

## The Promise of PD-1 Signaling Pathway for Cancer Immunotherapy

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Productive adaptive immune responses are the end product of a complex set of cellular and molecular interactions fine-tuned by various stimulators and inhibitors, also referred as immune checkpoint blockers, of T cells to ensure optimal immune response for elimination of external and internal threats followed by resolution of the effector T cells without collateral damage and autoimmune consequences. In this context, the CD28 and tumor necrosis factor receptor (TNFR) super family costimulatory and coinhibitory molecules are central to immune modulation and immune homeostasis. These signals bridge innate, adaptive, and regulatory immunity through a coordinated communication network that ensures the generation of productive immune responses against pathogens without collateral damage to the host. Costimulatory receptor ligand pairs, such as CD28/B7-1 (CD80) or B7-2 (CD86), 4-1BB/4-1BB ligand, OX-40/OX40 ligand, HVEM/LIGHT, and others in both families, act as intrinsic adjuvants for augmenting the T cell responses generated by the recognition of peptides derived from pathogens and presented in the context of major histocompatibility molecules (Signal 1) by providing a qualifier signal (Signal 2) for T cell activation [1]. Similarly coinhibitory receptor ligand pairs particularly GITR/GITRL, CTLA-4/B7-1 or B7-2, Fas/FasL, and PD-1/PD-L1 are evolved to contribute to peripheral tolerance to self-antigens as well as to ensure homeostatic control of unrestrained T cell activation/proliferation ensuing immune responses to pathogens [2]. Therefore, costimulatory and coinhibitory signaling pathways work in balance to ensure physiological immune responses to infection and cancer in the absence of collateral damage and autoimmunity.

The discovery of tumor specific and/or associated antigens (TAAs) expressed by cancerous cells taken together with numerous preclinical and clinical studies providing evidence for the importance of immunosurveillance in controlling cancer revitalized enthusiasm of tumor immunologists to formulate approaches where intrinsic TAA specific antitumor responses can be potentiated for durable therapeutic responses against cancer. Varieties of cancer vaccine (active immunization) and adoptive T cell therapies approaches are being currently developed in preclinical and clinical studies [3]. The past decade can be viewed as golden era for cancer immunotherapies as a preventive vaccine, Gardasil, was successfully developed and approved by food and drug administration (FDA) for cervical cancer in 2006. This exciting development was followed by FDA approval of a dendritic cell based vaccine, Provenge, as first therapeutic cancer vaccine for end stage prostate cancer patients in 2010. Particularly, the approval of Provenge is perceived a breakthrough for the development of therapeutic cancer vaccines from scientific as well as regulatory standpoints.

While these important discoveries led to a boost of enthusiasm in the field for the development of various cancer vaccines and immunotherapies in preclinical models and translation to the clinic, a new class of immunotherapy is focused on unleashing the breaks of immune system by inhibiting the signaling of immune check point blockers, such as CTLA-4 and PD-1. These two immune checkpoint receptors have been most extensively studied. The importance of CTLA-4 signaling in controlling adaptive immune responses was

hinted by observations that mice deficient for this receptor displayed massive generalized lethal autoimmune/hyper immune like syndrome due to their inability to limit the amplitude of naïve and memory T cells systemically [4]. Decades of studies by Allison and colleagues and others first in preclinical models and then in the clinic led to FDA approval in 2011 of an antibody to CTLA-4 (ipilimumab) after its historic success in phase III clinical trial demonstrating survival benefit in melanoma patients [5]. HLA-A\*0201-positive patients (n=676) with stage III or IV un-resectable metastatic melanoma which have gone through standard of care therapy, were arbitrarily assigned, in 3:1:1 ratio, and received ipilimumab with gp100 (403 patients), ipilimumab only (137), or gp100 alone (136). The primary end point for the study was overall survival. Ipilimumab, with or without gp100 peptide, improved overall survival in these patients as compared to gp100 peptide alone group. Grade 3 or 4 immune-associated adverse effects occurred in a small fraction of patients that received ipilimumab. These adverse side effects can be severe but most can be mitigated with appropriate treatments.

The success of anti-CTLA-4 Ab in the clinic diverted attention to other coinhibitory molecules, in particular PD-1. PD-1 is expressed on activated T cells and has two well characterized ligands; PD-L1 (also known as B7-H1) and PD-L2 (B7-DC) [6,7]. The ligands of PD-1 are upregulated in response to inflammation. PD-L1 is upregulated on hematopoietic, endothelial, and epithelial cells in response to pro-inflammatory cytokines, particularly IFN- $\gamma$ , whereas PD-L2 upregulation is restricted to dendritic cells (DCs) and macrophages in response to IL-4 [8,9]. Signaling through PD-1 was shown to limit T cell responses in peripheral organs during inflammatory responses to infections, and as such limit organ specific autoimmunity [10,11]. For example, PD-1 deficient mice on C57BL/6 background developed lupus like syndrome involving joints and kidneys [10], whereas PD-1 deficient mice on BALB/c background developed myocarditis [11]. Initial disappointment due to delayed (6-9 months) manifestation of mild deleterious phenotype of PD-1 knockout mice seems advantageous for targeting cancer immunotherapy with this pathway. These findings established PD-1 as an inhibitory receptor for control of organ specific autoimmunity. Phenotype of PD-1 KO mice became more clear by further studies demonstrating that acceleration of organ specific autoimmunity in autoimmune prone mice [12].

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Further studies demonstrated that signaling through PD-1 receptor indeed have crucial role in the inhibition of T cell responses [6,7]. The discovery that PD-L1 can bind to B7-1 in addition to PD-1 to inhibit the co-stimulatory signaling as well as inhibit T cell activation in PD-1 independent manner [13] introduced another complexity with added opportunity for targeting this pathway for immunotherapy.

The importance of PD-1 signaling in the control of peripheral immune response to cancer was demonstrated by forced expression of PD-L1 in mouse tumor cells, which conferred resistance to immune attack [14,15]. Subsequent studies demonstrated selective upregulation of PD-L1 in many human cancers [16]. PD-1 is expressed on majority of tumor infiltrating lymphocytes (TILs) and enhanced expression of PD-1 on CD8<sup>+</sup> T cells against tumors and chronic viral infections correlated with their nonresponsive/anergic state [17,18]. In a chronic viral infection model, blockade of PD/PD-L1 signaling restored the function of anergic virus specific CTLs [18]. Like CD8<sup>+</sup> T cells, expression of PD-1 was also observed on antigen-non-responsive CD4<sup>+</sup> T cells in chronically infected patients with hepatitis C virus. The blockade of PD-1 partially restored the CD4<sup>+</sup> T cell function [19]. In addition to inducing anergy in T effector cells, PD-1 expression on CD4<sup>+</sup> T effector cells in the PD-L1 enriched tumor microenvironment actively converts these cells into induced CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T regulatory (Treg) cells, which play critical roles in tumor immune evasion mechanisms [20]. Importantly, PD-1 signaling on B cells was shown to mediate depletion of activated memory B cells during simian immunodeficiency virus infection in a primate model and PD-1 blockade reversed this effect, restoring antibody titers [21]. Therefore, the pleiotropic effects of PD-1 signaling on multiple immune cell types, such as CD4<sup>+</sup> T cell, CD8<sup>+</sup> T cells, Treg cells, and B cells, and its demonstrated role in immune evasion mechanisms presents this receptor as an important target for cancer immunotherapy.

These insights into the biology of PD-1 and its ligand reinforce the notion that PD-1 and PD-L1 blockade may synergize multiple arms of adaptive immunity within tumor microenvironment for a productive and durable antitumor response required for clinical efficacy. There are currently four agents, MDX-1106, CT-011, MK-3475 and AMP-224, in clinical trials targeting the PD-1 pathway for cancer immunotherapy. The first three are anti-PD-1 monoclonal antibodies (mAbs) while the last is a B7-DC/IgG1 protein. MDX-1106 is a fully human IgG4mAb that was first tested in humans with various metastatic solid tumors, such as melanoma, renal cell carcinoma (RCC), colorectal cancer (CRC), and non-small cell lung cancer (NSCLC). The trials culminated in demonstration of impressive clinical efficacy in terms of objective and durable responses in advanced melanoma (~35%), RCC (~50%) and NSCLC (~25%) patients. The overall efficacy of the partial or complete responses of durable nature collectively in all of these cancer types was over 33%, which far exceeds the responses observed by standard chemotherapy in these cancer settings [22].

The humanized second mAb, CT-011, was first tested in a single and well-tolerated dose regimen of advanced hematologic malignancies [23]. Encouraging results from this trial led to its testing in additional clinical trials for patients with advanced hematologic malignancies and solid tumors. This Ab *in vitro* enhanced human NK cell function against autologous, primary multiple myeloma (MM) cells, seemingly through effects on NK cell trafficking, immune complex formation with MM cells, and cytotoxicity specifically toward PD-L1 expressing MM tumor cells, but not normal cells. These observations demonstrate the function of CT-011 on the innate immune system [24], and as such indicate the

potential utility of this agent for elimination of MHC class I negative tumor cells. The third humanized MK-3475 mAb is being tested in a phase I clinical trial. Finally AMP-224, a recombinant protein made by fusing the extracellular domain of PD-L2 to IgG1, is presently being tested in a phase I clinical trial against refractory metastatic cancer cells. The clinical performance of this agent is of significant interest due to the use of a natural ligand, rather than an Ab, given the toxicity and efficacy differences observed between natural ligands and agonistic Ab to costimulatory molecules, such as 4-1BB [25,26].

Moving forward, it is anticipated that one or more anti-PD-1 mAbs will be approved by 2015 based on the impressive early clinical responses in various tumor models. The anti-PD-1 mAbs may show even more impressive clinical benefits when used in combination with other checkpoint blockers, such as CTLA-4, or immune costimulatory molecules, such as agonists of 4-1BB, OX-40, HVEM, CD40 receptors. These costimulatory molecules may work with checkpoint blockers in true synergy, which provide strong rationale for the development of costimulatory agonists for cancer immunotherapy. Another level of synergy can be achieved by using the agonists of costimulatory molecules and antagonist of coinhibitory molecules in combination with TAAs as novel vaccine candidates. In this configuration, TAAs may prime tumor-specific T effector cells while the costimulatory agonists and coinhibitory antagonist augment the ongoing T cell response for a robust and durable anti-tumor response with therapeutic efficacy.

The incorporation of agents that target innate immunity and antigen presentation, such as DCs, into the costimulatory/coinhibitory immunotherapeutic configuration may further improve immunotherapies, particularly cancer vaccines. In this context, the use of toll like receptor agonists, such as FDA approved agent monophosphoryl lipid A (MPL-A), CpG-ODNs, or flagellins, is a logical choice. These agonists are expected to jump start the adaptive immunity by first activating DCs for antigen uptake, processing, and presentation to T cells in addition to the elaboration of various inflammatory cytokines, such as IL-12, required for up regulation of costimulatory receptors and initiation/expansion of T cell responses with therapeutic efficacy and long-term immune memory controlling micrometastases. However, given the potent nature and pleiotropic effects of these immune modulators, the combinatorial use of these molecules for cancer immunotherapy will require homework well done for achieving clinical benefit in the absence or with tolerable toxicity. Regardless, the field of tumor immunotherapy is finally ripe to deliver the long-anticipated promise of controlling/curing cancer and the years ahead will assess the feasibility of this promise.

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#### References

1. Croft M (2003) Co-stimulatory members of the TNFR family: keys to effective T-cell immunity? Nat Rev Immunol 3: 609-620.
2. von Boehmer H, Melchers F (2010) Checkpoints in lymphocyte development and autoimmune disease. Nat Immunol 11: 14-20.
3. Schlom J (2012) Therapeutic cancer vaccines: current status and moving forward. J Natl Cancer Inst 104: 599-613.
4. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, et al. (1995) Lymphoproliferative disorders with early lethality in mice deficient in CtlA-4. Science 270: 985-988.
5. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, et al. (2010)

- Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 363: 711-723.
6. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, et al. (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 192: 1027-1034.
  7. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, et al. (2001) PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2: 261-268.
  8. Shin T, Yoshimura K, Shin T, Crafton EB, Tsuchiya H, et al. (2005) In vivo costimulatory role of B7-DC in tuning T helper cell 1 and cytotoxic T lymphocyte responses. *J Exp Med* 201: 1531-1541.
  9. Wilke CM, Wei S, Wang L, Kryczek I, Kao J, et al. (2011) Dual biological effects of the cytokines interleukin-10 and interferon- $\gamma$ . *Cancer Immunol Immunother* 60: 1529-1541.
  10. Nishimura H, Nose M, Hiai H, Minato N, Honjo T (1999) Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 11: 141-151.
  11. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, et al. (2001) Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 291: 319-322.
  12. Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ (2007) The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol* 8: 239-245.
  13. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ (2007) Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* 27: 111-122.
  14. Wang S, Chen L (2011) Immunobiology of cancer therapies targeting CD137 and B7-H1/PD-1 cosignal pathways. *Curr Top Microbiol Immunol* 344: 245-267.
  15. Flies DB, Chen L (2007) The new B7s: playing a pivotal role in tumor immunity. *J Immunother* 30: 251-260.
  16. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, et al. (2002) Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 8: 793-800.
  17. Ahmadzadeh M, Johnson LA, Heemskerck B, Wunderlich JR, Dudley ME, et al. (2009) Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* 114: 1537-1544.
  18. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, et al. (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439: 682-687.
  19. Raziorrouh B, Ulsenheimer A, Schraut W, Heeg M, Kurktschiev P, et al. (2011) Inhibitory molecules that regulate expansion and restoration of HCV-specific CD4+ T cells in patients with chronic infection. *Gastroenterology* 141: 1422-1431, 1431.
  20. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, et al. (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med* 206: 3015-3029.
  21. Titanji K, Velu V, Chennareddi L, Vijay-Kumar M, Gewirtz AT, et al. (2010) Acute depletion of activated memory B cells involves the PD-1 pathway in rapidly progressing SIV-infected macaques. *J Clin Invest* 120: 3878-3890.
  22. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, et al. (2010) Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 28: 3167-3175.
  23. Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, et al. (2008) Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clin Cancer Res* 14: 3044-3051.
  24. Benson DM Jr, Bakan CE, Mishra A, Hofmeister CC, Efebera Y, et al. (2010) The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. *Blood* 116: 2286-2294.
  25. Schabowsky RH, Elpek KG, Madireddi S, Sharma RK, Yolcu ES, et al. (2009) A novel form of 4-1BBL has better immunomodulatory activity than an agonistic anti-4-1BB Ab without Ab-associated severe toxicity. *Vaccine* 28: 512-522.
  26. Sharma RK, Elpek KG, Yolcu ES, Schabowsky RH, Zhao H, et al. (2009) Costimulation as a platform for the development of vaccines: a peptide-based vaccine containing a novel form of 4-1BB ligand eradicates established tumors. *Cancer Res* 69: 4319-4326.