand and the xenograft models do not have the normal repertoire of additional inhibitor cells often found in human tumors (e.g., Treg, MDSC). Furthermore, PD-L1 is often not expressed on tumors, yet is upregulated when an inflammatory response is initiated (e.g., by interferon gamma) and these kinetics of inhibitor response may impact the activity of switch receptor-engineered cells. As also pointed out, tumors tend to have multiple inhibitory factors and it is not known if focusing on one element, such as PD-1/PD-L1, will be sufficient to over a tumor microenvironment that produces inhibitor cytokines (e.g.,TGF-beta) and regulatory cells (e.g., Treg). Overall, the results reported by Liu *et al*. are encouraging and support continuing research into methods to enhance the activity of anti-tumor engineered T cells as therapy for patients with advanced malignancies.

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Footnote

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Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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