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Significance of TIM3 expression in cancer: From biology to the clinic

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ABSTRACT

Targeting inhibitory immune checkpoint molecules has dramatically changed treatment paradigms in medical oncology. Understanding the best strategies to unleash a pre-existing immune response or to induce an efficient immune response against tumors has emerged as a research priority. In this work, we focus on a novel target for cancer immunotherapy, the inhibitory receptor T-cell immunoglobulin and mucin domain 3 (TIM3). This narrative review describes TIM3 biology in different (tumor-infiltrating) immune cells, particularly in the immunosuppressive regulatory T cells and dysfunctional/exhausted cytotoxic T lymphocytes, but also in cells that confer innate immunity – natural killer and dendritic cells. We discuss the functional role of TIM3, its expression and its clinical significance in a variety of tumors, and confront the heterogeneous results emerging from different studies, including clinical trials of immunotherapy. Finally, this work summarizes the principal early-phase clinical trials exploring TIM3 blockade and discusses some future perspectives.

Introduction

The recent success of immune checkpoint blockade (ICB), which acts by harnessing the function of a patient's own T cells against tumors, has focused attention on the role played by the immune system of the host in its defense against cancer.[1] Spontaneous T cell responses can be generated against tumor antigens (Ags) and a variety of cosignaling receptors (both inhibitory and costimulatory) tightly regulate every step of T cell mediated immunity. These receptors are usually expressed on the surface of immune cells and are named immune checkpoint molecules.[2]

Inhibitory immune checkpoint pathways have been widely studied as possible mechanisms of immune escape in cancer, and for some of them, particularly the cytotoxic T lymphocyte antigen (Ag)-4 (CTLA-4), and the programmed cell death-1 (PD-1) pathways, inhibitory antibodies (Abs) have garnered regulatory approvals worldwide – with Abs targeting CTLA-4, PD-1, and programmed cell death-ligand 1 (PD-L1) having achieved United States (US) Food and Drug Administration (FDA) and European Medicines Agency (EMA) approvals for the treatment of a broad spectrum of neoplastic diseases including melanoma, non-small cell lung cancer (NSCLC), head and neck (HN) cancer, lymphomas, microsatellite instable-high (MSI-H) solid tumors, urothelial carcinoma, renal cell carcinoma, gastric cancer, hepatocellular carcinoma (HCC) and Merkel cell carcinoma, in both early and advanced settings, often resulting in durable clinical responses in a small fraction of patients (reviewed in[3]). However, a relevant proportion of patients do not respond to these treatments, or they experience progression of disease after an initial benefit. Intense research efforts are ongoing to identify subsets of patients more responsive to ICB, for example those with MSI-H colorectal cancer[4]; and NSCLC with high expression of PD-L1 by tumor cells[5] along with the growing need to elucidate and identify the biological mechanisms underlying response or resistance to ICB.

Resistance to ICB may be due to multiple and some partially unknown factors that can confer intrinsic, naturally acquired, or therapy-induced acquired resistance.[6] In this context, the expression of additional modulatory inhibitory immune checkpoint molecules including lymphocyte activation gene 3 (LAG3),[7] T-cell immunoglobulin and mucin domain 3 (TIM3), and others[2] are emerging as important in dysfunctional lymphocytes, playing a possible role both in dampening the inherent immune response

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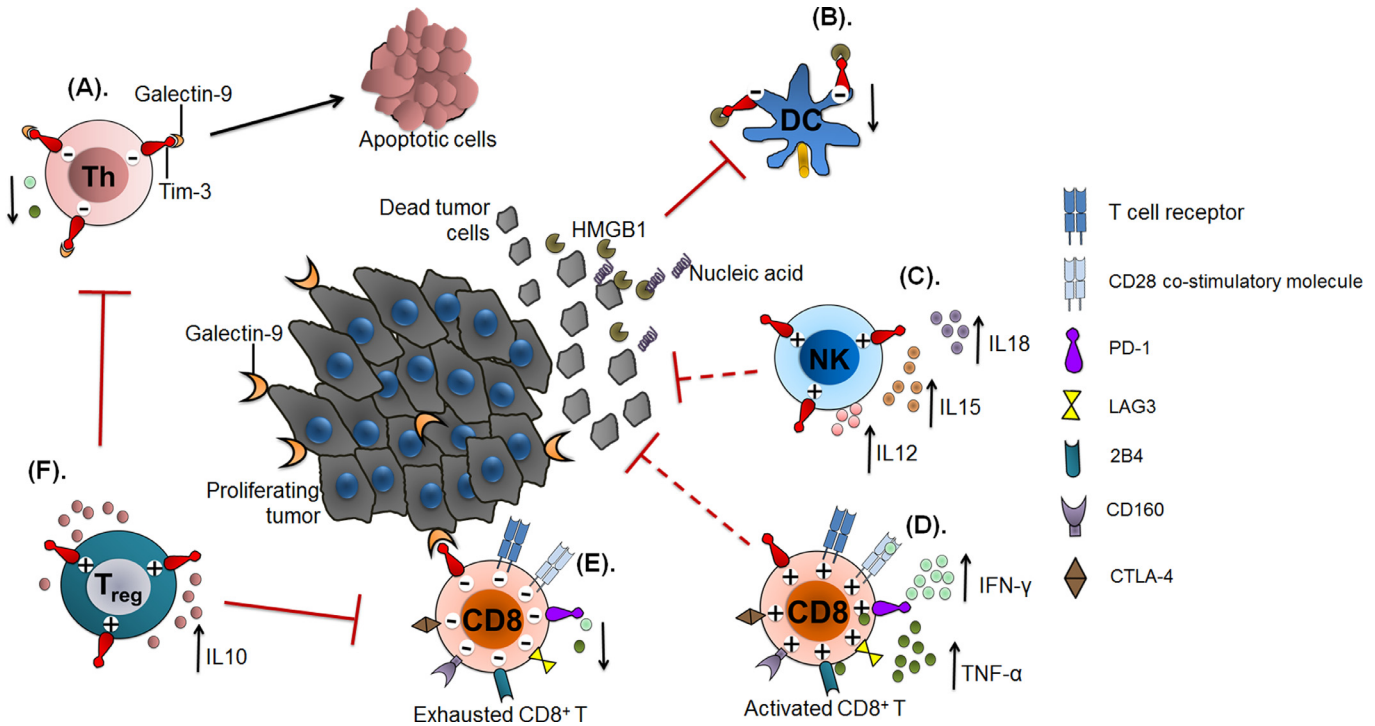

Fig. 1. The complex interplay of the expression of TIM3 and its ligands on immune cells. (A). Binding of TIM3 to its main ligand, galectin-9, results in the death of T helper cell (Th1/Th17) by apoptosis. (B) In the tumor microenvironment (TME), TIM3 expressed on tumor-associated dendritic cells (DCs) binds to High Mobility Group Box 1 (HMGB1) molecules released from apoptotic tumor cells. This interaction blocks the transport of nucleic acids into endosomes of DCs, thereby suppressing the innate immune response to tumor-derived nucleic acids that would normally be mediated by DCs. (C) Upregulation of TIM3 in mature natural killer cells (NK) usually induces release of cytokines IL12, IL15, and/or IL18 and confers increased cytotoxicity to tumor cells. (D) In $CD8^+$ T cells, expression of TIM3 together with other inhibitory immune checkpoint molecules, such as PD-1, CD160; 2B4, LAG3 and others is thought to be linked to T cell differentiation and activation with increased levels of IFN-γ and TNF-α. (E) However, the binding of TIM3 on $CD8^+$ T cells with its ligand galectin-9 expressed on tumor cells, or on other immune cells in the TME, leads to a state of exhaustion with reduced levels of IFN-γ and TNF-α that can block antitumor responses. (F) Furthermore, $TIM3^+FoxP3^+$ regulatory T cells (Tregs) present within the tumor express high amounts of IL10 and other cytokines that can inhibit effector T cells. Thus, blocking the TIM3 pathway may abrogate Treg activation.

and in contributing to a tumor's ability to acquire resistance to ICB. Indeed, TIM3 expression in patients' lymphocytes has been implicated in resistance to ICB in patients with a diagnosis of melanoma and NSCLC,[8] representing a potential novel target for cancer immunotherapy. Similar to the PD-1/PD-L1 axis, it is important to emphasize that these additional checkpoints are not peculiar to specific tumors, but instead belong and contribute to the physiological homeostasis of the immune response against pathogens.[9]

In the present work, we review and discuss the heterogeneous biological role of TIM3 at the interface between cancer and the immune response, highlighting issues and initial data that support its explorative targeting in clinical immunotherapy settings. We divided the work into 3 parts, ideally moving from the biology of TIM3, to its role in cancer and finally to its potential clinical relevance as a biomarker and as an immunotherapy target.

TIM3: biology

TIM3, belonging to the immunoglobulin (Ig) superfamily, is a member of a relatively newly described T-cell Ig and TIM family, comprised of type-I cell surface glycoproteins having a signal peptide, an extracellular IgV domain, a mucin-like domain, a transmembrane domain, and an intracellular cytoplasmic tail, that are involved in apoptosis.[10-12] TIM3 has been shown to regulate both innate and adaptive immune responses, potentially exerting either positive or negative effects[13] thus acting as an immune modulator. The different roles of TIM3 probably depend on the different cells expressing this receptor and on the various immunologic profiles of the tumor microenvironment. TIM3 expression has been reported on interferon (IFN)-gamma (γ) secreting helper T (Th)1 cells, Th17, regulatory T cells (Tregs), $CD8^+$ T cells, dendritic cells (DCs), monocytes, and other leukocyte subsets, including natural killer (NK) cells (Fig. 1).[14-20]

Four ligands that interact with the TIM3 IgV domain have been described. They are (1) galectin-9,[21] probably the most relevant; (2) phosphatidylserine (PtdSer), exposed on the surfaces of apoptotic cells[12]; (3) high mobility group box 1 (HMGB1), binding to nucleic acids released from dying tumor cells, that activates the innate immune responses mediated by DCs[22]; and (4) carcinoembryonic Ag-related cell adhesion molecule 1 (CEACAM1), a negative regulator of T cell responses that functions as a self-ligand on T cells.[23] Binding of TIM3 to its main ligand, galectin-9 results in Th1 cell death by apoptosis (Fig. 1). This effect is mediated by an influx of calcium to the intracellular region of Th1 cells, followed by aggregation and death *in vitro*.[21] Further, suppression of Th1 mediated autoimmunity and reduction of IFN-γ producing cells were observed after galectin-9 binding to TIM3.[23] Conversely, the levels of IFN-γ secreting T cells and the proliferation of Th1 cells increased after *in vitro* TIM3 blockade,[24] confirming the role of TIM3 in preventing/controlling excessive immune activation in the $CD4^+$ Th cell compartment.

By producing high levels of interleukin (IL)10 as well as by expressing higher FoxP3 $TIM3^+$ Tregs are potent inhibitors of the effector T cell function when compared to $TIM3^-$ Treg cells, suggesting TIM3 expression characterizes highly immunosuppressive Tregs.[15,25,26] IL2 stimulation significantly improves the proliferation of Tregs, increases the expression of TIM3, CTLA-4, and LAG3, and enhances Treg-mediated suppression of conventional T cell proliferation *in vitro*.[27] While the proliferation capacity of $TIM3^+$ Tregs appears to be comparable with that of $TIM3^-$ Tregs, they

have higher levels of CTLA-4 and LAG3 along with the enhanced ability to suppress conventional T cell proliferation.[26] The IL2-mediated proliferation of TIM3$^+$ Tregs is abrogated by the TIM3 blockade.[27] In human CD8$^+$ T cells, TIM3 expression, together with other immune checkpoint molecules including PD-1, CD160; 2B4, LAG3 and others is thought to be linked to T cell differentiation and activation, although its chronic/overtime persistence might be associated with an exhaustion status.[16,28,29] Constitutive expression of TIM3 is found on innate immune cells including monocytes/macrophages, DCs, mast cells, and mature NK cells. When present in a subset of macrophages, monocytes, and splenic CD8$^+$ DCs, TIM3 acts as a phagocytic receptor for apoptotic cells.[30] However, TIM3 may also negatively interfere with the role of DCs in the initiation of innate responses to nucleic acids, following its interaction with the endogenous danger signal HMGB1[22] (Fig. 1). In apparent contrast, other reports have observed that the stimulation of TIM3 on DCs by its ligands can result in the production of proinflammatory cytokines and DC maturation,[31-33] acting here as a modulator that promotes inflammation. A similar proinflammatory role has been described for TIM3 in other myeloid cells, such as murine bone marrow-derived mast cells, where it enhances IgE/Ag-induced cytokine production.[34]

NK are innate lymphoid cells able to kill tumor cells without major histocompatibility complex (MHC) specificity, which is complementary to the MHC-restricted tumor lysis mediated by CD8$^+$ T cells.[35,36] NK cell cytotoxicity is attenuated by TIM3 receptor expression. TIM3 is present at basal levels in mature NK cells, and variably expressed by immature NK cells, where it can be induced by cytokines[19] (Fig. 1). Upregulation of TIM3 in mature NK cells usually follows NK activation and cytokine release (IL12, IL15, and/or IL18)[19,37] (Fig. 1). The functional role of TIM3 activation in NK is still not completely clear, since the artificial blockade of the TIM3/galectin-9 pathway in preclinical models has given contrasting effects, either decreasing NK cytotoxicity[19] or stimulating the production of proinflammatory IFN-γ.[37]

These observations highlight the complexity and heterogeneity of the effects generated through the manipulation of the multiple immunomodulatory pathways which are regulated by TIM3 in the different cells of the adaptive and innate immune response.

TIM3 in cancer: Presence, role, and clinical relevance

In humans TIM3 expression has been reported in multiple tumor settings, including tumor Ag-specific (anti-Melan-A) CD8$^+$ tumor-infiltrating lymphocytes (TILs) in melanoma,[38] CD4$^+$ and CD8$^+$ TILs in breast[39] and kidney cancer[40] and in malignant ascites from patients with gastrointestinal tumors where TIM3 expression has been shown to correlate with a worse clinical outcome.[41] In patients with esophageal squamous cell cancer treated with ICB, elevations in the proportion of circulating TIM3$^+$ CD4$^+$ T cells after the first cycle of the anti-PD-1 Ab nivolumab were linked with better survival rates, supporting the further exploration of this T cell subpopulation as a potential biomarker for clinical responses to ICB.[42] While these results may appear to contrast with previous evidence associating TIM3 expression by TILs with a possible mechanism of resistance to PD-1 ICB[7] as mentioned above, multiple, dynamic parameters may contribute to the complex role of TIM3. For instance, TIM3 expression by itself may be a consequence of "positive" early lymphocyte activation, and could occur following successful treatment with an ICB. Furthermore, a generic report of CD4$^+$ TIM3$^+$ lymphocytes does not allow for a functional distinction between Tregs and Th cells. In general, a deeper interpretation should not be limited to a single lymphocyte subset but should include a comprehensive evaluation of TIM3 in all circulating immune components including CD8$^+$ and NK cells, as well as others.

Indeed, in NSCLC, high levels of TIM3 on tumor-infiltrating Tregs were reported to be associated with poor clinical outcomes[43] (Table 1). While in CRC, circulating Tregs coexpressing LAG3 and TIM3 represented approximately half of CD4$^+$CD25$^{+/hi}$ T cells and more than 60% of CD4$^+$CD25$^{+/hi}$FoxP3$^+$ Tregs. These cells were shown to be highly immunosuppressive and characterized by higher levels of transforming growth factor beta (TGF-β), IL10 secretion, and higher CTLA-4 and FoxP3 expression.[44] LAG3$^+$ TIM3$^+$ Tregs were potent suppressors of the activity of macrophages, with lower expression of MHC-II, CD80, CD86, and tumor necrosis factor alfa (TNF-α), but higher expression of IL10.[44] Further, in a murine model of HN cancer, anti-PD-1 ICB downregulated TIM3 expression in Treg TILs, and their suppressive activity was reversed by IFN-γ secreted by CD8$^+$ TILs upon PD-1 blockade.[45] In breast cancer (BC) TIM3 was found expressed by a subset of exhausted follicular helper T cells (Tfh). Tfh cells are known to be important regulators of Ag-specific B cell responses, potentially triggering tertiary lymphoid structure formation and generating B cell responses at the tumor site.[46] TIM3$^+$ Tfh cells expressed high levels of PD-1, reduced levels of the B cell chemoattractant chemokine (C-X-C motif) ligand 13 (CXCL13) and IL21. They were characterized by reduced proliferation and displayed minor Tfh-mediated B cell help with respect to the TIM3$^-$ counterpart, supporting their exhausted profile.[47] CD8$^+$ T cells expressing CXCR5 (CXCR5$^+$), the receptor for CXCL13, localized in metastatic lymph nodes of patients diagnosed with thyroid cancer presented significantly higher PD-1 expression and lower or comparable TIM3 and CTLA-4 expression, with respect to CXCR5$^-$CD8$^+$ T cells, signifying that the degree of PD-1 expression might identify different functional profiles of these CD8$^+$ T cell subpopulations.[29] Indeed, CXCR5$^+$ CD8$^+$ T cells presented higher expression of proinflammatory cytokines including IL2, IFN-γ, and TNF-α, higher proliferation capacity and higher expression of cytotoxic molecules (granzyme A, B and perforin) than CXCR5$^-$CD8$^+$ T cells.[48] However, PD-1^{high} CD8$^+$ T cells isolated from patients with HCC expressed higher levels of genes linked to T cell dysfunction (namely TIM3 and LAG3), which signal exhaustion.[49] These cells produced lower amounts of IFN-γ and TNF-α following anti-CD3 stimulation, probably representing another distinct subpopulation with reduced proliferative and cytokine production. Indeed, incubation of PD-1^{high} CD8$^+$ T cells with Abs against PD-1 and TIM3 or LAG3 further restored proliferation and production of IFN-γ and TNF-α in response to an anti-CD3 proliferative *stimulus*, highlighting the potential activity of mAbs blocking additional modulatory inhibitory immune checkpoint molecules other than PD-1.[49] The clinical relevance of the coexpression of PD-1 and TIM3 by CD8$^+$ TIL has been confirmed in renal cell carcinoma where it is associated with a poor prognosis.[50] It is emerging how TIM3 is part of a complex coinhibitory genetic module involving multiple receptors (including PD-1 and LAG3) and transcription factors; a circuit expressed by T lymphocytes in both chronic infections and cancers, at least partially driven by the regulatory cytokine IL27.[51]

A very relevant and still open issue is whether CD8$^+$ TILs expressing (or coexpressing) inhibitory molecules are able to elicit antitumor effector functions. In NSCLC, a molecular signature of cytotoxic TILs revealed that Ag specific CD8$^+$ TILs had high levels of PD-1, 4-1BB, and TIM3, probably indicating that the functional profile of these cells is skewed toward exhaustion following a chronic activation in the context of a long lasting inflammatory environment. Remarkably, tumors with a high density of cytotoxic TILs had enrichment in transcripts linked to the presence of tissue-resident memory cells that were associated with improved survival.[52]

Heterogeneity in the expression of multiple inhibitory immune checkpoint molecules by TILs is another crucial and emerging issue to be considered, as recently reported in BC[39] and in melanoma.[38] These findings render the question whether targeting concurrently

Table 1
TIM3 expression in cancer: clinical significance.

TIM3$^+$ cells	Tumor type	Function	Outcome	Other immune checkpoint molecules expressed
CD8$^+$ TILs	Patients with metastatic melanoma vaccinated with CpG and the melanoma antigen Melan-A/MART-1 peptide	Antigen-specific cells	No correlations	CTLA-4, LAG3, PD-1, CD160, 2B4...
CD4$^+$ and CD8$^+$ TILs	Early-stage breast cancer	NA	Better outcome in basal-like (*TIM3* gene expression)	PD-1, LAG3
CD4$^+$ and CD8$^+$ TILs	Localized clear cell renal carcinoma	CD8$^+$ PD-1$^+$ TIM3$^+$ LAG3$^+$ TILs were polyclonal/poorly cytotoxic CD8$^+$ PD-1$^+$ TIM3$^+$ TILs were oligoclonal/cytotoxic	Worse outcome	PD-1, LAG3
CD4$^+$ and CD8$^+$ TILs	Metastatic gastrointestinal tumors (gastric, pancreatic, colorectal) with ascites	NA	Worse outcome if PD-1$^+$ and TIM3$^+$ CD4$^+$ and CD8$^+$ TILs	PD-1
Circulating CD4$^+$ T cells	Esophageal squamous cell cancer	NA	CD4$^+$ TIM3$^+$ better survival in patients treated with anti-PD-1	-
CD4$^+$ and CD8$^+$ TILs	Non-small cell lung cancer	Frequency of IFN-γ^+ cells was lower in CD8$^+$ TIM3$^+$ cells About 60% of FoxP3$^+$ TILs were TIM3$^+$	NA NA	PD-1
Circulating regulatory T cells	Colorectal cancer	Immunosuppression: ↓macrophages ↑TNF-β; ↑IL10; ↑CTLA-4; ↑FoxP3	NA	LAG3; CTLA-4
Tumor-infiltrating regulatory T cells	Head and neck squamous cell carcinoma	Inhibition of *naïve* T cell proliferation (PD-1^{hi} TIM3$^+$ regulatory T cells); regulatory T cells have an effector like phenotype; treatment with INF-γ reversed the suppressive function of regulatory T cells	NA	PD-1, CTLA-4, CD39, and IFN-γ receptor
Exhausted follicular helper T cells	Breast cancer	↓CXCL13; ↓IL21; ↓ proliferating cells	NA	PD-1
CXCR5$^+$ CD8$^+$ TILs	Thyroid cancer	↑IL2, ↑IFN-γ and ↑TNF-α; ↑ proliferation capacity with respect to CXCR5$^-$; ↑expression of granzyme A, B and perforin	NA	PD-1, CTLA-4
PD-1^{high} CD8$^+$ TILs	Hepatocellular carcinoma	↓IFN-γ and TNF-α production following anti-CD3 stimulation; incubation of CD8$^+$ T cells with a discrete population of PD-1^{high} cells with antibodies against PD-1 and TIM3 or LAG3 restored proliferation and production of IFN-γ and TNF-α in response to anti-CD3.	NA	PD-1, LAG3
CD8$^+$ TILs	Renal cell carcinoma	NA	Poor outcome	PD-1
CD8$^+$ TILs	Non-small cell lung cancer	Antigen specific TIL; presence of tissue-resident memory cells (CD103^{high}) in TIL^{hi} tumors	NA for TIM3$^+$ (better in CD8^{high} and CD103^{high} tumors)	PD-1, CTLA-4, 4-1BB; CD27; LAG3
Tumor-derived CCR1$^+$CD14$^+$ monocytes	Hepatocellular carcinoma	Upregulation of immune checkpoints, indoleamine and arginase, inflammatory/pro-angiogenic cytokines, matrix remodeling proteases, and inflammatory chemokines (immunosuppressive profile)	poor survival	PD-L1, B7-H3
Circulating natural killer	Gastric cancer	NA	NA	NA
Circulating natural killer	Lung adenocarcinoma	Exhaustion and dysfunction, anti-TIM3 antibodies restored the cytotoxicity and the IFN-γ production	Worse survival	NA
Circulating natural killer	Metastatic melanoma	Exhausted phenotype; anti-TIM3 antibodies: ↑cytotoxicity, ↑IFN-γ, internalization of the receptor, ↑IL2R	NA	NA
Circulating natural killer	Bladder cancer	↓cytotoxicity; TIM3 blockade restored cytotoxicity; exogenous Gal-9 reduced the cytotoxicity of TIM3$^+$ NK cells; exogenous IL2 + IL15 and IL2 + IL21 significantly enhanced, but could not completely restore, the cytotoxicity of NK cells	Worse survival	NA
Circulating natural killer	Anaplastic thyroid cancer	Lower cytotoxicity if CD56^{hi}CD16$^{hi/lo}$ cells PD-1$^+$	NA	PD-1
Tumor cells	Myeloid leukemia, sarcoma, gastric, cervical and lung cancer, and osteosarcoma	NA	Poor outcome	NA

CXCL13 = chemokine (C-X-C motif) ligand 13; CXCR5 = C-X-C chemokine receptor type 5; IFN = interferon; IL = interleukin; IL2R = interleukin 2 receptor; TIL = tumor-infiltrating lymphocytes; TNF = tumor necrosis factor.

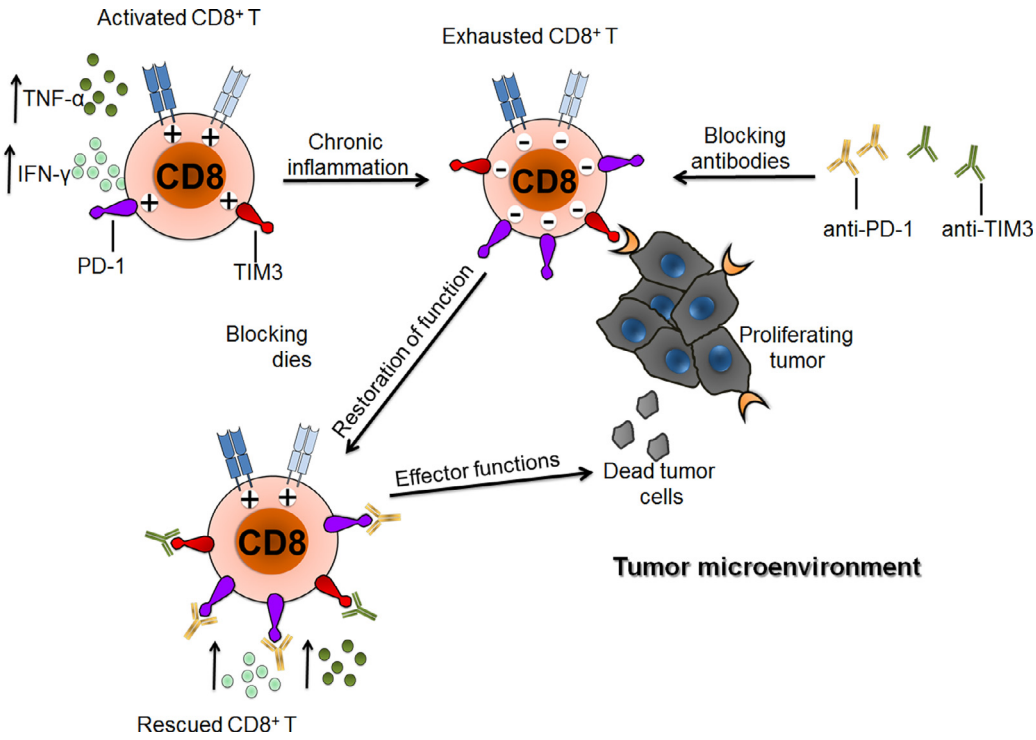

Fig. 2. TIM3 and the development of T cell exhaustion. TIM3 and PD-1 are highly expressed on dysfunctional or exhausted $CD8^+$ T cells in chronic diseases such as cancer. In these diseases, combinational receptor blockade has strong synergistic effects, resulting in improved effector $CD8^+$ T cells activity against the growing tumor.

or sequentially more than one inhibitory targetable immune checkpoint molecule is a reasonable approach, from a biological and a clinical point of view. Expression of TIM3, together with PD-1, CD163, and CD206 was also shown to characterize M2-like tumor-associated macrophages (TAM) having a protumor (immunosuppressor) profile. This phenotype can be induced by a variety of soluble factors (ie, TGF-β, IL4, IL10, IL13, monocyte colony stimulating factor) released by lymphocytes and cancer cells [reviewed in[53]]. Indeed, increased expression of TIM3 (at mRNA level) was observed in TAM M2a ($IL4^+$ and $IL13^+$) and M2c (TGF-β^+ and $IL10^+$), which were characterized by a significantly lower production of IL6, IL12, and TNF-α in mice.[54]

In humans, higher levels of TIM3 were observed in tumor-derived $CCR1^+CD14^+$ monocytes from patients with HCC, together with PD-L1 and B7-H3, suggesting a possible immunosuppressive role of these cells.[55] These observations were confirmed by transcriptome sequencing, revealing that these $CCR1^+CD14^+$ monocytes were reprogrammed to upregulate inhibitory immune checkpoint molecules, immune tolerogenic metabolic enzymes (indoleamine 2,3-dioxygenase and arginase), inflammatory/proangiogenic cytokines, matrix remodeling proteases, and inflammatory chemokines. Furthermore, the high-density of marginal $CCR1^+CD14^+$ monocytes was an independent marker of poor survival.[56] The biologic effects of TIM3 on myeloid cells depend upon the context where its stimulation occurs.[57]

Upregulation of TIM3 in circulating NK cells from cancer patients[58-62] was associated with their exhaustion and dysfunction. From a different angle, in some diseases the expression of TIM3 has been described directly on tumor cells (ie, myeloid leukemia, sarcoma, gastric, cervical and lung cancer, and osteosarcoma)[63-66] while in other diseases tumor cells resulted as $TIM3^-$ (ie, in BC).[39,67] TIM3 expression by tumor cells was associated with poor clinical outcomes.[63-66] Nevertheless, the intrinsic biological role of this receptor in tumor cells still remains not clearly defined; requiring dedicated translational studies and exploration in controlled clinical settings.

Targeting TIM3 in cancer

Current research efforts are exploring the possible beneficial effects of blocking or modulating TIM3 as a therapy for cancer. Understanding the effects of anti-TIM3 mAbs on the different leukocyte subsets, in different contexts, will be important for the future development of this approach. Noteworthy, TIM3 manipulation might have differential effects on effector/exhausted T cells *vs* Tregs and the role(s) of TIM3 can likely vary depending on the tumor type.[68] In patients with melanoma or NSCLC, for example, TIM3 expression has been implicated in resistance to blockade of the PD-1/PD-L1 axis.[8]

In experimental murine models[69] and patients with a diagnosis of advanced melanoma,[70] therapeutic blockade of TIM3 and the PD-1 axis, was reported to positively restore the functionality of exhausted antitumor $TIM3^+$ $PD\text{-}1^+$ $CD8^+$ T lymphocytes, improving cytokine production, antitumor activity and T cell proliferation. While in patients with gastric cancer vaccinated with tumor-Ag specific DCs, combined blockade of TIM3, TIGIT, and PD-1 was reported to promote proliferation and reverse the dysfunction of tumor-Ag specific T lymphocytes.[71] Dual immunomodulation is under investigation, with MCLA-134, a compound targeting PD-1 and TIM3, having been tested in preclinical models.[72]

It is not clear whether the activity of anti-TIM3 mAbs is completely due to blocking the interaction of the receptor with one or more of its ligands (ie, galectin-9 and/or HMGB1 or others) (Fig. 2). As noted above, one must consider that TIM3 is expressed by multiple cell types, with potentially different roles, including Ag presenting cells (APCs), Th1, Th17, $CD8^+$, and Tregs, making any interpretation difficult.

While TIM3 appears to be a potentially promising target for cancer immunotherapy, most of the research thus far suggests the therapeutic outcome of its blockade may depend on the impact of any intervention on its multiple biological circuits, including its modulation of both $CD4^+$ and $CD8^+$ T lymphocytes, the production of IFN-γ by $CD8^+$ T cells and the inhibition of $TIM3^+$ Tregs.[15,73]

Table 2
Ongoing trials of TIM3 blockade in cancer.

Reference	Drug(s)	Phase	Tumor type	Main objectives	Status
NCT02817633	TSR-022 (anti-TIM3) alone or in combination with nivolumab or TSR-042 (anti-PD-1); or TSR-042 (anti-PD-1) and TSR-033 (anti-LAG3)	I	Advanced or metastatic solid tumors	Safety and tolerability, antitumor activity, RP2D and schedule as monotherapy and in combination with an anti-PD-1 antibody, ORR, DOR, DCR, PFS, OS, PK parameters	Recruiting
NCT03489343	Sym023 (anti-TIM3)	I	Locally advanced/unresectable, metastatic solid tumors, or lymphomas that are refractory to available therapy or for which no standard therapy is available	Safety, tolerability, DLTs, MTD and/or RP2D. If an MTD is not identified, a MAD will be determined.	Recruiting
NCT02608268	MBG453 (anti-TIM3) alone or in combination with PDR001 (anti-PD-1)	I/II	Histologically documented advanced or metastatic solid tumors	Safety and tolerability of MBG453 alone and in combination with PDR001; incidence and severity of AEs; expression of immunological markers, time of maximum observed serum concentration, AUC, etc	Recruiting
NCT03652077	INCAGN02390 (anti-TIM3)	I	Cervical cancer, gastric cancer, gastroesophageal junction cancer, esophageal cancer, HCC, melanoma, uveal melanoma, Merkel cell carcinoma, mesothelioma, NSCLC, ovarian cancer, SCC of the head and neck, SCLC; renal cell carcinoma, triple-negative breast cancer, urothelial carcinoma, mismatch repair deficiency	Number of treatment-emergent AEs, MTD, pharmacologically active dose, Cmax, Tmax, Cmin, AUC, ORR, DOR, DCR, PFS, level of binding of INCAGN02390 to TIM3, immunogenicity of INCAGN02390	Recruiting
NCT03099109	LY3321367 (anti-TIM3) alone or in combination with LY3300054 (anti-PD-L1)	I	Solid tumors	Number of participants with DLTs, PK: Cmax of LY3321367, PK: Cmax of LY3321367 in combination with LY3300054, ORR, PFS, DOR, DCR	Recruiting
NCT03708328	RO7121661 (PD-1 and TIM3 bispecific antibody)	I	Solid tumors, metastatic melanoma, NSCLC	DLTs, MTD, percentage of participants with at least one AE, percentage of participants with at least one Grade ≥3 AE, ORR, DCR, DOR, PFS, AUC, Cmax, etc	Recruiting
NCT03680508	TSR-022 (anti-TIM3) *plus* TSR-042 (anti-PD-1)	II	Advanced, localized/unresectable HCC	ORR, DOR, TTP, PFS, OS, AFP response	Not yet recruiting

AFP = alpha-fetoprotein; AE = adverse event; AUC = area under the curve; Cmax = maximum plasma concentration; Cmin = minimum plasma concentration; DCR = disease control rate; DLT = dose limiting toxicity; DOR = duration of response; HCC = hepatocellular carcinoma; MAD = maximum administered dose; MTD = maximum tolerated dose; NSCLC = non-small cell lung cancer; ORR = objective response rate; OS = overall survival; PFS = progression free survival; PK = pharmacokinetic; RP2D = recommended phase II dose; SCC = small cell carcinoma; SCLC = small cell lung cancer; Tmax = time to reach maximum plasma concentration.

Preclinical data support the notion that anti-TIM3 therapy alone has a modest effect, with concomitant blockade of the PD-1/PD-L1 axis providing the best therapeutic effects[69,73] (Fig. 2). An increase in antitumor effect in experimental preclinical models has also been achieved by combining anti-TIM3 with anti-CTLA-4 mAbs.[73] Finally, antitumor activity with the anti-TIM3/anti-CTLA-4/anti-PD-1 combination has been reported after complete Treg depletion,[74] an intervention that stimulated both the innate and the adaptive arms of antitumor immunity.

Probably the most relevant target cell population of anti-TIM3 therapy may be TIM3$^+$ PD-1$^+$ TILs.[69,70] In preclinical models, tumors with higher fractions of TIM3$^+$ PD-1$^+$ CD8$^+$ T cells responded best to anti-TIM3. However, it is not possible to distinguish or selectively segregate the direct and indirect activity TIM3 blockade exerts on TIM3$^+$ PD-1$^-$ and TIM3$^+$ PD-1$^+$ T lymphocytes or even the TIM3$^-$ PD-1$^-$ subsets. A study investigating combined blockade of PD-1/PD-L1 and TIM3/galectin-9 in mice with acute myeloid leukemia showed that combined blockade was able to rescue the dysfunctional phenotype of TIM3$^+$ PD-1$^+$ T cells.[75] Similarly, data regarding combined anti-PD-1 and anti-TIM3 treatment of established fibrosarcoma suggests this combination might be effective against at least a fraction of tumors.[73] On the other hand, recent elegant data underscored how combined TIM3/PD-1 blockade may induce indirect, but relevant dynamic changes in the TIM3$^-$ PD-1$^-$ subset of TILs.[76] In particular, within multiple cancer models, it was reported that TIM3$^-$ PD-1$^-$ CD8$^+$ TILs underwent profound transcriptional changes, with proliferation and expansion of tumor-Ag specific precursors belonging to memory-precursor and effector-like TIL subsets.[76] As mentioned above it is possible that in addition to a direct effect of the anti-TIM3 Ab, other indirect activities may contribute to these positive effects on TIM3$^-$ PD-1$^-$ CD8$^+$ T lymphocytes. For example, anti-TIM3 and anti-PD-1/PD-L1 Abs can modulate multiple myeloid cells in the tumor microenvironment, impairing the acquisition of an M2-like phenotype in TAMs[77] or promoting the production of type 1 IFN, IL12, and IFN-γ by CD103$^+$ DCs in BC.[67]

The effect of targeting TIM3 on CD11c$^+$ DCs is not yet completely clear and in part controversial.[73] In mice implanted with T-cell lymphoma cell lines, treatment with an anti-TIM3 mAb delayed tumor progression and inhibited splenic CD11b$^+$ Ly6G$^+$ cells.[32] However, in other models direct targeting of TIM3 on CD11c$^+$ DCs did not have a significant impact, suggesting the importance of these cells is modest and model dependent.[73] Furthermore, some biological considerations appear counterintuitive, such as TIM3 ligation *per se* may favor the expansion of CD11b$^+$ Ly6G$^+$ cells, along with DC maturation and the production of proinflammatory cytokines.[18,21,32]

In regard to NK cells, TIM3 blockade in patients with advanced melanoma, lung adenocarcinoma, and bladder cancer had the effect of rescuing the function of exhausted NK cells, increasing their cytotoxicity and IFN-γ production.[59-62,78]

Targeting TIM3: Ongoing early phase clinical trials

Currently, early phase clinical trials are investigating anti-TIM3 mAbs in. A summary is provided in Table 2.

The principal intended mechanism of action of anti-TIM3 therapy is abrogation of T-cell inhibition/exhaustion, with the consequent rescue and activation of Ag-specific T lymphocytes and enhancement of cytotoxic T–cell-mediated antitumor activity.[79] Based on preclinical data, most trials are exploring the activity of anti-TIM3 treatments in synergy with PD-1/PD-L1 blockade.

A number of agents are now being used in early phase clinical trials as single or dual ICB (Table 2). The anti-TIM3 mAbs TSR-022 and MBG453 are being administered as monotherapy or in combination with anti-PD-1 or anti-PD-L1 Abs in patients with advanced solid tumors (NCT02817633; NCT02608268; NCT03099109). While the anti-TIM3 Abs Sym023 and INCAGN02390 are being given as single agents in patients with advanced solid tumors or lymphomas refractory to available treatments or for which no standard therapy is available (NCT03489343; NCT03652077). Furthermore, RO7121661, a new bispecific Ab targeting both TIM3 and PD-1 is being tested in a phase 1 trial of metastatic/advanced solid tumors (NCT03708328).

In addition to anti-TIM3 therapeutics in ongoing clinical trials, new agents are under preclinical development and may emerge as potential novel candidates for cancer immunotherapy.[80] One such example is DCB-8, a humanized Ab against human TIM3 that has been shown to significantly enhance cytokine secretion by treated human T cells, inhibiting tumor growth in *in vitro* and *in vivo* assays. Expected effects from the TIM3 blockade include the promotion of Th1 antitumor responses and release of the inhibition of dysfunctional $CD8^+$ T cells.

Conclusions and perspectives

TIM3 is emerging as a potentially important immune-checkpoint molecule, involved at different levels in the regulation of antitumor immune responses. Its therapeutic blockade is currently being investigated to unleash cell-mediated antitumor immune responses even if the exact mechanisms underlying the antitumor activity of anti-TIM3 mAbs are still not well understood. Part of the complexity in the exploration and therapeutic modulation of TIM3 resides in its being constitutively expressed, with different roles, on several cell components of the myeloid and lymphoid lineages. Immunological and clinical results from its therapeutic inhibition depend on these diverse factors. For instance, TIM3 Abs were shown to activate signals in cells involved in innate immunity – including macrophages, DCs and NK cells – while rescuing dysfunctional T cells and inhibiting Tregs. Current evidence suggests TIM3 blockade will be optimal if used in combination with other immunotherapeutic agents, as its efficacy is increased in rational combinations with anti-PD-1 and/or anti-CTLA-4 mAbs.

Considering the results from diverse preclinical studies investigating TIM3 blockade with mAbs and the results from early phase clinical trials yet to mature, it is hoped that TIM3 ICB will emerge as a potential new approach for cancer immunotherapy, particularly in combination with other ICB agents. However, recognizing an increasing number of attempts to enhance the activity of agents targeting the PD-1/PD-L1 axis have failed, it is also important to underline the strong need to incorporate reliable translational studies in the design of emerging next-generation clinical trials. It will be important that these studies include innovative endpoints that will allow one to better comprehend the diverse immunological interplay, both at the cellular and molecular levels, behind observed responses and treatment failures.

Declaration of Competing Interest

Authors declare they have no conflict of interest.

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