Review

Discovery Medicine 2025; 37(195): 631–646
https://doi.org/10.24976/Discov.Med.202537195.55

Immunomodulatory Role of T Cell Immunoglobulin and Mucin Domain-3 in Cancer

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Published: 20 April 2025

Immune checkpoint inhibitors are one of the most promising areas in oncoimmunology research. T cell immunoglobulin and mucin domain-3 (TIM-3) expression has been linked to the advanced stages with reduced survival in several types of cancer, primarily due to its association with the dysfunction in T cells. Thus, TIM-3 is an interesting target in designing advanced therapy for cancer. TIM-3 has been implicated in resistance to immunotherapy on account of its involvement in T cell exhaustion. Identifying small molecule inhibitors targeting TIM-3 with high affinity, either alone or in combination with either chemotherapy or other types of immunotherapies could significantly enhance the life span, overcoming the resistance and overall immune response in therapy. TIM-3 pathway is multidimensional in terms of canonical signaling with varied expression of immune cells and diverse ligands and modulates the immune response. This may include restoration of the functioning of killer T lymphocytes and natural killer cells (NK cells) and likely promise better results in cancer immunotherapy. In this review, we will discuss the immunomodulatory role of TIM-3 in cancer, with special emphasis on lymphoma and solid tumors, and their role in diverse immune cells in tumorigenesis and inflammation.

Keywords: checkpoint inhibitors; TIM-3; programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1); dendritic cells (DCs); T cell; NK cells; signaling; immunomodulation

Introduction

Immunotherapy is considered as relatively novel method and constitutes a vital part of present-day cancer therapy. Immunotherapy with multifarious protocols has been found to enhance the immune response to human cancer cells, hindering tumor growth, and has attained remarkable success in overall therapy in certain cancers. Immune checkpoint inhibitors (ICIs), such as programmed cell death-1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4), have been accomplished a significant milestone in several types of cancers [1–3]. Also, many patients do not respond to checkpoint immunotherapy, suggesting its limited efficiency in various cancer types. T cell immunoglobulin and mucin domain-3 (TIM-3) encoded by *Hepatitis A virus cellular receptor 2* (*Havcr2)* is constituted with an immunoglobulin and mucin domain and was originally reported as a surface marker on interferon-γ (IFN-γ) producing $CD4^+$ and $CD8^+$ T cells. TIM-3 represents a member of the *TIM* family of genes in syntenic chromosomal regions in humans (*5q33.2*) and mice (*11B1.1*) associated with autoimmune and allergic diseases [4,5]. TIM-3 was reported to be expressed on the type I T helper (Th1) cells and later also documented on the surface of monocytes, macrophages, and T helper 17 (Th17) cells [4,6]. Several studies demonstrated that TIM-3 acts as a negative regulator following its binding to its ligand Galectin-9 (Gal-9) and causes depletion of Th1 cells [7–9]. TIM-3 and its ligand prompted peripheral immune tolerance, and inhibiting TIM-3 could eliminate the emergence of Th1 tolerance [10,11]. The immunomodulatory role of TIM-3 has been documented in the experimental graft vs host disease model, where it negatively regulates the generation of IFN-γ by the $CD8^+$ T cells [12].

The function of TIM-3 was first reported by Monney *et al*. [4], who demonstrated the exacerbation of autoimmune encephalomyelitis in the central nervous system after *in vivo* TIM-3 monoclonal antibodies (mAbs) administration. Later, other studies showed the immunoregulatory function of TIM-3 by disrupting the TIM-3–TIM-3-ligand interactions via administration of either TIM-3–immunoglobulin (Ig) or antibody against TIM-3, which results in significantly elevated Th1 responses and exacerbation of autoimmune diabetes in non-obese diabetic mice [11,13]. Further, germline loss-of-function mutations in *Havcr2* and its connection with conditions like subcutaneous panniculitis-like T cell lymphoma (SPTCL) and hyperactivated T and myeloid cells in diseases like hemophagocytic lymphohistiocytosis (HLH) proves the "immune checkpoint" or negative regulatory role of TIM-

3 [14,15]. In cancer, TIM-3 expression is specifically observed in most of the dysfunctional or terminally exhausted subset of $CD8^+$ T cells [16,17]. TIM-3 is also co-expressed and co-regulated with other immune checkpoint receptors including l ymphocyte activation gene 3 (Lag-3), PD-1, and T cell immunoreceptor with Ig and ITIM domains (TIGIT) on $CD4^+$ and $CD8^+$ T lymphocytes [18,19]. Co-inhibition of PD-1 and TIM-3 pathways has shown significant tumoricidal potential both in solid and hematologic malignancies [17,20,21].

TIM-3 has multifarious roles in diseases such as immune diseases, chronic viral infections, and cancer. However, its underlying mechanisms in pathogenesis are unclear. Negative regulation of immune response by TIM-3 has attracted much attention in chronic viral infections involving exhausted or dysfunctional T cells. Reports suggest that blocking the TIM-3 signaling pathway restores the antigenic response of exhausted T cells, and preclinical studies indicate that TIM-3 plays a critical role in antitumor immune response [22,23]. These data strongly support TIM-3 as an immunomodulatory agent for targeting immunotherapy in cancer and numerous human diseases. Ongoing trials on binary application of anti-TIM-3 and anti-PD-1 are in progress against solid tumors. In this review, we will elucidate the immunomodulatory role of TIM-3 in the pathogenesis of cancer, specifically in solid tumors like lymphoma, renal cell carcinoma, etc., providing a potential description for a comprehensive understanding of clinical treatment.

T Cell Immunoglobulin and Mucin Domain-3

Immunotherapy with antibodies targeted to ICIs has emerged as a novel therapeutic tool. It has been applied in clinical trials, with encouraging results in many types of cancer, including malignant lymphoma. The ICIs regulate the immune system's response to cancer at multiple levels, ensuring adequate T cell homeostasis, activation, and differentiation [24].

Structure of TIM-3

A plethora of human and murine cell types express TIM-3. Murine and human TIM-3 consist of 281 and 302 amino acid residues respectively with 63% identity between them [4]. As a member of the immunoglobulin superfamily (IgSF), TIM-3 consists of an extracellular domain with a membrane-distal N-terminal immunoglobulin domain (IgV domain) which is followed by a membrane-proximal mucin domain having potential sites for O-linked glycosylation. The stalk region between the mucin and transmembrane domain contains the sites for N-linked sugars, which is subsequently followed by a transmembrane domain and a cytoplasmic tail [25]. In humans, a soluble form of TIM-3 is released by a disintegrin and metalloprotease (ADAM)-mediated ectodomain shedding. ADAM10 and ADAM17 are two major shedders that specifically cut glutamate (Glu) 181- aspartate (Asp) 190 of the stalk region of TIM-3 [26]. The crystal structure of the IgV domain of TIM-3 has two anti-parallel β sheets tethered by a disulfide bond. Additionally, 2 extra disulfide bonds stabilized the IgV domain and re-orient a CC loop toward an FG loop, thereby forming a "cleft" like structure, thought to be responsible for ligand binding and unique to TIM-3. This "cleft" assembly is present in all the TIM family member proteins, including TIM-1 and TIM-4 [27–29]. IgV domain engagement with appropriate ligands is important for the immune modulatory role of TIM-3 and for the induction of peripheral tolerance and suppression of anti-tumor immunity [30,31]. The TIM-3 cytoplasmic tail lacks immune receptor tyrosine-based inhibitory motifs (ITIMs) or immune receptor tyrosine-based switches (ITSMs). Still, it contains a conserved region of five tyrosine (Tyr,Y) residues, two of which (Tyr 256 & 263 in mice and Tyr 265 & 272 in humans) are critically important for coupling downstream signaling events. The sequence of peptides surrounding the above two tyrosine residues are highly conserved between mice and humans and function as Src-homology 2 (SH2) domain-binding motifs and bind to several SH2 domain-containing kinases, including lymphocyte-specific protein tyrosine kinase (LCK), FYN, phosphoinositide 3-kinase regulatory subunit p85 (PI3K p85), and IL2 Inducible T Cell Kinase (Itk) [32]. SH2 domain binding motif is also a trans-regulatory site that controls the TIM-3 signal transduction [33] (Fig. 1). As depicted in Fig. 1, the TIM-3 structure is presented including glycosylations sites and the cytoplasmic tail for binding to its ligands. All the ligands and their corresponding cellular occurrence are presented for initiating signaling events.

TIM-3 Ligands

Various ligands have been identified for this receptor which include phosphatidylserine (PS), high-mobility group box-1 (HMGB-1), Gal-9 (C-type lectin), and carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM-1) [34]. The PS is a common ligand for all three subtypes of TIM. PS is a non-protein ligand for TIM-3 and binds to the 'FG-CC' loops of the IgV domain of TIM-3. PS is also a ligand for other TIM family proteins. This interaction of TIM-3 with PS may result in the co-expression of interleukin (IL) -10 in T cells in association with TIM-3 and binds to FG-CC loops of the IgV domain of TIM-3 [35]. PS is also a ligand for other TIM family proteins [28,29,35]. Allelic variants of murine TIM-3 are functionally different in their recognition of PS and removal of apoptotic cells [35]. This interaction of TIM-3 with PS may result in the co-expression of IL-10 in T cells in association with TIM-3 (Fig. 1).

HMGB1 is another TIM-3 ligand [30]. TIM-3 in the tumor microenvironment (TME) binds to HMGB1 and hinders the transport of nucleic acids into endosomes. This suppresses pattern recognition receptor (PRR) mediated in-

nate immune responses to tumor-derived nucleic acids [30]. Regions of TIM-3 that bind HMGB1 overlap with those for CEACAM-1, particularly at the 'FG-CC' loop in the IgV domain, suggesting potential competition for binding between HMGB1 and CEACAM-1. Liver-primed $CD8^+$ T cells downregulate antiviral adaptive immunity through Gal-9 independent TIM-3 engagement of HMGB1 in mice [36]. TIM-3 acts as a "sink" to sequester HMGB1 on these T cells which prevents HMGB1 induced activation of hepatic $CD8^+$ T cells [36]. TIM-3 inhibits immune responses (IR) via its interaction with HMGB1 and prevents HMGB1/cell-free DNA collection binding to toll-like receptors (TLRs) [30].

C-type lectin Gal-9, a 35 kilodalton (kDa) protein was identified as the TIM-3 ligand [10]. Gal-9 is a soluble protein having two tandemly linked carbohydrate recognition domains, which recognizes N-linked sugar chains in the TIM-3 IgV domain but not those of TIM-2 and TIM-4. Gal-9 and TIM-3 interaction trigger cell death in Th1 effector cells, and thereby reduces tissue inflammation leading to the inhibition of experimental autoimmune encephalomyelitis (EAE) [10]. Gal-9 and TIM-3 interaction on monocytes/macrophages results in a change in cytokine production, affecting Th1 or Th17 response involving variations in IL-12 and IL-23 production [37,38]. Gal-9 also enhances the killing of $TIM\text{-}3^+$ $CD8^+$ tumor-infiltrating lymphocytes (TILs) in colon cancer [39]. Besides that, Gal-9 also increases TIM-3-induced IFN-γ synthesis in an NK cell line [40].

A 60 kDa protein called CEACAM-1 is another TIM-3 ligand [31]. The membrane-distal IgV domains of both CEACAM-1 and TIM-3 are congruent in structure and interact with the 'FG-CC' interface, which was predicted as a ligand-binding site [27,31]. The TIM-3 glycosylation requires the co-expression of CEACAM-1 for stabilization, and the inhibitory function of TIM-3 gets compromised due to the loss of CEACAM-1 expression. This relationship is based on the presence of cis interaction between TIM-3 and CEACAM-1. A CEACAM-1-TIM-3 trans interaction inhibits T effector cell function and maintains T cell tolerance. Co-expression of CEACAM-1 with TIM-3 on dysfunctional T cells enhances and stabilizes TIM-3 inhibitory functions. Different regions within the IgV domain of TIM-3 interact with Gal-9 and CEACAM-1. These interactions lead to the same downstream events when a negative regulator of TIM-3, known as HLA-B associated transcript 3 (BAT3), is released from its binding sites located in the cytoplasmic tail of TIM-3 [33]. Gal-9 and CEACAM-1 perform cooperative effects in regulating TIM-3 signaling, indicating that coordinate interactions may regulate the functions of specific immune responses (Fig. 1).

Unique Features of TIM-3

TIM-3 is constitutively present on many types of cells belonging to the myeloid lineage and serves as a receptor for HMGB-1 and PS, which are primarily expressed on innate immune cells. Unlike CTLA-4, Lag-3, PD-1, and B and T lymphocyte attenuator (BTLA), TIM-3 interaction with ligands expressed on antigen-presenting cells has not been reported. Besides its traditional expression on activated and exhausted T cells, TIM-3 is also reported in myeloid cells derived from breast cancer samples, suggesting its role in antigen-presenting cells like macrophages and dendritic cells (DCs) [41]. Antibodies targeting human TIM-3 augment T cell responses either alone or in blend with PD-1 inhibitors, offering a rationale for exploring new strategies against anti-cancer immunity via TIM-3 [42–45]. TIM-3 antibodies induce activating signals in human DCs [6,46].

Signaling of TIM-3

TIM-3 is considered a negative regulator in IR and regulates IFN-γ-secreting $CD4^+$ Th1 and $CD8^+$ T lymphocytes and is responsible for the exhaustion and dysfunction of T cells in multiple settings. TIM-3 does not have either ITIMs or ITSMs motif in its cytoplasmic tail and is not accompanied by structural outfits for the recruitment of phosphatases (inhibitory), likely to be involved in the phosphorylated protein inactivation for proximal T cell signaling events. Cytoplasmic tails of TIM-3 in mice and humans possess five conserved Tyr residues. Phosphorylation of two Tyr, Y256 and Y263 in mice and Y265, 272 in humans, is critically important for coupling to downstream signaling pathways. The interaction of TIM-3 and T cell receptor (TCR) signaling pathway, including the zeta chain associated protein kinase 70 (ZAP-70) and SH2 domain-containing leukocyte protein 76 (SLP-76) enhances nuclear factor of activated T cells (NFAT) and nuclear factor kappa b (NF-κB) activation, leading to amplified T cell signaling [32]. TIM-3 positive human $CD4^+$ T lymphocytes generate less IL-2 following stimulation with phorbol ester and a calcium ionophore mediated by inhibition of NFAT dephosphorylation and activator protein-1 (AP-1) transcription [47]. Clayton *et al.* [48] demonstrated that TIM-3 is recruited to the immune synapse between $CD8^+$ T lymphocytes and the target cells, hinders stable synapse formation events, and associates with receptor phosphatases that suppress TCR signaling. Thus, TIM-3 interaction with multiple partners promotes crosstalk with immune cell signaling components, resulting in immune activation or immunosuppression (Fig. 2).

The TIM-3 ligand Gal-9 negatively regulates Th1 type immunity with intracellular calcium flux, aggregation and death of Th1 cells [10]. Gal-9 introduction *in vivo* causes suppression of Th1 dependent IR with selective elimination of IFN-γ producing cells [10]. BAT3 enhances T cell responses and autoimmunity by obstructing TIM-3-induced exhaustion and cell death. BAT3 binds to the C-terminal end of TIM-3 via Y256 and Y263, forming a molecular complex via recruitment of LCK and enhances T cell signaling and downregulates TIM-3-aggravated cellular ex-

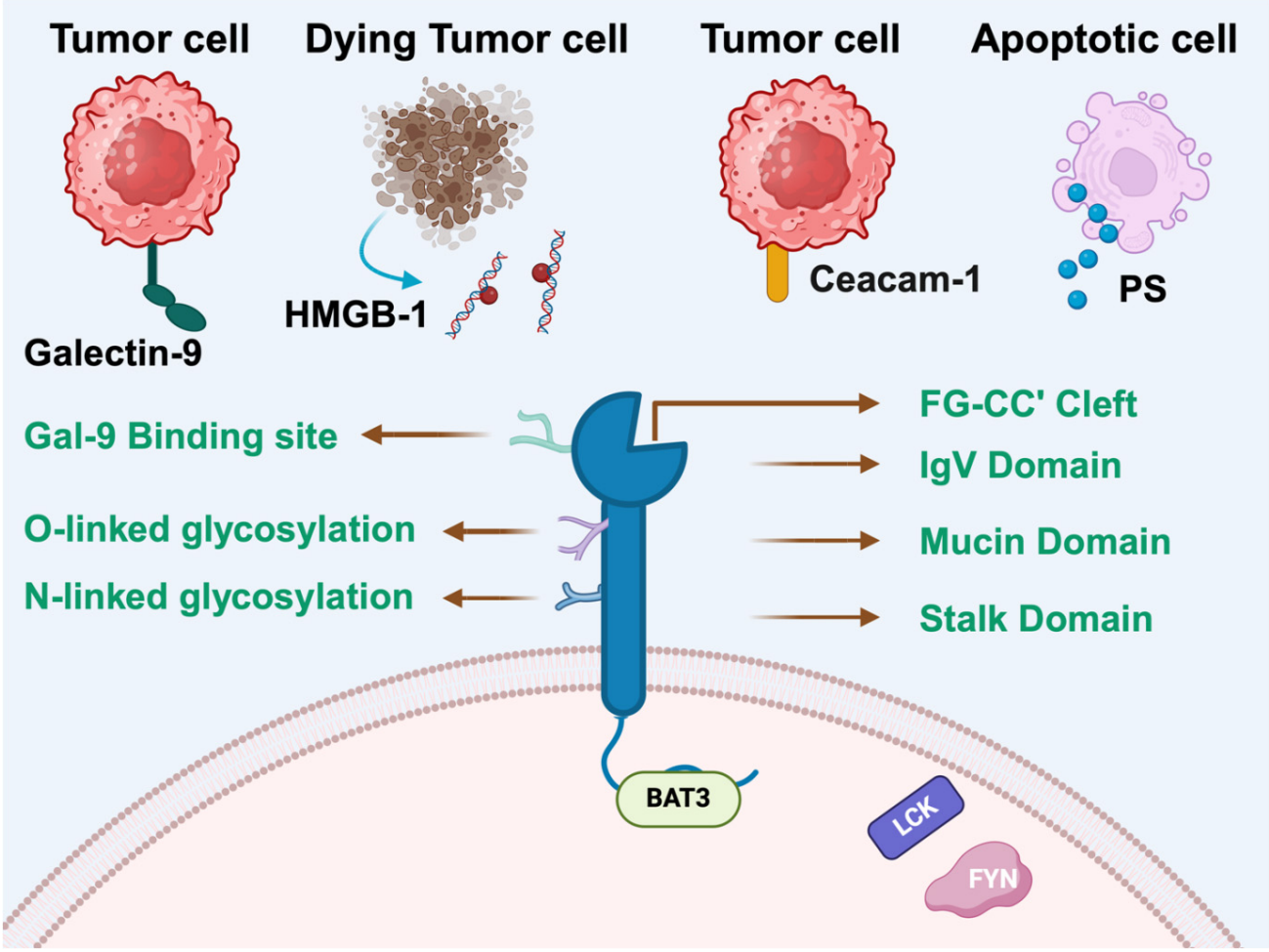

Fig. 1. The TIM-3 molecular structure and ligands. T cell immunoglobulin and mucin domain-3 (TIM-3) is present on mouse chromosome *11B1.1* and human chromosome *5q33.2* and have four parts: the immunoglobulin-like domain (IgV domain), mucin domain, transmembrane region (stalk domain) and cytoplasmic region. The TIM-3 protein has an extracellular region with an IgV domain, a mucin-like domain, and a stalk domain. It also has a cytoplasmic tail with tyrosine-based signaling motifs. The mucin and stalk domains contain N-linked and O-linked glycosylations. The ligands for TIM-3 include Galectin-9 (Gal-9), carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM-1), high-mobility group box-1 (HMGB-1), and phosphatidylserine (PS). The IgV domain of TIM-3 has an FG-CC cleft that is thought to interact with its ligands, such as CEACAM-1, HMGB-1 and PS. Gal-9 binds to the N-linked sugar chain present in the immunoglobulin variable-like domain. The binding of these ligands to the extracellular region of TIM-3 initiates signaling cascades that modulate T cell function. Created with https://www.biorender.com.

haustion and apoptosis [33]. TIM-3 interaction with Gal-9 and CEACAM-1 phosphorylate Y256 and Y263 and releases BAT-3 from the TIM-3 tail, which promotes TIM-3-mediated T cell inhibitory functions by allowing binding of Src kinases having SH2 domain and with subsequent regulation of TCR signaling. Higher BAT3 expression hinders TIM-3's orchestrated inhibitory regulation in signaling and augments T cell effector functions. On the other hand, attenuated BAT3 expression enhances puissant TIM-3-induced inhibitory signaling. BAT3 expression was reduced in TIM-3^{+} PD-1^{+} TILs but not in TIM-3^{-} PD-1^{+} TILs of murine CT26 colorectal carcinomas. Besides T cells, TIM-3 expression on monocytic cells, including DCs and microglia, increases their expression of co-stimulatory receptors and cytokines [6]. Thus, TIM-3-induced stimulation facilitates hyperactivation signaling in T cells, causing extensive immunomodulation leading to immune dysfunction and exhaustion (Fig. 2).

Biological Unique Characteristics of TIM-3

CTLA-4 is reportedly upregulated on the effector T cells and T regulatory cells (T_{regs}). Thus, blockade of CTLA-4 inhibits the effector T cells or T_{regs} functions [2,49, 50]. This is important because the blockade of widely expressed checkpoint receptors could promote autoimmune-like side effects. Similar to CTLA-4, PD-1 is also upregulated on all T effector cells, and autoimmune-like toxicities are observed in patients following treatment with antibod-

ies against PD-1 [51,52]. TIM-3, on the other hand, is not expressed in all types of T cells and is selectively present in T lymphocytes with differentiated IFN-γ producing phenotypes [53]. In cancer patients, TIM-3 is expressed primarily in intra-tumoral T lymphocytes [54,55]. Thus, it is less likely that TIM-3 blockade would interfere with the regulation of T cell mediated IR outside the tumor tissue compared with either CTLA-4 or PD-1. Unlike PD-1 or CTLA-4 deficient mice, TIM-3 deficiency does not exhibit autoimmunity [13,56,57] and tumor-bearing mice treated with anti-TIM-3 antibodies also do not show signs of autoimmunity [20]. The lack of ITIMs or ITSMs in the intracellular portion of TIM-3 does not result in functional redundancy compared with other ITIMs/ITSMs bearing checkpoint receptors. The cytoplasmic segment of TIM-3 binds to BAT3, which is released and triggered by the Gal-9-mediated phosphorylation of the TIM-3 cytoplasmic section, resulting in the production of the catalytically inactive form of the LCK and aberrant synthesis of IFN-γ and IL-2 [33]. Thus, BAT3 acts as a negative regulator of TIM-3. Unlike ITIMs/ITSMs-containing molecules, which recruit phosphatases and de-phosphorylate key TCR signaling intermediates, TIM-3 affects the signaling events downstream of TCR activation differently. This observation suggests that TIM-3 may improve clinical efficacy in cancer treatment, especially when combined with PD-1 blockade, leading to enhanced outcomes in preclinical cancer models.

TIM-3 as a Target in Cancer Immunotherapy

Besides expressing on non-myeloid and myeloid immune cells, a wide variety of cancer cells also express TIM-3, which include melanoma, osteosarcoma, clear cell renal cell carcinoma (ccRCC) and cervical cancer [58–60]. In acute myeloid leukemia (AML) patients, TIM-3 expression is elevated in leukemia stem cells (LSCs) and not in normal hematopoietic stem cells (HSCs), suggesting that TIM-3 could be a potential prognostic marker for the presence of LSCs in AML patients [61,62]. TIM-3 was also found in lymphoma-derived endothelial cells [63]. TIM-3 promotes tumor progression by facilitating tumor cell migration and invasion via suppressing $CD4^+$ T lymphocytes and by activating the IL-6/signal transducer and activator of transcription 3 (STAT3) pathway which prevents Th1 polarization [63]. TIM-3 also activates the mammalian target of rapamycin (mTOR) functions in AML cells [64]. TIM-3 expression on TILs and tumor cells may play a key role in immune escape for the tumor via immunosuppressive myeloid-derived suppressor cells (MDSCs) through the Gal-9-dependent mechanism.

Rational for Targeting TIM-3 in Cancer

TIM-3 expression and $CD8^+$ T lymphocyte exhaustion are closely connected to diverse pathogenesis. Preclinical studies in cancer have documented that TIM-3 acts as a critical checkpoint inhibitor of antitumor immune surveillance. TIM-3 expression in exhausted T lymphocytes is also accompanied by PD-1 presence in human and animal studies, suggesting a correlation between PD-1 and TIM-3 in developing T lymphocyte exhaustion [16,17,21]. Combined blockade of TIM-3 and PD-1 is more effective, leading to markedly higher tumor regression compared with monotherapy with either combination [17,20]. Failure of PD-1 monotherapy blockade or PD-1 adaptive resistance in a mouse lung adenocarcinoma model is associated with the upregulation of TIM-3 [65]. This study indicates that binary therapy involving simultaneous blocking of PD-1 and TIM-3 may abrogate adaptive resistance to PD-1 monotherapy and offer an effective alternative therapeutic solution for patients with the above condition [65]. In cancers like melanoma, key mutations related to acquired resistance and immune escape, accompanied by PD-1 blockade may offer potential benefits of using anti-TIM-3 alternatives in preventing the progression of the disease associated with adaptive resistance to specific checkpoint inhibitors [66]. TIM-3 was also targeted for a positive response in preclinical colorectal cancer models, refractory to the combination of CTLA-4 and PD-1 blockade.

Patients with melanoma treated with either anti-CTLA-4 or anti-PD-1 antibodies develop autoimmune-like toxicities [52,67]. In these conditions, targeting TIM-3 is a valuable option since TIM-3 is only expressed selectively in T lymphocytes and intratumoral T cells [54,68]. Unlike CTLA-4 or PD-1 deficient mice, TIM-3 null mice do not show autoimmunity, suggesting TIM-3 is a safer choice as a checkpoint inhibitor compared with targeting either CTLA-4 or PD-1 or both [13,56,57]. Also, tumor-bearing animals treated with anti-TIM-3 antibodies do not develop autoimmunity [20]. Combination therapy with TIM-3 blockade with agonistic antibodies against pro-stimulatory molecules on tumor specific T lymphocytes, such as CD137, showed long-term anti-tumor protection in an experimental ovarian cancer model [69]. All the above studies surely suggest that the TIM-3 blockade could offer novel immunotherapeutic opportunities to treat cancers. The presence of a mutation in a tumor disables the power of immunogenicity, and immunosuppression in TME and renders intrinsic resistance to therapy with immune checkpoint inhibitors. Despite some positive clinical outcomes, the low patient response rate remains a significant problem, likely due to the enhanced expression of programmed cell death-ligand 1 (PD-L1) and other checkpoint receptors such as TIM-3, LAG-3 and TIGIT [68,70,71].

Immunomodulatory Role of TIM-3 in Cancer: Emphasis on Lymphoma and Solid Tumors

Immunomodulatory roles of TIM-3 aiming at therapeutic targeting, including CTLA-4 and PD-1, are immensely important in clinical trials for treating selected

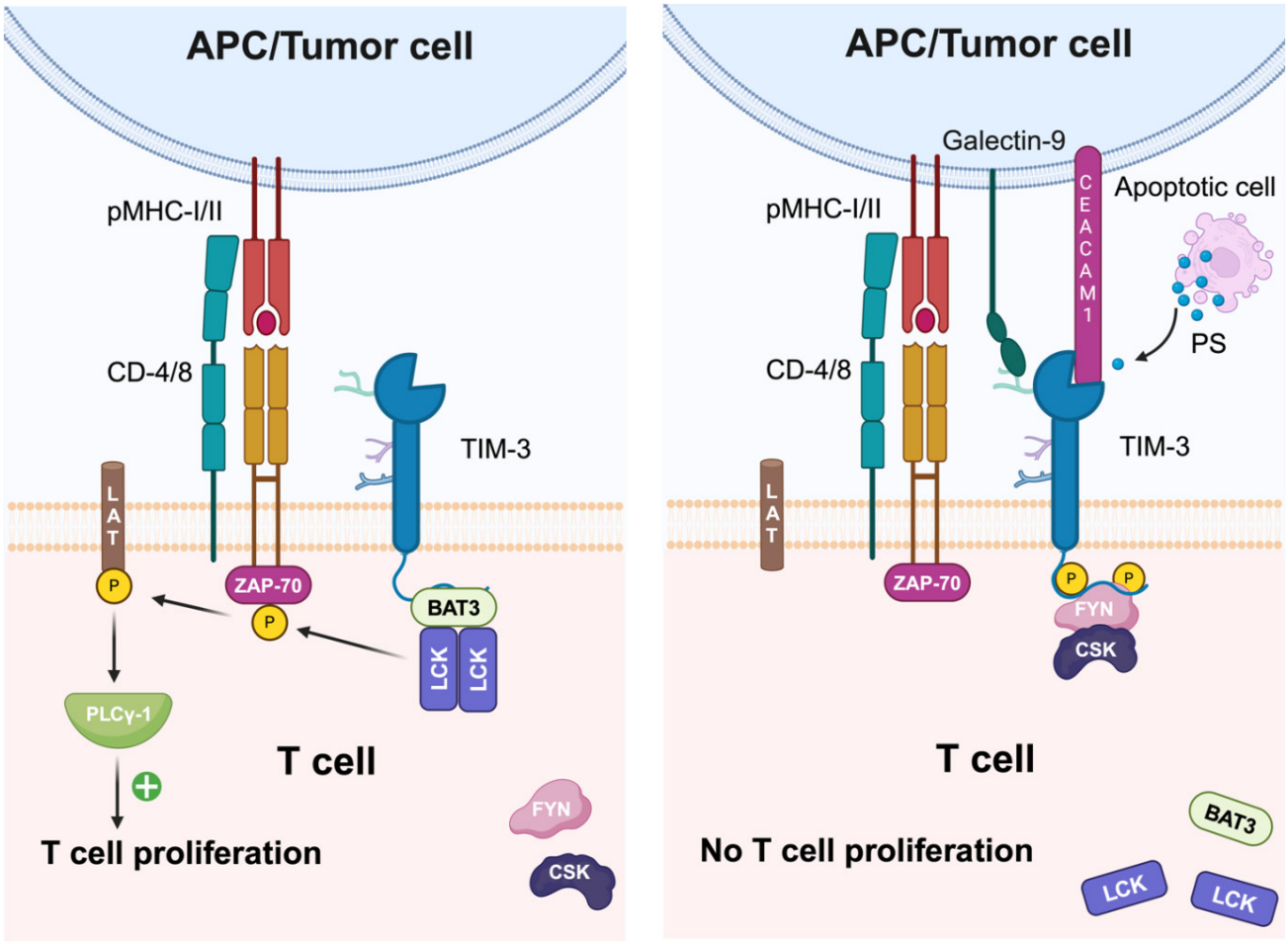

Fig. 2. TIM-3 signaling in T lymphocyte activations. In the absence of a ligand, the cytoplasmic tail of TIM-3 remains unphosphorylated and interacts with HLA-B-associated transcript 3 (BAT3). This interaction facilitates the recruitment of the active form of lymphocyte-cell specific protein tyrosine kinase (LCK), a Src family tyrosine kinase. LCK then phosphorylates various tyrosine residues located within the immunoreceptor tyrosine-based activation motifs (ITAMs) found in the cytosolic domains of the T cell receptor zeta and CD3 chains. The phosphorylated tyrosine residues on the ITAMs serve as docking sites for zeta-associated protein 70 (ZAP-70), a member of the Syk family of tyrosine kinases, which binds to these phosphorylated residues through its SH2 domain. LCK subsequently phosphorylates and activates ZAP-70. The active ZAP-70 further phosphorylates tyrosine residues on the linker for activation of T cells (LAT), which in turn leads to the recruitment of downstream signaling molecules such as phospholipase C-gamma (PLC-γ), ultimately promoting T cell proliferation. The binding of Gal-9, CEACAM-1, and PS to TIM-3 induces the phosphorylation of specific Tyr residues in the cytosolic tail of TIM-3, thereby inhibiting its interaction with BAT3. Following the dissociation from BAT3, the TIM3 protein engages with FYN, a member of the Src family of protein kinases. This interaction facilitates the recruitment of the C-terminal c-Src kinase, which subsequently phosphorylates the C-terminal Tyr residue of LCK. This phosphorylation event leads to a negative regulatory effect on LCK activity, ultimately inhibiting T cell functions. Created with https://www.biorender.com.

types of human cancer. TIM-3 constitutes an attractive therapeutic target in cancer immunotherapy since it exerts its effect on key regulatory circuits of innate and acquired immune responses for restoring antitumor immunity. The immunomodulatory role of TIM-3 in lymphoma and various forms of solid tumors is described below (Fig. 3).

Lymphoma

Diffuse large B cell lymphoma (DLBCL) is the most prevalent form of non-Hodgkin lymphoma (NHL), characterized by a diverse array of biologically distinct subtypes that lead to the clonal expansion of malignant B cells, either from the germinal center or post-germinal center. The conventional therapeutic approach for DLBCL continues to be chemo-immunotherapy, specifically utilizing the R-CHOP regimen, which includes rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone. Recent investigations into the genomic and transcriptomic profiles of DLBCL have revealed specific patient subsets that exhibit unfavorable responses to chemo-immunotherapy [72].

The prevalence of FoxP3$^+$ CXCR5$^+$ CD4$^+$ T follicular regulatory (Tfr) cells in the peripheral blood of patients with DLBCL is markedly elevated in comparison to healthy control individuals, particularly among those with less advanced stages of DLBCL. Furthermore, these Tfr cells may play a role in the IR within the TME [73].

TIM-3$^+$ Foxp3$^+$ T_{reg} (TFT) cells are found in elevated quantities in lung and colorectal cancers, with their prevalence linked to disease advancement and unfavorable outcomes. In patients with DLBCL, TFT cells are significantly abundant within the TME and are associated with poor prognosis. These cells contribute to the progression of DLBCL, partly by releasing IL-10 within the TME, a process that anti-TIM-3 antibodies can inhibit [74]. DLBCL tissues demonstrate elevated levels of TIM-3 expression, which is associated with a poor prognosis [75,76]. In DLBCL, Gal-9 produced by M2 macrophages within the TME facilitates the exhaustion of CD8$^+$ T lymphocytes via the TIM-3/Gal-9 signaling pathway, resulting in immune evasion [77]. Activated DLBCL is characterized by elevated levels of PD1$^+$ TIM3$^+$ TILs that exhibit diminished functionality. Notably, these exhausted PD-1$^+$ TIM-3$^+$ TILs can regain functional activity following the inhibition of either PD-1 or TIM-3 [78].

TIM-3 and LAG-3 are present in Hodgkin and Reed/Sternberg (HRS) cells and within the TME associated with classical Hodgkin lymphoma (cHL). This observation may provide a rationale for the binary targeting of LAG-3 or TIM-3 alongside anti-PD-1 antibodies in the therapeutic approach for relapsed cHL [79]. TIM-3 is expressed positively in 95% of patients with extranodal NK/T cell lymphoma (ENKTL) and may serve as a prognostic indicator for this patient population [80]. IL-12 promotes T cell dysfunction through the upsurge in TIM-3 expression on T lymphocytes within the tumor microenvironment. This mechanism significantly affects the adverse effects observed in follicular B cell non-Hodgkin lymphoma (FL). Notably, there is an increased prevalence of TIM-3 expressing T lymphocytes in FL, which exhibit diminished signaling capabilities and functional responses [55]. The transcriptional analysis of the endothelium associated with lymphoma indicates that TIM-3 is predominantly expressed in endothelium-derived lymphoma. This TIM-3-expressing endothelium engages with and inhibits the activation of CD4$^+$ T lymphocytes, thereby promoting lymphoma progression [63]. In an *ex vivo* study, it was observed that CD8$^+$ TIM-3 T lymphocytes are significantly enriched in FL lymph node samples. These cells demonstrate impaired cytokine synthesis and reduced activation of the lytic potential in comparison to their CD8/TIM-3 negative counterparts [81].

TIM-3 is significantly upregulated in tumor cells of DLBCL and TILs. A robust association exists between elevated TIM-3 protein expression and poorer overall survival outcomes. This indicates that the TME in DLBCL is likely influenced by TIM-3 expression in both tumor cells and TILs, resulting in diminished immune surveillance and reduced tumor clearance [82]. The assessment of TIM-3 within a group of peripheral T cell lymphomas (PTCL), which encompasses peripheral T cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma (ALCL), angioimmunoblastic T cell lymphoma (AITL), extranodal natural killer T cell lymphoma (ENKTCL), and enteropathy-associated T cell lymphoma (EATL), revealed that TIM-3 was either absent or exhibited minimal expression in the context of PTCL and their associated TME [83]. In subcutaneous panniculitis-like T cell lymphoma (SPTCL), a rare and uncommon cytotoxic T cell NHL, is associated with hemophagocytic lympho histiocytosis (HLH). This life threatening immunological activation adversely affects survival of the patients [84]. Mutations leading to TIM-3 alterations are considered as a cause for genetical defect in SPTCL. SPTCL patients with TIM-3 mutation benefit from immunomodulation, and therapeutic repression of TIM-3 may have adverse consequences [15]. DLBCL patients express high levels of PD-1 and TIM-3, which are correlated to the staging of the disease. PD-1 and TIM-3 existence is also associated with the efficiency of chemotherapy, suggesting that their expression could be used as a potential biomarker for chemotherapeutic efficacy in DLBCL patients [85] (Fig. 3).

Prostate Cancer

TIM-3 expression in prostate cancer was markedly increased in tumor tissues, indicating their possible role as important biomarkers for the diagnosis of this disease [86]. In the mouse model of prostate cancer, TIM-3 negatively regulates the synergistic effects of anchored granulocyte-macrophage colony-stimulating factor (GM-CSF) vaccine and anti-PD-1 antibodies. TIM-3 expression was found to be enhanced and is resistant to the binary therapy mentioned above. Sequential administration of antibodies against PD-1 and TIM-3 improves the efficacy of the anchored GM-CSF therapy resulting in significant reduction in the tumor volume for over 60% of the treated animals [87]. Functional prostate-specific antigen (PSA) responsive CD8$^+$ T cells in prostate cancer patients was reduced to $<$50% compared to healthy individual males. PSA146-154 responsive CD8$^+$ T showed elevated expression of CD38 (activation marker) and TIM-3 (exhaustion marker) in these patients suggesting that PSA-specific cells are immunologically exhausted [88].

Glioma

TIM-3 and also PD-1 were markedly upregulated on T lymphocytes that infiltrate gliomas. Binary therapy with antibodies against PD-1 and TIM-3 in conjunction with stereotactic radiosurgery enhances antitumor immune responses and results in long-term survival in an experimental model of murine glioma [89]. TIM-3 expression is

relatively high in glioma and unlike other tumors, TIM-3 is predominantly present in macrophages residing in the glioma microenvironment [90]. TIM-3 is expressed significantly higher in tumor cells and microglia and macrophages of TME in diffuse intrinsic pontine glioma (DIPG). DIPG is an aggressive brainstem tumor and considered as the leading cause of paediatric cancer-related death. Abrogation of TIM-3 in syngeneic models of DIPG prolong disease free survival and harbour immune memory cells, indicating clinical relevance of TIM-3 inhibition in tumor cells of DIPG [91]. TIM-3 mRNA and protein have been detected in glioma cells, and knocking down TIM-3 expression improved the potential of temozolomide (TMZ) treatment [92]. Glioma cell-intrinsic TIM-3 regulates malignant behaviours of glioma cells and induces migration of macrophages and its conversion to pro-tumorigenic and anti-inflammatory phenotype by TIM-3/IL-6 signaling [93]. PTEN-deficient glioblastoma multiforme (GBM) cells secrete elevated levels of Gal-9 through the Ak strain transforming (AKT)-Glycogen Synthase Kinase 3 Beta (GSK3β)-Interferon Regulatory Factor 1 (IRF1) pathway. This promotes the polarization of macrophages towards the M2 phenotype by activating the TIM-3 receptor and its associated downstream signaling pathways. Consequently, these polarized macrophages secrete vascular endothelial growth factor A (VEGFA), which stimulates angiogenesis and supports the enhanced proliferation of gliomas [94]. Enhanced TIM-3/Gal-9 signaling forecasts poor outcomes in glioma patients, and blockade of its expression inhibits M2 polarization of macrophages and impair tumor growth [94].

TIM-3/Gal-9 axis is linked with the poor prognosis and induces NOD-like receptor family CARD domain-containing 4 (NLRC4) inflammasome formation and activation in glioma patients. TIM-3/Gal-9 blockade in glioma could reduce the inflammatory microenvironment by abrogating the NLRC4 inflammasome [95]. Gliomas with chromosome *1p/19q* co-deletion were considered as a specific tumor entity that promotes the antitumor IR by downregulating the expression of TIM-3 and Gal-9 [96]. A concomitant increase in TIM-3 expression and significantly higher levels of C-C Motif Chemokine Ligand (CCL)-7, CCL18, and C-X-C Motif Chemokine Ligand (CXCL)-13 were observed in glioblastoma tissues compared with the tissues with low or marginal TIM-3 expression. TIM-3 expression was positively correlated to significant immune cell infiltration [97]. Isocitrate dehydrogenase (IDH) mutation in diffuse glioma is associated with low levels of TIM-3$^+$ cells and fewer interactions with Gal-9$^+$ microglia/macrophages, indicating reduced Gal-9/TIM-3 interactions in IDH-mutant astrocytic gliomas [98]. Glial TIM-3 is unique and responds distinctively to brain tumors by playing distinct intracellular and intercellular immunoregulatory roles which differ from TIM-3 on T lymphocytes in the TME. In glioblastoma, TIM-3 also plays a role in mediating myeloid cell responses. TIM-3 expression was found to be lower in tumor-infiltrating CD11b$^+$ CD45$_{mid}$ glial cells but higher in tumor-infiltrating CD8$^+$ T cells [99]. In malignant glioma patients, TIM-3 expression is higher in peripheral CD3$^-$ CD56$^+$ NK cells and CD14$^+$ monocytes. TIM-3$^+$ monocytes demonstrate M2-like phenotype. TIM-3 expression on both monocytes and NK cells positively correlates with the ratio of Ki-67$^+$ tumor cells and a high risk of recurrence or death [100]. Deficiency of miR-15a/16, a micro RNA (miRNA) cluster located at chromosome *13q14* in a mouse glioma model, showed tumor-infiltrating CD8$^+$ T cells with reduced expression of PD-1, TIM-3 and LAG-3, and have more vigorous secretion of IL-2, IFN-γ, and tumor necrosis factor alpha (TNF-α) than wild type tumor-infiltrating CD8$^+$ T cells, alleviating glioma progression via targeting mTOR [101]. Binary therapy of anti-TIM-3 antibody plus stereotactic radiosurgery or combination of anti-TIM-3 and anti-PD-1 antibodies enhanced the life span of the patients compared with treatment with anti-TIM-3 antibody only. Triple combination of the above therapies resulted in 100% overall survival with increased infiltration, and activation of immune cells and establishment of immune memory responses [89]. TIM-3 expression was significantly increased in both CD4$^+$ and CD8$^+$ T lymphocytes in glioma patients compared to control. Patients with advanced cancer and higher tumor grade have abundance of TIM-3 in CD8$^+$ T lymphocytes compared with patients with less advanced tumor grade. The Karnofsky score of glioma patients was negatively correlated with TIM-3$^+$ CD8$^+$ T cells percentage, suggesting the involvement of TIM-3 for the emergence and development of glioblastoma [102].

Hepatocellular Carcinoma (HCC)

Gal-9, the ligand for TIM-3, is significantly up-regulated in Kupffer cells located within the TME of hepatocellular carcinoma. This up-regulation of Gal-9 in liver Kupffer cells is facilitated by IFN-γ, secreted by T lymphocytes present in the TME. The *ex-vivo* interaction between Kupffer cells and T lymphocytes obtained from HCC patients results in T cell anergy, demonstrating a direct involvement of TIM-3 in the immunosuppression process [103]. A critical factor contributing to immune evasion in HCC is the exhaustion of CD8$^+$ T lymphocytes mediated by TIM-3. Research has indicated that long noncoding RNA associated with TIM-3 promotes CD8$^+$ T cell exhaustion by interacting with the intracellular domain of TIM-3, which disrupts the binding of BAT3. This disruption inhibits the formation of the TIM-3/BAT3 signaling complex, which is essential for preventing cell death and exhaustion. When BAT3 remains unbound, it translocates to the nucleus, enhancing the transcription of anti-apoptotic genes and thereby supporting the survival of exhausted CD8$^+$ T lymphocytes in HCC patients [104]. NK cells are also implicated in the immune evasion of HCC. The expression

levels of TIM-3 and TIGIT on NK cells have been found to correlate directly with accelerated tumor progression in patients with hepatitis B virus (HBV)-related HCC. The presence of these cells is also associated with poor prognosis. NK cells expressing both TIGIT and TIM-3 produce lower levels of TNF-α, IFN-γ, and CD107a compared to their TIGIT and TIM-3-negative counterparts, indicating characteristics of an exhausted phenotype [105]. Over-expression of TIM-3 in SMMC-7721 cells correlates with decreased E-cadherin levels and escalated the expression of N-cadherin, Twist 1, matrix metalloproteinases (MMPs), Slug, Snail, and Sma and Mad-related proteins (Smad). This suggests that TIM-3 has a role in epithelial-mesenchymal transition (EMT), and thereby facilitating metastasis in HCC [106]. Blocking of TIM-3 on $CD4^+$ and $CD8^+$ T lymphocytes enhances their effector capabilities, resulting in reduction of T cells exhibiting an exhausted phenotype [107]. Combining TIM-3 inhibition with radiation therapy has been demonstrated to trigger apoptosis in tumor cells, leading to enhanced survival rates in mouse models of HCC [108] (Fig. 3).

Gastric Cancer

Gastric cancer ranks fourth in most common cancer types and accounts for the second leading cause of cancer-related deaths. $CD4^+$ and $CD8^+$ T lymphocytes of gastric cancer express TIM-3 markedly higher compared with gastritis. The expression of TIM-3 in $CD4^+$ T cells was related to the depth of invasion and lymph node metastasis [109]. TIM-3 plays an essential role in the invasion and metastasis, leading to adverse prognosis in gastric cancer. TIM-3 gene polymorphisms was also responsible for gastric cancer. Individual genotype analysis confirmed that the *1516G/T*, *574G/T* and *882C/T* polymorphic loci of the TIM-3 gene promoter were connected with the susceptibility to gastric cancer. The *1516G/T* locus promotes distant metastasis [110]. The survival of TIM-3 positive gastric tumor patients was substantially lower compared with patients having low or negative TIM-3 expression. TIM-3 over-expression in tumor cells influence poor gastric cancer prognosis [111].

Breast Cancer

Tumor-infiltrating $CD8^+$ T cells from breast tumors exhibit a notable increase in TIM-3 expression and a significantly elevated level of the apoptotic marker Annexin V when treated with IL-15. Blocking TIM-3 significantly enhances the effector functions of $CD8^+$ T lymphocytes, induced by IL-15. Therefore, combining IL-15 with TIM-3 blocking antibodies may represent a viable alternative to IL-2 for the expansion of tumor-associated $CD8^+$ T cells *in vitro*, a critical component of adoptive T cell therapy [112]. TIM-3 in breast tumor tissues plays a pivotal role in disrupting the integrity of tight junctions by modulating the expression of proteins associated with these junctions. Furthermore, TIM-3 enhances tumor-associated angiogenesis, and its overexpression affects molecules related to EMT and facilitates metastasis [113]. The regional lymph nodes in breast cancer patients harbour follicular T cells similar to exhausted phenotypes of cytotoxic T lymphocytes, expressing high PD-1 and TIM-3 molecules [114]. Dual inhibition of PD-1 and TIM-3 modulate survival, proliferation, and transformation of T lymphocytes. Cells treated with antibodies against PD-1 and TIM-3 increased the cytotoxic potential of melanoma-associated antigen A11 (MAGE-A11) antigen-specific CTLs against breast tumor cells and may be considered as a potential therapeutic approach against breast cancer [115]. Different types of breast cancer cells express TIM-3 and have critical roles in tumorigenesis, tumor progression and predicting prognosis. TIM-3 blockade induces an antitumor immune response, which includes tumor growth and enhanced chemotherapeutic potential [116]. TIM-3 blockade augments anti-tumor immunity by upregulating genes, suppressing tumor angiogenesis and limiting malignant cell growth via Janus kinases (JAK)-STAT signaling and Wingless-related integration site (Wnt) signaling [117]. Enhanced TIM-3 expression on TILs of triple-negative breast cancer patients is associated with poor response to neoadjuvant chemotherapy [118]. TIM-3 is expressed by $CD4^+$, $CD8^+$ and regulatory T cells in breast tumor-draining lymph nodes and was primarily related to poor prognosis involving invasiveness and higher tumor grade [119] (Fig. 3).

Renal Cell Carcinoma (RCC)

TIM-3 is also expressed in tumor cells of RCC across an extensive series of tumor tissues. Elevated levels of TIM-3 expression in RCC patients are associated with tumor-associated macrophages. They are directly linked to reduced progression-free survival in RCC patients and are considered as a potential biomarker for RCC [59]. TIM-3 expression was found to be different between the local and metastatic tumors in RCC patients. Expression of TIM-3 in primary and metastatic tumors was related to overall progression-free survival for longer periods [120]. Infiltrated $TIM\text{-}3^+$ $PD1^+$ $CD8^+$ T lymphocytes act as a mediator for generating an aggressive phenotype in RCC patients [121]. Variation in TIM-3 and Gal-9 polymorphisms influence susceptibility and overall survival of clear cell RCC patients [122].

Ovarian Cancer (OC)

The importance of TIM-3 in OC deserves further exploration. Aberrant TIM-3 expression in OC indicates its potential importance as a biomarker for the disease. Occlusion of TIM-3 reverses T_{reg} mediated CD8+ T lymphocyte inhibition, suggesting that TIM-3 could be a potential therapeutic target in order to overcome immunotherapy related resistance in OC [123]. Occurrence of TIM-3/CXCL13 positive tissue-resident memory T lympho-

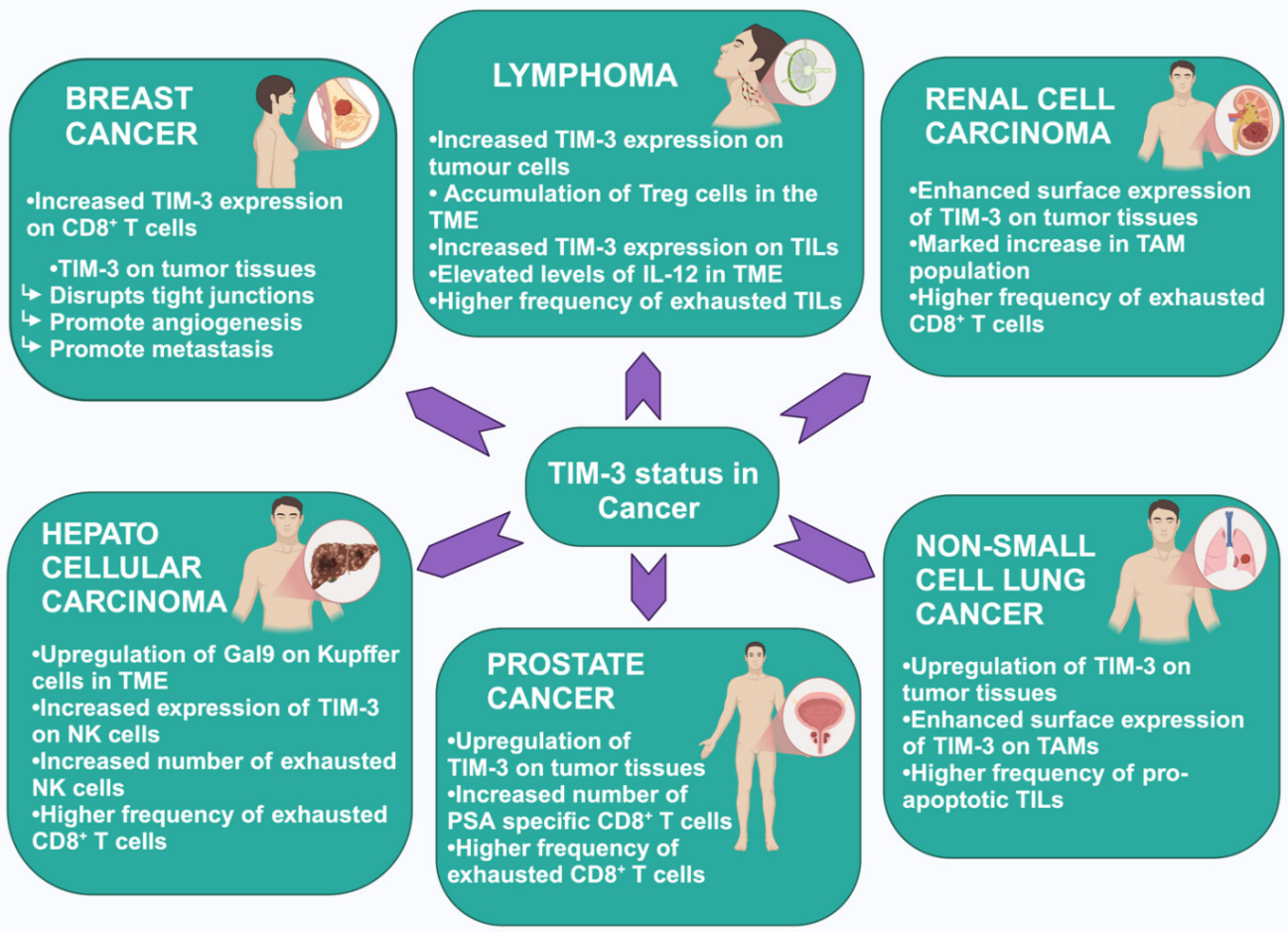

Fig. 3. Immunomodulation of TIM-3 in lymphoma and solid tumors. TIM-3 expression and its interaction with immune cells are depicted in lymphoma and solid tumors. Created with https://www.biorender.com.

cytes (Trm) (CD8/CD103 positive) population in high-grade epithelial ovarian cancer (EOC) has been reported in 175 patients. This study revealed that TIM-3$^+$ Trms were intimately associated with improved patient survival. CXCL13$^+$ CD8$^+$ T lymphocytes were also linked to the anti-PD1 therapy, suggesting a binary inhibition involving TIM-3 and PD-1 may usher an advancement in therapy for the (re)activation of anti-cancer immunity in EOC [124].

Non-Small Cell Lung Cancer (NSCLC)

TIM-3, a key negative regulator in T cell-mediated response, plays a critical role in the clinicopathological characteristics of NSCLC. TIM-3 expresses on NSCLC cells and TILs and covers all the NSCLC pathological types. Degree of TIM-3 expression on NSCLC TILs is correlated with the levels of PD-1 and its ligand PD-L1. High TIM-3 expression in NSCLC patients indicates poor prognosis [125]. TIM-3 expression on tumor-associated macrophages (TAMs) in NSCLC acts as a prognostic biomarker for NSCLC or adenocarcinoma with a less than promising prognosis in patients with the disease [126]. TIM-3 showed distinct tissue/cell distribution, functional implications, and genomic correlation in NSCLC. TIM-3 expression in TILs is associated with activation and proapoptotic phenotypes of T cells [127]. Exosome TIM-3 (Exo-T) and Exosome Gal-9 (Exo-G) were markedly increased in NSCLC plasma compared to that in the healthy control group and positively correlated with larger tumor size, advanced stages, and metastasis in distant organs. This is also associated with extensive lymph node involvement and its metastasis. Plasma from lung squamous cell carcinoma demonstrated a surge in Exo-T and Exo-G than lung adenocarcinoma. Thus, plasma TIM-3 and Gal-9 exosomes are biomarkers and modulate antitumor immune response in patients with lung carcinoma [128].

Soluble TIM-3 (sTIM-3) in humans is a cleaved product from TIM-3 by ADAM10/17, potentially involved in anti-PD-1 resistance in NSCLC. Serum sTIM-3 is upregulated in NSCLC and various digestive tumors, specifically in patients not responding to anti-PD-1 antibody therapy for NSCLC and in cholangiocarcinoma patients who are resistant to anti-PD-1 therapy. sTIM-3 drives exhaustion of terminal T lymphocytes and inhibits CD8$^+$ T lymphocytes response to PD-1 blockade via CEACAM-1. ADAM10 in-

hibitor GI254023X inhibits generation of sTIM-3; abrogate tumor development in TIM-3 humanised mice, and reverses anti-PD-1 mediated resistance in human TILs. This study indicates that human sTIM-3 is a critical immunoregulator with predictive and therapeutic potential in NSCLC [129]. $CD8^+$ T cell exhaustion in NSCLC patients attributed to the TME with the occurrence of PD1, Hematopoietic progenitor kinase 1 (HPK1) and TIM-3. High infiltration of $HPK1^+$ $PD\text{-}1^+$ $TIM\text{-}3^+$ $CD8^+$ T lymphocytes has been identified as an independent risk factor responsible for poor clinical outcome affecting overall response rate, progression-free survival, and eventual life span. This study indicates a comprehensive multilevel immunotherapy strategy is required to ablate the effect of negative modulators [130]. Immunohistochemical analysis of TIM-3 in NSCLC patients showed overwhelming positivity for TIM-3 in tissue samples. Also, TIM-3 presence in NSCLC tumor cells was correlated with tissue histology and pathologic T classification of the disease. TIM-3 positive NSCLC patients have markedly shorter survival times than TIM-3 negative counterparts [131]. The immunomodulatory function of TIM-3 in NSCLC patients was further strengthened by the fact that this regulatory molecule is upregulated substantially on both $CD4^+$ and $CD8^+$ TILs in tumor tissues but rarely observed on T lymphocytes derived from the patient's peripheral blood. Also, $TIM\text{-}3^+$ $CD8^+$ cells have significantly low frequency of $IFN\text{-}\gamma^+$ TILs compared to $TIM\text{-}3^-$ $CD8^+$ TILs. Nearly 70% of $TIM\text{-}3^+$ $CD4^+$ TILs are with low IFN-γ level expressed on FOXP3, and 60% of the $FOXP3^+$ TILs were found to be $TIM\text{-}3^+$. Abundance of TIM-3 on $CD4^+$ T lymphocytes in NSCLC patients directly correlates with poor clinicopathological parameters, associated with nodal involvement and advanced stage cancer. This data showed that TIM-3 is an immunomodulator in TME of NSCLC with its predominant presence in T_{regs} [54] (Fig. 3).

Oral & Esophageal Carcinoma

Oral squamous cell carcinoma (OSCC) is one of the most common types of oral cancer in India for people who consume tobacco. Higher sTIM-3 and squamous cell carcinoma antigen in OSCC patients offer better diagnostic potential, suggesting a positive correlation of sTIM-3 levels with clinicopathological factors of the disease state [132]. Papillomavirus-positive human oropharyngeal head and neck squamous cell carcinoma have reduced TIM-3-expressing macrophages owing to the expression of virus-induced Gal-9. Gal-9 expressing $CD4^+$ T lymphocytes were increased in patients with virus-induced head and neck squamous cell carcinoma. Higher frequency of Gal-9 secreting $CD4^+$ T lymphocytes poses significant risk coupled with an array of immunosuppressive effects. The Gal-9 positive $CD4^+$ T lymphocytes expand $TIM\text{-}3^+$ monocytes, which in turn suppress IFN-γ synthesis by activated $CD8^+$ T lymphocytes [133]. TIM-3 mRNA and protein expression were increased in esophageal squamous cell carcinoma (ESCC) patients. Downregulation of TIM-3 in ESCC cell lines resulted in reduced cell motility, invasion and a reversal of EMT through the inhibition of the Akt/GSK-3β/Snail signaling pathways [134].

Future Perspectives

The complex biological features of TIM-3 are linked with tumorigenesis and immune regulation in inflammation and chronic viral infections. Besides its role in T cells, the expression of TIM-3 on DCs, macrophages, MDSCs, mast cells, and NK cells suggests a broad spectrum of significance in the progression and outcome of neoplasias. Although several aspects of TIM-3 biology have been extensively studied, several elements of a combination regimen involving immunomodulation of TIM-3 may be significant for a better understanding of the tumor immune response. The following points are essential to consider.

(1) Effective targeting of immunosuppressive mechanisms of TIM-3, expressed in diverse cell types (Tregs, and innate immune cells, effector T lymphocytes) for converting TME status to immune-activating state from a suppressive type.

(2) Combinational targeting of TIM-3 with other checkpoint blockers or binary application of TIM-3 inhibition with new immunotherapeutics in order to prime tumor antigen specific T-lymphocytes with potentially durable clinical benefit.

(3) Devising reliable new strategies for combination therapies targeting TIM-3 for better anti-tumor IR and mediating tumor regression in conditions where anti-PD-1 or anti-CTLA-4 failed to deliver desired outcomes, such as in colorectal carcinomas.

(4) Occurrence of TIM-3 upregulation in PD-1 blockade requires TIM-3 inhibition following the development of PD-1 induced adaptive resistance. This issue need to be studied for an effective binary regimen in which TIM-3 abrogation is a key component.

(5) The role of TIM-3 in non-T immune cells, like myeloid cells, needs further investigation. Unlike other checkpoint inhibitors, TIM-3 lacks inhibitory signaling motifs in its structure. A detailed study on the actual behaviour of TIM-3, whether it is an inhibitory or a stimulatory receptor in a varied spectrum of cancer deserves attention.

(6) Multiple combination therapies incorporating TIM-3 can be attempted to achieve synergistic effects and enhanced clinical efficacy compared to monotherapy. This could reduce drug resistance and provide therapeutic anticancer benefits, including attenuating tumor growth, downscaling metastatic potential, triggering apoptosis and elimination of cancer stem cells.

(7) Harnessing TIM-3 for cancer immunotherapy for elucidation of both extracellular and intracellular signals which could drive TIM-3 upregulation. Understanding

the signals responsible for TIM-3-driven suppressive IR and the receptor-ligand interplay operational in immune-suppressive TME is essential.

(8) Exploring novel strategies that combine patient-tailored approaches to enhance IR to specific neoantigens, which could improve the effectiveness of checkpoint inhibitors in cancer treatment. Additionally, searching the application of adoptive cell therapy alongside targeted checkpoint inhibition may offer promising results for anti-cancer immunotherapy.

(9) Molecular analysis of predictive biomarkers, resistance mechanisms, tumor hyperprogression, treatment dynamics, adverse pathological events, and binary therapy strategies is critical for optimizing the benefits and minimizing the risks of checkpoint immunotherapies.

Conclusion

TIM-3 has emerged as a novel second-generation ICI in cancer. Its selective, yet versatile expression in tumor and immune cells ranging from T lymphocytes, macrophages, and DCs makes TIM-3 unique among checkpoint inhibitors. TIM-3 plays a critical role in immunosuppression, highlighting its value as a novel target for tumor immunotherapy. The essential future steps will be investigating and harnessing its role in anticancer immunotherapy to elucidate extracellular and intracellular signals that drive TIM-3 in upregulating immunesuppression and receptor-ligand interactions that are operational in various cancers. The complex biological features of TIM-3, endowed with diverse functions and preclinical applications enable TIM-3 to be linked with targeted therapy in combination modalities with other checkpoint inhibitors for achieving objective response with higher frequency in solid tumors and lymphoma. Unlike other checkpoint receptors, TIM-3 has no inhibitory motif in its structure, which provokes a debate about whether it functions as an inhibitory or stimulatory receptor in cancer. The occurrence of sTIM-3 in diverse malignancies indicates that it has some critical role in neoplasias, including the role of a molecular sink to hinder its interaction with the ligand. The possible role of sTIM-3 as a prognostic biomarker and its impact on specific malignancies deserve close attention and detailed study. Inhibition of TIM-3 in association with other checkpoint inhibitors like PD-1 has produced noteworthy results in preclinical studies and clinical trials. Yet, additional efforts may be incorporated, including adoptive cell therapy-based targeted therapy in combination with TIM-3, which may constitute a novel binary approach for next-generation therapeutic exploration. Future investigation on the molecular mechanisms of TIM-3 in diverse contexts, including poorly managed aggressive cancer is critically important.

Availability of Data and Materials

Not applicable.

Author Contributions

PPM conceptualise the idea. PPM, AC, PY, PC and SS contributed in conception and writing of the manuscript. AC made the diagrams. PPM edited and finalised the manuscript. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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