

# Subverting the adaptive immune resistance mechanism to improve clinical responses to immune checkpoint blockade therapy

Young J Kim<sup>1,2,\*</sup>

<sup>1</sup>Department of Otolaryngology – Head & Neck Surgery; Johns Hopkins University; School of Medicine; Baltimore, MD USA; <sup>2</sup>Department of Oncology and the Sidney Kimmel Comprehensive Cancer Center; Johns Hopkins University; School of Medicine; Baltimore, MD USA

**Keywords:** adaptive immune resistance, anti-PD-1, cancer vaccine, tumor microenvironment, TLR agonists

The correlation between tumor-infiltrating lymphocyte (TIL)-expression of programmed cell death ligand 1 (PD-L1) and clinical responsiveness to the PD-1 blocking antibody nivolumab implicates adaptive immune evasion mechanisms in cancer. We review our findings that tumor cell PD-L1 expression is induced by interferon  $\gamma$  (IFN $\gamma$ ) producing TILs. We provide a mechanistic rationale for combining IFN $\gamma$ <sup>+</sup> T helper type 1 (Th1)-inducing cancer vaccines with PD-1 immune checkpoint blockade.

Clinical oncology and immunology have now converged onto the 2-signal model of T-cell activation comprising stimulation presaging attendant inhibitory pathways as the second signal and the future of cancer treatment. Antibody-mediated blockade of cytotoxic T lymphocyte associated protein 4 (CTLA-4) with ipilimumab and programmed cell death 1 (PDCD1, better known as PD-1) with nivolumab, have both demonstrated clinical efficacy in various advanced malignancies.<sup>1,2</sup> For the nivolumab trial, the tight correlation between clinical response and PD-L1 (B7-H1) expression has emphasized the importance of PD-L1 expression on tumor cells as a mechanism of immune escape.<sup>3,4</sup> Constitutive PD-L1 on the surface tumor cells has been linked to tumor suppressor gene PTEN,<sup>5</sup> but correlative studies of the human tumor microenvironment have shown that PD-L1 expression is significantly associated with tumor infiltrating T cells and interferon  $\gamma$  (IFN $\gamma$ )<sup>6</sup>. These latter findings support an induced, adaptive mechanism of PD-L1 expression in human melanoma. Although these 2 mechanisms of PD-L1 expression on the tumor cells are not mutually exclusive, both constitutive and adaptive mechanisms have important clinical implications going forward with

the design of clinical trials with PD-1 or PD-L1 blockade.

In order to study the mechanism by which PD-L1 is induced by tumor-infiltrating lymphocytes (TILs), both we and Spranger et.al. used the B16 melanoma model.<sup>7,8</sup> These complementary reports showed that PD-L1 expression on these cells were both IFN $\gamma$  and CD8<sup>+</sup> T-cell dependent. While Spranger et.al. used IFN $\gamma$ <sup>-/-</sup> mice, our lab used IFN $\gamma$  neutralizing antibodies as well as CD8 depleting antibodies to show that this induction of PD-L1 on cancer cells within the tumor microenvironment was driven by the infiltrating CD8<sup>+</sup> T-cells. These findings supported previous work from Liepeng Chen's lab that initially dissected this PD-L1 dependent immune resistance mechanism *in vivo* through an artificial overexpression of PD-L1 on P815 mastocytoma cell line.<sup>4</sup> In addition to these mechanistic studies, we were also intent on developing immunotherapeutic agents that could potentially be translated for clinical trials.

Toward these goals, we first engineered a cell-based vaccine formulated with adjuvants that can activate both conventional and plasmacytoid dendritic cells.<sup>8</sup> We formulated glucopyranosyl lipid A (GLA), a Toll-like receptor 4 (TLR4) agonist, and resiquimod (R848), a TLR7/8 agonist,

2 agents found to be safe in patients – with a tumor cell based vaccine to create TLR agonists enhanced GM-CSF secreting vaccine – termed TEGVAX. We next sought to address its antitumor effects in an established, palpable B16 treatment model. Application of the TEGVAX vaccine using a prime-boost method, significantly enhanced tumor growth *in vivo* in a T cell and MyD88-TRIF dependent manner.<sup>8</sup> TEGVAX clearly induced increased tumor-specific cytotoxic T lymphocyte (CTL) responses as well as increased the presence of TILs into the tumor microenvironment. Nevertheless, despite the presence of a clear antitumor immune response we did not see any regression of the tumor in this poorly immunogenic B16 model.

We sought to determine whether the antitumor activity of TEGVAX was potentially being dampened by the induction of PD-L1 in the tumor cells responding to IFN $\gamma$  secreting tumor-specific TILs in the tumor microenvironment. In fact, TEGVAX did increase IFN $\gamma$ -dependent PD-L1 upregulation, and further, we also noted co-localization of PD-L1 and CD8<sup>+</sup> T-cells in the tumor microenvironment. The final demonstration of adaptive immune resistance mechanism came from the finding that the combination of

\*Correspondence to: Young J Kim; Email: ykim76@jhmi.edu

Submitted: 07/07/2014; Accepted: 07/09/2014

<http://dx.doi.org/10.4161/21624011.2014.954868>

TEGVAX and anti-PD-1 blockade induced regression of established B16 tumors.<sup>8</sup> In contrast, control experiments with anti-PD-1 blockade alone and anti-PD-1 blockade + GM-CSF secreting vaccine did not display comparable antitumor effects as the combination of TEGVAX and anti-PD-1 blockade.

One important feature of our experiments was the use of palpable, established B16 murine model. While others used non-palpable B16 tumors (which may not model clinical scenarios with established tumor) or more immunogenic tumors before starting treatments,<sup>9</sup> our therapeutic assay was much more stringent in that we initiated treatment 7–10 days after B16 inoculation, at which point an organized immune inhibitory tumor microenvironment is established rendering these tumors resistant to most previously tested strategies of active immunotherapy. Secondly, we showed that combinatorial therapy was broadly applicable as we found this approach to be an effective therapy against multiple tumor models aside from B16 melanoma. Another relevant feature of our work is the fact that anti-PD-1

blockade alone failed to induce B16 regression, similar to reports in some of the patients in clinical trials. Aside from the heterogeneity of human tumors, one explanation is that the palpable B16 model utilizes other mechanisms of tumor immune evasion, such that an endogenous immune system cannot generate a potent IFN $\gamma$  associated T helper type 1 (Th1) response in the tumor microenvironment. Our B16 tumors had minimal baseline expression of PD-L1, which may model certain human tumors that do not express PD-L1. Only upon TEGVAX-dependent induction of sufficient tumor-specific CTLs with immune checkpoint molecule blockade did we observe regression *in vivo*. Lastly, our report describes a newly formulated cancer vaccine that can be easily translated into cancer patients as all the components have been found to be safe.

The implication from our findings is that vaccines should be coupled with anti-PD-1 blockade in future clinical trials. The objective responses achieved in the treatment of advanced cancer patients with anti-PD-1 monotherapy ranged from 18–28%, which suggests that there is

room for clinical improvements with the addition of IFN $\gamma$ -producing vaccines. Currently, the clinical trials with anti-PD1 blocking antibodies have screened human tumor specimens for PD-L1 expression, but our finding that IFN $\gamma$  inducing vaccines can increase PD-L1 in the tumor microenvironment provides both mechanistic and clinical rationale for combining Th1-inducing vaccines with anti-PD-1 blockade. An important area of future research is to better define the patterns of PD-L1 expression in the tumor microenvironment both in the murine system and in human tissues. Conceptually, immunotherapeutic strategies to increase tumor-infiltrating CTL anticancer activities with immune checkpoint inhibitors may convert anti-PD-1 blockade non-responders to responders, thus circumventing immune evasion.<sup>10</sup>

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### References

- Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; 363:711-23; PMID:20525992; <http://dx.doi.org/10.1056/NEJMoa1003466>
- 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; PMID:22658128; <http://dx.doi.org/10.1056/NEJMoa1200694>
- 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; PMID:22658127; <http://dx.doi.org/10.1056/NEJMoa1200690>
- Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nat Med* 2002; 8:793-800; PMID:12091876; <http://dx.doi.org/10.1038/nm730>
- Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, Cachola KE, Murray JC, Tihan T, Jensen MC, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* 2007; 13:84-8; PMID:17159987; <http://dx.doi.org/10.1038/nm1517>
- Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, Chen S, Klein AP, Pardoll DM, Topalian SL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Sci Transl Med* 2012; 4:127ra37; PMID:22461641; <http://dx.doi.org/10.1126/scitranslmed.3003689>
- Fu J, Malm IJ, Kadayakkara DK, Levitsky H, Pardoll D, Kim YJ. Preclinical evidence that PD-1 blockade cooperates with cancer vaccine TEGVAX to elicit regression of established tumors. *Cancer Res* 2014; 74:4042-52; PMID:24812273; <http://dx.doi.org/10.1158/0008-5472.CAN-13-2685> [pii]
- Spranger S, Spaepen RM, Zha Y, Williams J, Meng Y, Ha TT, Gajewski TF. Up-regulation of PD-L1, IDO, and tregs in the melanoma tumor microenvironment is driven by CD8+ T cells. *Sci Transl Med* 2013; 5:200ra116; PMID:23986400; <http://dx.doi.org/10.1126/scitranslmed.3006504>
- Duraiwamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T cell rejection function in tumors. *Cancer Res* 2013; 73:3591-603; PMID:23633484; <http://dx.doi.org/10.1158/0008-5472.CAN-12-4100>
- Lutz ER, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, Solt S, Dorman A, Wamwea A, Yager A, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. *Cancer Immunol Res* 2014; 2:616-31; PMID:24942756; <http://dx.doi.org/10.1158/2326-6066.CIR-14-0027>