



Cell intrinsic PD-1 checkpoint blockade releases the brake on human chimeric antigen receptor (CAR) T cells for solid tumors

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The immune system plays an important role in controlling and eliminating cancer. Nevertheless, tumors often evade endogenous immune responses due to tolerogenic mechanisms which prevent the rejection of malignant cells that are recognized as ‘self’ or alternatively, not ‘dangerous.’ The complex network of biological pathways that maintain tolerance involves several mediators, including myeloid and lymphoid-derived regulatory cells, immunosuppressive cytokines and chemokines, as well as immune checkpoint molecules that down-modulate anti-tumor immunity. The programmed cell death protein 1 (PD-1)—PD-1 ligand 1 (PD-L1) receptor-ligand pair is a principal immune checkpoint axis operative in the tumor microenvironment that attenuates T cell receptor (TCR)-mediated activation, leading to inhibition of T cell expansion and cytokine production (1-3). Although the genetic re-direction of T cells with chimeric antigen receptors (CARs) is an attractive approach to break tolerance in the setting of cancer, there is a paucity of experimental evidence to suggest that CAR T cells are optimally functional within solid tumors and additionally, that blockade of immune checkpoints can potentiate their activity. Cherkassky *et al.* (4) address this long-standing question by engineering mesothelin-targeted CAR T cells to be intrinsically resistant to PD-1-induced inhibition. These findings should spur future investigations focused on more carefully defining the determinants of CAR T cell exhaustion and assessing the therapeutic potential of individual or combined checkpoint blockade in augmenting anti-tumor T cell responses.

In the case of most human cancers, the TCR repertoire is

either lacking or inadequate for tumor control (5-7), which has paved the way for the concept of using CAR-redirection T cells for adoptive immunotherapy. Currently, we and others have demonstrated that CAR T cells can eradicate leukemia and induce long-term durable remissions in patients (8-10). In the setting of solid tumors, however, this approach has not achieved the same degree of clinical success, and this may be attributed to unique barriers imposed by solid tumors that are absent in hematological malignancies. Following the trafficking of CAR T cells to tumor tissues and infiltration through stromal elements, these engineered lymphocytes must overcome challenges present within a profoundly immunosuppressive landscape which, in addition to the aforementioned tolerogenic mechanisms, may include nutrient deprivation, oxidative stress, low pH and hypoxia [reviewed in (11)]. Furthermore, in this microenvironment where CAR T cells must function, there is evidence that TCR signaling becomes uncoupled independently of the effects of tumors on TCR ζ expression (12,13). This led us and other groups to construct “bipartite receptors” comprised of TCR ζ and intracellular signaling modules such as CD28, 4-1BB, OX40, ICOS or CD27 (14-19) to substantially improve the function and proliferation of adoptively transferred T cells. While the induction of costimulatory pathways can overcome some degree of solid tumor-mediated inhibitory signaling, the extent to which T cells expressing these “second generation” CARs could be inhibited upon *in vivo* antigen exposure within the solid tumor microenvironment is not well-characterized.

The studies by Cherkassky *et al.* (4) highlight the effects

of specific types of co-stimulatory signaling on CAR T cell potency, which have been largely unappreciated due the use of immune sensitive model systems and the infusion of large numbers of T cells that do not accurately reflect effector to target cell ratios attained in patients with high tumor burdens. Similar to previous studies using different *in vivo* experimental systems, T cells expressing anti-mesothelin chimeric receptors bearing TCR ζ signaling modules alone were not sufficient to drive sustained antitumor activity in an orthotopic model of malignant pleural mesothelioma (4,14). In contrast, the administration of high doses of CAR T cells incorporating CD28 or 4-1BB endodomains resulted in tumor clearance, regardless of the costimulatory pathway induced. Interestingly, upon transfer of low doses of T cells, only 4-1BB ζ CAR T cells resulted in long-term tumor eradication, despite an intratumoral accumulation and persistence comparable to that of their CD28 ζ counterparts. These findings were supported by the superior ability of 4-1BB ζ CAR T cells to retain effector cytokine production and cytotoxic capacity following *in vivo* antigen exposure (4). In addition, when recall response was assessed (the so-called ability to function as “serial killers”), 4-1BB ζ CAR T cells were more resistant to ongoing immune inhibition induced by repeated antigen stimulation *in vitro* or tumor re-challenge *in vivo* (4). These findings underscore that when selecting an optimal costimulatory signaling strategy, mere assessment of CAR T cell engraftment particularly as measured through peripheral blood sampling may not be a sufficient indication of potency in the setting of solid tumor indications. Pre-clinical studies that will ultimately inform clinical trial designs should thus move toward the evaluation of functional correlates of persistence in model systems that more accurately recapitulate tumor antigen load in patients.

Despite the improved anti-tumor activities of second generation CAR T cells in which enhanced persistence is mainly attributed to the engagement of costimulatory pathways, these engineered lymphocytes also undergo an activation-induced upregulation of co-inhibitory receptors and pathways to naturally control the magnitude of immune responses. Accordingly, the balance between positive and negative signals is a critical determinant of the outcome of T cell-mediated immunity. However, the increased expression of inhibitory receptors following antigen encounter coupled with the overexpression of their cognate ligands by cancer cells greatly limits overall anti-tumor activity. Although blockade of negative regulatory checkpoint pathways has recently demonstrated promise in restoring defective T cell function (20-22), the clinical

success of these approaches often relies on tumor mutation burden (23) and the localization of T cells within the microenvironment. Further, unabridged immune activation following checkpoint blockade is often accompanied by a number of toxicities and autoimmune sequelae (24). In patients with tumors of insufficient immunogenicity (i.e., “non-inflamed” tumors), treatment with CAR-redirected T cells may address these limitations. Nevertheless, adoptively-transferred T cells are susceptible to immune inhibition, and therefore, the removal of inhibitory checkpoint signals may potentiate their full anti-tumor effects.

Cherkassky and colleagues (4) focused on repairing the defect observed in tumor-infiltrating CD28 ζ -signaling CAR T cells that was largely characterized by PD-1 overexpression. Signaling through PD-1 results in the recruitment of phosphatases SHP-2 and to a lesser extent SHP-1 to the inhibitory receptor cytoplasmic domain that initiates dephosphorylation of antigen receptor proximal signaling molecules including ZAP70, PKC θ , and CD3 ζ (1), leading presumably to attenuation of the CAR/CD28 ζ signal. Co-expression of a PD-1 with a truncated intracellular signaling domain counteracted the inhibitory signaling conferred by endogenous PD-1 (4), which was likely attributed to competition for PD-L1 by the dominant negative receptor (*Figure 1*). This strategy resulted in enhanced *in vitro* CD28 ζ CAR T cell activity and significantly improved *in vivo* anti-tumor efficacy with a single administration (4). The strength of this approach is elimination of the requirement for repeated anti-PD-1/PD-L1 antibody dosing, which the authors also demonstrate to be efficacious when administered concurrently with CD28 ζ CAR T cells. Indeed, this genetic engineering strategy may offer an enhanced safety profile as well as a therapeutic benefit over PD-1/PD-L1-targeting antibodies due to pathway inhibition that is restricted to adoptively-transferred T cells. This permits tumor-targeted T cell reinvigoration within the microenvironment without dose-limiting toxicities that are inherent to broadly applied antibody-based checkpoint blockade (24). In future investigations focused on extending this approach into clinical development, consideration should be given to the incorporation of inducible suicide genes into CAR T cells that co-express a dominant negative PD-1 receptor. This “safety switch”-type engineering may ultimately prevent or limit the potential for adverse lymphoproliferative or autoimmunity.

How might these findings improve treatment strategies

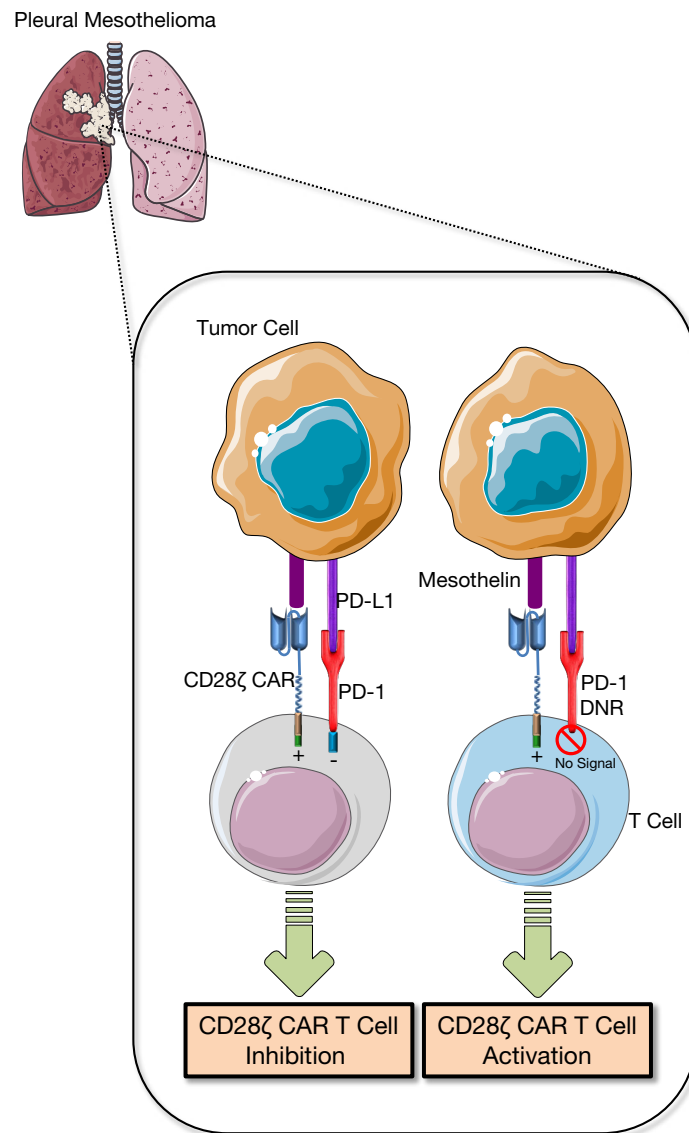


Figure 1 Inhibition of PD-1 signaling in CD28 ζ CAR T cells potentiates anti-tumor function in a model of metastatic pleural mesothelioma. Schematic representation of CD28 ζ CAR T cells associating with their cognate antigen (mesothelin) and also binding to PD-L1 via endogenous PD-1 (co-inhibitory signal) leads to functional inhibition of these redirected T lymphocytes (left). Abrogation of PD-1/PD-L1 signaling by co-expression of a PD-1 dominant negative receptor (DNR) with CAR renders T cells resistant to immune inhibition and potentiates activation and resultant anti-tumor activity (right). PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand 1; CAR, chimeric antigen receptor.

based on adoptive T cell transfer? Cherkassky *et al.* (4) illustrated the functional significance of a central mechanism underlying T cell exhaustion in an antigen-dependent model system that recapitulates certain features of inhibitory signaling in the tumor microenvironment. In addition, the authors demonstrate the importance of selecting an optimal

costimulatory strategy that is capable of withstanding immune inhibition. It is intriguing to speculate that CAR T cells containing tripartite signaling domains may be more resistant to immune inhibitory effects imposed by this model, due to their “hardwired” costimulatory signals that are triggered upon encounter with tumor antigen.

There are theoretical reasons to suggest that three signaling modules comprising a “third generation” chimeric receptor might have additive or synergistic effects. Although 4-1BB can function independently of CD28 (25), it is also possible that one of these co-stimulatory signals will be dominant. Nevertheless, deep immunophenotyping in addition to relevant functional analyses of pre- and post-infusion CAR T cells may provide clues about which T cells can proliferate and elicit superior anti-tumor activity following inhibition of the PD-1 inhibitory pathway. For example, it has been recently demonstrated that a certain PD-1+ CD8+ T cell population that proliferates following blockade of PD-1/PD-L1 signaling expresses several costimulatory molecules (e.g., ICOS, CD28) and is capable of self-renewal as well as effector differentiation (26). Thus, selection of such a population of T cells for CAR-based strategies that combine checkpoint blockade may optimize PD-1-directed immunotherapy. Finally, it is becoming increasingly evident that T cell exhaustion is regulated by co-expression of multiple inhibitory receptors including PD-1 (27). A systematic exploration of all inhibitory pathways that could limit CAR T cell potency in the setting of the solid tumor microenvironment may elucidate the therapeutic potential of individual or combined blockade of additional inhibitory receptors in restoring anti-tumor T cell function.

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