Journal for ImmunoTherapy of Cancer

Tim-3 finds its place in the cancer immunotherapy landscape

Nandini Acharya,[1,2] Catherine Sabatos-Peyton,[3] Ana Carrizosa Anderson [1,2]

To cite: Acharya N, Sabatos-Peyton C, Anderson AC. Tim-3 finds its place in the cancer immunotherapy landscape. *Journal for ImmunoTherapy of Cancer* 2020;**8**:e000911. doi:10.1136/jitc-2020-000911

Accepted 09 May 2020

[1]Department of Neurology, Evergrande Center for Immunologic Diseases and Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA
[2]Harvard Medical School, Boston, Massachusetts, USA
[3]Exploratory Immuno-oncology, Novartis Institutes for BioMedical Research, Cambridge, Massachusetts, USA

Correspondence to
Ana Carrizosa Anderson; acanderson@bwh.harvard.edu

ABSTRACT

The blockade of immune checkpoint receptors has made great strides in the treatment of major cancers, including melanoma, Hodgkin's lymphoma, renal, and lung cancer. However, the success rate of immune checkpoint blockade is still low and some cancers, such as microsatellite-stable colorectal cancer, remain refractory to these treatments. This has prompted investigation into additional checkpoint receptors. T-cell immunoglobulin and mucin domain 3 (Tim-3) is a checkpoint receptor expressed by a wide variety of immune cells as well as leukemic stem cells. Coblockade of Tim-3 and PD-1 can result in reduced tumor progression in preclinical models and can improve antitumor T-cell responses in cancer patients. In this review, we will discuss the basic biology of Tim-3, its role in the tumor microenvironment, and the emerging clinical trial data that point to its future application in the field of immune-oncology.

INTRODUCTION

T-cell immunoglobulin and mucin domain 3 (Tim-3) (encoded by *Havcr2*) is an immunoglobulin (Ig) and mucin domain-containing cell surface molecule that was originally discovered as a cell surface marker specific to interferon (IFN-γ) producing $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic 1 (Tc1) cells.[1] Tim-3 is a member of the TIM family of genes which is located in syntenic chromosomal regions in human (5q33.2) and mouse (11B1.1) that have been linked to both allergy and autoimmune disease.[2 3] That Tim-3 may function as a T-cell inhibitory receptor was initially demonstrated by Monney *et al* who showed that *in vivo* administration of Tim-3 monoclonal antibodies (mAbs) exacerbated disease in the experimental autoimmune encephalomyelitis model of central nervous system autoimmunity.[1] Later, two studies showed that disruption of Tim-3–Tim-3-ligand interactions either by administration of Tim-3–Ig or Tim-3 mAb resulted in exacerbated Th1 responses and promotion of autoimmune diabetes in nonobese diabetic mice.[4 5] However, despite these studies, the lack of a canonical inhibitory signaling motif in the cytoplasmic tail of Tim-3 called into question the inhibitory role of Tim-3. Two recent studies that demonstrate an association of germline loss-of-function mutations in *HAVCR2* with two diseases that result from hyperactivated T and myeloid cells, hemophagocytic lymphohistiocytosis (HLH) and subcutaneous panniculitis-like T-cell lymphoma (SPTCL), solidify the role of Tim-3 as a negative regulator or "immune checkpoint".[6 7] Indeed, Tim-3 is coregulated and coexpressed along with other immune checkpoint receptors (PD-1, Lag-3, and TIGIT) on $CD4^+$ and $CD8^+$ T cells[8,9] . In cancer, Tim-3 expression specifically marks the most dysfunctional or terminally exhausted subset of $CD8^+$ T cells[1011] In preclinical cancer models, coblockade of the Tim-3 and PD-1 pathways has shown remarkable efficacy in both solid[11 12] and hematologic tumors.[13] This led to the investigation of Tim-3 blockade in the clinic. Ongoing clinical trials are largely investigating anti-Tim-3 in combination with anti-PD-1 in solid tumors. However, striking early trial data show efficacy of TIM-3 in combination with chemotherapy in myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML)[14] indicating its potential value in the treatment of hematologic malignancy and disorders.

TIM-3 STRUCTURE AND SIGNALING

The TIM family of proteins are type I membrane proteins that share a similar structure: a variable Ig domain (IgV), a glycosylated mucin domain of varying length, and a single transmembrane domain. All TIM molecules, except for Tim-4, contain a C-terminal cytoplasmic tail with a conserved tyrosine-based signaling motif. Interestingly, in contrast to other checkpoint receptors like PD-1 and TIGIT, Tim-3 lacks classical inhibitory immunoreceptor tyrosine-based inhibition or immunoreceptor tyrosine-based switch signaling motifs in its cytoplasmic tail.

Although much remains to be learned about Tim-3 signaling, it is known that HLA-B-associated transcript 3 (Bat3)[15] and SH2 (Src homology 2) domain-containing protein Fyn[16] interact with the conserved tyrosines

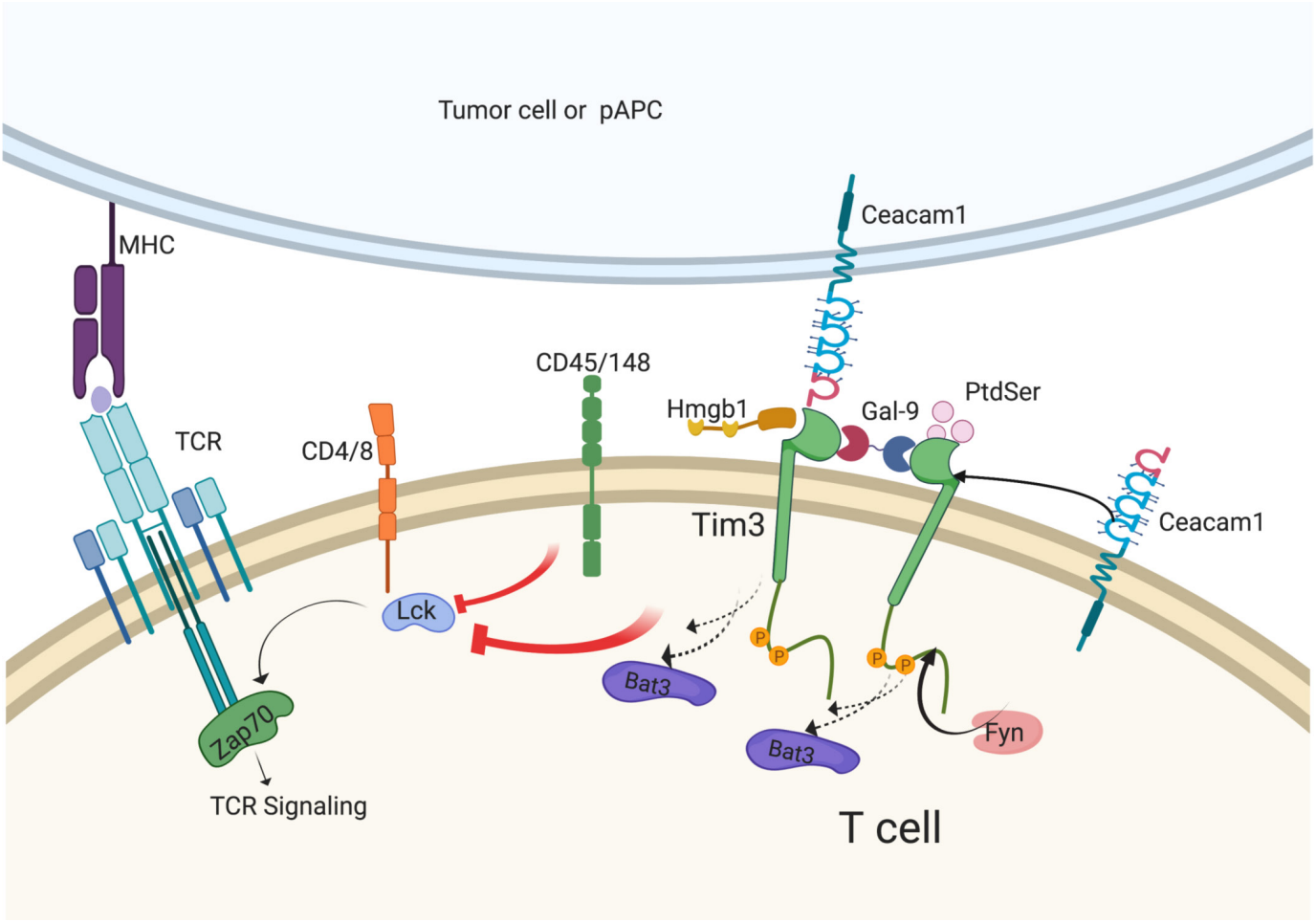

Figure 1 Model of Tim-3 signaling in T cells. In the absence of Tim-3 ligand, Bat-3 is bound to the cytoplasmic tail of Tim-3 and to the catalytically active form of Lck. Lck then phosphorylates the CD3ζ subunit of the T Cell receptor (TCR) complex which is then followed by subsequent recruitment of Zeta-chain-associated protein kinase (ZAP70) to the TCR complex. This recruitment results in the activation of ZAP70/Linker for Activation of T cells (LAT)/Phospholipase C gamma 1 (PLCγ1)/Ca2+ to promote T-cell proliferation and survival. However, Tim-3 ligation by ligand displaces Bat-3 from the Tim-3 tail, resulting in the recruitment of tyrosine phosphatases (CD45 and CD148) which lead to dephosphorylation (inactivation) of Lck, and downregulation of ZAP70/LAT/PLCγ1/Ca2+ TCR signaling and suppression of T-cell proliferation and survival. Bat-3, HLA-B-associated transcript 3; Ceacam1, carcinoembryonic antigen-related cell adhesion molecule-1; Gal-9, galectin-9; Hmgb1, high-mobility group protein B1; PtdSer, phosphatidylserine; Tim-3, T-cell immunoglobulin and mucin domain 3.

Y256 and Y263 in its cytoplasmic tail. The current model of Tim-3 signaling is that on T-cell activation, Tim-3 is recruited to the immunological synapse[17] where Bat3 binds to the cytoplasmic tail of Tim-3 and recruits the active, catalytic form of Lymphocyte-specific protein tyrosine kinase (Lck)[15] (figure 1). However, when Tim-3 is engaged by ligand, the conserved tyrosine residues in the cytoplasmic tail become phosphorylated, leading to the release of Bat3, thereby allowing Tim-3 to exert its inhibitory function. Both galectin-9 and carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1), two ligands described for Tim-3 (discussed below), have been shown to trigger phosphorylation of Y256 and Y263 by the tyrosine kinase Interleukin-2-inducible T-cell Kinase (ITK),[18 19] leading to the release of Bat3. Further, one study has reported that the expression of a long-non-coding RNA that binds Tim-3 (Lnc-Tim-3) was upregulated in dysfunctional $CD8^+$ T cells from patients with hepatocellular carcinoma (HCC) and that binding of Lnc-Tim-3 to Tim-3 leads to the release of Bat3, which then diminishes T-cell activation and antitumor immunity.[20] Of note, increased Bat3 expression blocks Tim-3-mediated inhibitory signaling and enhances effector T-cell function.[15] By contrast, reduced Bat3 expression leads to stronger Tim-3-mediated inhibitory signaling. Accordingly, analysis of Bat3 mRNA in $CD8^+$ tumor-infiltrating lymphocytes (TILs) isolated from CT26 colorectal carcinomas revealed that terminally dysfunctional Tim-3^+PD-1^+ $CD8^+$ TILs displayed a greater than 50% reduction in Bat3 mRNA levels relative to Tim-3^-PD-1^+ $CD8^+$ TILs that still retain effector function.[15] However, it is important to note that Bat3-mediated regulation of Tim-3 signaling is described only for T cells. It remains to be determined if Tim-3 employs the same downstream signaling molecules in other cells such as dendritic cells (DCs). Indeed, one study has demonstrated that ligation of Tim-3 on DCs

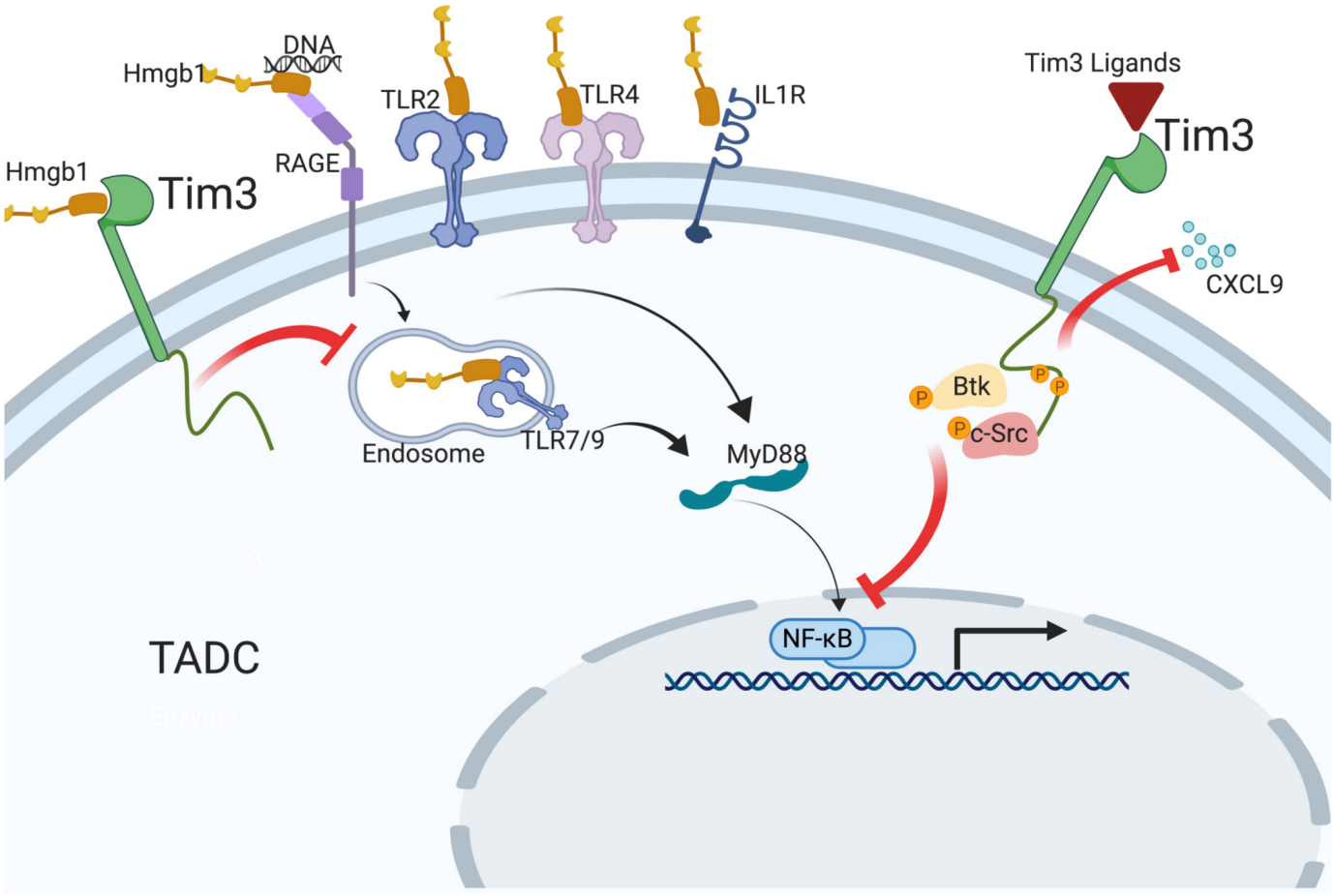

Figure 2 Model of Tim-3 signaling in DCs HMGB1 can interact with several receptors either alone or in a complex with DNA or Lipopolysaccharide (LPS). HMGB1 receptors include Receptor for Activated Glycation End products (RAGE), TLR4, TLR2, and IL-1R. HMGB1–DNA complexes bind to RAGE, leading to internalization and activation of TLR9 and TLR7 in the endosome. This leads to the activation of several downstream transcription factors, such as NF-κB, and activation of tumor-associated dendritic cells (TADCs). Tim-3 can sequester HMGB1, resulting in suppression of NF-kB-mediated activation of DCs. Ligation of Tim-3 on DCs also activates Btk and c-Src, which also inhibit the activation of NF-kB. Tim-3-mediated suppression of DCs dampens the production of CXCL9 thereby reducing $CD8^+$ T-cell recruitment to the TME. Bat-3, HLA-B-associated transcript 3; Btk, Bruton's tyrosine kinase; DCs, dendritic cells; HMGB1, high-mobility group protein B1; Tim-3, T-cell immunoglobulin and mucin domain 3; TME, tumor microenvironment.

activates the SH2 domain-containing signal transducers Bruton's tyrosine kinase and c-Src which results in inactivation of Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and subsequently leads to inhibition of DC activation[21] (figure 2).

That Tim-3 may function as an activating receptor comes primarily from *in vitro* studies showing that ectopic expression of Tim-3 on Jurkat T cells led to T-cell activation resulting from increased NFAT/AP-1 activation.[16] These activities of Tim-3 occurred without the addition of exogenous ligand, and structure/function studies suggested that cell surface expression of Tim-3 may be sufficient for its ability to augment T-cell activation. The requirements for Src kinases and for ZAP-70 and SLP-76 in Tim-3-mediated activation suggested that Tim-3 intersects closely with TCR signaling pathways. However, as discussed further below, the association of naturally occurring loss-of-function mutations in Tim-3 with pathologic inflammation now solidify the function of Tim-3 as an inhibitory receptor.

TIM-3 LIGANDS

Thus far, four distinct ligands for Tim-3 have been identified: galectin-9, phosphatidylserine (PtdSer), high-mobility group protein B1 (HMGB1), and CEACAM-1. All of these have been described in the context of cancer and have relevance in disease progression as discussed below.

Galectin-9

Galectin-9, a 36 kDa β-d-galactoside mammalian C-type lectin, was the first ligand identified for Tim-3.[22 23] Galectin-9 is a secreted protein that binds to a carbohydrate structure on the IgV domain of mouse Tim-3, which has two N-linked glycosylation sites.[24] While the exact structure of the target carbohydrates recognized by

galectin-9 is unclear, galectin-9 has an enhanced affinity for larger poly-*N*-acetylactosamine-containing structures.[25] Galectin-9 is produced broadly by immune cells including mast cells, T cells, B cells, macrophages, and also by non-immune cells, including the epithelium of the gastrointestinal tract, endothelial cells, and fibroblasts. Galectin-9 production is upregulated by IFN-γ,[26 27] and thus may be part of a negative feedback loop similar to PD-L1, which is also upregulated by IFN-γ.[28] Given that galectin-9 binds carbohydrate structures, it has multiple target molecules. In fact, Tim-3 deficiency only reduces galectin-9-mediated Th1 cell death by about 40%,[24] suggesting that some of the effects of *in vivo* administration of galectin-9 may be mediated by galectin-9 binding to receptors other than Tim-3. Indeed, galectin-9 has also been reported to exert various biological functions via interaction with CD44[29] and IgE.[30]

As discussed above, galectin-9 binding results in the oligomerization of Tim-3 on the cell surface, resulting in the release of Bat3 from the intracellular tail of Tim-3 (figure 1). This, in turn, leads to T-cell inhibition,[15] and may be one of the mechanisms by which T cells enter the state of dysfunction or exhaustion. Many lines of evidence indicate a critical role for the Tim-3–galectin-9 interaction in the context of cancer. A study in patients with hepatitis B virus (HBV)-associated HCC showed that galectin-9 was highly expressed by antigen-presenting cell subsets including Kupffer cells, myeloid DCs, and plasmacytoid DCs and that the Tim-3–galectin-9 interaction contributed to immune dysfunction and poor prognosis.[27] In human AML, an autocrine TIM-3–galectin-9 loop drives the self-renewal of AML stem cells by activating the NF-kB and β-catenin pathways[31] and the secretion of both TIM-3 and galectin-9 allows cancer cells to evade immune surveillance.[32] Further, transgenic overexpression of Tim-3 was shown to lead to increased frequency of $CD11b^{+}Ly\text{-}6G^{+}$ myeloid suppressor cells, which was lost on deletion of galectin-9.[33] Finally, administration of anti-Tim-3 and anti-galectin-9 antibodies was shown to be equivalent in their ability to improve the response to paclitaxel (PTX) chemotherapy in models of breast cancer.[34] Collectively, these data show that the Tim-3–galectin-9 interaction can suppress immune responses and facilitate tumor growth.

However, *in vitro* studies have suggested that in breast cancer cells, galectin-9 suppresses metastatic potential by promoting cancer cell aggregation, thereby limiting invasion, detachment from the tumor, and attachment to the vascular endothelium.[35 36] Galectin-9 has also been shown to induce apoptosis and inhibit the growth of HCC cells.[37] The opposing effects of galectin-9 in tumor immunity make the prognostic value of galectin-9 in cancer patients unclear. Indeed, positive galectin-9 expression predicted a worse clinical outcome in patients with urinary tumors[38] and non-small cell lung cancer (NSCLC).[39] However, several other studies have indicated that high expression of galectin-9 contributes to a better outcome for various solid tumors such as breast cancer,[36] melanoma,[40] HCC,[41–43] colon cancer,[44] and bladder urothelial carcinoma.[44] Whether these various effects involve galectin-9 binding to Tim-3 or carbohydrate structures on other proteins is not known.

Phosphatidylserine

PtdSer, a phospholipid that is exposed on the surface of apoptotic cells, serves as a ligand for all Tim family members.[45 46] Despite the fact that Tim-3 binds PtdSer with at least five times lower affinity compared with other TIM family members,[47] it has been demonstrated that the Tim-3–PtdSer interaction is important for clearance of apoptotic cells *in vivo* and that mice treated with anti-Tim-3 mAb have increased numbers of apoptotic cells in splenic follicles and increased serum levels of anti-dsDNA antibodies.[47] How the Tim-3–PtdSer interaction operates in the context of cancer is unclear especially given increased exposure of PtdSer in the tumor microenvironment (TME) on cancer cells due to multiple factors, including oxidative stress[48] and the effects of chemotherapy and radiotherapy.[49] It is possible that the Tim-3–PtdSer interaction could be important for mediating phagocytosis of apoptotic cells by Tim-3-expressing $CD8^{+}$ DCs and subsequent cross-presentation of the apoptotic cell-associated antigens to $CD8^{+}$ T cells. However, it is important to note that PtdSer also binds to other receptors, including Mertk and Axl, which are expressed on infiltrating macrophages and DCs and also frequently expressed on tumor cells themselves.[50] Further, it has been shown that B16 melanoma tumor cells produce microvesicles that express PtdSer on the outer surface and can promote metastasis.[51] Whether Tim-3 has a role in this mechanism remains unknown.

High-mobility group protein B1

HMGB1, an alarmin, was also reported to serve as a ligand for Tim-3.[52] HMGB1, which can be secreted by tumor cells among other cell types,[53] can interact with several receptors either alone or in a complex with DNA or LPS . HMGB1 receptors include RAGE, TLR4, TLR2, and IL-1R.[54] HMGB1–DNA complexes bind to RAGE, leading to internalization and activation of TLR9 and TLR7 localized in the endosome. Such interactions can stimulate proinflammatory and immunostimulatory pathways and, hence, HMGB1 constitutes a major cellular danger signal. However, complex formation of HMGB1 with nucleic acids and with other molecules can be inhibited by direct interaction with Tim-3 (figure 2). In murine cancer models, Chiba *et al* showed that Tim-3 on DCs serves as a molecular trap for HMGB1 and thus inhibits the recruitment of nucleic acids into endosomes, subsequently preventing activation of DCs in the TME.[52] Accordingly, they showed that Tim-3 blockade could improve the efficiency of responsiveness to cisplatin chemotherapy, which is known to increase HMGB1 expression in human cervical carcinoma HeLa cells[55] and in MC38 colon carcinoma cells.[56] Importantly, this effect was found to be independent of galectin-9. This finding is of interest in light of the demonstration that in murine breast cancer both

anti-galectin-9 and anti-Tim-3 improve the response to PTX,[34] which is also known to induce HMGB1 release.[57] The relative roles of Tim-3–PtdSer and Tim-3–HMGB1 interactions in the regulation of the response to different chemotherapeutic agents remain to be determined.

Carcinoembryonic antigen-related cell adhesion molecule-1

CEACAM1, which is expressed at high levels on activated but not naïve T cells, is also a ligand for Tim-3.[19 58 59] In addition to its expression by T cells, CEACAM1 is expressed by DCs,[60] monocytes,[61] macrophages[62] and tumor cells, such as melanoma. CEACAM1 can bind Tim-3 both intracellularly and extracellularly. The intracellular binding is important for maturation of Tim-3 protein. Accordingly, in a mouse model of colitis, CEACAM1$^{-/-}$ T cells expressed reduced surface levels of Tim-3, concomitant with a higher production of the effector cytokines IFN-γ, TNFα, and IL-17A.[19] The extracellular binding can trigger the release of Bat3 from Tim-3, thus allowing Tim-3-mediated inhibition of TCR signaling.[19] CEACAM1 and Tim-3 are highly expressed on dysfunctional CD8$^+$ T cells in the TME. Thus, the Tim-3–CEACAM1 interaction can potentially inhibit immune responses either in cis or trans in these cells (figure 1). It is important to note that CEACAM1 has been shown to bind itself[63] and crystallography data show that the CEACAM1 homotypic interaction is stronger than the CEACAM1–TIM-3 interaction.[19] Although, further studies are required to delineate the physiological contexts where CEACAM1–CEACAM1 and CEACAM–Tim-3 interactions operate, anti-Tim-3 antibodies that have demonstrated functional efficacy *in vivo* have been shown to interfere with Tim-3 binding to CEACAM1 and PtdSer.[64]

It is possible that Tim-3 can bind to several ligands at the same time. Binding of Tim-3 to PtdSer or CEACAM-1 does not exclude binding to Galectin-9 as the binding sites are on opposite faces of the Tim-3 IgV domain. A recently published crystal structure of human TIM-3 determined distinct potential glycosylation sites between murine (Thr44, Asn74, and Asn100) and human (Asn33, Asn100, and Asn124) TIM-3.[65] The authors propose that carbohydrate side-chain modifications at Asn124 might alter the human TIM-3 interaction with ligands that bind the GFCC' face, such as PtdSer, and by extension, antibodies that block human TIM-3 interactions with ligands at the GFCC' face may also block the TIM-3–galectin-9 interaction. Further, given that galectin-9 has two identical carbohydrate recognition domains, it has been proposed that galectin-9 may serve to aggregate Tim-3–PtdSer or Tim-3–CEACAM1 complexes, thus promoting Tim-3 signaling. Whether CEACAM-1, PtdSer, or galectin-9 expression predominates may be the key determinant of Tim-3 signaling in a given tissue.

ROLE OF TIM-3 IN THE TME

The TME is heterogenous and comprises different cell types. What is known about the expression and function of Tim-3 in various tumor-infiltrating cells types is discussed below.

Tim-3 on T cells

CD8$^+$ T cells are key mediators of tumor clearance. However, chronic activation in the presence of suppressive signals in the TME pushes CD8$^+$ T cells into a cellular state commonly described as dysfunction or exhaustion. Dysfunctional T cells are characterized by deficits in cytotoxicity, the production of pro-inflammatory cytokines, and high expression of several checkpoint receptors. Notably, Tim-3 marks the most terminally dysfunctional subset of CD8$^+$ TILs.[11 66] Although the exact mechanism by which Tim-3 contributes to terminal dysfunction in CD8$^+$ T cells is unclear, it is tempting to speculate that Tim-3 reduces the stemness of CD8$^+$ T cells by antagonizing TCF-1, which is known to maintain stemness and restrain effector differentiation.[67] Unlike PD-1, which is expressed together with TCF-1 on stem-like CD8$^+$ T cells in the TME, Tim-3 expression is strongly anticorrelated with TCF-1 expression.[68] The potential regulatory relationship between Tim-3 and TCF-1 is clinically relevant given the positive correlation of TCF-1$^+$ CD8$^+$ T cells with response to checkpoint blockade immunotherapy in melanoma patients,[69] that loss of TCF-1$^+$ in CD8$^+$ T cells limits the response to checkpoint blockade in preclinical cancer models,[70 71] and that Tim-3 expression is a negative prognostic marker in several cancers (discussed below).

In addition to CD8$^+$ TILs, Tim-3 is also expressed at higher levels by CD4$^+$ regulatory T cells (T_{reg}) in both human and murine tumors compared with T_{reg} present in the tumor draining lymph node, spleen, or blood.[72–74] Importantly, Tim-3$^+$ Treg exhibit a more suppressive phenotype.[74] Gao *et al* demonstrated that approximately 70% of Tim-3$^+$CD4$^+$ TILs expressed Foxp3 and about 60% of Foxp3$^+$ TILs were TIM-3$^+$ and that the presence of Tim-3$^+$ Treg correlated with advanced tumor stage and the presence of nodal metastasis in patients with NSCLC.[73] How Tim-3 signaling impacts on the functional phenotype of CD8$^+$ T cells and Treg in the TME is not fully known, and investigation will require the use of lineage-specific mutant mice.

Tim-3 in non-T cells

Dendritic cells

Tim-3 is constitutively expressed on DCs. In particular, Tim-3 expression is highest in cDC1 cells[34] (CD103$^+$ in mouse, CD141$^+$ in human) that cross-present antigen and license CD8$^+$ T cells.[75 76] Although the role of Tim-3 in DCs is still unclear, studies in preclinical models have shown that Tim-3 can suppress intracellular TLR-induced activation as described above[52] and that the effect of Tim-3 blockade in improving the response to chemotherapy requires DCs.[34 52] In a murine model of breast cancer, anti-Tim-3 treatment is associated with the promotion of CXCL9 production by tumor cDC1, which in turn increases lymphocyte infiltration and activation.[34] Interestingly, antibodies directed against galectin-9, but not

HMGB1 or CEACAM-1, promoted CXCL9 secretion by tumor cDC1s.[34] In this regard, ligation of Tim-3 was shown to dampen activation of NF-kB and thus inhibit the maturation of murine DCs[21] (figure 2). How Tim-3 affects the functional phenotype of DCs and the role of interactions with galectin-9, CEACAM-1, and HMGB1 in this process will require careful dissection and the use lineage-specific conditional knockout mice as well as reagents that block specific ligand interactions.

Macrophages

One study has demonstrated that increased Tim-3 expression favors M2 macrophage polarization in a mouse model of colitis associated cancer. Using RAW264.7 cells, the authors demonstrated that STAT1 is a signaling adaptor of Tim-3 in macrophages and that Tim-3 controls macrophage polarization by inhibiting the STAT1-miR-155 signaling axis.[77] Thus, Tim-3 may additionally promote tumor progression by promoting suppressive macrophage phenotype.

NK cells

Natural Killer (NK) cells constitutively express Tim-3. Blockade of TIM-3 in NK cells derived from patients with metastatic melanoma led to reduction in cytotoxicity and IFN-γ production *in vitro*.[78] In patients with lung adenocarcinoma, high expression of TIM-3 on both $CD3^{-}CD56^{+}$ NK cells and CD56(dim) NK-cells were independently correlated with shorter overall survival (OS) of patients with lung adenocarcinoma, indicating that TIM-3 expression in Nk cells can function as a prognostic biomarker in this Disease.[79] Again, blockade of Tim-3 signaling with anti-TIM-3 Mab resulted in increased cytotoxicity and IFN-γ production of peripheral NK cells in these patients. Although TIM-3 blockade appears to augment the cytolytic function of circulating NK cells, the role of TIM-3 in reinvigorating tumor-infiltrating NK cells remains to be demonstrated. Collectively, these studies show that TIM-3 may also function as a checkpoint receptor on NK cells.

REGULATION OF TIM-3 EXPRESSION

Several transcription factors have been implicated in promoting the expression of Tim-3 on T cells. The first one identified was T-bet; indeed, it was demonstrated that T-bet binds to the promoter of Tim-3.[80] Subsequently, it was demonstrated that Nfil3 (Nuclear Factor, Interleukin 3 Regulated) can further augment the effect of t-bet on Tim-3 expression by remodeling the Tim-3 locus and making it more permissive to T-bet.[81] Interestingly, Nfil3 is induced by IL-27,[81] which also induces c-maf and prdm1 to drive the expression of a module of checkpoint receptors including Tim-3.[8] Further, other signals in the TME can cooperate with IL-27 to drive Tim-3 expression. Our unpublished data indicate that glucocorticoid signaling can cooperate with IL-27 to promote Tim-3 expression on $CD8^{+}$ T cells. Finally, one study has shown that IL-35, which shares the Ebi3 subunit with IL-27, can also induce expression of Tim-3 along with other checkpoint receptors.[82] Notably, all of these studies examined T cells. Whether Tim-3 expression is similarly regulated in other cell types is not known.

GENETIC TIM-3 ALTERATIONS

Human TIM-3 is localized at chromosome 5q33.3, which contains a large number of single nucleotide polymorphisms.[3] TIM-3 polymorphisms (−1516 G/T (rs10053538) and −574 G/T (rs10515746) in the promoter region and +4259 T/G (rs1036199) in the coding region) have been associated with increased cancer risk. TIM-3 promoter region polymorphisms (−1516 G/T, −882 C/T, and −574 G/T) have shown association with increased susceptibility to gastric cancer.[83] TIM-3–574 G/T polymorphism has shown association with the risk of developing myasthenia gravis-associated thymoma[84] and TIM-3–1516 G/T has shown association with increased breast cancer susceptibility and breast cancer progression.[85]

Further, two recent studies demonstrate that germline loss-of-function mutations in *HAVCR2* lead to a hyperactivation of T and myeloid cells resulting in two inflammatory diseases, HLH and SPTCL.[6 7] The mutations are located in the Tim-3 IgV domain and result in misfolding of Tim-3 protein. Misfolded protein aggregates intracellularly resulting in loss of Tim-3 expression on the cell surface of both T cells and myeloid cells. Patients harboring these mutations exhibit a severe autoimmune phenotype characterized by excessive production of the proinflammatory molecules CXCL10, IL-1β, IL-18, and soluble CD25. That Tim-3 loss-of-function mutations result in disease promoting inflammation confirms the inhibitory function of Tim-3.

TIM-3 AS A PROGNOSTIC MARKER IN CANCER

Given its inhibitory effects on multiple cell types, it is not surprizing that several studies have shown that Tim-3 expression is a negative prognostic biomarker in several tumor types. As discussed above, the presence of $TIM\text{-}3^{+}$ Treg has been correlated with poor clinical parameters in NSCLC.[73] Similarly, Komohara *et al* demonstrated that TIM-3 was highly expressed on $CD204^{+}$ tumor-associated macrophages and tumor cells in patients with clear cell renal cell carcinoma and that a higher expression level of TIM-3 was positively correlated with shorter progression-free survival in these patients.[86] Li *et al* reported that TIM-3 expression was increased on both $CD4^{+}$ and $CD8^{+}$ T cells in HBV-associated HCC as compared with the adjacent tissues and that the numbers of $TIM\text{-}3^{+}$ tumor-infiltrating cells were negatively associated with patient survival. Additionally, TIM-3 expression has been associated with advanced tumor node metastasis (TNM) stage in several different types of cancers including gastric cancer,[87] colon cancer,[88] and cervical cancer.[89] Of note, a meta-analysis of the OS of patients with solid tumors

demonstrated that higher expression of TIM-3 was significantly correlated with shorter OS.[90]

TIM-3 IN AML AND MDS

Two independent groups identified that TIM-3 is expressed on the majority of $CD34^+CD38^-$ leukemic stem cells (LSCs) and $CD34^+CD38^+$ leukemic progenitors in human AML, but not in $CD34^+CD38^-$ normal hematopoietic stem cells (HSCs).[91 92] TIM-3 expression has also been described on blasts in MDS and found to correlate with disease progression.[93] Upregulation of TIM-3 is also associated with leukemic transformation of preleukemic disease, including MDSs and myeloproliferative neoplasms, such as chronic myelogenous leukemia.[31]

Functional evidence for a key role for TIM-3 in AML was established by the use of an-ADCC (antibody-dependent cellular cytotoxicity) and CDC (complement-dependent cellular cytotoxicity)-competent anti-TIM-3 antibody which inhibited engraftment and development of human AML in immune-deficient murine hosts.[31] In line with observations in preclinical solid tumor models, dual blockade of TIM-3 and PD-1 has been shown to significantly reduce tumor burden and prolong survival in a mouse syngeneic model of AML.[13] TIM-3 is reported to promote an autocrine stimulatory loop via the TIM-3–galectin-9 interaction which supports LSC self-renewal[31] (figure 3). $TIM\text{-}3^+$ LSCs and blasts were shown to actively secrete galectin-9. Galectin-9 ligation of primary patient $TIM\text{-}3^+$ AML cells was shown to stimulate the NF-κB pathway by inducing phosphorylation of extracellular signal-regulated kinases (ERK) and AKT, and also increase nuclear translocation of β-catenin.[31] This TIM-3–galectin-9 autocrine feedback loop may support clonal selection of preleukemic HSCs which outgrow normal HSCs and may promote transformation to myeloid LSCs/promote their self-renewal.

CLINICAL DEVELOPMENT OF TIM-3 ANTIBODIES

Extensive data in preclinical cancer models[11–13] and *in vitro* cultures with patient samples[10] showing the advantage of blocking Tim-3, particularly in conjunction with PD-1 blockade, in improving antitumor immunity supported the development of Tim-3 as an immunotherapeutic target. Further, upregulation of TIM-3 has been associated with the development of resistance to PD-1 blockade in both lung cancer patient samples and in lung cancer models as well as in samples from head and neck cancer patients.[94 95] First-in-human phase 1/2 clinical

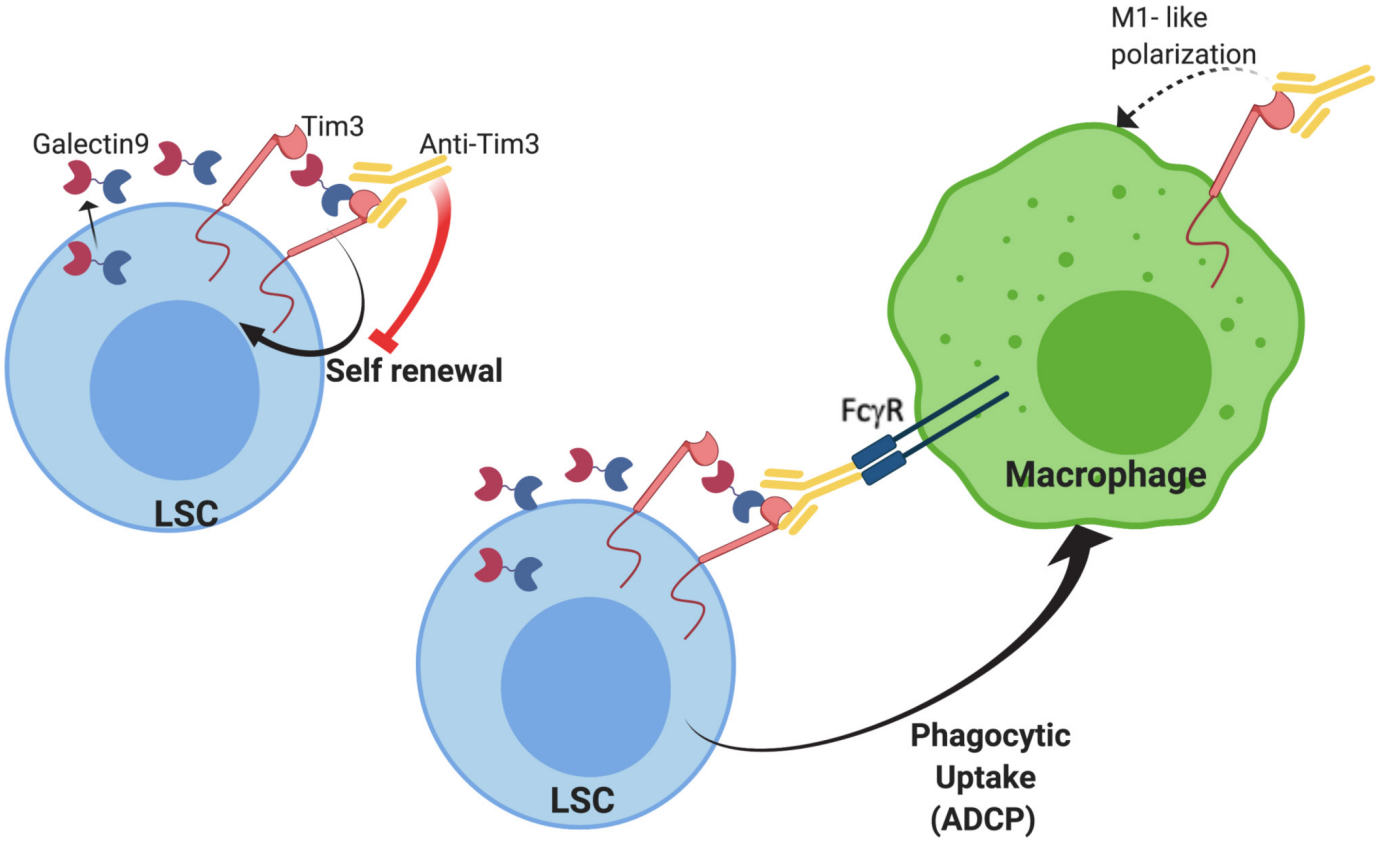

Figure 3 Model for Tim-3 mAb mechanism of action in AML/MDS. The Tim-3–galectin-9 interaction promotes autocrine leukemic stem cell (LSC) self-renewal. Blockade of the Tim-3–galectin-9 interaction may directly inhibit downstream signaling pathways that foster stem cell self-renewal, including the NF-kB and β-catenin pathways. Alternatively and/or additionally, binding of an anti-TIM-3 antibody to TIM-3 on the surface of LSCs/blasts may facilitate antibody-dependent cellular phagocytosis (ADCP) by myeloid cells/macrophages expressing FcγRs and promotion of M1 phenotype. Tim-3, T-cell immunoglobulin and mucin domain 3.

Table 1 Anti-Tim-3 clinical trials

Reagent name (manufacturer)	Isotype	ClinicalTrials.gov identifier	Phase	Coblockade	Cancer type	Further reading
MGB453 (Novartis Pharmaceuticals)	IgG4 (S228P)	NCT02608268	I/IIb	Anti-PD-1	Advanced malignancies	99 100
MGB453 (Novartis Pharmaceuticals)	IgG4 (S228P)	NCT03066648	I	Monotherapy or anti-PD-1 or Hypomethylating Agent (HMA) (decitabine or azacitidine)	AML, MDS	14 99 100
MGB453 (Novartis Pharmaceuticals)	IgG4 (S228P)	NCT03946670	II	Randomized; HMA (decitabine or azacitidine)	MDS	14 99 100
TSR-022 (Tesaro)	IgG4	NCT02817633	I	Anti-PD-1	Advanced solid tumors	101–104
TSR-022 (Tesaro)	IgG4	NCT030680508	II	Anti-PD-1	Liver cancer	101–103
Sym023 (Symphogen A/S)	?	NCT03489343	I	Monotherapy	Solid tumors and lymphomas	105
Sym023 (Symphogen A/S)	?	NCT03311412	I	Anti-PD-1	Solid tumors and lymphomas	105
BGBA425 (BeiGene)	IgG1 (variant, engineered to remove FcγR binding)	NCT03744468	I	Anti-PD-1	Solid tumors	106
R07121661 (Hoffmann-La Roche)	Bispecific antibody	NCT03708328 (development halted)	I	Targets both TIM-3 and PD-1	Solid tumors metastatic melanoma, NSCLC	107
LY3321367 (Eli Lilly and Company)	?	NCT03099109 (development halted)	Ia/Ib	Anti-PD-L1	Advanced relapsed/ refractory solid tumors	108
ICAGN02390 (Incyte Corporation)	IgG1k, N297A (Fc-engineered silent)	NCT03652077	I	Monotherapy	Solid tumors	109
BMS-986258 (Bristol-Myers Squibb)	IgG1, silent	NCT03446040	I	Anti-PD-1, human recombinant hyaluronidase	Advanced cancer	N/A

AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; N/A, not applicable; NSCLC, non-small cell lung cancer; TIM-3, T-cell immunoglobulin and mucin domain 3.

trials have been initiated with many TIM-3 antibodies (table 1), including TSR-022 (NCT02817633), MBG453 (NCT02608268), and LY3321367 (NCT03099109), for which early clinical data have been reported. Many of these anti-TIM-3 antibodies are being tested in combination with anti-PD-1/L1 mAbs. Importantly, early data have shown that this combination is broadly safe and well tolerated. In line with preclinical data showing the efficacy of anti-Tim-3+anti-PD-1, TSR-022 in combination with anti-PD-1 (TSR-042) has shown activity in NSCLC patients who had progressed on previous anti-PD-1 therapy. Further, LY3321367 has demonstrated single agent activity with a partial response in a small cell lung cancer patient at 1200 mg Q2W.

Given the expression of TIM-3 on LSCs and blasts in AML and MDS, and the absence of expression on HSCs, anti-TIM-3 antibody MBG453 was tested in combination with standard of care hypomethylating agents decitabine or azacitidine in a multicenter, open label phase Ib dose-escalation study (NCT03066648) in patients with high-risk MDS or AML and no prior hypomethylating agent therapy. Preliminary data presented by Borate and colleagues showed that MBG453 plus decitabine demonstrated encouraging preliminary efficacy in these patient populations with an overall response rate in high-risk MDS of 58%, including 47% CR/mCR, with responders continuing on study for up to 2 years.[14] A phase II multicenter, randomized study of MBG453 or placebo added to hypomethylating agents (azacitidine or decitabine) in adult subjects with intermediate, high, or very high risk MDS (NCT03946670) and no prior hypomethylating agent therapy is currently underway. Potential

mechanisms of action of MBG453 include disruption of Tim-3–galectin-9-mediated autocrine LSC self-renewal, promotion of antibody-dependent cellular phagocytosis (ADCP), and/or promotion of M1 phenotype in macrophages (figure 3).

Clinical anti-TIM-3 antibodies: isotype

In humans, there are four isotypes of IgG (IgG1-4), differing in their binding profiles to various Fcγ receptors (FcγR) and to complement subunits, such as C1q. IgG1 has the highest affinity to all FcγRs and C1q, leading to significant effector functions, such as ADCC, ADCP, and CDC, whereas IgG2 and IgG4 induce significantly weaker or no ADCC and CDC. The majority of anti-TIM-3 antibodies in early clinical development are Fc-receptor silent, with the exception of Sym023, which is a wild-type IgG1 antibody, currently in testing in advanced solid tumors and lymphoma (NCT03489343).[96] Some anti-TIM-3 mAbs (table 1) are hIgG4 isotype with hinge stabilization (S228P) to eliminate fab-arm exchange. Recent data have demonstrated that hIgG4 antibodies with a S228P mutation can bind FcγRI and mediate ADCP.[97 98] It remains to be seen whether clinical anti-TIM-3 antibodies do mediate ADCP and if this could have utility in the AML/MDS setting where TIM-3 expression on LSCs or blasts may lead to direct anticancer activity (figure 3). Of note, the surrogate anti-TIM-3 mAb which demonstrated activity in preventing leukemic engraftment in an immune-deficient murine host was both ADCC-competent and CDC-competent,[92] suggesting that optimization of FcR engagement may be a desirable property for anti-TIM-3 mAbs in AML/MDS.

CONCLUSION AND PERSPECTIVE

Given that Tim-3 is expressed by a wide variety of immune cells as well as LSCs and is activated by several different ligands, much remains to be learned about the molecular and cellular circuitry by which Tim-3 operates to mediate its biological effects in the TME. Despite initial contradictory observations suggesting that Tim-3 may function as a costimulatory receptor, the recent reports demonstrating that germline loss-of-function mutations in *HAVCR2* lead to diseases that result from a hyperactivated immune system establishes Tim-3 as an inhibitory receptor. Currently, the therapeutic potential of anti-Tim-3 antibodies is being tested in different types of cancer, with activity in combination with hypomethylating agents in AML/MDS suggesting that its role on LSCs may be critical, in addition to its role in immune regulation. Further elucidation of these key functions for TIM-3 will help guide clinical development.

Contributors NA, CS-P, and ACA wrote the manuscript.

Funding Work in the authors' laboratory (ACA) is supported by grants from the National Institutes of Health (P01AI073748, R01CA229400, R01CA187975) and a Drug Discovery Program grant from Novartis.

Competing interests ACA is a member of the SAB for Tizona Therapeutics, Compass Therapeutics, and Zumutor Biologics, and Astellas Global Pharma Development, which have interests in cancer immunotherapy. ACA and CS-P are inventors on patents related to Tim-3. CS-P is an employee of Novartis.

Patient consent for publication Not required.

Provenance and peer review Commissioned; externally peer reviewed.

ORCID iD

Ana Carrizosa Anderson http://orcid.org/0000-0002-0877-2932

REFERENCES

1 Monney L, Sabatos CA, Gaglia JL, *et al*. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 2002;415:536–41.
2 McIntire JJ, Umetsu SE, Akbari O, *et al*. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked TIM gene family. *Nat Immunol* 2001;2:1109–16.
3 Lee J, Phong B, Egloff AM, *et al*. TIM polymorphisms—genetics and function. *Genes Immun* 2011;12:595–604.
4 Sabatos CA, Chakravarti S, Cha E, *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.
5 Sánchez-Fueyo A, Tian J, Picarella D, *et al*. Tim-3 inhibits T helper type 1–mediated auto- and alloimmune responses and promotes immunological tolerance. *Nat Immunol* 2003;4:1093–101.
6 Polprasert C, Takeuchi Y, Kakiuchi N, *et al*. Frequent germline mutations of HAVCR2 in sporadic subcutaneous panniculitis-like T-cell lymphoma. *Blood Adv* 2019;3:588–95.
7 Gayden T, Sepulveda FE, Khuong-Quang D-A, *et al*. Germline HAVCR2 mutations altering Tim-3 characterize subcutaneous panniculitis-like T cell lymphomas with hemophagocytic lymphohistiocytic syndrome. *Nat Genet* 2018;50:1650–7.
8 Chihara N, Madi A, Kondo T, *et al*. Induction and transcriptional regulation of the co-inhibitory gene module in T cells. *Nature* 2018;558:454–9.
9 DeLong JH, O'Hara Hall A, Rausch M, *et al*. Il-27 and TCR stimulation promote T cell expression of multiple inhibitory receptors. *ImmunoHorizons* 2019;3:13–25.
10 Fourcade J, Sun Z, Benallaoua M, *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.
11 Sakuishi K, Apetoh L, Sullivan JM, *et al*. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010;207:2187–94.
12 Ngiow SF, von Scheidt B, Akiba H, *et al*. Anti-TIM3 Antibody Promotes T Cell IFN- -Mediated Antitumor Immunity and Suppresses Established Tumors. *Cancer Res* 2011;71:3540–51.
13 Zhou Q, Munger ME, Veenstra RG, *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.
14 Borate U, Esteve J, Porkka K, *et al*. Phase Ib study of the Anti-TIM-3 antibody MBG453 in combination with decitabine in patients with high-risk myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). *Blood* 2019;134:570.
15 Rangachari M, Zhu C, Sakuishi K, *et al*. Bat3 promotes T cell responses and autoimmunity by repressing Tim-3–mediated cell death and exhaustion. *Nat Med* 2012;18:1394–400.
16 Lee J, Su EW, Zhu C, *et al*. Phosphotyrosine-Dependent coupling of Tim-3 to T-cell receptor signaling pathways. *Mol Cell Biol* 2011;31:3963–74.
17 Clayton KL, Haaland MS, Douglas-Vail MB, *et al*. T cell Ig and mucin Domain–Containing protein 3 is recruited to the immune synapse, disrupts stable synapse formation, and associates with receptor phosphatases. *J Immunol* 2014;192:782–91.
18 van de Weyer PS, Muehlfeit M, Klose C, *et al*. A highly conserved tyrosine of Tim-3 is phosphorylated upon stimulation by its ligand galectin-9. *Biochem Biophys Res Commun* 2006;351:571–6.

19 Huang Y-H, Zhu C, Kondo Y, *et al*. Ceacam1 regulates TIM-3-mediated tolerance and exhaustion. *Nature* 2015;517:386–90.
20 Ji J, Yin Y, Ju H, *et al*. Long non-coding RNA Lnc-Tim3 exacerbates CD8 T cell exhaustion via binding to Tim-3 and inducing nuclear translocation of BAT3 in HCC. *Cell Death Dis* 2018;9:478.
21 Maurya N, Gujar R, Gupta M, *et al*. Immunoregulation of Dendritic Cells by the Receptor T cell Ig and Mucin Protein-3 via Bruton's Tyrosine Kinase and c-Src. *J Immunol* 2014;193:3417–25.
22 Türeci O, Schmitt H, Fadle N, *et al*. Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease. *J Biol Chem* 1997;272:6416–22.
23 Wada J, Kanwar YS. Identification and characterization of galectin-9, a novel beta-galactoside-binding mammalian lectin. *J Biol Chem* 1997;272:6078–86.
24 Zhu C, Anderson AC, Schubart A, *et al*. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* 2005;6:1245–52.
25 Nagae M, Nishi N, Murata T, *et al*. Structural analysis of the recognition mechanism of poly-N-acetyllactosamine by the human galectin-9 N-terminal carbohydrate recognition domain. *Glycobiology* 2009;19:112–7.
26 Imaizumi Tet *al*. Interferon-Gamma stimulates the expression of galectin-9 in cultured human endothelial cells. *J Leukoc Biol* 2002;72:486–91.
27 Li H, Wu K, Tao K, *et al*. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. *Hepatology* 2012;56:1342–51.
28 Garcia-Diaz A, Shin DS, Moreno BH, *et al*. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. *Cell Rep* 2017;19:1189–201.
29 Katoh S, Ishii N, Nobumoto A, *et al*. Galectin-9 inhibits CD44–Hyaluronan interaction and suppresses a murine model of allergic asthma. *Am J Respir Crit Care Med* 2007;176:27–35.
30 Niki T, Tsutsui S, Hirose S, *et al*. Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE-antigen complex formation. *J. Biol. Chem.* 2009;284:32344–52.
31 Kikushige Y, Miyamoto T, Yuda J, *et al*. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. *Cell Stem Cell* 2015;17:341–52.
32 Gonçalves Silva I, Yasinska IM, Sakhnevych SS, *et al*. The Tim-3-galectin-9 secretory pathway is involved in the immune escape of human acute myeloid leukemia cells. *EBioMedicine* 2017;22:44–57.
33 Dardalhon V, Anderson AC, Karman J, *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.
34 de Mingo Pulido Álvaro, Gardner A, Hiebler S, *et al*. TIM-3 Regulates CD103^{+} Dendritic Cell Function and Response to Chemotherapy in Breast Cancer. *Cancer Cell* 2018;33:e66–74.
35 Yamauchi A, Kontani K, Kihara M, *et al*. Galectin-9, a novel prognostic factor with antimetastatic potential in breast cancer. *Breast J* 2006;12:S196–200.
36 Yamauchi A, Kontani K, Kihara M, *et al*. Galectin-9, a novel prognostic factor with antimetastatic potential in breast cancer. *Breast J* 2006;12:S196–200.
37 Fujita K, IWAMA H, OTO T, *et al*. Galectin-9 suppresses the growth of hepatocellular carcinoma via apoptosis in vitro and in vivo. *Int J Oncol* 2015;46:2419–30.
38 Fu H, Liu Y, Xu L, *et al*. Galectin-9 predicts postoperative recurrence and survival of patients with clear-cell renal cell carcinoma. *Tumor Biol.* 2015;36:5791–9.
39 Schulkens IA, Heusschen R, van den Boogaart V, *et al*. Galectin expression profiling identifies galectin-1 and Galectin-9Δ5 as prognostic factors in stage I/II non-small cell lung cancer. *PLoS One* 2014;9:e107988.
40 Holtan SG, Mansfield AS, Creedon DJ, *et al*. An organ system based approach to prognosis in advanced melanoma. *Front Biosci* 2012;4:2723–33.
41 Zhang Z-Y, Dong J-H, Chen Y-W, *et al*. Galectin-9 acts as a prognostic factor with antimetastatic potential in hepatocellular carcinoma. *Asian Pac J Cancer Prev* 2012;13:2503–9.
42 Gu CJ, Wu H, Sheng CY, *et al*. Expression and prognostic value of galectin-9 in hepatocellular carcinoma patients]. *Zhonghua Yi Xue Za Zhi* 2013;93:2025–8.
43 Sideras K, Biermann K, Verheij J, *et al*. PD-L1, Galectin-9 and CD8^{+} tumor-infiltrating lymphocytes are associated with survival in hepatocellular carcinoma. *Oncoimmunology* 2017;6:e1273309.
44 Wang Y, Sun J, Ma C, *et al*. Reduced expression of galectin-9 contributes to a poor outcome in colon cancer by inhibiting NK cell chemotaxis partially through the Rho/ROCK1 signaling pathway. *PLoS One* 2016;11:e0152599.
45 Kobayashi N, Karisola P, Peña-Cruz V, *et al*. Tim-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. *Immunity* 2007;27:927–40.
46 DeKruyff RH, Bu X, Ballesteros A, *et al*. T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells. *J.i.* 2010;184:1918–30.
47 Nakayama M, Akiba H, Takeda K, *et al*. Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. *Blood* 2009;113:3821–30.
48 Fadok VA, Voelker DR, Campbell PA, *et al*. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol* 1992;148:2207–16.
49 Vallabhapurapu SD, Blanco VM, Sulaiman MK, *et al*. Variation in human cancer cell external phosphatidylserine is regulated by flippase activity and intracellular calcium. *Oncotarget* 2015;6:34375–88.
50 Graham DK, DeRyckere D, Davies KD, *et al*. The TAM family: phosphatidylserine-sensing receptor tyrosine kinases gone awry in cancer. *Nat Rev Cancer* 2014;14:769–85.
51 Lima LG, Chammas R, Monteiro RQ, *et al*. Tumor-Derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. *Cancer Lett* 2009;283:168–75.
52 Chiba S, Baghdadi M, Akiba 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.
53 Curtin JF, Liu N, Candolfi M, *et al*. Hmgb1 mediates endogenous TLR2 activation and brain tumor regression. *PLoS Med* 2009;6:e10.
54 Bertheloot D, Latz E. Hmgb1, IL-1α, IL-33 and S100 proteins: dual-function alarmins. *Cell Mol Immunol* 2017;14:43–64.
55 Xia J, Yu X, Song X, *et al*. Inhibiting the cytoplasmic location of HMGB1 reverses cisplatin resistance in human cervical cancer cells. *Mol Med Rep* 2017;15:488–94.
56 Tesniere A, Schlemmer F, Boige V, *et al*. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* 2010;29:482–91.
57 Liu P, Zhao L, Loos F, *et al*. Identification of pharmacological agents that induce HMGB1 release. *Sci Rep* 2017;7:14915.
58 Gray-Owen SD, Blumberg RS. Ceacam1: contact-dependent control of immunity. *Nat Rev Immunol* 2006;6:433–46.
59 Nakajima A, Iijima H, Neurath MF, *et al*. Activation-Induced expression of carcinoembryonic antigen-cell adhesion molecule 1 regulates mouse T lymphocyte function. *J Immunol* 2002;168:1028–35.
60 Kammerer R, Stober D, Singer BB, *et al*. Carcinoembryonic antigen-related cell adhesion molecule 1 on murine dendritic cells is a potent regulator of T cell stimulation. *J Immunol* 2001;166:6537–44.
61 Horst AK, Bickert T, Brewig N, *et al*. CEACAM1+ myeloid cells control angiogenesis in inflammation. *Blood* 2009;113:6726–36.
62 Coutelier J-P, Godfraind C, Dveksler GS, *et al*. B lymphocyte and macrophage expression of carcinoembryonic antigen-related adhesion molecules that serve as receptors for murine coronavirus. *Eur J Immunol* 1994;24:1383–90.
63 Hunter I, Sawa H, Edlund M, *et al*. Evidence for regulated dimerization of cell-cell adhesion molecule (C-CAM) in epithelial cells. *Biochem J* 1996;320:847–53.
64 Sabatos-Peyton CA, Nevin J, Brock A, *et al*. Blockade of Tim-3 binding to phosphatidylserine and CEACAM1 is a shared feature of anti-Tim-3 antibodies that have functional efficacy. *Oncoimmunology* 2018;7:e1385690.
65 Gandhi AK, Kim WM, Sun Z-YJ, *et al*. High resolution X-ray and NMR structural study of human T-cell immunoglobulin and mucin domain containing protein-3. *Sci Rep* 2018;8:17512.
66 Jin H-T, Anderson AC, Tan WG, *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.
67 Gattinoni L, Zhong X-S, Palmer DC, *et al*. Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. *Nat Med* 2009;15:808–13.
68 Im SJ, Hashimoto M, Gerner MY, *et al*. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* 2016;537:417–21.
69 Sade-Feldman M, Yizhak K, Bjorgaard SL, *et al*. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 2018;175:998–1013.

70 Kurtulus S, Madi A, Escobar G, *et al*. Checkpoint blockade immunotherapy induces dynamic changes in PD-1–CD8+ tumor-infiltrating T cells. *Immunity* 2019;50:181–94.
71 Siddiqui I, Schaeuble K, Chennupati V, *et al*. Intratumoral Tcf1^{+}PD-1^{+}CD8^{+} T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy. *Immunity* 2019;50:195–211.
72 Yan J, Zhang Y, Zhang J-P, *et al*. Tim-3 expression defines regulatory T cells in human tumors. *PLoS One* 2013;8:e58006.
73 Gao X, Zhu Y, Li G, *et al*. Tim-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. *PLoS One* 2012;7:e30676.
74 Sakuishi K, Ngiow SF, Sullivan JM, *et al*. TIM3^{+}FOXP3^{+} regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. *Oncoimmunology* 2013;2:e23849.
75 Broz ML, Binnewies M, Boldajipour B, *et al*. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* 2014;26:938.
76 Roberts EW, Broz ML, Binnewies M, *et al*. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. *Cancer Cell* 2016;30:324–36.
77 Jiang X, Zhou T, Xiao Y, *et al*. Tim-3 promotes tumor-promoting M2 macrophage polarization by binding to STAT1 and suppressing the STAT1-miR-155 signaling axis. *Oncoimmunology* 2016;5:e1211219.
78 da Silva IP, Gallois A, Jimenez-Baranda S, *et al*. Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade. *Cancer Immunol Res* 2014;2:410–22.
79 Xu L, Huang Y, Tan L, *et al*. Increased Tim-3 expression in peripheral NK cells predicts a poorer prognosis and Tim-3 blockade improves NK cell-mediated cytotoxicity in human lung adenocarcinoma. *Int Immunopharmacol* 2015;29:635–41.
80 Anderson AC, Lord GM, Dardalhon V, *et al*. T-Bet, a Th1 transcription factor regulates the expression of Tim-3. *Eur J Immunol* 2010;40:859–66.
81 Zhu C, Sakuishi K, Xiao S, *et al*. An IL-27/NFIL3 signalling axis drives Tim-3 and IL-10 expression and T-cell dysfunction. *Nat Commun* 2015;6:6072.
82 Turnis ME, Sawant DV, Szymczak-Workman AL, *et al*. Interleukin-35 limits anti-tumor immunity. *Immunity* 2016;44:316–29.
83 Zhu ST CB, CQ X, *et al*. The correlation between the Tim-3 gene promoter polymorphisms and the risk of gastric cancer. *Journal of Capital Medical University* 2010;31:299–303.
84 Xu G, ZHENG KAI, LU X, *et al*. Association between polymorphisms in the promoter region of T cell immunoglobulin and mucin domain-3 and myasthenia gravis-associated thymoma. *Oncol Lett* 2015;9:1470–4.
85 Wang Z, Liu X, Wang X, *et al*. Polymorphisms in *TIM-3* and breast cancer susceptibility in Chinese women: A case-control study. *Oncotarget* 2016;7:43703–12.
86 Komohara Y, Morita T, Annan DA, *et al*. The coordinated actions of Tim-3 on cancer and myeloid cells in the regulation of tumorigenicity and clinical prognosis in clear cell renal cell carcinomas. *Cancer Immunol Res* 2015;3:999–1007.
87 Jiang J, Jin M-S, Kong F, *et al*. Decreased galectin-9 and increased Tim-3 expression are related to poor prognosis in gastric cancer. *PLoS One* 2013;8:e81799.
88 Zhou E, Huang Q, Wang J, *et al*. Up-Regulation of Tim-3 is associated with poor prognosis of patients with colon cancer. *Int J Clin Exp Pathol* 2015;8:8018–27.
89 Cao Y, Zhou X, Huang X, *et al*. Tim-3 expression in cervical cancer promotes tumor metastasis. *PLoS One* 2013;8:e53834.
90 Zhang Y, Cai P, Liang T, *et al*. TIM-3 is a potential prognostic marker for patients with solid tumors: a systematic review and meta-analysis. *Oncotarget* 2017;8:31705–13.
91 Jan M, Chao MP, Cha AC, *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.
92 Kikushige Y, Shima T, Takayanagi S-ichiro, *et al*. Tim-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell* 2010;7:708–17.
93 Asayama T, Tamura H, Ishibashi M, *et al*. Functional expression of Tim-3 on blasts and clinical impact of its ligand galectin-9 in myelodysplastic syndromes. *Oncotarget* 2017;8:88904–17.
94 Koyama S, Akbay EA, Li YY, *et al*. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun* 2016;7:10501.
95 Shayan G, Srivastava R, Li J, *et al*. Adaptive resistance to anti-PD1 therapy by Tim-3 upregulation is mediated by the PI3K-Akt pathway in head and neck cancer. *Oncoimmunology* 2017;6:e1261779.
96 Trine Lindsted MG, Grandal MV, Frölich C, *et al*. Preclinical characterization of Sym023 a human anti-TIM3 antibody with a novel mechanism of action. *Cancer Research* 2018.
97 Zhang T, Song X, Xu L, *et al*. The binding of an anti-PD-1 antibody to FcγRI has a profound impact on its biological functions. *Cancer Immunol Immunother* 2018;67:1079–90.
98 Chen X, Song X, Li K, *et al*. FcγR-Binding is an important functional attribute for immune checkpoint antibodies in cancer immunotherapy. *Front Immunol* 2019;10:292.
99 Curigliano G GH, Mach N, Doi T. Wai menG David tai, Patrick Forde, John Sarantopoulos, Philippe L. Bedard, Chia-Chi Lin, Stephen Hodi, Sofie Wilgenhof, Armando Santoro, Catherine Sabatos-Peyton, Tyler Longmire, Kitty Wan, Panagiotis Nikolopoulos, Luigi Manenti and Aung Naing. phase I/II study of MBG453 ± spartalizumab (PDR001) in patients with advanced malignancies. *Cancer Res* 2019;79:CT183.
100 N. Mach, G. C, Santoro, D A, Kim DWM, *et al*. Phase (pH) II study of MBG453 + SPARTALIZUMAB in patients (PTS) with non-small cell lung cancer (NSCLC) and melanoma pretreated with ANTI–PD-1/L1 therapy. *ESMO* 2019.
101 BMC. 32Nd annual meeting and Pre-Conference programs of the Society for immunotherapy of cancer (SITC 2017): Part one. *J Immunother Cancer* 2017;5:86.
102 BMC. 33rd Annual Meeting & Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2018). *Journal for ImmunoTherapy of Cancer* 2018;6:115.
103 Murtaza A, Laken H, Da Silva Correia J, *et al*. Discovery of TSR-022, a novel, potent anti-human Tim-3 therapeutic antibody. *Eur J Cancer* 2016;69:S102.
104 Rubuffet L*et al*. Phase II trial of neoadjuvant nivolumab (Nivo) and Intra-Tumoral (it) CMP-001 in high risk resectable melanoma (MEL).. *SITC* 2019.
105 Lindsted T. Abstract 5629: preclinical characterization of Sym023 a human anti-TIM3 antibody with a novel mechanism of action. *Cancer Res* 2018;78:5629.
106 Zhang T. Abstract 2628: BGB-A425: a humanized anti-human Tim-3 antibody that exhibits strong immune cell activation. *Cancer Res* 2017;77:2628.
107 Klein C, Schaefer W, Regula JT, *et al*. Engineering therapeutic bispecific antibodies using CrossMab technology. *Methods* 2019;154:21–31.
108 Harding JJ PA, Moreno V, *et al*. A phase Ia/Ib study of an anti-TIM-3 antibody (LY3321367) monotherapy or in combination with an anti-PD-L1 antibody (LY3300054): interim safety, efficacy, and pharmacokinetic findings in advanced cancers. *ASCO-SITC Clinical Immuno-Oncology Symposium* 2019;12.
109 Waight J*et al*. Abstract 3825: INCAGN02390, a novel antagonist antibody that targets the co-inhibitory receptor Tim-3. *Cancer Res* 2018;78:3825.