

Tim-3: An Emerging Target in the Cancer Immunotherapy Landscape

Ana C. Anderson

Abstract

The cancer immunotherapy field has grown exponentially in the past few years, largely driven by the success of immune checkpoint blockade. Therapies targeting the immune checkpoint molecules CTLA-4 and PD-1 have achieved objective responses in melanoma, renal cancer, and lung cancer; however, a large number of patients are still suffering with these cancers that are not benefiting from these therapies. Moreover, several cancers have proved to be largely refractory to therapies that target CTLA-4 and PD-1. This has catalyzed interest in targeting novel immune checkpoint receptors with the goal of realizing the full potential of checkpoint blockade for treating cancer. In this regard, the immune checkpoint receptor Tim-3 exhibits several unique features that make it an intriguing candidate for the next wave of therapies that target immune checkpoints in cancer. *Cancer Immunol Res*; 2(5); 393–8. ©2014 AACR.

Introduction

In 2010, the reported success of anti-CTLA-4 antibody in prolonging survival in approximately 20% of treated patients with advanced metastatic melanoma catalyzed the opening of the flood gates for a new class of antibody-based biologics that block T-cell inhibitory or immune checkpoint receptors for cancer immunotherapy (1). Since then, the field has been working feverishly toward harnessing the promise of immune checkpoint blockade with the goal of achieving even greater success than that initially observed with CTLA-4 blockade. Approaches that combine CTLA-4 blockade with other immune-modulatory agents as well as approaches that target other immune checkpoint receptors are being investigated. Indeed, antibodies that block PD-1 are already showing greater success than anti-CTLA-4 antibody in clinical trials, with objective response rates reaching up to 52% of treated patients (2, 3). Despite these successes, approximately one half of treated patients remain unresponsive to these therapies. The field has, therefore, started looking toward other inhibitory receptors with the hope that they hold the key to increasing the frequency of objective responses as well as achieving efficacy in cancer indications that have thus far been largely refractory to CTLA-4 and PD-1 blockade, such as colorectal cancer. In this regard, the T-cell inhibitory receptor Tim-3 (T-cell immunoglobulin and mucin-domain containing-3) is currently receiving

much attention due to its demonstrated success in multiple preclinical cancer models. This "Cancer Immunology at the Crossroads" article discusses the current knowledge of the role of Tim-3 in regulating antitumor immunity and highlights why Tim-3 is likely the next hot ticket in cancer immunotherapy.

Tim-3 Is an Immune Checkpoint Receptor

Tim-3 was first identified 12 years ago as a molecule selectively expressed on IFN- γ -producing CD4⁺ T helper 1 (Th1) and CD8⁺ T cytotoxic 1 (Tc1) T cells (4). At that time, Th1 cells were believed to be the main drivers of autoimmune disease, and initial studies primarily investigated the role of Tim-3 in the setting of autoimmunity. These studies showed that anti-Tim-3 antibody exacerbated experimental autoimmune encephalomyelitis (EAE; ref. 4), a T-cell-mediated autoimmune disease of the central nervous system. These data provided the first indication that Tim-3 may function as a T-cell inhibitory receptor. Indeed, the inhibitory function of Tim-3 became clear upon the identification of the C-type lectin galectin-9 as a Tim-3 ligand. Galectin-9 triggering of Tim-3 was shown to induce cell death in Tim-3⁺ Th1 cells and ameliorate EAE (5). Further studies showed that Tim-3 is required for the induction of tolerance, as both Tim-3-deficient mice and mice treated with a Tim-3-Ig fusion protein exhibited defects in the induction of antigen-specific tolerance (6, 7). Collectively, these studies demonstrate that Tim-3 is an immune checkpoint receptor that functions specifically to limit the duration and magnitude of Th1 and Tc1 T-cell responses.

Tim-3 in T Cells in Cancer

It is now well accepted that tumors have co-opted immune checkpoint pathways as a mechanism of immune suppression. Evidence that Tim-3 may be a key immune checkpoint in tumor-induced immune suppression came from the demonstration that Tim-3 marks the most

Author's Affiliation: Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Harvard Institutes of Medicine, Boston, Massachusetts

Corresponding Author: Ana C. Anderson, Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Harvard Institutes of Medicine, Room 784, Boston, MA 02115. Phone: 617-525-5850; Fax: 617-525-5566; E-mail: acanderson@partners.org

doi: 10.1158/2326-6066.CIR-14-0039

©2014 American Association for Cancer Research.

suppressed or dysfunctional population of CD8⁺ T cells in preclinical models of both solid and hematologic malignancy (8, 9). In these models, all of the CD8⁺ Tim-3⁺ T cells coexpress PD-1, and these dual-expressing cells exhibit greater defects in both cell-cycle progression and effector cytokine production [interleukin (IL)-2, TNF, and IFN- γ] than cells that express PD-1 alone. Together, these data indicate that the Tim-3 pathway may cooperate with the PD-1 pathway to promote the development of a severe dysfunctional phenotype in CD8⁺ T cells in cancer.

Tim-3 is also found on T cells in patients with cancer. In patients with advanced melanoma, approximately 30% of NY-ESO-1-specific CD8⁺ T cells express Tim-3 (10). In patients with non-small cell lung cancer (NSCLC), approximately one third of CD8⁺ tumor-infiltrating lymphocytes (TIL) express Tim-3 (11). In patients with follicular B-cell non-Hodgkin lymphoma (FL; ref. 12), approximately one third of lymph node CD4⁺ and CD8⁺ T cells express Tim-3. In these three cancers, all Tim-3⁺ T cells coexpress PD-1 and exhibit defects in proliferation and effector cytokine production. Thus, Tim-3 marks dysfunctional T cells in multiple cancer types both in experimental models and in humans.

The dysfunctional phenotype of the CD8⁺Tim-3⁺ T cells observed in preclinical tumor models as well as the CD8⁺ Tim-3⁺ T cells in patients with cancer resembles most closely the dysfunctional or exhausted CD8⁺ T cells that have been described in chronic viral infections (reviewed in ref. 13). Indeed, exhausted virus-specific CD8⁺ T cells also exhibit upregulation of Tim-3 in chronic lymphocytic choriomeningitis virus (LCMV) infection in mice (14) and in both HIV and hepatitis C virus infection in humans (15–17). However, whether the CD8⁺Tim-3⁺ T cells in cancer are truly analogous to the exhausted T cells in chronic viral infection or whether they represent a different state of T-cell dysfunction that is unique to cancer remains an open question. Irrespective of these considerations, it is important to note that the Tim-3⁺ T cells in cancer are not senescent T cells, as has been suggested in a recent review (18). Unlike senescent T cells, the CD8⁺Tim-3⁺ T cells in cancer are not irreversibly cell-cycle arrested as their proliferation can be restored by blockade of Tim-3 together with PD-1 (10). Moreover, the CD8⁺Tim-3⁺ T cells in cancer are severely impaired in their ability to secrete cytokines and other effector molecules (8–10), whereas senescent T cells are known to secrete multiple factors that constitute the senescence-associated secretory phenotype (SASP; ref. 19).

In addition to its key role in regulating CD8⁺ T-cell effector function in cancer, recent studies further implicate Tim-3 in the biology of intratumoral FoxP3⁺ regulatory T cells (Treg). In patients with NSCLC, approximately 60% of CD4⁺FoxP3⁺ TILs express Tim-3. Interestingly, these Tim-3⁺ Tregs are infrequent in the peripheral blood of patients with NSCLC, and the presence of Tim-3⁺ Tregs correlates with the presence of nodal metastases and advanced cancer stage (11). Importantly, the presence of Tim-3⁺ Tregs seems to be a common feature across multiple different cancers as Tim-3⁺ Tregs have been reported in hepatocellular, ovarian, colon, and cervical carcinomas (20).

The unique presence of Tim-3⁺ Tregs in tumor tissue has also been observed in multiple preclinical models, including both transplantable and *de novo* tumors (21). In these models, the Tim-3⁺ Treg population has been shown to predominate in tumor tissue and to be more immunosuppressive than the Tim-3⁻ Treg population, likely as a result of increased production of IL-10 and other key effector molecules such as perforin and granzymes. Moreover, it has been suggested that Tim-3⁺ Tregs have a specific role in actively promoting the development of a dysfunctional phenotype in CD8⁺ TILs (21).

Recent studies have shown that the Tregs present in specific anatomic compartments express unique factors and mediate distinct nonimmune functions. For example, adipose tissue Tregs express the transcription regulator PPAR- γ and regulate metabolism (22), and the Tregs that accumulate in muscle tissue after muscle injury express amphiregulin, which mediates repair of damaged tissue (23). The observation that Tim-3⁺ Tregs are found uniquely in tumor tissue raises the important question of whether the Tim-3⁺ Tregs in tumor tissue express a unique factor that influences and shapes the tumor microenvironment.

Targeting Tim-3 for Cancer Immunotherapy

The Tim-3 pathway is perfectly poised as a target for anticancer immunotherapy due to its expression on both dysfunctional CD8⁺ T cells and Tregs—two key immune cell populations that constitute immunosuppression in tumor tissue. Indeed, targeting the Tim-3 pathway has shown very promising results in preclinical cancer models. In the Wilms tumor-3 (WT3) sarcoma and the transgenic adenocarcinoma of the mouse prostate C-1 (TRAMP-C1) cancer models, Tim-3 blockade alone is effective in a dose-dependent manner. In preclinical models of colon carcinoma (CT26 and MC38), Tim-3 blockade alone exhibits similar efficacy to PD-1 pathway blockade (24). However, the combination of Tim-3 blockade with PD-1 pathway blockade is remarkably more effective in these models, such that tumor regression is more complete and observed with higher frequency than with blockade of either the Tim-3 or PD-1 pathway alone (8, 24). This synergistic effect of Tim-3 pathway blockade with PD-1 pathway blockade has also been observed in B16F10 melanoma, which is known to be poorly immunogenic and resistant to treatment (24). Importantly, a combination regimen blocking the Tim-3/PD-1 pathway is also effective in mice with established CT26 or B16F10 tumors, and in mice with methylcholanthrene-induced fibrosarcoma (24). Finally, the dual blockade of the Tim-3/PD-1 pathway in mice with acute myelogenous leukemia (AML) prolongs survival to a higher degree than the PD-1 pathway blockade alone (9). Collectively, these data strongly support the potential of Tim-3 pathway blockade for the treatment of various cancer types and suggest that combinatorial approaches involving the PD-1 pathway may be most effective in the clinical setting.

The remarkable synergy of Tim-3/PD-1 pathway coblockade in controlling tumor growth in preclinical models of cancer could reflect the combined effects of coblockade on modulating the functional phenotype of dysfunctional CD8⁺ T cells

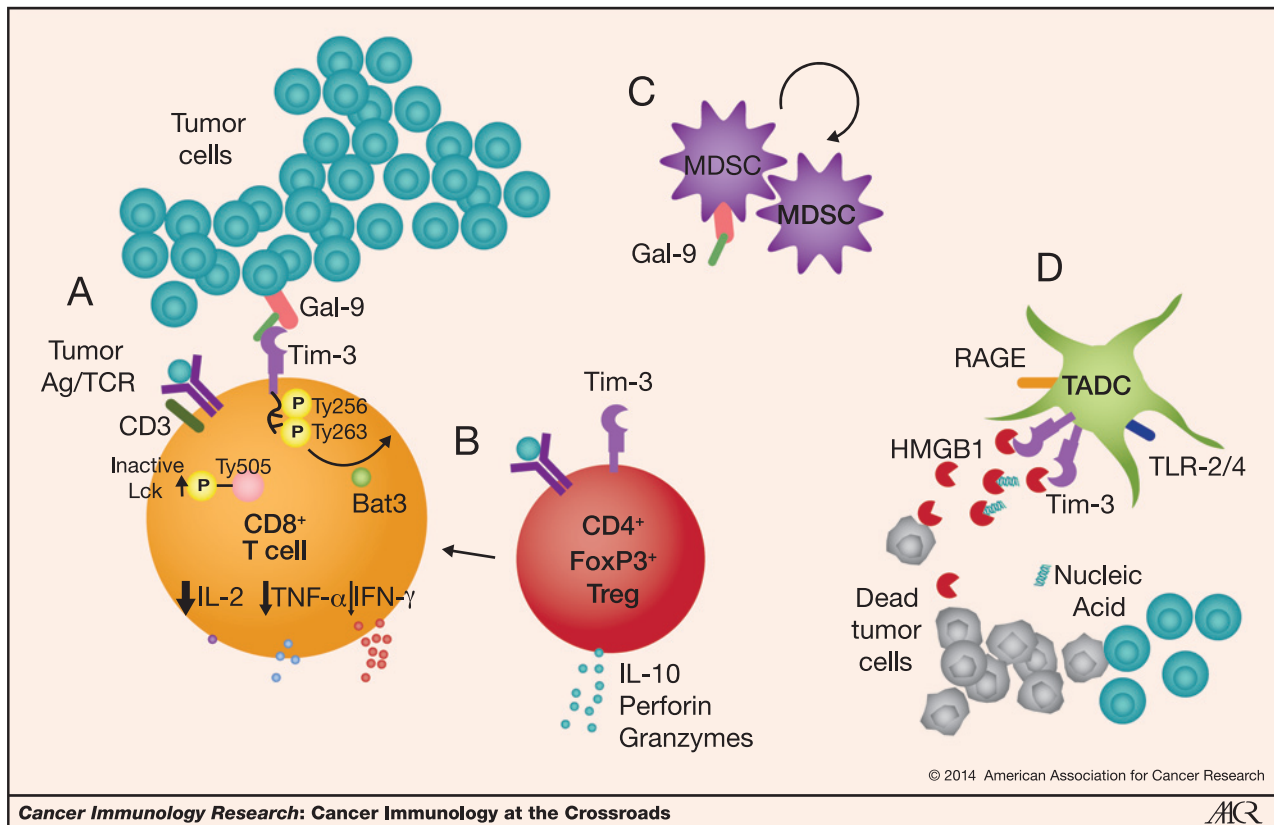


Figure 1. The Tim-3 pathway in cancer. A, dysfunctional CD8⁺ T cells in the tumor microenvironment express Tim-3. Triggering of Tim-3 by galectin-9 on tumor cells results in the phosphorylation of the Tim-3 cytoplasmic tail at tyrosines 256 and 263, releasing Bat3 from the Tim-3 cytoplasmic tail, and the accumulation of inactive Lck (phosphorylated at tyrosine 505; Ty505). B, Tim-3⁺FoxP3⁺ Tregs are present within the tumor and express high amounts of Treg effector molecules (IL-10, perforin, and granzymes). Tim-3⁺ Tregs are potent suppressors of immune responses in tumor tissue and may promote the development of dysfunctional phenotype in intratumoral CD8⁺ T cells. C, Tim-3 expression on T cells promotes myeloid-derived suppressor cells (MDSC), which suppress intratumoral immune responses. D, tumor-associated dendritic cells (TADC) express Tim-3, which binds to HMGB1 and decreases the free HMGB1 available to bind nucleic acid released by dying tumor cells. Tim-3–HMGB1 binding further interferes with HMGB1 binding to RAGE and/or Toll-like receptors (TLR) 2 and 4 and activation of the innate immune response. Blockade of Tim-3 signals in the tumor microenvironment will interfere with all of these immune-suppressive functions of Tim-3.

and/or Tregs (Fig. 1A and B). Indeed, Tim-3/PD-1 pathway cblockade is more effective than either Tim-3 or PD-1 pathway blockade alone at restoring tumor antigen-specific IFN- γ production in CD8⁺ T cells from tumor-bearing mice (25). Moreover, Tim-3/PD-1 pathway cblockade also drives the downmodulation of several genes associated with potent Treg-suppressor function in Tim-3⁺ Tregs (21). Thus, Tim-3/PD-1 cblockade knocks out two major mechanisms of immune suppression in tumor tissue, restoring function to dysfunctional CD8⁺ T cells and deprogramming potent intratumoral Tregs. The molecular mechanisms by which Tim-3/PD-1 pathway cblockade achieves these effects have not yet been elucidated.

Non-T-cell Functions of Tim-3 in Cancer

Aside from its role in T cells, Tim-3 has been noted to have effects in the myeloid compartment, which seem to be driven by distinct Tim-3–Tim-3 ligand interactions. T-cell expression of Tim-3 has been shown to promote CD11b⁺Gr-1⁺ myeloid-derived suppressor cells (MDSC) in a galectin-9–

dependent manner (Fig. 1C; ref. 26). Recent data also suggest that Tim-3 is specifically upregulated on tumor-associated dendritic cells (TADC), in which it interferes with the sensing of DNA released by cells undergoing necrotic cell death. Tim-3 binds to high mobility group protein 1 (HMGB1), thereby preventing HMGB1 from binding to DNA from dying cells and mediating delivery to innate cells via interactions with receptor for advanced glycation end (RAGE) products and/or Toll-like receptors (TLR) 2 and 4 (Fig. 1D; ref. 27). Thus, Tim-3 binding to HMGB1 interferes with the alarmin function of HMGB1 and dampens activation of the innate immune response in tumor tissue. This interaction of Tim-3 with HMGB1 could be particularly relevant in dampening immune activation in the context of chemotherapy approaches that trigger immunogenic cell death. Collectively, these data show that Tim-3 can further suppress antitumor T-cell responses by T-cell extrinsic mechanisms involving myeloid cells and different Tim-3–Tim-3 ligand interactions. Tim-3 blockade would be predicted to additionally interfere with these T-cell extrinsic mechanisms of immunosuppression.

In the past few years, two studies have identified Tim-3 as a surface molecule specifically expressed on leukemic stem cells (LSC) but not on normal hematopoietic stem cells (HSC) in AML (28, 29). Importantly, anti-Tim-3 antibody was successful in blocking the engraftment of AML after xenotransplantation and eliminating the reconstitution of human AML by LSCs in secondary recipients without interfering with the reconstitution of normal human HSCs. These observations raise the intriguing possibility of using a depleting anti-Tim-3 antibody or an anti-Tim-3 antibody-drug conjugate to selectively eliminate LSCs in patients with AML (29). However, whether this is a viable approach in AML awaits experimental data. Moreover, whether Tim-3 is expressed on cancer stem cells in other hematologic malignancies remains to be determined.

Why Target Tim-3?

Increasing data support the relevance of targeting Tim-3 in human cancer. As mentioned above, Tim-3⁺ NY-ESO-1-specific CD8⁺ T cells in patients with melanoma exhibit dysfunctional phenotype (10). Tim-3 pathway blockade alone restores IFN- γ and TNF- α production as well as the frequency and proliferation of NY-ESO-1-specific CD8⁺ T cells in response to tumor antigen stimulation. Coblockade of Tim-3 and PD-1 further restores IL-2 production in NY-ESO-1-specific CD8⁺ T cells. In NSCLC, Tim-3 is uniquely expressed on the CD4⁺ and CD8⁺ T cells that infiltrate tumor (11). The CD8⁺Tim-3⁺ TILs in NSCLC coexpress PD-1 and exhibit dysfunctional phenotype. Moreover, the presence of CD4⁺Tim-3⁺ Tregs in NSCLC correlates strongly with the presence of nodal metastases and advanced tumor grade. In patients with FL, the CD8⁺Tim-3⁺ T cells in lymph node biopsies coexpress PD-1 and exhibit dysfunctional phenotype. Interestingly, and in contrast with what is observed in NSCLC and in all preclinical cancer models, the CD4⁺Tim-3⁺ T cells in lymph node biopsies of patients with FL are not Tregs as they lack the expression of both CD25 and FoxP3 (12). Instead, these CD4⁺Tim-3⁺ T cells coexpress PD-1 and exhibit dysfunctional phenotype, indicating that they are dysfunctional CD4⁺ T cells. Whether the presence of dysfunctional CD4⁺Tim-3⁺ effector T cells in patients with FL cancer reflects a unique feature of lymphoid tumors remains to be addressed. Interestingly, the presence of these dysfunctional CD4⁺Tim-3⁺ T cells in patients with FL correlates strongly with poor survival (12). Collectively, these studies support that Tim-3 is a relevant immune checkpoint receptor in multiple human cancers.

Distinguishing Features of Tim-3

As the field moves toward targeting novel checkpoint receptors for anticancer immunotherapy, it is important to consider what characteristics distinguish these receptors from other checkpoint receptors that are currently being targeted. One very important consideration is the expression of the checkpoint receptor in the host. This is of importance because blockade of checkpoint receptors that are widely expressed could promote autoimmune-like side effects. CTLA-4 is known to be upregulated on all effector T cells

and is also expressed on all Tregs. Consequently, blockade of CTLA-4 could disrupt CTLA-4-driven regulation of effector T-cell responses or interfere with the function and/or number of Tregs, as has been suggested by recent studies (30–32). Indeed, autoimmune-like toxicities are commonly observed in patients treated with anti-CTLA-4 antibody (1). PD-1 is similarly upregulated on all effector T cells, and autoimmune-like toxicities also are observed in patients treated with anti-PD-1 antibodies (2, 3). In this regard, targeting Tim-3 is advantageous as Tim-3 is not expressed on all T cells; rather, it is selectively expressed on T cells that have differentiated toward an IFN- γ -producing phenotype (4), and in patients with cancer, Tim-3 seems to be expressed primarily in intratumoral T cells (11, 12). Thus, Tim-3 blockade is less likely to interfere with the regulation of T-cell responses outside of tumor tissue than blockade of either CTLA-4 or PD-1. Tim-3-deficient mice do not exhibit autoimmunity (6), unlike both CTLA-4-deficient (33, 34) and PD-1-deficient mice (35), and tumor-bearing mice treated with anti-Tim-3 antibody do not exhibit autoimmunity (24), supporting the notion that targeting Tim-3 is less likely to be associated with adverse autoimmune-like toxicities.

A second important consideration is the mechanism of action by which the checkpoint receptor inhibits T-cell responses. Unlike PD-1, the intracellular tail of Tim-3 does not contain any immunoreceptor tyrosine-based inhibition motifs (ITIM) or immunoreceptor tyrosine-based switch motifs (ITSM). Thus, Tim-3 is not likely to be functionally redundant with other ITIM/ITSM-containing checkpoint receptors. Currently, very little is known about the signaling pathway downstream of Tim-3. Investigators from a recent study reported that human leukocyte antigen B-associated transcript 3 (Bat3) binds to the cytoplasmic tail of Tim-3. Galectin-9-induced phosphorylation of the Tim-3 cytoplasmic tail triggers the release of Bat3, resulting in the accumulation of the catalytically inactive form of the lymphocyte-specific protein tyrosine kinase (Lck) and defective IL-2 and IFN- γ production (25). These data suggest that Bat3 acts as a negative regulator of the inhibitory function of Tim-3. Although much more work is required to fully elucidate the Tim-3 signaling pathway, at this time it is clear that Tim-3 affects the signaling pathway downstream of T-cell receptor (TCR) activation in a very different way from ITIM/ITSM-containing molecules that recruit phosphatases and dephosphorylate key TCR signaling intermediates. The fact that the mechanism by which Tim-3 signals differs from that of ITIM/ITSM-containing receptors suggests that Tim-3 would be a good partner for ITIM/ITSM-containing molecules. Indeed, this could partly underlie the remarkable clinical efficacy of the Tim-3/PD-1 pathway coblockade that has been observed in preclinical models of cancer.

Perspective

Tim-3 has emerged as a unique immune checkpoint receptor in cancer. Its selective expression in tumor tissue, along with its role in multiple mechanisms of immunosuppression, highlights its value as a target for cancer immunotherapy. Important future steps toward harnessing Tim-3 for anticancer

immunotherapy will be to elucidate the signals, both extracellular and intracellular, that drive the upregulation of Tim-3, the signals that underlie Tim-3-driven immune suppression, and the receptor–ligand relationships that are operative in each immune-suppressive mechanism. The unique features of Tim-3, together with accumulating preclinical data, strongly support the use of Tim-3-targeted therapies in combinatorial modalities with other checkpoint-based therapies to achieve objective responses in a higher frequency

of patients and responses in cancers that have been thus far been refractory to CTLA-4 or PD-1 blockade.

Disclosure of Potential Conflicts of Interest

A.C. Anderson is a consultant/advisory board member for CoStim Pharmaceuticals, Inc.

Received March 7, 2014; accepted March 13, 2014; published online May 2, 2014.

References

- Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711–23.
- Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366:2443–54.
- Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013;369:134–44.
- Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, et al. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 2002;415:536–41.
- Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* 2005;6:1245–52.
- Sabatos CA, Chakravarti S, Cha E, Schubart A, Sanchez-Fueyo A, Zheng XX, et al. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat Immunol* 2003;4:1102–10.
- Sanchez-Fueyo A, Tian J, Picarella D, Domenig C, Zheng XX, Sabatos CA, et al. TIM-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. *Nat Immunol* 2003;4:1093–101.
- Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010;207:2187–94.
- Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood* 2011;117:4501–10.
- Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. *J Exp Med* 2010;207:2175–86.
- Gao X, Zhu Y, Li G, Huang H, Zhang G, Wang F, et al. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. *PLoS ONE* 2012;7:e30676.
- Yang ZZ, Grote DM, Ziesmer SC, Niki T, Hirashima M, Novak AJ, et al. IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma. *J Clin Invest* 2012;122:1271–82.
- Wherry EJ. T cell exhaustion. *Nat Immunol* 2011;12:492–9.
- Jin HT, Anderson AC, Tan WG, West EE, Ha SJ, Araki K, et al. Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. *Proc Natl Acad Sci U S A* 2010;107:14733–8.
- Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *J Exp Med* 2008;205:2763–79.
- Golden-Mason L, Palmer BE, Kassam N, Townshend-Bulson L, Livingston S, McMahon BJ, et al. Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. *J Virol* 2009;83:9122–30.
- McMahan RH, Golden-Mason L, Nishimura MI, McMahon BJ, Kemper M, Allen TM, et al. Tim-3 expression on PD-1+ HCV-specific human CTLs is associated with viral persistence, and its blockade restores hepatocyte-directed *in vitro* cytotoxicity. *J Clin Invest* 2010;120:4546–57.
- Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol* 2013;25:214–21.
- Rodier F, Campisi J. Four faces of cellular senescence. *J Cell Biol* 2011;192:547–56.
- Yan J, Zhang Y, Zhang JP, Liang J, Li L, Zheng L. Tim-3 expression defines regulatory T cells in human tumors. *PLoS ONE* 2013;8:e58006.
- Sakuishi K, Ngiew SF, Sullivan JM, Teng MWL, Kuchroo VK, Smyth MJ, et al. TIM3+FOXP3+ regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. *Oncol Immunology* 2013;2:e23849.
- Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelson SE, et al. PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* 2012;486:549–53.
- Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, et al. A special population of regulatory T cells potentiates muscle repair. *Cell* 2013;155:1282–95.
- Ngiew SF, von Scheidt B, Akiba H, Yagita H, Teng MW, Smyth MJ. Anti-TIM3 antibody promotes T cell IFN- γ -mediated anti-tumor immunity and suppresses established tumors. *Cancer Res* 2011;71:3540–51.
- Rangachari M, Zhu C, Sakuishi K, Xiao S, Karman J, Chen A, et al. Bat3 protects T cell responses by repressing Tim-3-mediated exhaustion and death. *Nat Med* 2012;18:1394–400.
- Dardalhon V, Anderson AC, Karman J, Apetoh L, Chandwaskar R, Lee DH, et al. Tim-3/galectin-9 pathway: regulation of Th1 immunity through promotion of CD11b+Ly-6G+ myeloid cells. *J Immunol* 2010;185:1383–92.
- Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. *Nat Immunol* 2012;13:832–42.
- Jan M, Chao MP, Cha AC, Alizadeh AA, Gentles AJ, Weissman IL, et al. Prospective separation of normal and leukemic stem cells based on differential expression of TIM3, a human acute myeloid leukemia stem cell marker. *Proc Natl Acad Sci U S A* 2011;108:5009–14.
- Kikushige Y, Shima T, Takayanagi S, Urata S, Miyamoto T, Iwasaki H, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell* 2010;7:708–17.
- Selby M, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, et al. Anti-CTLA-4 antibodies of IgG2a isotype enhance anti-tumor activity through reduction of intratumoral regulatory T cells. *Cancer Immunol Res* 2013;1:32–42.
- Bulliard Y, Jolicoeur R, Windman M, Rue SM, Ettenberg S, Knee DA, et al. Activating Fc gamma receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies. *J Exp Med* 2013;210:1685–93.

32. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* 2013;210:1695–710.
33. Waterhouse PW, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. *Science* 1995;270:985–8.
34. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. CTLA-4 deficient mice exhibit massive lymphoproliferation and multi-organ lymphatic infiltration: a critical negative immunoregulatory role of CTLA-4. *Immunity* 1995;3:541–7.
35. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999;11:141–51.