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A novel combinatorial cancer immunotherapy
poly-IC and blockade of the PD-1/PD-L1 pathway

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A non-antigen specific immunotherapy consisting of repeated co-administration of poly-IC and blocking antibodies targeting the programmed cell death-1 (PD-1) pathway dramatically inhibits tumor development in several mouse models of cancer. Tumor-reactive CD8+ T cells mediate the antitumor effects mediated by PD-1 blockade. This therapeutic avenue can be readily translated to cancer patient treatment regimens.

CD8+ cytotoxic T lymphocytes (CTLs) are the most efficient mediators of the adaptive immune system. Among the myriad of immune cell responses to cancer, CTLs are capable of both recognizing and destroying tumor cells. CTLs recognize antigens on the surface of tumor cells that are presented as MHC class I-peptide complexes. These peptides, known as CTL epitopes, are commonly derived from processed proteins. When derived from cancer cells, these CTL epitopes correspond to tumor-associated antigens (TAAs). In order to develop effective epitope-based cancer vaccines, a necessary step is to identify TAAs containing peptide epitopes that potently solicit tumor-reactive CTLs.

Numerous investigators are working to develop epitope-based vaccination strategies for immunization against cancer. These include melanoma and human papilloma virus (HPV)-mediated cervical cancer, particularly since these malignancies have defined TAAs that can be used to stimulate tumor-reactive CTL responses. In B16 melanoma and HPV cervical cancer mouse models, we recently investigated vaccines consisting of synthetic peptides representing minimal CTL epitopes administered intravenously in a mixture with polyinosinic-polycytidylic acid (poly-IC) and anti-CD40 monoclonal antibody (mAb)—a combinatorial agent called TriVax—or with poly-IC alone (termed BiVax). We reported that such combinatorial treatments were capable of eliciting robust T cell responses that were highly effective against established tumors, demonstrating that these strategies are useful approaches for the treatment of tumor types in which reliable TAAs are known.[1-5]

However, for many other tumors such as lung and colon cancers, few if any dependable TAAs inducing tumor-specific CTL responses have been identified to date, limiting our ability to develop epitope-based vaccines. Thus, we feel that there is a great need to explore novel immunotherapeutic approaches to induce tumor-reactive CTLs without the use of defined TAAs. Recently, while performing vaccination experiments using the murine B16 melanoma model and peptides administered in combination with poly-IC and anti-programmed death-ligand 1 (PD-L1) mAb, we noticed surprising and dramatic antitumor effects in control mice that received an irrelevant synthetic peptide.[5] These results suggested that the repeated co-administration of these immune adjuvants alone, even in the absence of peptide epitope, might provide a therapeutic benefit against established tumors. This approach might be especially useful for the treatment of lung and colon cancers for which no known CTL-activating TAAs have been identified so far.

To test this hypothesis, we explored the therapeutic efficacy of the combined administration of poly-IC and anti-PD-L1 mAb in 3 cancer mouse models: B16 melanoma, Lewis lung carcinoma, and MC38 colon carcinoma.[6] B16 tumor growth was delayed by the poly-IC/anti-PD-L1 mAb combination therapy, although none of the host mice completely rejected their tumors. On the other hand, treatment with the combination of poly-IC and anti-PD-L1 mAb completely eradicated tumors in 60–80% of mice bearing the lung and colon cancer models, respectively. Furthermore, anti-PD-1 mAb alone was found to be equally effective in controlling MC38 colon tumor growth as compared with anti-PD-L1 mAb co-administration with poly-IC. Next, to assess the generation of long-term systemic immunity, mice that rejected lung or colon tumors were rechallenged with same tumor. In the colon cancer xenograft model, none of survivor mice developed tumors after the rechallenge, whereas in the lung cancer model one half of the host mice were able to reject the second tumor challenge, suggesting that this combinatorial therapy could potentially induce durable immunity.

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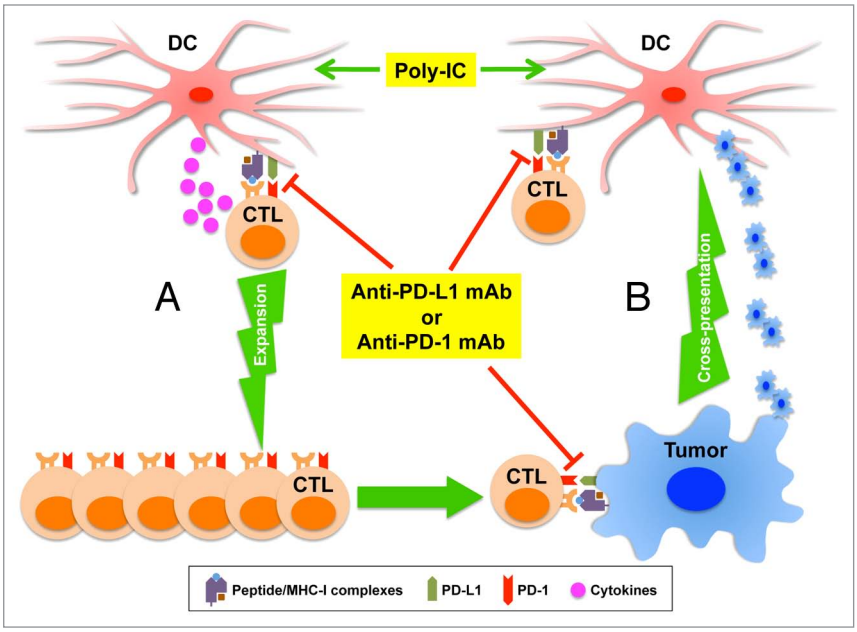

Figure 1. Co-administration of poly-IC and blocking antibodies of the PD-1/PD-L1 pathway may enhance anticancer cytotoxic T lymphocyte responses. (**A and B**) Potential mechanisms accounting for the therapeutic benefit of polyinosinic-polycytidylic acid (poly-IC) and programmed cell death-1 (PD-1/PD-L1) blockade. (**A**) Poly-IC stimulates dendritic cells (DCs) to increase expression of peptide/MHC complexes and secrete cytokines, including type-I interferon (IFN) that enhances cytotoxic T lymphocyte (CTL) activation, proliferation, and maturation. The blockade of PD-1/PD-L1 pathway using monoclonal antibodies (mAbs) improves T cell activation, and CTLs expand and become more potent effector cells. (**B**) Poly-IC activates DCs at the tumor site and enhances tumor antigen cross-presentation, leading to the generation of new CTL responses. The blockade of PD-1/PD-L1 pathway enhances the effector phase of CTL responses. Green arrows, stimulatory; red arrows, inhibitory.

We also sought to study the role the various lymphocyte subsets in tumor rejection. To this end, we evaluated the antitumor efficacy of the poly-IC/anti-PD-L1 mAb combinatorial therapy in mice depleted of $CD8^+$ T cells, $CD4^+$ T cells, or natural killer (NK) cells. The therapeutic benefits of the combination therapy disappeared when $CD8^+$ T cells, but not $CD4^+$ T cells or NK cells, were depleted, indicating that the antitumor effects of this therapy are mediated by $CD8^+$ CTLs. Additionally, using the EliSPOT assay we confirmed that tumor-reactive CTLs were induced by this combinatorial therapy as evinced by the tumor cell elicitation of interferon-γ (IFNγ) producing T cells obtained from treated wild-type animals. However, in the experiments using genetically deficient mice, we found that the efficacy of this combinatorial therapy partly depended upon the participation of type-I IFN, whereas IFNγ did not appear to play a major role. As with our previous experience using peptide vaccines,[1] we assume that CTLs mediate their antitumor effects via perforin-granzyme-mediated cytotoxicity.

In conclusion, our data suggest that the administration of poly-IC and blocking mAbs of the PD-1/PD-L1 pathway may improve CTL responses naturally generated against TAAs, or alternatively, could foster new CTL responses (**Fig. 1**). Poly-IC is known to stimulate antigen-presenting cells (APCs) including dendritic cells (DCs) that may enhance tumor antigen cross-presentation to CTLs. Thus, tumor-infiltrating DCs exposed to poly-IC that capture dead tumor cells or tumor debris containing TAAs become potent APCs capable of priming new tumor-reactive CTLs or expanding preexistent CTLs. Moreover, it is also known that antigen-exposed CTLs may express the inhibitory PD-1 receptor. The blockade of the PD-1/PD-L1 pathway between these T cells and DCs, that express PD-L1, rescues the T cells to expand and become more potent effector cells. Since tumor cells also express PD-L1, the blockade of the PD-1/PD-L1 pathway may also enhance the effector phase of the CTL response, promoting T cell survival and proliferation at the tumor site.

Finally, we believe that our observations in mice could readily be translated into a novel cancer treatment, especially in those instances where no reliable TAAs have been identified. Both poly-IC and several humanized mAbs targeting the PD-1/PD-L1 pathway are currently undergoing clinical development.[7-10]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Cho HI, Celis E. Optimized peptide vaccines eliciting extensive CD8 T-cell responses with therapeutic antitumor effects. Cancer Res 2009; 69:9012-9; http://dx.doi.org/10.1158/0008-5472.CAN-09-2019; PMID:19903852
2. Cho HI, Lee YR, Celis E. Interferon γ limits the effectiveness of melanoma peptide vaccines. Blood 2011; 117:135-44; http://dx.doi.org/10.1182/blood-2010-08-298117; PMID:20889921
3. Cho HI, Reyes-Vargas E, Delgado JC, Celis E. A potent vaccination strategy that circumvents lymphodepletion for effective antitumor adoptive T-cell therapy. Cancer Res 2012; 72:1986-95; http://dx.doi.org/10.1158/0008-5472.CAN-11-3246; PMID:22367213
4. Barrios K, Celis E. TriVax-HPV: an improved peptide-based therapeutic vaccination strategy against human papillomavirus-induced cancers. Cancer Immunol Immunother 2012; 61:1307-17; http://dx.doi.org/10.1007/s00262-012-1259-8; PMID:22527249
5. Cho HI, Barrios K, Lee YR, Linowski AK, Celis E. BiVax: a peptide/poly-IC subunit vaccine that mimics an acute infection elicits vast and effective anti-tumor CD8 T-cell responses. Cancer Immunol Immunother 2013; 62:787-99; http://dx.doi.org/10.1007/s00262-012-1382-6; PMID:23266830
6. Nagato T, Lee YR, Harabuchi Y, Celis E. Combinatorial Immunotherapy of Polyinosinic-Polycytidylic Acid and Blockade of Programmed Death-Ligand 1 Induce Effective CD8 T-cell Responses against Established Tumors. Clin Cancer Res 2014; 20:1223-34; PMID:24389326; http://dx.doi.org/10.1158/1078-0432.CCR-13-2781
7. Okada H, Kalinski P, Ueda R, Hoji A, Kohanbash G, Donegan TE, Mintz AH, Engh JA, Bartlett DL, Brown CK, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with alpha-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol 2011; 29:330-6; http://dx.doi.org/10.1200/JCO.2010.30.7744; PMID:21149657

8. Sabbatini P, Tsuji T, Ferran L, Ritter E, Sedrak C, Tuballes K, Jungbluth AA, Ritter G, Aghajanian C, Bell-McGuinn K, et al. Phase I trial of overlapping long peptides from a tumor self-antigen and poly-ICLC shows rapid induction of integrated immune response in ovarian cancer patients. Clin Cancer Res 2012; 18:6497-508; http://dx.doi.org/10.1158/1078-0432.CCR-12-2189; PMID:23032745

9. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366:2443-54; http://dx.doi.org/10.1056/NEJMoa1200690; PMID:22658127

10. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012; 366:2455-65; http://dx.doi.org/10.1056/NEJMoa1200694; PMID:22658128