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Better Performance of CARs Deprived of the PD-1 Brake

Aizea Morales-Kastresana, Sara Labiano, José I. Quetglas, and Ignacio Melero

Immunotherapies often permit combinations to increase efficacy. Two approaches are currently leading our field: adoptive therapy with T cells transfected with chimeric antigen receptors and monoclonal antibodies blocking the PD-1/PD-L1 (B7-H1) axis. In this issue of *Clinical Cancer Research*, preclinical evidence for a synergistic combination of such approaches is reported. *Clin Cancer Res*; 19(20); 5546-8. ©2013 AACR.

In this issue of *Clinical Cancer Research*, John and colleagues (1) provide preclinical evidence for a synergistic combination of adoptive T-cell therapy with T cells expressing chimeric antigen receptors (CAR) and anti-PD-1 monoclonal antibodies (mAb). Both of these strategies are in the limelight of translational research due to their amazing reported efficacy in early-phase clinical trials. The most successful cases reported with T cells transduced with CARs have taken place against hematologic lymphoid malignancies, whereas blockade of the PD-1/PD-L1 (B7-H1) pathway with mAbs has so far shown signs of clear efficacy against solid tumors.

Adoptive T-cell therapy has been at the workbench of immunotherapists for decades (2). Results in patients with melanoma using cultured tumor-infiltrating lymphocytes are showing very interesting efficacy, provided that the patients are heavily preconditioned with myelosuppressive chemotherapy to induce lymphopenia. Technical complexity makes it difficult to extend the approach to many clinical centers and relies on the endogenous repertoire of antigen T-cell receptors (TCR) recognizing cancer antigens. As an alternative, retroviral *ex vivo* gene therapy has been used to transfer the chains of the TCR from CD8 T-lymphocyte clones with high affinity for a tumor antigen presented by HLA class I molecules. This approach is progressing, and the proof of principle in patients with melanoma has been established (2). The potency of these treatments has inherent risks if the tumor antigen is not uniquely expressed by the tumor tissue and by dispensable cells such as melanocytes (3). The transgenic TCR chains mispaired with endogenous TCRs may give rise to autoimmune clones. All of these issues are being cleverly addressed with biotechnology tricks.

Cultured lymphocytes can also be gene-transduced to express CARs on their surface. Such chimeric proteins include a single-chain antibody for antigen recognition on the surface of tumor cells, a membrane domain and intracellular signaling domains borrowed from the CD3 ζ chain and costimulatory receptors such as CD28, CD137 (4-1BB), and CD27 (2, 4). *Ex vivo* retroviral gene therapy is used to introduce such constructions into preactivated T-lymphocytes in culture. The most remarkable results have been observed with an anti-CD19-CD3 ζ -4-1BB chimera that has shown complete responses in a handful of chronic lymphocytic leukemia (5) and acute lymphocytic leukemia (6) cases, including pediatric patients. The treatment works at the expense of inducing a lifelong tolerable B-cell aplasia and cytokine storms or tumor lysis syndromes. Other malignancies are being pursued with similar approaches, but there is well-justified fear about the tumor selectivity of the antigens because of reported clinical experience (7). Industrial development has started for these overall strategies, and the technology is being refined through introduction of inducible suicide genes to treat potentially serious side effects and cotransfection of two different CARs that recognize two different tumor surface antigens, one providing TCR-like signals and the other costimulation (4). Need for simultaneous recognition of two antigens should add more specificity and safety.

The PD-1/PD-L1 (B7-H1) pathway inhibits signals at the cell-to-cell contacts of lymphocytes (8). PD-1 plays its inhibitory role by recruiting the tyrosine phosphatase SHP-2 to the surface area, where the TCRs and costimulatory receptors are signaling (9). When T cells are activated, they start expressing surface PD-1 that is overexpressed in exhausted/anergic T lymphocytes. Many tumors express PD-L1 (B7-H1), and that is a negative prognostic correlate in many instances (8). It is also interesting that tumors upregulate PD-L1 (B7-H1) when under immune attack mainly in response to IFN- γ (8). PD-1 has another ligand termed PD-L2 (B7-DC) with a pattern of expression restricted to hematopoietic cells and mainly to dendritic cells. The importance of PD-L2 in tumor immunology remains to be assessed but it is likely also to contribute to downsizing T-cell responses and thus to become an obstacle for cancer immunotherapy (8).

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Antibodies against PD-1 and PD-L1 are under active clinical development and showing impressive clinical activity in melanoma, renal cell carcinoma, and lung cancer cases (10, 11). A number of pharmaceutical companies are pursuing these targets with humanized mAbs and chimeric proteins. Indeed, at least five agents of this kind have entered clinical trials. Three features allow us to rate the approach as extremely exciting: (i) clinical objective responses are frequent and long lasting, (ii) safety profiles seem to be sufficiently mild, and (iii) PD-1 blockade in combination with CTLA-4 blockade with mAbs seems to be highly synergistic in patients with melanoma (12).

In this issue of *Clinical Cancer Research*, John and colleagues (1) report on their preclinical test of a combination of T cells with a CAR recognizing HER-2 on the surface of tumor cells and a mouse anti-PD-1 mAb in the treatment of HER-2⁺ breast carcinoma-transplanted tumors. The CAR chosen includes the signaling domains of CD28 and CD3 ζ . Each of the elements in the two-pronged combination shows signs of monotherapy efficacy, but there are very interesting signs of synergistic, rather than additive, effects on the HER-2-transfected mouse models.

It is conceivable that PD-1 limits the signal transduction capabilities of the artificial chimeric receptors at the immune synapses between CAR-transduced cells and tumor cells. The tumor cell lines in culture constitutively expressed PD-L1 and presumably keep expressing this molecule in the tumor microenvironment. Even with PD-L1-negative cell lines, it is very likely that IFN- γ will elicit PD-L1 precisely where and when the CAR-transduced lymphocytes are attacking. The mode of action could be simplistically envisioned as a result of displacement of PD-1

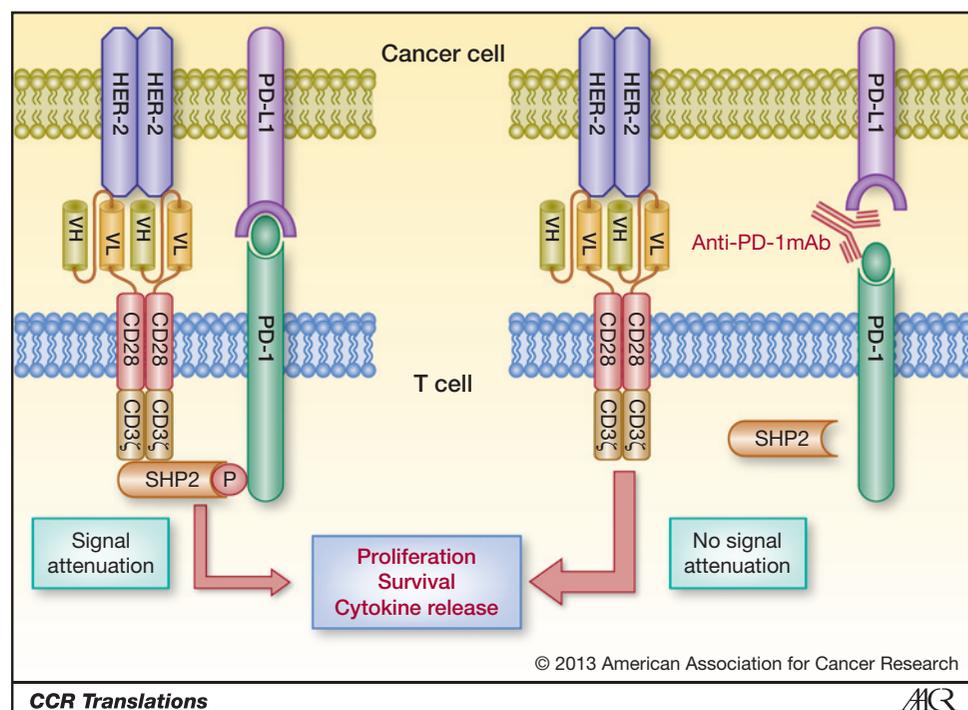
and its recruited SHP-2 from the synapses between CAR-expressing T lymphocytes and PD-L1 on tumors (Fig. 1). Enhanced CAR signals induce stronger proliferation and cytolytic functions. However, as reported by the authors, other intriguing mechanisms may be important, such as the observed deleterious effects on myeloid-derived suppressor cells and regulatory T cells (1).

While anti-PD-1 and anti-PD-L1 antibodies rely on an endogenous and weak antitumor immune response that is to be amplified, infusion of CAR-transduced cells tilts this situation toward a much more favorable proportion of high-affinity tumor-reactive T cells. It remains to be investigated if the exhausted endogenous immune response by nontransfected T lymphocytes, when unrestrained by anti-PD1 mAb, also plays a role. The CAR used might be improved by introducing the CD137 or CD27 intracellular domains because of the unique costimulatory properties of this receptor family for memory maintenance, survival, and *in vivo* expansion (5). In this regard, anti-CD137 and anti-PD-L1 mAbs are highly synergistic. It is also likely that CD137-related signaling from the CAR would bypass the need for pretreatment with total body irradiation (5).

Combinations of more than two immunotherapy agents will eventually take the stage to maximize efficacy. For instance, we have observed that spontaneous hepatocellular carcinoma in oncogene transgenic mice can only be cured by combining three immunostimulatory mAbs and adoptive T-cell therapy (13).

The translational potential of the combination is further highlighted by the observation that no observable autoimmunity takes place in endogenous breast and central nervous system tissues expressing the targeted antigen in the

Figure 1. CARs and PD-1 at tumor-T cell immune synapses. Schematic representation of immune synapses of CARs—transduced T cells recognizing HER-2 on the surface of tumor cells. The state of affairs in the presence (right) or absence (left) of blockade of the PD-1/PD-L1 (B7-H1) axis with mAbs is represented. Anti-PD-1 mAbs determine no relocation of PD-1 to the lymphocyte contacts with tumor cells. As a consequence, PD-1-associated SHP-2 tyrosine phosphatase cannot be recruited to these immune synapses, thereby allowing for unrestrained tyrosine phosphorylation signals from CARs.



tumor-rejecting mice. No matter how reassuring, caution should be exercised by careful dose escalation when translating this approach to patients.

This report from the Australian group (1) brings excellent news: Our best players in immunotherapy can play together passing the ball to each other well with synergistic results. If translated to the clinic, PD-1 blockade and CAR-transduced T cells could very well be the dream team.

Disclosure of Potential Conflicts of Interest

I. Melero has a commercial research grant from Bristol-Myers Squibb and is a consultant/advisory board member of Bristol-Myers Squibb, AstraZeneca, Merck Serono, and Miltenyi. No potential conflicts of interest were disclosed by the other authors.

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