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Out of the bitter came forth sweet
Activating CD28-dependent co-stimulation via PD-1 ligands

Chen Ankri and Cyrille J Cohen*

Laboratory of Tumor Immunology and Immunotherapy; The Goodman Faculty of Life Sciences; Bar-Ilan University; Ramat Gan, Israel

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Programmed cell death 1 (PDCD1, best known as PD-1) is a central negative regulator of effector T cells that is involved in the etiology of chronic inflammatory conditions, viral diseases, and cancer. We have recently sought to improve T-cell functions by means of a novel chimeric co-stimulatory molecule that could divert the negative signals normally transmitted by PD-1 into positive ones. Human T cells transduced to express a fusion protein encompassing the extracellular domain of PD-1 and the intracellular portion of the co-stimulatory molecule CD28, which we named PD-1/28, exhibited an increase in cytokine secretion, the upregulation of activation markers, an improved proliferative potential and superior antineoplastic activity in xenograft models of human melanoma.

Inhibitory (or negative) co-stimulatory molecules such as programmed cell death 1 (PDCD1, best known as PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA4) have been shown to actively modulate T-cell responses upon activation.[1] Interestingly, they have also been implicated in the escape of malignant cells from immunosurveillance, as the signal they convey can impair T-cell functions, often leading to exhaustion, decreased secretion of multiple cytokines including interleukin-2 (IL-2), interferon γ (IFNγ) and tumor necrosis factor α (TNFα), dampened proliferation and limited cytotoxic activity.[2] PD-1, which is expressed on effector T cells shortly after T-cell receptor (TCR)-dependent activation, can negatively regulate T-cell function by itself. PD-1 binds to 2 different ligands, CD274 (best known as PD-L1 or B7-H1) and PD-1 ligand 2 (PDL2, also known as B7-DC), that can be expressed by professional antigen-presenting cells as well as by tumor cells of distinct histological origin (e.g., breast, kidney, ovarian, pancreatic, bladder, and gastric cancer cells).[3] Because of its critical immunosuppressive role, PD-1 has been extensively studied and therapeutic approaches aimed at eliminating its negative impact on T cell-dependent antitumor responses have been devised, mostly based on the blockade of PD-1 signaling with anti-PD-1 or anti-PD-L1 antibodies. These agents can reverse T-cell exhaustion ex vivo and in vivo, hence inducing durable tumor regressions or prolonged disease stabilization in patients with advanced cancers.[4] In contrast to PD-1, several co-stimulatory molecules, such as CD28, provide positive signals that are required for the full activation and effector activity of naïve T cells. Upon binding to their cognate ligands, these receptors—which belong to either the B7/CD28 family or the TNFα receptor (TNFR) family—convey TCR-independent intracellular signals that can lead to T-cell expansion as well as to the acquisition of effector functions. Thus, the balance between co-stimulatory and co-inhibitory signals regulate the response, function and expansion of T cells in multiple pathophysiological scenario.

The adoptive transfer of tumor-infiltrating lymphocytes (TILs) or genetically engineered T cells has received increasing attention over the past decade as this approach appears to mediate impressive tumor regressions in some patients bearing advanced neoplasms.[5] In addition to receptors that endow T cells with a new specificity (including TCRs and so-called chimeric antigen receptors, CARs), co-stimulatory receptors such as CD28 can be genetically introduced into T cells in order to enhance their effector functions, persistence and antitumor activity.[6-8] However, due to the paucity of some activatory ligands (e.g., B7 family members) and the overexpression of inhibitory ligands (such as PD-L1) in the tumor microenvironment, T cells expressing co-stimulatory receptors are expected to function inadequately within neoplastic lesions. To circumvent this issue and generate T cells that are supposed to exhibit robust effector functions in the tumor microenvironment, we designed and optimized a re-targeting molecule that we termed "co-stimulatory converter," which comprises the extracellular domain of PD-1 fused to the signaling domains of CD28 and/or TNFR superfamily, member 9 (TNFRSF9, best known as 4–1BB).[9] The rationale of this approach was to take advantage of the elevated levels of PD-L1 found on malignant cells to stimulate genetically-engineered T cells (**Fig. 1**). Moreover, to emulate clinical conditions, we designed a tripartite retroviral vector that encodes

*Correspondence to: Cyrille J Cohen, Ph.D; Email: cohency@mail.biu.ac.il
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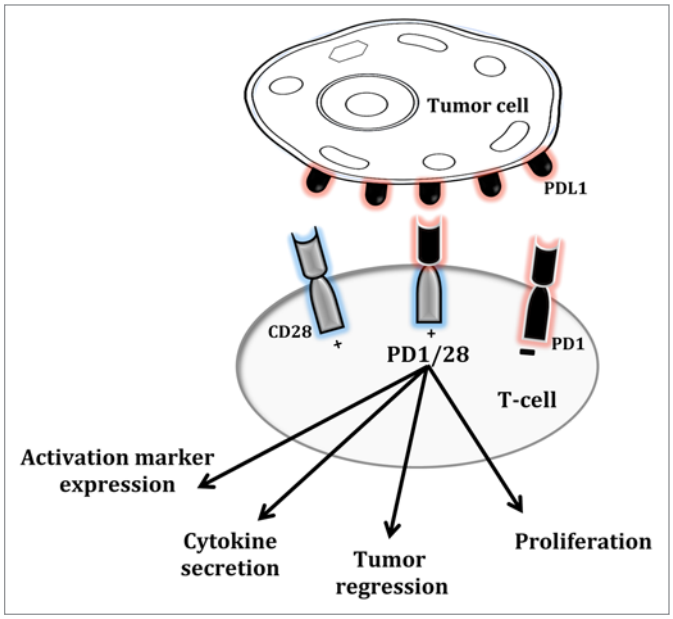

Figure 1. PD-1/28, a chimeric co-stimulatory converter. PD-1/28 is composed of the extracellular domain of the co-inhibitory receptor programmed cell death 1 (PDCD1, best known as PD-1) and the intracellular domain of the co-stimulatory molecule CD28. Upon binding to PD-1 ligands expressed on the surface of cancer cells, PD-1/28 results in increased cytokine secretion, upregulation of T-cell activation markers, improved proliferative potential and superior antitumor activity in xenograft models of human melanoma.

the α and β chains of a clinically-tested melan A (MLANA)-specific TCR (F4) as well as one of our chimeric receptors, the PD-1/28 molecule. Following transduction, we were able to achieve high levels of expression of both PD-1/28 and F4 TCR in primary human T cells. We then evaluated the function of human T cells co-expressing PD-1/28 and F4 exposed to different melanoma cell lines, and we found that PD-1/28-engineered human T-cells secreted high amounts of various cytokines (including IL-2, IFNγ and TNFα) and expressed increased levels of activation markers including CD25, CD69, and 4–1BB. PD-1/28-expressing T cells also manifested an improved proliferative response as compared with control cells. These observations prompted us to investigate the cytotoxic functions of PD-1/28-expressing T cells in 2 xenograft models of human melanoma. First, we took advantage of a system that we recently adopted for adoptive T-cell transfer studies,[8] which that is based on the growth of human tumors on the chick embryo chorioallantoic membrane (CAM). Following the intravenous transfer of PD-1/28-transduced T cells, we observed improved tumor regression as compared with control conditions, and we were able to detect by flow cytometry the accumulation of adoptively transferred T cells within neoplastic lesions. In addition, by using an immunodeficient mouse model, we demonstrate that PD-1/28-transduced T cells are highly efficient at delaying the growth of human melanoma in vivo.

Using other co-inhibitory[10] and co-stimulatory molecules for the generation of additional co-stimulatory converters is an attractive perspective. We believe that this type of strategy could be useful in circumstances in T cells undergo exhaustion owing to the PD-1/PD-L1 signaling axis, such as in the course of chronic viral diseases. As malignant cells that escape T-cell responses could be selected in vivo over time based on their high levels of PD-L1, co-stimulatory converters may be useful for reverting this situation, reducing immunosuppression and hence enabling a robust T cell-mediated antitumor response.

In summary, our results suggest that the PD-1/28 co-stimulatory converter improves the antitumor activity of adoptively transferred antigen-specific T cells, resulting to tumor regression. We trust that our findings highlight the importance of manipulating co-stimulatory pathways for the improvement of T cell-based treatments using gene transfer approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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