



The TIM3/Gal9 signaling pathway: An emerging target for cancer immunotherapy

Sashi Kandel^a, Pratik Adhikary^a, Guangfu Li^b, Kun Cheng^{a,*}

^a Division of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO, 64108, USA

^b Department of Molecular Microbiology & Immunology, School of Medicine, University of Missouri, One Hospital Drive, Columbia, MO, 65212, USA

ARTICLE INFO

Keywords:

TIM3/Gal9

Immuno-oncology

Immune checkpoint receptors

Immune checkpoint blockade

Immune suppression

ABSTRACT

Immune checkpoint blockade has shown unprecedented and durable clinical response in a wide range of cancers. T cell immunoglobulin and mucin domain 3 (TIM3) is an inhibitory checkpoint protein that is highly expressed in tumor-infiltrating lymphocytes. In various cancers, the interaction of TIM3 and Galectin 9 (Gal9) suppresses anti-tumor immunity mediated by innate as well as adaptive immune cells. Thus, the blockade of the TIM3/Gal9 interaction is a promising therapeutic approach for cancer therapy. In addition, co-blockade of the TIM3/Gal9 pathway along with the PD-1/PD-L1 pathway increases the therapeutic efficacy by overcoming non-redundant immune resistance induced by each checkpoint. Here, we summarize the physiological roles of the TIM3/Gal9 pathway in adaptive and innate immune systems. We highlight the recent clinical and preclinical studies showing the involvement of the TIM3/Gal9 pathway in various solid and blood cancers. In addition, we discuss the potential of using TIM3 and Gal9 as prognostic and predictive biomarkers in different cancers. An in-depth mechanistic understanding of the blockade of the TIM3/Gal9 signaling pathway in cancer could help in identifying patients who respond to this therapy as well as designing combination therapies.

1. Introduction

Immune checkpoint proteins are stimulatory or inhibitory regulators that play a key role in maintaining immune homeostasis and preventing the onset of autoimmunity [1]. Stimulatory checkpoint proteins like the cluster of differentiation 28 (CD28), OX40, glucocorticoid-induced tumor necrosis factor receptor (GITR), CD137, and CD27 enhance T cell functions, whereas inhibitory checkpoint proteins such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), B and T lymphocyte attenuator (BTLA), V-domain immunoglobulin suppressor of T cell activation (VISTA), and lymphocyte-activation gene 3 (LAG3) suppress T cell immunity [2]. Immune checkpoint proteins help in maintaining a balance between positive and negative signals mediated by T cells. In cancers, inhibitory immune checkpoints are often activated, which protect the tumor cells from immune surveillance [1]. Targeting immune checkpoint proteins is, therefore, one of the most active areas of cancer research. Particularly, checkpoint inhibitors targeting the PD-1/PD-L1 axis and CTLA-4 have been approved for the treatment of different types of cancer.

Among these checkpoint proteins, TIM3 has attracted much attention and is being vigorously studied. TIM3 biology and its suppressing role of antitumor immunity upon interaction with its ligands like Galectin 9 (Gal9) [3], phosphatidylserine (Ptdser) [4], high mobility group box 1 (HMGB1) [5], and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) [6] are being actively studied. A recent review by Wolf Y et al. focused on the four ligands of TIM3, the role of TIM3 in cancer and chronic viral infections, and ongoing clinical trials of anti-TIM3 antibodies [7]. Another review by Solinas C et al. described TIM3 biology in different immune cells, T helper cells (Th1 and Th17), regulatory T cells (Tregs), dysfunctional cytotoxic T lymphocytes (CD8⁺ T cells), natural killer (NK) cells, and dendritic cells (DCs) along with several anti-TIM3 antibodies in clinical trials [8]. These articles discussed the heterogeneous biological role of TIM3 in cancer and the application of TIM3 as a therapeutic target regardless of its specificity to any particular ligand. Our review focuses on the role of the TIM3/Gal9 interaction in various cancers and implications of this pathway in cancer immunotherapy. As the Gal9 binding site on TIM3 is different from other ligands, the anti-TIM3 antibodies that have been investigated in clinical trials may not block the TIM3/Gal9 pathway. A

* Corresponding author. Division of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO, 64108, USA.

E-mail address: chengkun@umkc.edu (K. Cheng).

<https://doi.org/10.1016/j.canlet.2021.04.011>

Received 3 September 2020; Received in revised form 31 March 2021; Accepted 15 April 2021

Available online 22 April 2021

0304-3835/© 2021 Elsevier B.V. All rights reserved.

handful of anti-TIM3 mAbs (TSR022, Sym023, ICAGN02390, BGB-A425, and MBG453) that prominently block the TIM3/Ptdser interaction are investigated in clinical trials. Among them, LY3321367 is the only anti-TIM3 mAb that partially blocks the TIM3/Gal9 interaction [9–13]. Even though Gal9 is the most relevant ligand for TIM3 [8], drugs that specifically block the TIM3/Gal9 pathway have not entered clinical trials yet. As a result, there is a great need to fully understand the TIM3/Gal9 interaction and its role in tumor pathogenesis. The current review highlights the importance of the TIM3/Gal9 pathway in cancer development and how the blockade of this pathway can be exploited for the treatment of different types of cancer. We also discuss the potential applications of the TIM3/Gal9 pathway in other diseases along with the possibilities of using TIM3 or Gal9 as a prognostic or predictive biomarker for cancer.

2. Structure and functions of TIM3

The immune checkpoint protein TIM3 is a negative regulator of anti-tumor immunity [14] and a member of the TIM family, which comprises of eight members, TIM1-TIM8, among which TIM1, TIM3, and TIM4 are found in humans [15]. TIM3 is a type I membrane glycoprotein expressed on terminally differentiated CD4⁺ T cell subsets like Th1 cells, Th17 cells, Tregs, and CD8⁺ T cell subset type1 CD8⁺ T cell (Tc1) but not on Th2 cells. It is also expressed on B cells, macrophages, DCs, NK cells, mast cells, and monocytes [8,16–21].

TIM3 is composed of an N-terminal extracellular immunoglobulin variable (IgV) domain that has FG-CC' loop and N-linked glycans, a mucin domain-containing sites for O-linked glycosylation, a stalk domain-containing N-linked glycans, a transmembrane domain, and a cytoplasmic domain-containing tyrosine phosphorylation motifs (Fig. 1) [7,22,23]. There are four different ligands of TIM3 (Table 1). Binding of TIM3 with Gal9 dampens Th1-mediated immunity [3] and induces apoptosis of TIM3⁺ T cells [24]. TIM3 expressing T cells can recognize apoptotic cells by binding to another ligand Ptdser, which is expressed on the apoptotic cells, but cannot engulf the apoptotic cells. However, TIM3 expressing DCs, when bound to Ptdser, can recognize and clear the apoptotic bodies [25]. Another TIM3 ligand, HMGB1, upon binding to TIM3 expressed in DCs, inhibits the transportation of tumor-derived nucleic acids to the endosome, leading to the suppression of pattern recognition receptor (PPR) mediated immune response to these nucleic acids [5,26]. However, the interaction of TIM3 expressing T cells with HMGB1 is poorly understood [27]. The interaction of CEACAM1 with TIM3 expressed on T cell suppresses T-cell effector functions and helps maintain T-cell tolerance. Among the TIM3 ligands, Gal9 binds to the N-linked glycans in the IgV domain of TIM3, while other ligands bind to the unique cleft called the FG-CC' loop in the IgV domain [28]. Although the binding sites for Gal9 and CEACAM1 in the IgV domain are different, studies have shown that they induce the same downstream signaling events [23,29].

3. Structure and functions of Gal9

Galectins are soluble proteins composed of a characteristic structure, called the carbohydrate recognition domain (CRD), which binds to β galactose [30,31]. Based on the number of CRDs, galectins are subdivided into the prototype, tandem-repeat type, and chimeric-type galectins [32]. Gal9 is a member of a ‘tandem-repeat’ type of galectin superfamily that consists of two non-homologous CRDs joined by a flexible peptide linker (14–56 amino acids in length) that plays important roles in protein-protein interactions, membrane insertions, and presentation of the CRDs [33]. The peptide linker contains a metalloproteinase site which can assist with the secretion of Gal9 into the extracellular matrix [34,35]. Gal9 is highly expressed in epithelial cells, endothelial cells, T cells, B cells, mast cells, and macrophages [31,36]. Gal9 has an important function in eosinophil activation, hemagglutination and anti-metastasis, T cell apoptosis via the TIM3/Gal9 pathway,

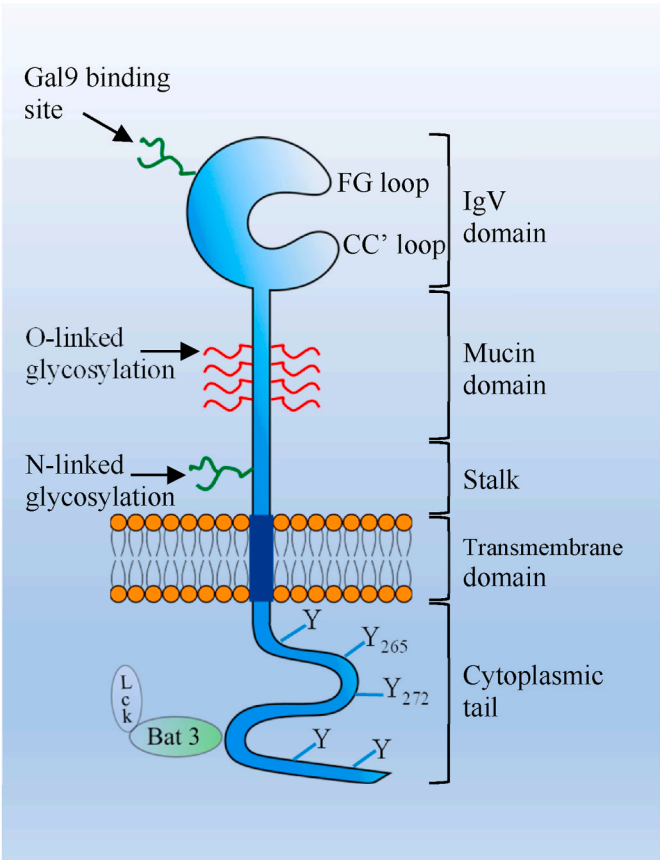


Fig. 1. The structure of TIM3. The extracellular region of T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) consists of an IgV domain, a mucin domain, and a stalk domain. The transmembrane region consists of a transmembrane domain and the intracellular region consists of a cytoplasmic tail with five tyrosine residues. The IgV domain contains binding sites for its ligands. Phosphatidylserine (Ptdser), carcinoembryonic antigen-related cell adhesion molecule (CEACAM1), and high mobility group box 1 (HMGB1) bind to the FG-CC' loop, while Gal9 binds to the N-linked glycan.

Table 1
Receptor and ligands for TIM3 and Gal9.

Receptor/Ligands		Biological function of Receptor-Ligand interaction	References
TIM3	Gal9	In T cells: Immunosuppression, T cell apoptosis	[43]
		In NK cells: Immunostimulatory effect	[39]
		DCs: Immunostimulatory effect	[44]
		Macrophages: Immunosuppression	[45]
	Ptdser	Apoptotic cells recognition, and clearance of apoptotic cells (in DCs)	[25]
	CEACAM1	Suppresses T cell immunity	[23,29]
	HMGB1	Inhibits tumor derived nucleic acid migration in DCs	[46]
Gal9	TIM3	As mentioned above	
	Dectin-1	Macrophage dependent T cell immunity suppression	[40]
	4-1BB	Enhances cytokine secretion, enhances immune response	[42]
	CD40	Immunosuppressive effect	[41]

T cell homeostasis and production of cytokines, and differentiation of Tregs and Th17 cells, making Gal9 a regulator of immune responses [37].

Four receptors with an affinity for Gal9 have been reported (Table 1). Among the receptors, TIM3 is a fully characterized receptor for Gal9 [3].

The TIM3/Gal9 interaction shows inhibitory or stimulatory effects in the immune system depending on the cell type that expresses TIM3. In normal physiology, TIM3 expressed on T cell subsets (Th1, Th17, and Tc1) and macrophages shows inhibitory effects upon Gal9 binding, whereas TIM3 expressed on NK cells and DCs shows stimulatory effects when it binds to Gal9 [38,39]. The other receptors for Gal9 are Dectin-1, 4-1BB, and CD40. Dectin-1 is highly expressed on macrophages and exerts an immunosuppressive effect after binding to Gal9 [40]. Similarly, the Gal9/CD40 interaction is known to prevent the proliferation and survival effects of CD40 on CD4^{lo}CD40⁺ effector T cells [41]. Gal9 binds to 4-1BB (an activating immune checkpoint protein) and promotes the clustering of 4-1BB, enhancing the proinflammatory pathway and immune response [42].

4. Physiological functions of the TIM3/Gal9 pathway

4.1. TIM3/Gal9 pathway in adaptive immunity

Unlike other immune checkpoint proteins such as PD-1, CTLA-4, TIGIT, and LAG-3, which have an inhibitory motif in their cytoplasmic tail, TIM3 has five tyrosine residues in its cytoplasmic tail. Of the five tyrosine residues, Y265 and Y272 (Y256 and Y263 in mice) play an important role in TIM3 signal transduction [47]. While the exact molecular mechanism of TIM3 signaling is not fully elucidated, it has been demonstrated that before TIM3 ligand engagement, the tyrosine residues present in the intracellular tail of TIM3 are necessary for the augmentation of T cell receptor (TCR) activation suggesting the coupling of TIM3 and TCR pathways [48].

TIM3 when not bound to any of its ligands, its cytoplasmic tail containing Y256 and Y263, interacts with HLA-B associated transcript 3 (Bat3) [49], which facilitates the recruitment of catalytically activated Lck [7], a member of the *Src* family protein kinase (Fig. 1). The formed

Lck clusters phosphorylate the CD3 domain of TCR, leading to the recruitment and activation of protein kinase Zap 70 [50], which phosphorylates the linker adaptor protein (LAT) [51,52]. Phosphorylated LAT recruits and activates the signaling effector phospholipase C γ 1 (PLC γ 1), which participates in the production of second messengers like inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) [53,54]. These second messengers subsequently activate NFAT, MEK/ERK, and NF- κ B pathways, resulting in T cell response and modulation, cell proliferation, and production of interleukin-2 (IL-2), Tumor Necrosis Factor (TNF α), and interferon γ (IFN γ) (Fig. 2A) [53,55].

Interaction between TIM3 and Gal9 induces phosphorylation of the cytoplasmic tail at Y265 and Y272 [47], resulting in the release of bound Bat3 from the cytoplasmic tail [49] (Fig. 2B). Bat3-released TIM3 binds to Fyn, one of the *Src* family protein kinases that share the same binding site with Bat3 [49]. Fyn recruitment in the cytoplasmic tail of TIM3 [7] phosphorylates the phosphoprotein associated with glycosphingolipid microdomains 1 (PAG), which promotes the recruitment of the C-terminal c-*Src* kinase (Csk). Csk then phosphorylates the C-terminal tyrosine of Lck, which negatively regulates the Lck activity and leads to the inhibition of T cell functions [56–58]. Davidson et al. have reported that PAG-associated Fyn is involved in increasing the Ca⁺⁺ flux intracellularly, which weakens the TCR signaling and leads to T cell anergy [59]. Thus, the TIM3/Gal9 signaling pathway mediates events like inhibition of T cell proliferation, reduction in cytokine production and potentially resulting in T cell death.

The TIM3/Gal9 interaction also affects proximal TCR signaling. The conserved tyrosine residues present in the cytoplasmic tail of TIM3 are also responsible for proximal signaling events. The TIM3/Gal9 interaction enhances the clustering of tyrosine receptor phosphatases CD45 and CD148 present in the immunological synapse [43]. The receptor phosphatase CD45 dephosphorylates the tyrosine residues Y505 and Y394 of Lck and reduces its catalytic activity [60]. Similarly, CD148

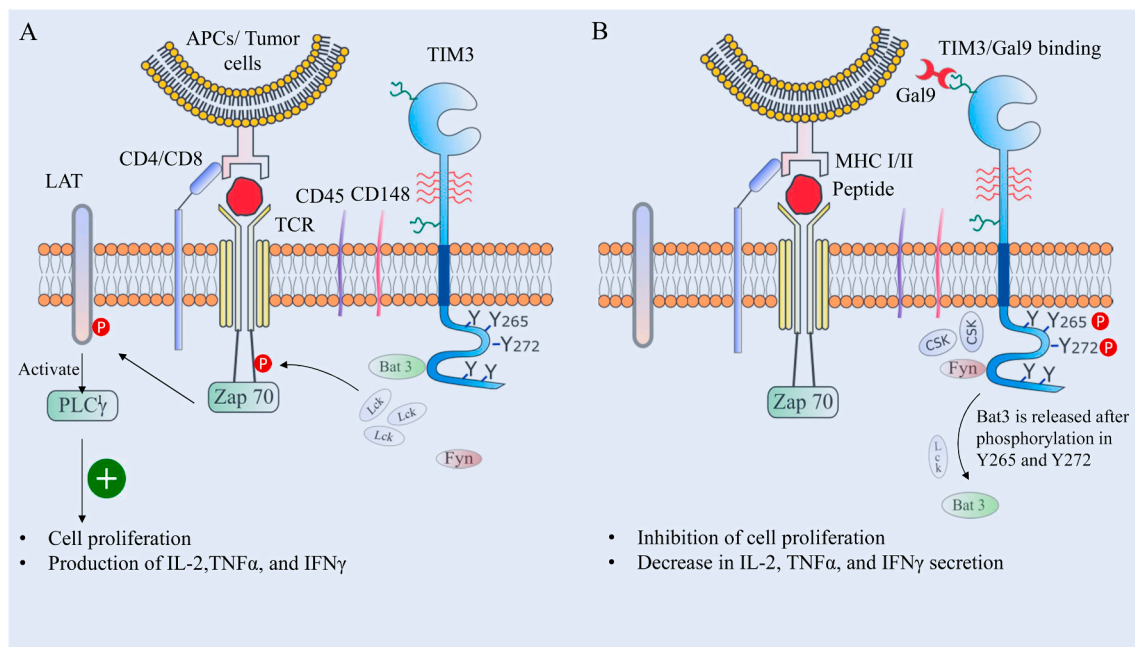


Fig. 2. Schematics of the TIM3/Gal9 signaling pathway in T cells. (A) In an unbound state, the cytoplasmic tail of T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) binds to HLA-B associated transcript 3 (Bat3) which helps to recruit the tyrosine kinase Lck. Lck clusters then phosphorylate the CD3 domain of TCR, leading to the recruitment of the tyrosine kinase Zap 70. Zap 70 then phosphorylates linker adaptor protein (LAT), which activates the phospholipase C γ 1 (PLC γ 1) to produce second messengers like Inositol trisphosphate (IP3) and diacylglycerol (DAG), leading to the production of cytokines and T cell proliferation. (B) Upon the TIM3/Gal9 interaction, Y265 and Y272 in the cytoplasmic tail of TIM3 are phosphorylated, resulting in the release of Bat3 and Lck. The tyrosine kinase Fyn is recruited to the cytoplasmic tail of TIM3 and helps in the clustering of C-terminal c-*Src* kinase (Csk). Csk clusters promote phosphorylation of the C-terminal tyrosine of Lck and inhibit its catalytic activity. Subsequently this leads to the inhibition of T cell proliferation and suppression of the production of IL-2, TNF α , and IFN γ .

hypophosphorylates LAT and PLC γ 1, leading to the inhibition of the downstream T cell functions [61]. In conclusion, the TIM3/Gal9 interaction inhibits immunity mediated by Th1, Th17, and Tc1 cells through inhibiting the production of IFN γ , TNF α , and IL-2 (Fig. 3A, B and 3D) (Table 2).

In Tregs, numerous studies have reported the downstream effects of the TIM3/Gal9 interaction, but the exact intermediate steps of this pathway are yet to be explored. The TIM3/Gal9 interaction is believed to maintain and regulate Tregs function and development. One study has shown that TIM3/Gal9 ligation results in overexpression of cytokines like IL-10 and TGF β , which facilitates the proliferation of CD4⁺CD25⁺Foxp3⁺ Treg cells. The proliferation of Tregs then suppresses the activity of effector T cells [62]. A study by Yan et al. has shown that TIM3 expression is significantly higher in CD4⁺Foxp3⁺ T cells (70%) as compared to CD4⁺Foxp3⁻ T cells (20%). They also found that Foxp3 is expressed in tumor-infiltrating TIM3⁺CD4⁺ T cells which exhibit Treg properties in the tumor microenvironment [63]. A similar study using CT26 cells demonstrated that 50% of CD4⁺Foxp3⁺ T cells

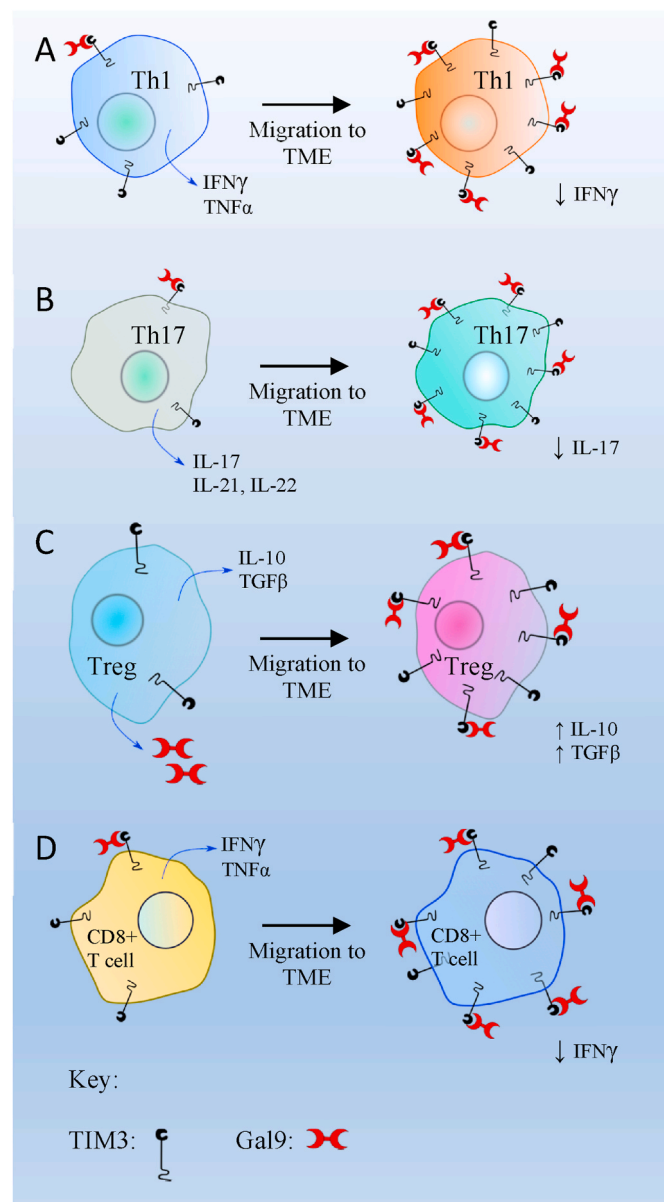


Figure 3. The role of the TIM3/Gal9 interaction on different T cell subsets. (A) T helper 1 cell (Th1), (B) T helper 17 cell (Th17), (C) T regulatory cells (Tregs), and (D) CD8⁺ T cell.

Table 2

The mechanisms and physiological functions of TIM3/Gal9 in different immune cells.

TIM3 expression in immune cells	Mechanisms	Physiological functions	References
Th1, Th17 and Tc1 cells	- Inhibit Lck-mediated phosphorylation of TCR - Decrease production of TNFα, IFNγ, and IL-17	- Decrease cell proliferation, migration, - T cell apoptosis	[43,80]
T regs	Increase production of IL-10 and TGFβ	- Enhancement of suppressive effects of Tregs - Proliferation of Tregs	[63]
NK cells	- Increase IFNγ production in non-tumor cells - Decrease in cytokine release by dNKs	- Enhance innate immune response - Maintain pregnancy	[39,72]
Dendritic cells	Synergize with LPS/TLR4 to increase TNFα release	Enhance innate immune response	[18]
Monocytes	- Crosstalk with TLR4 - Decrease pro production of TNFα and IL-6	Suppress innate immunity	[45,73]
Macrophages	- Trans-association inhibits TLR4 mediated cytokine production (decreases TNFα and IL-6 levels) - Cis-association enhances TLRs	- Downregulate innate immunity against diseased condition. - Increase secretion of Gal9	[81]

express TIM3, whereas only 10% of CD4⁺Foxp3⁻ TILs express TIM3. In line with this *in vitro* study, an *in vivo* study showed that TIM3⁺ Tregs produced two-fold production of IL-10 and had twice the immune-suppressive activity compared to TIM3⁻ Tregs in B16 melanoma and CT26 colon carcinoma [64].

Additionally, Tregs also express Gal9 (Fig. 3C), which may bind to TIM3 expressed on Th1/Th17 cells, and suppress T cell-mediated immunity and induce apoptosis of Th1/Th17 cells [65,66]. In tumor-associated Tregs, TIM3 is overexpressed as a marker of infiltrating Tregs [63]. TIM3⁺ Tregs are prominent in the tumor nest but are significantly low in peripheral blood. The TIM3/Gal9 interaction was found to enhance the immunosuppressive function of Tregs and participate in the development of allograft tolerance [67,68]. The TIM3/Gal9 interaction enhances the immunosuppressive activity of Tregs in the tumor microenvironment (TME) by increasing the secretion of IL-10 and TGF β .

4.2. TIM3/Gal9 pathway in innate immunity

The expression of TIM3 on innate immune cells like NK cells, DCs, macrophages, and monocytes is prominent (Table 2). For example, TIM3 is highly expressed in mature CD56^{dim} CD16⁺ NK cells and acts as a maturation marker [69]. Even though the role of the TIM3/Gal9 pathway in NK cells is still not fully understood, Jost et al. has shown that Gal9 binding to TIM3 present on NK cells has a stimulatory effect when NK cells are pre-treated with cytokines IL-12, IL-15 and IL-18. But constant exposure to Gal9 leads to the downregulation of TIM3 on NK cells (Fig. 4A) [70]. In addition, Gleason et al. reported that coupling of TIM3 and Gal9 increases IFN γ production in NK92 NK cells, suggesting that TIM3 is an NK-cell coreceptor which enhances IFN γ production [39]. In contrast, during pregnancy, the TIM3/Gal9 pathway plays an important role in the differentiation of NK cells to decidual NK (dNK)-like cells to promote fetal tolerance by maintaining the anti-inflammatory function of dNK cells [71]. The binding of Gal9 to

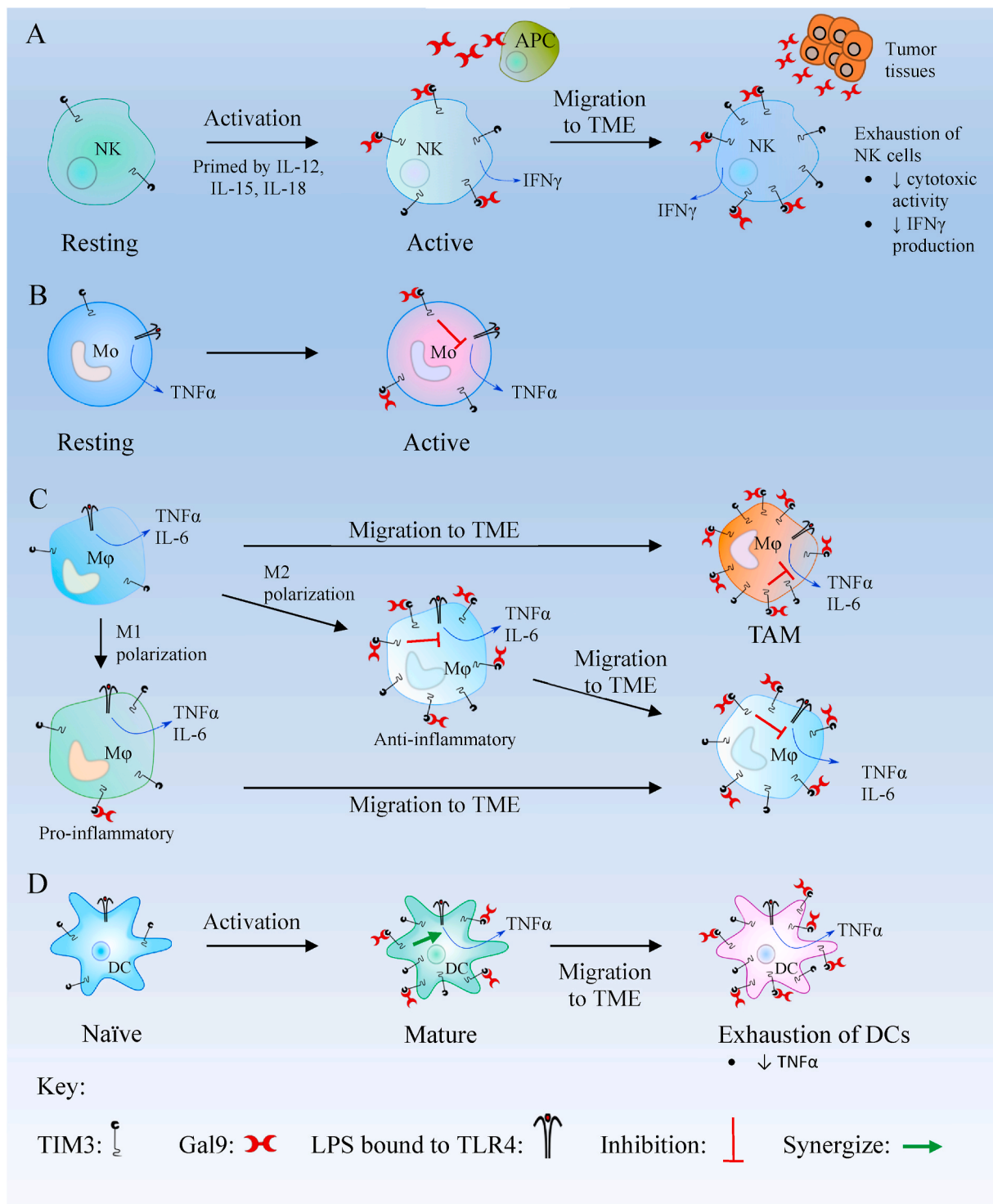


Fig. 4. Schematics of the TIM3/Gal9 pathway in innate immune cells. (A) Resting natural killer (NK) cells become active in the presence of IL-12, IL-15, and IL-18 and express more T cell immunoglobulin and mucin-containing protein 3 (TIM3). In active NK cells, TIM3/Galactin 9 (Gal9) binding enhances interferon γ (IFN γ) secretion. After migration to the tumor microenvironment (TME), NK cells are exposed to high levels of Gal9 which leads to their exhaustion. (B) Active monocytes (Mo) express a high number of TIM3 receptors. The TIM3/Gal9 interaction inhibits the Lipopolysaccharide (LPS)/Toll like receptor 4 (TLR4) pathway and decreases the secretion of TNF α . (C) Macrophages (M ϕ) in the TME overexpress TIM3. Macrophages are polarized to M1 and M2 phenotypes. M1 macrophages are pro-inflammatory and express a low level of TIM3. M1 macrophages can migrate to the TME and may transform to M2 macrophages. M2 macrophages are anti-inflammatory and express high levels of TIM3. TIM3/Gal9 binding in M2 macrophages inhibits LPS/TLR4 pathway and decreases the production of inflammatory cytokines like Tumor Necrosis Factor α (TNF α), interleukin-6 (IL-6), and IL-12. After migrating to the TME, M2 macrophages overexpress TIM3, and the TIM3/Gal9 binding leads to the inhibition of the LPS/TLR4 pathway to suppress the immune response. (D) Mature DCs express a high level of TIM3, and the TIM3/Gal9 binding synergizes with the LPS/TLR4 pathway which produces TNF α . In Dendritic cells (DCs) within the TME, the TIM3/Gal9 interaction suppresses CXCL9 expression, thus inhibiting innate immunity and promote tumor growth.

TIM3 expressed on dNK cells inhibits lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines and perforin, thus maintaining a normal pregnancy [72]. In addition, the TIM3/Gal9 pathway in NK cells also has been reported in other diseases. For instance, blockade of the TIM3/Gal9 interaction leads to increased IFN γ production resulting in increased cytotoxicity of NK cells in chronic hepatitis B virus infection [19]. Under normal physiological condition, the binding of Gal9 to TIM3 present on NK cells induces immune-stimulatory activity, whereas, in diseased conditions like cancers or viral infections, the TIM3/Gal9 interaction leads to overactivation of NK cells, resulting in exhaustion of NK cells. The possible reason could be that the TIM3/Gal9 pathway may crosstalk with other pathways like IL-2/IL-2 Receptor (IL-2R) and LPS/Toll-like Receptor 4 (TLR4) to produce immune-stimulatory or inhibitory effects.

In monocytes and macrophages (M/M ϕ), Gal9 bound TIM3 crosstalks with TLR4 receptors, hence increasing the production of IL-10 but reducing the production of cytokines like TNF α , IL-6, and IL-12 (Fig. 4B and C) [45,73]. Similar findings were reported in human CD14⁺M/M ϕ , TIM3 acts as a brake that controls TLR4 and TLR7, thus inhibiting TLR-mediated IL-2 production [44]. In active monocytes, the inhibition of LPS/TLR4 pathway is found to be reversed after treatment with mAbs that block the TIM3/Gal9 interaction [74]. Zhang and colleagues demonstrated that the TIM3/Gal9 interaction inhibits the function of macrophages by decreasing the production of inflammatory mediators. Furthermore, TIM3/Gal9 binding, in an autocrine fashion, regulates M1/M2 polarization via LPS stimulation. Short term LPS exposure in macrophages upregulates TIM3/Gal9 signaling, subsequently inhibiting M1 polarization. Conversely, long term LPS stimulation results in downregulation of TIM3/Gal9 pathway, resulting in inhibition of M2 polarization [75]. The upregulation of TIM3 expression is correlated with M2 polarization while downregulation is correlated with the M1 polarization of macrophages (Fig. 4C). However, a study by Ma et al. reported a different finding regarding the TIM3/Gal9 interaction on M/M ϕ . Trans ligation of Gal9 with TIM3 negatively regulates M/M ϕ and decreases the TLR-mediated IL-12 expression, whereas cis ligation of Gal9 with TIM3 increases the expression of IL-12 and IL-23 via STAT3 phosphorylation resulting in enhanced inflammatory responses [76].

In DCs, the TIM3/Gal9 interaction synergizes with TLRs to promote inflammation [18]. Although the role of the TIM3/Gal9 interaction in DCs has not been fully elucidated, ligation of TIM3 with another ligand HMGB1 suppresses DC function. A recent study has shown that within the TME, Gal9 binds to TIM3 present on DCs, inhibiting anti-tumor immunity by suppression of the CXCL9 expression [77]. The TIM3/Gal9 interaction on DC is assumed to be in synergy with the TLRs and activates their innate immunity (Fig. 4D) [18].

In summary, several studies have demonstrated the inhibitory role of TIM3/Gal9 interaction as it down-regulates Th1-mediated anti-tumor immunity as well as inhibits the autoimmune and alloimmune responses [3,78]. On the contrary, Gal9 binding with TIM3 expressed on innate immune cells like DCs and NK cells enhances the immune response promoting inflammation [18,79]. These intriguing findings complicate the comprehension of the TIM3/Gal9 pathway. Further studies are necessary to understand the definitive molecular mechanism of the TIM3/Gal9 pathway in the innate immune system as well as the adaptive immune system.

5. TIM3/Gal9 pathway in cancers

Perturbation of the TIM3/Gal9 pathway results in various disease conditions like autoimmune diseases, infections, cancers, and complications of pregnancy. Numerous studies have shown increased levels of TIM3 and Gal9 in cancers including Acute Myeloid Leukemia (AML), prostate cancer, non-small cell lung cancer (NSCLC), esophageal squamous cell carcinoma (ESCC), breast cancer, glioma, head and neck cancer, colon cancer, melanoma, and lung adenocarcinoma (Table 3).

5.1. Hematological malignancy

Unlike healthy white blood cells, primary human AML cells and primary human CD34⁺ cells express latrophilin 1 (LPHN1) on their surface. LPHN1 binds to its receptor fibronectin leucine-rich repeat transmembrane protein (FLRT3) and activates the PKC α and mTOR pathways, which help in synthesis and secretion of TIM3 and Gal9 [82]. Intracellularly synthesized Gal9 lacks the signal peptide that is responsible for Gal9 secretion. TIM3 is known to help traffic Gal9 from the intracellular region to the extracellular region, which is facilitated by the SNARE complex [83]. The released TIM3 and Gal9 interact with each other and may result in a conformational change of Gal9. This conformational change enhances the binding ability of the unbound CRD of Gal9 to interact with TIM3 present in NK cells [84]. This interaction of Gal9 with TIM3 on NK cells leads to the inhibition of the cytotoxic effect of NK cells and their ability to kill AML cells [82]. Similarly, overexpression of TIM3 and Gal9 has also been reported in leukemic stem cells (LSCs) compared to normal healthy stem cells [85]. The *in vitro* study performed in CD34⁺ AML cells has shown that TIM3/Gal9 interaction via an autocrine loop on CD34⁺ AML cells activates the NF- κ B and Beta-catenin pathways [85]. Activation of these pathways is believed to play an important role in the self-renewal of human AML cells decreasing patient survival [86]. However, these signaling pathways are not active in normal HSCs. It suggests that the blockade of TIM3/Gal9 coupling could be a potential therapy for myeloid malignancies and to prevent the transformation of preleukemic disease to AML [85].

5.2. Solid malignancies

As the immunoinhibitory role of TIM3 when it binds to Gal9 is well known, blockade of TIM3 is a promising therapeutic approach for a wide range of cancers. For example, increased expression of TIM3 on T cells and Gal9 on tumor cells is observed in solid tumors such as breast cancer, prostate cancer, glioma, head and neck cancer, osteosarcoma, NSCLC, and ESCC.

In breast cancer cells, the expressions of TIM3 and Gal9 were high compared to healthy tissues. Latrophilin isoforms LPHN2 and LPHN3, which are expressed in breast cancer tissues, are responsible for the secretion of Gal9. Binding of FLRT3 to LPHN2/LPHN3 facilitates the secretion of Gal9 via the phospholipase C/phosphokinase C pathway. In various solid and liquid tumors, the FLRT3/LPHN/TIM3/Gal9 pathway is believed to be involved in the translocation of TIM3 and Gal9 onto the cellular surface [87].

Recent studies have shown the overexpression of TIM3 on TILs and Gal9 on tumor cells, and their expression is correlated with the advancement of prostate cancer [21,87]. The increased level of TIM3 expression on CD4⁺ T cells and CD8⁺ T cells were associated with a higher Gleason score and higher preoperative prostate-specific antigen (PSA) levels, suggesting TIM3 as an indicator of prostate cancer progression [21]. Similarly, increased expression of TIM3 on T cells and Gal9 on Kupffer cells was observed in hepatocellular carcinoma [88].

In glioma, there is a high expression of TIM3 and Gal9 compared to healthy brain cells. High TIM3 expression is associated with WHO grade II-IV glioma and is related to the progression of glioma. In an *ex vivo* study, TIM3 expression was found to be lower in healthy peripheral blood mononuclear cells (PBMCs), but TIM3 and Gal9 levels were elevated in TILs in glioma. As the severity of glioma progresses from Grade II/III to Grade IV, TIM3 expression on CD4⁺ and CD8⁺ T cells and Gal9 expression in tumors increase [89]. Likewise, in colorectal cancer, an *in vitro* study demonstrated that the treatment with anti-TIM3 antibody significantly decreased the Gal9-induced apoptosis of TILs, and an *in vivo* study showed that the use of anti-TIM3 antibody blocked the TIM3/Gal9 pathway, increasing the activity of cyclophosphamide in a CT26 mouse colon tumor model [90].

In human papilloma virus-positive oropharyngeal head and neck

Table 3

Roles of the TIM3/galectin9 pathway in various cancers.

Tumor type	Pathophysiology	Study nature/type	References
AML	In AML cells • Increase in TIM3 and Gal9 expression in AML cells. • Autocrine TIM3/Gal9 binding facilitates self-renewal of AML cells that leads to the progression of the disease. In NK cells • Gal9 released by AML cells binds to TIM3 present on NK cells decreasing the NK cytotoxicity activity.	• In vitro study [83] • Clinical study (blood samples from patients) [85]	
Prostate cancer	• Enhanced TIM3 expression on CD4 and CD8 T cells. TIM3/gal9 binding results in suppression of anti-tumor immunity. • TIM3 expression on T cells is related to the advancement of Prostate cancer.	• Clinical study (Tissue specimens from patients)	[21]
NSCLC	• High level of exosomal TIM3 and Gal9 present in the plasma that is correlated with the advanced stage of tumor and distant metastasis	• Clinical study (blood samples)	[95]
Esophageal squamous cell cancer	• Increased TIM3 expression indicates a poor prognosis of the disease. • High TIM3 and low Gal9 expression shortened the overall survival of patients.	• Clinical study (blood samples)	[96]
Breast cancer	• Overexpression of TIM3 and Gal9 is seen in TILs and tumor cells. • TIM3/Gal9 coupling leads to apoptosis of T cells leading to the suppression of host anti-tumor immunity.	• Clinical study (human tissue samples), and • In vitro study	[87]
Glioma	• Increased TIM3 expression on CD4 and CD8 T cells as well as high expression of Gal9 on tumor cells resulting in apoptosis of T cells.	• Clinical study (blood samples and glioma tissues)	[89]
Osteosarcoma	• Increased expression of TIM3 and Gal9 are found in tumor-associated macrophages and T cells. • TIM3/Gal9 binding results in decreased production of IFNγ subsequently compromising the anti-inflammatory immune activity.	• Clinical study (blood sample)	[74]
Colorectal cancer	• Elevated TIM3 expression and binding of TIM3 with Gal9 causes exhaustion of TILs leading to tumor progression.	• In vitro study, and • In vivo study	[90]
Gastrointestinal stromal tumors	• Increased TIM3 expression in infiltrated NK cells and Gal9 expression seen in gastrointestinal stromal tumors. • Interaction between TIM3/Gal9 is followed by a decrease in the cytotoxic activity of NK cells (dysfunctional NK cells).	• Clinical study (Cancer tissue specimen)	[94]
Head and neck cancer	• Increased TIM3 expression on Tregs increases the differentiation of Tregs (there is increased numbers of Tregs). • TIM3/Gal9 binding on Tregs increases the production of inhibitory cytokines (IL-10, IL-35, perforins, granzyme A and granzyme B) • Suppression of T cell activity is potentiated.	• Clinical study (Head and neck squamous cell carcinoma samples), and • In vitro study	[92]
Lung adenocarcinoma	• Increased TIM3 and Gal9 expression and their interaction result in suppression of T cell-mediated immunity. • PD-1 is co-expressed with TIM3. Co-blockade of TIM3 and PD-1 minimizes anti-PD-1 therapy resistance.	• In vitro study, • In vivo study, and Clinical study (biopsies and effusions)	[97]
Advanced melanoma	• Increased TIM3 on T cells. • TIM3 is co-expressed with PD-1 resulting in T cell exhaustion.	• Ex vivo study, and Clinical study (blood sample)	[80]
Hepatocellular carcinoma	• Increased TIM3 and gal9 expression found on CD4 and CD8 T cells along with Kupffer cells compared to adjacent normal cells. • TIM3/Gal9 binding decreases the number of TILs consequently suppressing the T cell mediated immunity.	• Clinical study (tumor samples)	[88]
Human papilloma virus positive oropharyngeal head and neck squamous cell carcinoma	• Increased TIM3 expression on monocytes and Gal9 expression by CD4 T cells observed. • Exogenous as well as endogenous Gal9 binding with TIM3 leads to suppression of anti-tumor immune response.	• Clinical study (blood samples)	[91]

squamous cell carcinoma, overexpression of TIM3 on monocytes and Gal9 on CD4 T cells were observed [91]. Another study reported that the TIM3/Gal9 interaction suppresses anti-tumor immunity in head and neck cancer. The expression of TIM3 and Gal9 in the tumor stroma is positively correlated with the expression of Foxp3 (a Treg marker), CD68, and CD163 (a macrophage marker) [92]. Hence, there is an increase in the level of Tregs and M2 macrophages. The expression of TIM3 on CD4⁺CD25⁺Foxp3⁺ Treg cells results in the higher secretion of inhibitory cytokines IL-10 and TGF β [93], perforins, granzyme A, and granzyme G [64]. Treatment with an anti-TIM3 mAb (RMT3-23) in a HNSCC mouse model reduced the number of CD4⁺CD25⁺Foxp3⁺ Treg cells and increased the percentage of CD8⁺ T cells, leading to high production of IFN γ [92]. Evidences have been reported that TIM3 blockade in early-stage tumors inhibits the immune suppressive function of TIM3⁺ Tregs while TIM3 blockade in advanced-stage tumors restores CD8⁺ T cell function [64,92].

Similarly, higher expression of TIM3 and Gal9 was observed in tumor-associated macrophages isolated from osteosarcoma patients compared to non-cancer patients [74]. As in other cancers, there is a

diminution of IFN γ production after the TIM3/Gal9 interaction in osteosarcoma, and the blockade of this interaction enhances the production of cytokines from CD4⁺ cells [74]. Like in other tumors, there is enhanced expression of TIM3 in TILs and Gal9 expression in gastrointestinal stromal tumors. After infiltration of NK cells into the tumor cells, NK cells express TIM3 whereas gastrointestinal stromal tumor cells express Gal9. When TIM3 and Gal9 interact, anti-tumor immunity is suppressed [94].

The level of exosomal TIM3 and Gal9 was found to be significantly higher in the plasma of NSCLC patients than in the plasma of healthy donor [95]. The increased expression of TIM3 and Gal9 correlates with tumor size, tumor metastasis as well as progression to advanced stages of NSCLC [95]. Also, in patients suffering from ESCC, a better survival rate post-operation was seen in tumor samples with low TIM3 expression whereas increased TIM3 expression showed poor survival rate [96]. The binding of TIM3 with Gal9 results to the suppression of anti-tumor immune response. Therefore, the blockade of TIM3 and Gal9 can be used as a therapeutic strategy for the treatment of various cancers.

5.3. Co-targeting TIM3/Gal9 and PD-1/PD-L1 pathways

Co-expression of TIM3 and PD-1 in cancers like colorectal cancer [98], mammary cancer [99], lung cancer [100], melanoma [80], and AML [101] have been reported. Co-expression of TIM3 and PD-1 on TILs decreases the production of IL-2, TNF α , and IFN γ , resulting in dysfunction or impairment of T cells in the tumors [24,80]. Immunotherapy treatment with anti-PD-1/PD-L1, along with an antibody that blocks TIM3, has been found to be beneficial in various chemotherapy-resistant cancers. In a clinical study, combination therapy of Selinexor and HiDAC (high dose cytarabine) + Mitoxantrone in AML patients showed overexpression of PD-1 and TIM3 in patients in the total failure group as compared to patients in the complete remission group. Chemotherapy resistance was found in cancers where immune checkpoint proteins like TIM3 and PD-1 are involved [102]. Increased expression of PD-1 and its ligand PD-L1 along with TIM3 and its ligand Gal9 could be the reason for the failure of the combination chemotherapy. The use of immunotherapy that intervene with TIM3/Gal9 and PD-1/PD-L1 together with chemotherapy could diminish the drug resistance. Expression of Gal9 and PDL1 is prominent in AML cells, and the coupling of these ligands with their respective receptors on T cells leads to T cells dysfunction. Thus, the co-expression of TIM3 and PD-1 on T cells is an indication of exhausted T cells [101,103]. Therefore, blocking either the TIM3/Gal9 pathway or the PD-1/PD-L1 pathway alone does not slow the progression of AML [101]. The combined blockade of two different immune checkpoints like TIM3/Gal9 and PD-1/PD-L1 could restore T cell function.

Evidence from previous studies and the ongoing clinical trial with combined immunotherapy targeting TIM3/Gal9 and PD-1/PD-L1 (NCT03066648) in myeloid leukemia provides insight into the use of immune checkpoint inhibitors in combination therapy [104]. It also suggests that co-blockade of different immune checkpoint proteins reduces drug resistance in tumors and restores the immunity of T cells against tumors. For example, the combination of anti-PD-L1 antibody or bintrafusp alpha (an anti-PD-L1 fusion peptide) with M6903 (an anti-human TIM3 antibody) increase the activity of M6903 [105]. Furthermore, in an *in-vivo* study of lung adenocarcinoma, Koyama et al. have shown that upregulation of TIM3 on TILs and Gal9 on KRAS-mutant tumors with anti-PD-1 resistance, proposing the idea that other immune checkpoint proteins could be expressed in tumors [97]. In an *ex vivo* study in advanced melanoma, expressions of TIM3 and PD-1 were found to be upregulated in NY-ESO-1-specific CD8⁺ T cells indicating the exhaustion of T cells. TIM3⁺PD-1⁺ NY-ESO-1-specific CD8⁺ T cells suppress the T cell-mediated immunity more significantly than TIM3⁺PD-1⁻ NY-ESO-1 CD8⁺ T cells and TIM3⁻PD-1⁻ NY-ESO-1 CD8⁺ T cells. Upon TIM3/Gal9 binding, the TIM3⁺PD-1⁺ NY-ESO-1-specific CD8⁺ T cells suppress the T cell immunity by decreasing the production of IFN γ , TNF α , and IL-2. TIM3 and PD-1 overexpression on T cells are prone to a higher percentage of dysfunctional T cells [80]. Elevated expression of TIM3 on T cells and Gal9 on Kupffer cells were observed in hepatocellular carcinoma leading to the decrease in effector T cell functions. Also, the suppression of anti-tumor immunity is displayed by the TIM3/Gal9 and B7-H (PD-L1)/PD-1 axis in hepatocellular carcinoma [88]. These studies suggest that the combined blockade of TIM3/Gal9 and PD-1/PD-L1 might restore CD4⁺ and CD8⁺ T cell function and thus be a breakthrough in the field of cancer immunotherapy. Blocking TIM3/Gal9 pathway along with the anti-PD-1 therapy would reduce the chances of drug resistance and improve the efficacy of anti-PD-1 therapy in lung cancer. In line with previous studies, a clinical study by Limagne et al. also supports the idea of combination immunotherapy [106]. Because of anti-PD-1 resistance, only about one-fourth of patients responded to the anti-PD-1 drug Nivolumab, approved for use in NSCLC [100]. Limagne et al. have demonstrated the possible underlying mechanism for the resistance to anti-PD-1 therapy in NSCLC and the resistance may be due to upregulation of TIM3/Gal9 and other immune checkpoint proteins. The primary and secondary resistance to

Nivolumab could be due to increased TIM3 expression on lymphoid cells and increased production of Gal9 on monocytic MDSCs which diminishes CD8⁺ T cell function. Genetic mutations like neoantigen removal or mutation adapter proteins like PTEN, JAK/STAT, or HLA genes also have been found to cause immunotherapy resistance [107, 108].

Principally, the TIM3/Gal9 and PD-1/PD-L1 pathway are important immune checkpoint pathways responsible for the pathogenesis of several carcinomas. Inhibiting TIM3/Gal9 binding along with PD-1/PD-L1 axis could be a valuable approach for the treatment of different cancers.

6. TIM3/Gal9 pathway in other diseases

6.1. Autoimmune diseases

In addition to cancers, TIM3/Gal9 interaction has also been documented in autoimmune diseases (multiple sclerosis, rheumatoid arthritis, and Type 1 diabetes), viral infections, and protozoal infections. In a clinical study, patients with multiple sclerosis were treated with glatiramer or IFN β . Increased TIM3 expression was observed in both glatiramer and IFN β -treated patients compared to untreated patients, suggesting the recovery and maintenance of a basal level of TIM3 [109]. TIM3/Gal9 interaction causes T cell exhaustion and death, which decreases autoreactive T cells and improves autoimmune diseases. Type 1 diabetes is an autoimmune disease, characterized by activation of innate immunity, proliferation of autoreactive CD4⁺ and CD8⁺ T cells that may damage β cells [110]. Gal9 activates DCs to induce the production of TNF α , and these activated DCs play a crucial role in the activation of Th1 and autoimmune response. Treating patients with RMT3-23 (anti-TIM3 mAb) resulted in decreased production of TNF α by DCs and inhibits the downstream Th1 functions. This result demonstrates that the TIM3/Gal9 pathway blockade on DCs could be a potential therapeutic target in type 1 diabetes mellitus [110,111].

Significant downregulation of TIM3 and Gal9 is found in peripheral blood cells in patients suffering from thyroid-associated ophthalmopathy (TAO), an autoimmune disease. It is thought that Th1, and Th17 play a role in the pathogenesis of TAO [112]. mRNA expression of TIM3 and Gal9 was found to be significantly lower in TAO patients compared to healthy subjects. In an *in vitro* study, treatment using Gal9 blocking antibody blocked TIM3/Gal9 downstream function and resulted in increased IFN γ , TNF α , and IL-17 levels showing TIM3/Gal9 has a negative correlation with disease severity. The TIM3/Gal9 pathway suppresses T cell-mediated cytokine release in TAO fibroblasts, suppresses the Akt/NF κ B pathway, and alleviates TAO [113]. The TIM3/Gal9 interaction has shown to suppress inflammation and immune responses in TAO.

6.2. Other diseases

In chronic hepatitis B infection, higher TIM3 expression on NK cells was observed. TIM3/Gal9 binding inhibits the cytotoxic function of NK cells, leading to the compromised antiviral immunity of the host. Blockade of the TIM3/Gal9 axis helped restore the cytotoxic function of NK cells [19]. Another study in a hepatitis B virus mouse model showed that TIM3/Gal9 coupling lowered the IFN γ production and led to the exhaustion of CD8⁺ T cells. This resulted in the inhibition of antiviral immunity of infiltrating CD8⁺ T cells [114]. Also, plasma endotoxin and the cytokine IL-10 are responsible for overexpression of TIM3 and PD-1 on acute alcoholic hepatitis leading to the suppression of host immunity [115]. Co-blockade of TIM3 and PD-1 pathways have shown to generate a synergistic effect in anti-viral immunity of host against hepatitis B virus and chronic hepatitis B infection [116].

In a murine malaria model, the number of Gal9-and TIM3-positive cells are increased in the lungs, liver, and spleen, suggesting an important role of the TIM3/Gal9 pathway in the damage of these organs [117,

[118]. In a clinical study, the plasma level of Gal9 was found higher in severe malaria patients compared to uncomplicated cases. Moreover, Gal9 is associated with the severity of malaria. It is postulated that Gal9 inhibits immune response in malaria patients by binding to TIM3 [119]. As a result, malarial infection could be treated by blocking the TIM3/-Gal9 pathway. In a PbANKA-infected mouse model, TIM3/Gal9 pathway blockade with anti-TIM3 antibody showed an increase in the mRNA level of cytokines, including IFN γ , TNF α , IL-10, and IL-4. This finding suggests that antibody-mediated blockade of TIM3 contributes to the therapeutic clearance of malaria infection [120].

7. TIM3 and Gal9 as potential biomarkers

The discovery and identification of noninvasive biomarkers help identify cancer in the early stages and develop treatment strategies as well. Identification of non-invasive biomarkers would be beneficial to patients as they could bypass invasive surgical procedures like biopsies. Numerous ongoing studies aim to identify the relevant biomarkers to predict the sensitivity, potency, and efficacy of the immune checkpoint therapy; however, the perplexing mechanism of immune checkpoints make biomarker identification more challenging. While the role of TIM3 and Gal9 in cancer development has been recognized and widely studied, TIM3 and Gal9 as biomarkers are still being explored. We have summarized the recent studies on the possible use of TIM3 and Gal9 as prognostic and predictive biomarkers in various diseases.

High TIM3 expression on tumor-infiltrating T cells and increased Gal9 level in tumor cells in the TME are associated with numerous tumors. For example, high levels of plasma exosomal TIM3 and Gal9 are related to advanced tumor stages, large tumor size, and metastasis in NSCLC [95]. Upregulated Gal9 expression on tumor cells and TIM3 expression on CD8⁺ T cells are observed in gastric cancer [121], glioma [89], chronic lymphocytic leukemia [122], and esophageal squamous cell carcinoma [96]. Increased infiltration of TIM3⁺ T cells in tumors and increased Gal9 expression by tumor cells are inversely proportional to overall survival in cancer patients. The presence of tumor DNA in blood circulation has been reported in various cancers [123,124]. Isolation of tumor DNA from blood samples to assess DNA methylation or histone distributions of TIM3 and Gal9 genes could be used for prognostic or diagnostic purposes. A study performed in primary breast cancer patients to examine the promoter CpG profile in various immune checkpoint receptor genes found TIM3 promoter to be hypomethylated, whereas the gene body of TIM3 was hypermethylated. In addition, the distribution of repressive histone H3k27me3 was also found to be reduced in the TIM3 promoter region [125]. Similarly, in melanoma patients, DNA methylation of the TIM3 and Gal9 promoter region was inversely correlated with the mRNA expression of TIM3 and Gal9. mRNA expression of TIM3 and Gal9 is positively correlated with tumor infiltrating leucocyte fraction, but DNA methylation of TIM3 and Gal9 gene is negatively correlated with infiltrating leucocyte fraction in the tumor environment [126].

In addition to cancers, TIM3 and Gal9 expressions are prominent in other diseases like systemic lupus erythematosus, antiphospholipid syndrome [127], allograft rejection dysfunction [128], endometriosis [129], and, hepatitis C [130]. Gal9 levels in the serum can be used clinically as a stable biomarker in detecting IFN signature in systemic lupus erythematosus and antiphospholipid syndrome [127]. Li et al. showed that the serum levels of soluble TIM3 (sTIM3) and soluble Gal9 (sGal9) were found to be elevated in patients with allograft rejection, making sTIM3 and sGal9 promising biomarker for detection of allograft rejection dysfunction [128]. Elevated Gal9 levels were observed in the serum in endometriosis patients. Recently, a pilot study suggested that Gal9 is a promising biomarker for the diagnosis of endometriosis with a sensitivity of 94% and a specificity of 93.75% [129]. By contrast, a meta-analysis showed that CA125, a gold standard biomarker for endometriosis, only exhibit a sensitivity of 52% and a specificity of 93% [131]. These results indicate that Gal9 could be a sensitive biomarker of

endometriosis and other gynecological disorders. Additionally, increased Gal9 level is found in the blood of patients with hepatitis C infection, making it a useful predictive biomarker for hepatitis C [130]. Although the TIM3/Gal9 pathway is not fully understood, the higher level of sTIM3 and sGal9 is observed in the plasma during cancers and other diseases which can be exploited as potential biomarkers.

8. Conclusion and perspective

Altogether, in CD4⁺ and CD8⁺ T cells, the TIM3/Gal9 interaction results in reduced production of cytokines (IFN γ , IL-2 and TNF α), inhibits T cell proliferation, and induces apoptosis of T cells, resulting in suppression of Th1 like immunity. The TIM3/Gal9 interaction is believed to have stimulatory or inhibitory activity in innate immunity depending on the type of innate immune cells. This pathway is critical for maintaining the immunity against self-antigens and important for preventing the onset of autoimmunity. In the TME, the interaction of TIM3 with its ligand Gal9 is believed to inhibit both adaptive and innate immunity, which favors tumor progression. Although there are perplexing physiological discoveries on the stimulatory and inhibitory effect of the TIM3/Gal9 pathway on different types of immune cells, these findings suggest that the TIM3/Gal9 pathway is involved in the pathogenesis of various types of cancers. Collective studies have highlighted the immunoinhibitory role of the TIM3/Gal9 signaling pathway in cancer progression. The inhibition of TIM3 interaction with Gal9 could be a potential therapeutic approach for the treatment of various cancers. In addition, monotherapy using a single immune checkpoint inhibitor is likely to develop resistance. As a result, co-blockade of more than one immune checkpoint protein, such as blocking the TIM3/Gal9 and PD-1/PD-L1 pathways simultaneously, reduces the possibility of developing drug resistance. More studies are needed to unravel the complete molecular mechanism of the TIM3/Gal9 pathway to broaden its therapeutic applications for cancer therapy.

Furthermore, overexpression of TIM3 or Gal9 in the TME and elevated serum levels of TIM3 or Gal9 can be used as a predictive or prognostic biomarker to help the optimization and selection of appropriate therapeutic options in cancers. The identification of TIM3 and Gal9 as biomarkers in the serum could accelerate the development of non-invasive and early-stage cancer diagnoses.

Author contributions

Sashi Kandel: Conceptualization and preparation of the original draft; **Pratik Adhikary:** Participate in the preparation of the original draft; **Guangfu Li:** Review and edit the draft; **Kun Cheng:** Conceptualization, writing, reviewing, and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work is partially supported by the awards (R01CA231099 and R01AA021510) from the National Institutes of Health, United States.

References

- [1] P. Darvin, S.M. Toor, V. Sasidharan Nair, E. Elkord, Immune checkpoint inhibitors: recent progress and potential biomarkers, *Exp. Mol. Med.* 50 (2018) 1–11.
- [2] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, *Nat. Rev. Canc.* 12 (2012) 252–264.

- [3] C. Zhu, A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, V.K. Kuchroo, The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, *Nat. Immunol.* 6 (2005) 1245–1252.
- [4] G.J. Freeman, J.M. Casasnovas, D.T. Umetsu, R.H. DeKruyff, TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity, *Immunol. Rev.* 235 (2010) 172–189.
- [5] S. Chiba, M. Baghdadi, H. Akiba, H. Yoshiyama, I. Kinoshita, H. Dosaka-Akita, Y. Fujioka, Y. Ohba, J.V. Gorman, J.D. Colgan, M. Hirashima, T. Uede, A. Takaoka, H. Yagita, M. Jinushi, Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1, *Nat. Immunol.* 13 (2012) 832–842.
- [6] Y.H. Huang, C. Zhu, Y. Kondo, A.C. Anderson, A. Gandhi, A. Russell, S.K. Dougan, B.S. Petersen, E. Melum, T. Pertel, K.L. Clayton, M. Raab, Q. Chen, N. Beauchemin, P.J. Yazaki, M. Pyzik, M.A. Ostrowski, J.N. Glickman, C.E. Rudd, H.L. Ploegh, A. Franke, G.A. Petsko, V.K. Kuchroo, R.S. Blumberg, CEACAM1 regulates TIM-3-mediated tolerance and exhaustion, *Nature* 517 (2015) 386–390.
- [7] Y. Wolf, A.C. Anderson, V.K. Kuchroo, TIM3 comes of age as an inhibitory receptor, *Nat. Rev. Immunol.* 20 (3) (2019) 173–185.
- [8] C. Solinas, P. De Silva, D. Bron, K. Willard-Gallo, D. Sangiolo, Significance of TIM3 expression in cancer: from biology to the clinic, *Semin. Oncol.* 46 (2019) 372–379.
- [9] T. Lindsted, M. Gad, M.V. Grandal, C. Frölich, V.K. Bhatia, T. Gjetting, J. Lantto, I. D. Horak, M. Kragh, K. Koefoed, M.W. Pedersen, Abstract 5629: preclinical characterization of Sym023 a human anti-TIM3 antibody with a novel mechanism of action, *Canc. Res.* 78 (2018), 5629–5629.
- [10] T. Zhang, L. Xue, J. Zhang, Q. Liu, J. Ma, Y. Zhang, Y. Shi, H. Hou, H. Peng, N. Liu, Y. Zhang, X. Song, Y. Li, J. Song, L. Wang, M. Wei, K. Li, Abstract 2628: BGB-A425: a humanized anti-human Tim-3 antibody that exhibits strong immune cell activation, *Canc. Res.* 77 (2017), 2628–2628.
- [11] J.N. Haidar, S. Antonyamsy, S. Mathew, L. Wu, Y. Zhang, M.C. Kearns, L. Shen, J. M. Sauder, D. Schaer, K.E. Driscoll, M. Kalos, Abstract 2753: the molecular basis of blocking the TIM-3 checkpoint with the LY3321367 mAb in cancer immunotherapy, *Canc. Res.* 79 (2019), 2753–2753.
- [12] A. Murtaza, H. Laken, J.D.S. Correia, P. McNeeley, L. Altbobell, J.-G. Zhang, P. M. Vancutsem, K.M. Wilcoxon, D. Jenkins, Discovery of TSR-022, a novel, potent anti-human TIM-3 therapeutic antibody, *Eur. J. Canc.* 69 (2016).
- [13] J. Wright, P. Iyer, E. Breous-Nystrom, C. Riordan, M. Findeis, D. Underwood, J. Connolly, M. Sanicola-Nadel, H. Natri, P. Scherle, G. Hollis, R. Huber, R. Stein, M.v. Dijk, N.S. Wilson, Abstract 3825: INCAGN02390, a novel antagonist antibody that targets the co-inhibitory receptor TIM-3, *Canc. Res.* 78 (2018), 3825–3825.
- [14] A.C. Anderson, Tim-3, a negative regulator of anti-tumor immunity, *Curr. Opin. Immunol.* 24 (2012) 213–216.
- [15] L. Cheng, Z. Ruan, Tim-3 and Tim-4 as the potential targets for antitumor therapy, *Hum. Vaccines Immunother.* 11 (2015) 2458–2462.
- [16] F. Liu, Y. Liu, Z. Chen, Tim-3 expression and its role in hepatocellular carcinoma, *J. Hematol. Oncol.* 11 (2018) 126.
- [17] R. Fernandez-Santamaria, F. Palomares, M. Salas, I. Dona, G. Bogas, A. Ariza, A. Rodriguez-Nogales, M.C. Plaza-Seron, C. Mayorga, M.J. Torres, T. D. Fernandez, Expression of the Tim3-galectin-9 axis is altered in drug-induced maculopapular exanthema, *Allergy* 74 (2019) 1769–1779.
- [18] A.C. Anderson, D.E. Anderson, L. Bregoli, W.D. Hastings, N. Kassam, C. Lei, R. Chandwaskar, J. Karman, E.W. Su, M. Hirashima, J.N. Bruce, L.P. Kane, V. K. Kuchroo, D.A. Hafler, Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells, *Science* 318 (2007) 1141–1143.
- [19] Y. Ju, N. Hou, J. Meng, X. Wang, X. Zhang, D. Zhao, Y. Liu, F. Zhu, L. Zhang, W. Sun, X. Liang, L. Gao, C. Ma, T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B, *J. Hepatol.* 52 (2010) 322–329.
- [20] S. Nakae, M. Iikura, H. Suto, H. Akiba, D.T. Umetsu, R.H. DeKruyff, H. Saito, S. J. Galli, TIM-1 and TIM-3 enhancement of Th2 cytokine production by mast cells, *Blood* 110 (2007) 2565–2568.
- [21] Y. Piao, X. Jin, Analysis of Tim-3 as a therapeutic target in prostate cancer, *Tumour Biol* 39 (2017), 1010428317716628.
- [22] A. Friedlaender, A. Addeo, G. Banna, New emerging targets in cancer immunotherapy: the role of TIM3, *ESMO Open* 4 (2019), e000497.
- [23] M. Das, C. Zhu, V.K. Kuchroo, Tim-3 and its role in regulating anti-tumor immunity, *Immunol. Rev.* 276 (2017) 97–111.
- [24] K. Sakuishi, L. Apetoh, J.M. Sullivan, B.R. Blazar, V.K. Kuchroo, A.C. Anderson, Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity, *J. Exp. Med.* 207 (2010) 2187–2194.
- [25] R.H. DeKruyff, X. Bu, A. Ballesteros, C. Santiago, Y.L. Chim, H.H. Lee, P. Karisola, M. Pichavant, G.G. Kaplan, D.T. Umetsu, G.J. Freeman, J.M. Casasnovas, T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells, *J. Immunol.* 184 (2010) 1918–1930.
- [26] Y. Lin, L. Zhang, A.X. Cai, M. Lee, W. Zhang, D. Neuberg, C.M. Canning, R. J. Soiffer, E.P. Alyea, J. Ritz, N. Hacohen, T.K. Means, C.J. Wu, Effective posttransplant antitumor immunity is associated with TLR-stimulating nucleic acid-immunoglobulin complexes in humans, *J. Clin. Invest.* 121 (2011) 1574–1584.
- [27] J.S. Dolina, T.J. Braciale, Y.S. Hahn, Liver-primed CD8+ T cells suppress antiviral adaptive immunity through galectin-9-independent T-cell immunoglobulin and mucin 3 engagement of high-mobility group box 1 in mice, *Hepatology* 59 (2014) 1351–1365.
- [28] C. Zhu, TIM-3 and its regulatory role in immune responses, *Current Topics in Microbiology and Immunology*, 2010.
- [29] W. Du, M. Yang, A. Turner, C. Xu, R.L. Ferris, J. Huang, L.P. Kane, B. Lu, TIM-3 as a target for cancer immunotherapy and mechanisms of action, *Int. J. Mol. Sci.* 18 (2017).
- [30] S. Fujihara, H. Mori, H. Kobara, K. Rafiq, T. Niki, M. Hirashima, T. Masaki, Galectin-9 in cancer therapy, *Recent Pat. Endocr. Metab. Immune Drug Discov.* 7 (2013) 130–137.
- [31] J.H. Lai, S.F. Luo, M.Y. Wang, L.J. Ho, Translational implication of galectin-9 in the pathogenesis and treatment of viral infection, *Int. J. Mol. Sci.* 18 (2017).
- [32] F.C. Chou, H.Y. Chen, C.C. Kuo, H.K. Sytwu, Role of galectins in tumors and in clinical immunotherapy, *Int. J. Mol. Sci.* 19 (2018).
- [33] M. Lu, X. Tian, X. Yang, C. Yuan, M. Ehsan, X. Liu, R. Yan, L. Xu, X. Song, X. Li, The N- and C-terminal carbohydrate recognition domains of Haemonchus contortus galectin bind to distinct receptors of goat PBMC and contribute differently to its immunomodulatory functions in host-parasite interactions, *Parasites Vectors* 10 (2017) 409.
- [34] K. Sideras, R.A. de Man, S.M. Harrington, W.G. Polak, G. Zhou, H.M. Schutz, A. Pedroza-Gonzalez, K. Biermann, S. Mancham, M. Ene, Hansen, R. Bart Takkenberg, A.J. van Vuuren, Q. Pan, J.N.M. Ijzermans, S. Sleijfer, D. Sprengers, H. Dong, J. Kwekkeboom, M.J. Bruno, Circulating levels of PD-L1 and Galectin-9 are associated with patient survival in surgically treated Hepatocellular Carcinoma independent of their intra-tumoral expression levels, *Sci. Rep.* 9 (2019) 10677.
- [35] S. Chabot, Y. Kashio, M. Seki, Y. Shirato, K. Nakamura, N. Nishi, T. Nakamura, R. Matsumoto, M. Hirashima, Regulation of galectin-9 expression and release in Jurkat T cell line cells, *Glycobiology* 12 (2002) 111–118.
- [36] S. Oomizu, T. Arikawa, T. Niki, T. Kadowaki, M. Ueno, N. Nishi, A. Yamauchi, T. Hattori, T. Masaki, M. Hirashima, Cell surface galectin-9 expressing Th cells regulate Th17 and Foxp3+ Treg development by galectin-9 secretion, *PLoS One* 7 (2012), e48574.
- [37] P. Moar, R. Tandon, Galectin-9 as a biomarker of disease severity, *Cell. Immunol.* 361 (2021) 104287.
- [38] K. Nagahara, T. Arikawa, S. Oomizu, K. Kontani, A. Nobumoto, H. Tateno, K. Watanabe, T. Niki, S. Katoh, M. Miyake, S. Nagahata, J. Hirabayashi, V. K. Kuchroo, A. Yamauchi, M. Hirashima, Galectin-9 increases Tim-3+ dendritic cells and CD8+ T cells and enhances antitumor immunity via galectin-9-Tim-3 interactions, *J. Immunol.* 181 (2008) 7660–7669.
- [39] M.K. Gleason, T.R. Lenvik, V. McCullar, M. Felices, M.S. O'Brien, S.A. Cooley, M. R. Verneris, F. Cichocki, C.J. Holman, A. Panoskaltsis-Mortari, T. Niki, M. Hirashima, B.R. Blazar, J.S. Miller, Tim-3 is an inducible human natural killer cell receptor that enhances interferon gamma production in response to galectin-9, *Blood* 119 (2012) 3064–3072.
- [40] D. Daley, V.R. Mani, N. Mohan, N. Akkad, A. Ochi, D.W. Heindel, K.B. Lee, C. P. Zambirinis, G.S.B. Pandian, S. Savadkar, A. Torres-Hernandez, S. Nayak, D. Wang, M. Hundeyin, B. Diskin, B. Aykut, G. Werba, R.M. Barilla, R. Rodriguez, S. Chang, L. Gardner, L.K. Mahal, B. Ueberheide, G. Miller, Dectin 1 activation on macrophages by galectin 9 promotes pancreatic carcinoma and peritumoral immune tolerance, *Nat. Med.* 23 (2017) 556–567.
- [41] G.M. Vaitaitis, D.H. Wagner Jr., Galectin-9 controls CD40 signaling through a Tim-3 independent mechanism and redirects the cytokine profile of pathogenic T cells in autoimmunity, *PLoS One* 7 (2012), e38708.
- [42] S. Madireddi, S.Y. Eun, S.W. Lee, I. Nemcovicova, A.K. Mehta, D.M. Zajonc, N. Nishi, T. Niki, M. Hirashima, M. Croft, Galectin-9 controls the therapeutic activity of 4-1BB-targeting antibodies, *J. Exp. Med.* 211 (2014) 1433–1448.
- [43] K.L. Clayton, M.S. Haaland, M.B. Douglas-Vail, S. Mujib, G.M. Chew, L. C. Ndhlovu, M.A. Ostrowski, T cell Ig and mucin domain-containing protein 3 is recruited to the immune synapse, disrupts stable synapse formation, and associates with receptor phosphatases, *J. Immunol.* 192 (2014) 782–791.
- [44] Y. Zhang, C.J. Ma, J.M. Wang, X.J. Ji, X.Y. Wu, J.P. Moorman, Z.Q. Yao, Tim-3 regulates pro- and anti-inflammatory cytokine expression in human CD14+ monocytes, *J. Leukoc. Biol.* 91 (2012) 189–196.
- [45] X. Yang, X. Jiang, G. Chen, Y. Xiao, S. Geng, C. Kang, T. Zhou, Y. Li, X. Guo, H. Xiao, C. Hou, R. Wang, Z. Lin, X. Li, J. Feng, Y. Ma, B. Shen, Y. Li, G. Han, T cell Ig mucin-3 promotes homeostasis of sepsis by negatively regulating the TLR response, *J. Immunol.* 190 (2013) 2068–2079.
- [46] D. Tang, M.T. Lotze, Tumor immunity times out: TIM-3 and HMGB1, *Nat. Immunol.* 13 (2012) 808–810.
- [47] P.S. van de Weyer, M. Muehlfeit, C. Klose, J.V. Bonventre, G. Walz, E.W. Kuehn, A highly conserved tyrosine of Tim-3 is phosphorylated upon stimulation by its ligand galectin-9, *Biochem. Biophys. Res. Commun.* 351 (2006) 571–576.
- [48] J. Lee, E.W. Su, C. Zhu, S. Hainline, J. Phuath, J.A. Moroco, T.E. Smithgall, V. K. Kuchroo, L.P. Kane, Phosphotyrosine-dependent coupling of Tim-3 to T-cell receptor signaling pathways, *Mol. Cell Biol.* 31 (2011) 3963–3974.
- [49] M. Rangachari, C. Zhu, K. Sakuishi, S. Xiao, J. Karman, A. Chen, M. Angin, A. Wakeham, E.A. Greenfield, R.A. Sobel, H. Okada, P.J. McKinnon, T.W. Mak, M. M. Addo, A.C. Anderson, V.K. Kuchroo, Bat3 promotes T cell responses and autoimmunity by repressing Tim-3-mediated cell death and exhaustion, *Nat. Med.* 18 (2012) 1394–1400.
- [50] N.S. van Oers, N. Killeen, A. Weiss, Lck regulates the tyrosine phosphorylation of the T cell receptor subunits and ZAP-70 in murine thymocytes, *J. Exp. Med.* 183 (1996) 1053–1062.
- [51] W. Zhang, J. Sloan-Lancaster, J. Kitchen, R.P. Tribble, L.E. Samelson, LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation, *Cell* 92 (1998) 83–92.

- [52] W.L. Lo, N.H. Shah, N. Ahsan, V. Horkova, O. Stepanek, A.R. Salomon, J. Kuriyan, A. Weiss, Lck promotes Zap70-dependent LAT phosphorylation by bridging Zap70 to LAT, *Nat. Immunol.* 19 (2018) 733–741.
- [53] M. Lovatt, A. Filby, V. Parravicini, G. Werlen, E. Palmer, R. Zamoyska, Lck regulates the threshold of activation in primary T cells, while both Lck and Fyn contribute to the magnitude of the extracellular signal-related kinase response, *Mol. Cell Biol.* 26 (2006) 8655–8665.
- [54] P.E. Paz, S. Wang, H. Clarke, X. Lu, D. Stokoe, A. Abo, Mapping the Zap-70 phosphorylation sites on LAT (linker for activation of T cells) required for recruitment and activation of signalling proteins in T cells, *Biochem. J.* 356 (2001) 461–471.
- [55] G. Gaud, R. Lesourne, P.E. Love, Regulatory mechanisms in T cell receptor signalling, *Nat. Rev. Immunol.* 18 (2018) 485–497.
- [56] D. Davidson, M.C. Zhong, P.P. Pandolfi, S. Bolland, R.J. Xavier, B. Seed, X. Li, H. Gu, A. Veillette, The csk-associated adaptor PAG inhibits effector T cell activation in cooperation with phosphatase PTPN22 and Dok adaptors, *Cell Rep.* 17 (2016) 2776–2788.
- [57] T. Vang, K.M. Torgersen, V. Sundvold, M. Saxena, F.O. Levy, B.S. Skallehgg, V. Hansson, T. Mustelin, K. Tasken, Activation of the COOH-terminal Src kinase (Csk) by cAMP-dependent protein kinase inhibits signaling through the T cell receptor, *J. Exp. Med.* 193 (2001) 497–507.
- [58] M. Bergman, T. Mustelin, C. Oetken, J. Partanen, N.A. Flint, K.E. Amrein, M. Autero, P. Burn, K. Alitalo, The human p50csk tyrosine kinase phosphorylates p56lck at Tyr-505 and down regulates its catalytic activity, *EMBO J.* 11 (1992) 2919–2924.
- [59] D. Davidson, B. Schraven, A. Veillette, PAG-associated FynT regulates calcium signaling and promotes anergy in T lymphocytes, *Mol. Cell Biol.* 27 (2007) 1960–1973.
- [60] U. D'Oro, J.D. Ashwell, Cutting edge: the CD45 tyrosine phosphatase is an inhibitor of Lck activity in thymocytes, *J. Immunol.* 162 (1999) 1879–1883.
- [61] J.E. Baker, R. Majeti, S.G. Tangye, A. Weiss, Protein tyrosine phosphatase CD148-mediated inhibition of T-cell receptor signal transduction is associated with reduced LAT and phospholipase Cgamma1 phosphorylation, *Mol. Cell Biol.* 21 (2001) 2393–2403.
- [62] J.P. Moorman, J.M. Wang, Y. Zhang, X.J. Ji, C.J. Ma, X.Y. Wu, Z.S. Jia, K.S. Wang, Z.Q. Yao, Tim-3 pathway controls regulatory and effector T cell balance during hepatitis C virus infection, *J. Immunol.* 189 (2012) 755–766.
- [63] J. Yan, Y. Zhang, J.P. Zhang, J. Liang, L. Li, L. Zheng, Tim-3 expression defines regulatory T cells in human tumors, *PLoS One* 8 (2013), e58006.
- [64] K. Sakushi, S.F. Ngiew, J.M. Sullivan, M.W. Teng, V.K. Kuchroo, M.J. Smyth, A. C. Anderson, TIM3(+)/FOXP3(+) regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer, *Oncol Immunology* 2 (2013), e23849.
- [65] F. Wang, L. Wan, C. Zhang, X. Zheng, J. Li, Z.K. Chen, Tim-3-Galectin-9 pathway involves the suppression induced by CD4+CD25+ regulatory T cells, *Immunobiology* 214 (2009) 342–349.
- [66] S. Gupta, T.B. Thornley, W. Gao, R. Larocca, L.A. Turka, V.K. Kuchroo, T. B. Strom, Allograft rejection is restrained by short-lived TIM-3+PD-1+Foxp3+ Tregs, *J. Clin. Invest.* 122 (2012) 2395–2404.
- [67] S. Shrawat, P.B. Reddy, N. Rajasagi, A. Suryawanshi, M. Hirashima, B.T. Rouse, Galectin-9/TIM-3 interaction regulates virus-specific primary and memory CD8 T cell response, *PLoS Pathog.* 6 (2010), e1000882.
- [68] X. Gao, Y. Zhu, G. Li, H. Huang, G. Zhang, F. Wang, J. Sun, Q. Yang, X. Zhang, B. Lu, TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression, *PLoS One* 7 (2012), e30676.
- [69] L.C. Ndhlovu, S. Lopez-Verges, J.D. Barbour, R.B. Jones, A.R. Jha, B.R. Long, E. C. Schoeffler, T. Fujita, D.F. Nixon, L.L. Lanier, Tim-3 marks human natural killer cell maturation and suppresses cell-mediated cytotoxicity, *Blood* 119 (2012) 3734–3743.
- [70] S. Jost, U.Y. Moreno-Nieves, W.F. Garcia-Beltran, K. Rands, J. Reardon, I. Toth, A. Piechocka-Trocha, M. Altfeld, M.M. Addo, Dysregulated Tim-3 expression on natural killer cells is associated with increased Galectin-9 levels in HIV-1 infection, *Retrovirology* 10 (2013) 74.
- [71] X.H. Hu, M.X. Tang, G. Mor, A.H. Liao, Tim-3: expression on immune cells and roles at the maternal-fetal interface, *J. Reprod. Immunol.* 118 (2016) 92–99.
- [72] Y.H. Li, W.H. Zhou, Y. Tao, S.C. Wang, Y.L. Jiang, D. Zhang, H.L. Piao, Q. Fu, D. J. Li, M.R. Du, The Galectin-9/Tim-3 pathway is involved in the regulation of NK cell function at the maternal-fetal interface in early pregnancy, *Cell. Mol. Immunol.* 13 (2016) 73–81.
- [73] H. Kerr, A. Richards, Complement-mediated injury and protection of endothelium: lessons from atypical haemolytic uraemic syndrome, *Immunobiology* 217 (2012) 195–203.
- [74] X. Li, Y. Chen, X. Liu, J. Zhang, X. He, G. Teng, D. Yu, Tim3/Gal9 interactions between T cells and monocytes result in an immunosuppressive feedback loop that inhibits Th1 responses in osteosarcoma patients, *Int. Immunopharm.* 44 (2017) 153–159.
- [75] W. Zhang, Y. Zhang, Y. He, X. Wang, Q. Fang, Lipopolysaccharide mediates time-dependent macrophage M1/M2 polarization through the Tim-3/Galectin-9 signalling pathway, *Exp. Cell Res.* 376 (2019) 124–132.
- [76] C.J. Ma, G.Y. Li, Y.Q. Cheng, J.M. Wang, R.S. Ying, L. Shi, X.Y. Wu, T. Niki, M. Hirashima, C.F. Li, J.P. Moorman, Z.Q. Yao, Cis association of galectin-9 with Tim-3 differentially regulates IL-12/IL-23 expressions in monocytes via TLR signaling, *PLoS One* 8 (2013), e72488.
- [77] A. de Mingo Pulido, A. Gardner, S. Hiebler, H. Soliman, H.S. Rugo, M.F. Krummel, L.M. Coussens, B. Ruffell, TIM-3 regulates CD103(+) dendritic cell function and response to chemotherapy in breast cancer, *Canc. Cell* 33 (2018) 60–74 e66.
- [78] A. Sanchez-Fueyo, J. Tian, D. Picarella, C. Domenig, X.X. Zheng, C.A. Sabatos, N. Manlongat, O. Bender, T. Kamradt, V.K. Kuchroo, J.C. Gutierrez-Ramos, A. J. Coyle, T.B. Strom, Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance, *Nat. Immunol.* 4 (2003) 1093–1101.
- [79] M. Nakayama, H. Akiba, K. Takeda, Y. Kojima, M. Hashiguchi, M. Azuma, H. Yagita, K. Okumura, Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation, *Blood* 113 (2009) 3821–3830.
- [80] J. Fourcade, Z. Sun, M. Benallaoua, P. Guillaume, I.F. Luescher, C. Sander, J. M. Kirkwood, V. Kuchroo, H.M. Zarour, Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients, *J. Exp. Med.* 207 (2010) 2175–2186.
- [81] R. Ocana-Guzman, L. Torre-Bouscoulet, I. Sada-Ovalle, Tim-3 regulates distinct functions in macrophages, *Front. Immunol.* 7 (2016) 229.
- [82] I. Goncalves Silva, I.M. Yasinska, S.S. Sakhnevych, F. Fiedler, J. Wellbrock, M. Bardelli, L. Varani, R. Hussain, G. Siligardi, G. Cececone, S.M. Berger, Y. A. Ushkaryov, B.F. Gibbs, E. Fasler-Kan, V.V. Sumbayev, The Tim-3-galectin-9 secretory pathway is involved in the immune escape of human acute myeloid leukemia cells, *EBioMedicine* 22 (2017) 44–57.
- [83] I. Goncalves Silva, L. Ruegg, B.F. Gibbs, M. Bardelli, A. Fruehwirth, L. Varani, S. M. Berger, E. Fasler-Kan, V.V. Sumbayev, The immune receptor Tim-3 acts as a trafficker in a Tim-3/galectin-9 autocrine loop in human myeloid leukemia cells, *Oncol Immunology* 5 (2016), e1195535.
- [84] M. Nagae, N. Nishi, T. Murata, T. Usui, T. Nakamura, S. Wakatsuki, R. Kato, Crystal structure of the galectin-9 N-terminal carbohydrate recognition domain from *Mus musculus* reveals the basic mechanism of carbohydrate recognition, *J. Biol. Chem.* 281 (2006) 35884–35893.
- [85] Y. Kikushige, T. Miyamoto, J. Yuda, S. Jabbarzadeh-Tabrizi, T. Shima, S. Takayanagi, H. Niuro, A. Yurino, K. Miyawaki, K. Takenaka, H. Iwasaki, K. Akashi, A TIM-3/Gal-9 autocrine stimulatory loop Drives self-renewal of human myeloid leukemia stem cells and leukemic progression, *Cell Stem Cell* 17 (2015) 341–352.
- [86] S. Schwallita, A.A. Fingerle, P. Cammareri, T. Nebelsiek, S.I. Goktuna, P. K. Ziegler, O. Canli, J. Heijmans, D.J. Huels, G. Moreaux, R.A. Rupec, M. Gerhard, R. Schmid, N. Barker, H. Clevers, R. Lang, J. Neumann, T. Kirchner, M.M. Taketo, G.R. van den Brink, O.J. Sansom, M.C. Arkan, F.R. Greten, Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties, *Cell* 152 (2013) 25–38.
- [87] I.M. Yasinska, S.S. Sakhnevych, L. Pavlova, A. Teo Hansen Selno, A.M. Teuscher Abeleira, O. Benlaouer, I. Goncalves Silva, M. Mosimann, L. Varani, M. Bardelli, R. Hussain, G. Siligardi, D. Cholewa, S.M. Berger, B.F. Gibbs, Y.A. Ushkaryov, E. Fasler-Kan, E. Klenova, V.V. Sumbayev, The Tim-3-galectin-9 pathway and its regulatory mechanisms in human breast cancer, *Front. Immunol.* 10 (2019) 1594.
- [88] H. Li, K. Wu, K. Tao, L. Chen, Q. Zheng, X. Lu, J. Liu, L. Shi, C. Liu, G. Wang, W. Zou, Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma, *Hepatology* 56 (2012) 1342–1351.
- [89] Z. Liu, H. Han, X. He, S. Li, C. Wu, C. Yu, S. Wang, Expression of the galectin-9-Tim-3 pathway in glioma tissues is associated with the clinical manifestations of glioma, *Oncol Lett* 11 (2016) 1829–1834.
- [90] C.W. Kang, A. Dutta, L.Y. Chang, J. Mahalingam, Y.C. Lin, J.M. Chiang, C.Y. Hsu, C.T. Huang, W.T. Su, Y.Y. Chu, C.Y. Lin, Apoptosis of tumor infiltrating effector TIM-3+CD8+ T cells in colon cancer, *Sci. Rep.* 5 (2015) 15659.
- [91] J. Dong, L. Cheng, M. Zhao, X. Pan, Z. Feng, D. Wang, Tim-3-expressing macrophages are functionally suppressed and expanded in oral squamous cell carcinoma due to virus-induced Gal-9 expression, *Tumour Biol* 39 (2017), 1010428317701651.
- [92] J.F. Liu, L. Wu, L.L. Yang, W.W. Deng, L. Mao, H. Wu, W.F. Zhang, Z.J. Sun, Blockade of TIM3 relieves immunosuppression through reducing regulatory T cells in head and neck cancer, *J. Exp. Clin. Oncol. Res.* 37 (2018) 44.
- [93] H. Sun, W. Gao, W. Pan, Q. Zhang, G. Wang, D. Feng, X. Geng, X. Yan, S. Li, Tim3(+) Foxp3(+) Treg cells are potent inhibitors of effector T cells and are suppressed in rheumatoid arthritis, *Inflammation* 40 (2017) 1342–1350.
- [94] H. Komita, S. Koido, K. Hayashi, S. Kan, M. Ito, Y. Kamata, M. Suzuki, S. Homma, Expression of immune checkpoint molecules of T cell immunoglobulin and mucin protein 3/galectin-9 for NK cell suppression in human gastrointestinal stromal tumors, *Oncol. Rep.* 34 (2015) 2099–2105.
- [95] J. Gao, X. Qiu, X. Li, H. Fan, F. Zhang, T. Lv, Y. Song, Expression profiles and clinical value of plasma exosomal Tim-3 and Galectin-9 in non-small cell lung cancer, *Biochem. Biophys. Res. Commun.* 498 (2018) 409–415.
- [96] N. Hou, J. Ma, W. Li, L. Zhao, Q. Gao, L. Mai, T-cell immunoglobulin and mucin domain-containing protein-3 and galectin-9 protein expression: potential prognostic significance in esophageal squamous cell carcinoma for Chinese patients, *Oncol Lett* 14 (2017) 8007–8013.
- [97] S. Koyama, E.A. Akbay, Y.Y. Li, G.S. Herter-Sprie, K.A. Buczkowski, W. G. Richards, L. Gandhi, A.J. Redig, S.J. Rodig, H. Asahina, R.E. Jones, M. M. Kulkarni, M. Kuraguchi, S. Palakurthi, P.E. Fecci, B.E. Johnson, P.A. Janne, J. A. Engelman, S.P. Gangadharan, D.B. Costa, G.J. Freeman, R. Bueno, F.S. Hodi, G. Dranoff, K.K. Wong, P.S. Hammerman, Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints, *Nat. Commun.* 7 (2016) 10501.
- [98] W. Kuai, X. Xu, J. Yan, W. Zhao, Y. Li, B. Wang, N. Yuan, Z. Li, Y. Jia, Prognostic impact of PD-1 and Tim-3 expression in tumor tissue in stage I-III colorectal cancer, *BioMed Res. Int.* 2020 (2020) 5294043.

- [99] H. Du, Z. Yi, L. Wang, Z. Li, B. Niu, G. Ren, The co-expression characteristics of LAG3 and PD-1 on the T cells of patients with breast cancer reveal a new therapeutic strategy, *Int. Immunopharm.* 78 (2020) 106113.
- [100] S.L. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D. F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, P. D. Leming, D.R. Spigel, S.J. Antonia, L. Horn, C.G. Drake, D.M. Pardoll, L. Chen, W.H. Sharfman, R.A. Anders, J.M. Taube, T.L. McMiller, H. Xu, A.J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G.D. Kolli, A. Gupta, J.M. Wigginton, M. Sznol, Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, *N. Engl. J. Med.* 366 (2012) 2443–2454.
- [101] Q. Zhou, M.E. Munger, R.G. Veenstra, B.J. Weigel, M. Hirashima, D.H. Munn, W. J. Murphy, M. Azuma, A.C. Anderson, V.K. Kuchroo, B.R. Blazar, Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia, *Blood* 117 (2011) 4501–4510.
- [102] P. Dama, M. Tang, N. Fulton, J. Kline, H. Liu, Gal9/Tim-3 expression level is higher in AML patients who fail chemotherapy, *J. Immunother. Cancer* 7 (2019) 175.
- [103] J. Tan, Z. Yu, J. Huang, Y. Chen, S. Huang, D. Yao, L. Xu, Y. Lu, S. Chen, Y. Li, Increased PD-1+Tim-3+ exhausted T cells in bone marrow may influence the clinical outcome of patients with AML, *Biomark Res* 8 (2020) 6.
- [104] M.A. Kursunel, G. Esendagli, A Co-inhibitory alliance in myeloid leukemia: TIM-3/Galectin-9 complex as a new target for checkpoint blockade therapy, *EBioMedicine* 23 (2017) 6–7.
- [105] D. Zhang, F. Jiang, R. Zaynagetdinov, H. Huang, V.D. Sood, H. Wang, X. Zhao, M. H. Jenkins, Q. Ji, Y. Wang, D.P. Nannemann, D. Musil, J. Wesolowski, A. Paoletti, T. Bartholomew, M.G. Derner, Q. An, C. Iffland, J.P. Halle, Identification and characterization of M6903, an antagonistic anti-TIM-3 monoclonal antibody, *Oncolimmunology* 9 (2020) 1744921.
- [106] E. Limagne, C. Richard, M. Thibaudin, J.D. Fumet, C. Truntzer, A. Lagrange, L. Favier, B. Coudert, F. Ghiringhelli, Tim-3/galectin-9 pathway and mMDSC control primary and secondary resistances to PD-1 blockade in lung cancer patients, *Oncolimmunology* 8 (2019), e1564505.
- [107] V. Anagnostou, K.N. Smith, P.M. Forde, N. Niknafs, R. Bhattacharya, J. White, T. Zhang, V. Adleff, J. Phallen, N. Wali, C. Hruban, V.B. Guthrie, K. Rodgers, J. Naidoo, H. Kang, W. Sharfman, C. Georgiades, F. Verde, P. Illei, Q.K. Li, E. Gabrielson, M.V. Brock, C.A. Zahnow, S.B. Baylin, R.B. Scharpf, J.R. Brahmer, R. Karchin, D.M. Pardoll, V.E. Velculescu, Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer, *Canc. Discov.* 7 (2017) 264–276.
- [108] C.M. Fares, E.M. Van Allen, C.G. Drake, J.P. Allison, S. Hu-Lieskovan, Mechanisms of resistance to immune checkpoint blockade: why does checkpoint inhibitor immunotherapy not work for all patients? *Am Soc Clin Oncol Educ Book* 39 (2019) 147–164.
- [109] L. Yang, D.E. Anderson, J. Kuchroo, D.A. Hafler, Lack of TIM-3 immunoregulation in multiple sclerosis, *J. Immunol.* 180 (2008) 4409–4414.
- [110] J.A. Bluestone, K. Herold, G. Eisenbarth, Genetics, pathogenesis and clinical interventions in type 1 diabetes, *Nature* 464 (2010) 1293–1300.
- [111] M. Kanzaki, J. Wada, K. Sugiyama, A. Nakatsuka, S. Teshigawara, K. Murakami, K. Inoue, T. Terami, A. Katayama, J. Eguchi, H. Akiba, H. Yagita, H. Makino, Galectin-9 and T cell immunoglobulin mucin-3 pathway is a therapeutic target for type 1 diabetes, *Endocrinology* 153 (2012) 612–620.
- [112] S. Fang, Y. Huang, S. Zhong, Y. Zhang, X. Liu, Y. Wang, P. Gu, H. Zhou, X. Fan, IL-17A promotes RANTES expression, but not IL-16, in orbital fibroblasts via CD40-CD40L combination in thyroid-associated ophthalmopathy, *Invest. Ophthalmol. Vis. Sci.* 57 (2016) 6123–6133.
- [113] L.H. Luo, D.M. Li, Y.L. Wang, K. Wang, L.X. Gao, S. Li, J.G. Yang, C.L. Li, W. Feng, H. Guo, Tim3/galectin-9 alleviates the inflammation of TAO patients via suppressing Akt/NF- κ B signaling pathway, *Biochem. Biophys. Res. Commun.* 491 (2017) 966–972.
- [114] Y. Ju, N. Hou, X.N. Zhang, D. Zhao, Y. Liu, J.J. Wang, F. Luan, W. Shi, F.L. Zhu, W.S. Sun, L.N. Zhang, C.J. Gao, L.F. Gao, X.H. Liang, C.H. Ma, Blockade of Tim-3 pathway ameliorates interferon-gamma production from hepatic CD8+ T cells in a mouse model of hepatitis B virus infection, *Cell. Mol. Immunol.* 6 (2009) 35–43.
- [115] L.J. Markwick, A. Riva, J.M. Ryan, H. Cooksley, E. Palma, T.H. Tranah, G. K. Manakkat Vijay, N. Vergis, M. Thursz, A. Evans, G. Wright, S. Tarff, J. O'Grady, R. Williams, D.L. Shawcross, S. Chokshi, Blockade of PD1 and TIM3 restores innate and adaptive immunity in patients with acute alcoholic hepatitis, *Gastroenterology* 148 (2015) 590–602, e510.
- [116] G. Nebbia, D. Peppas, A. Schurich, P. Khanna, H.D. Singh, Y. Cheng, W. Rosenberg, G. Dusheiko, R. Gilson, J. ChinAleong, P. Kennedy, M.K. Maini, Upregulation of the Tim-3/galectin-9 pathway of T cell exhaustion in chronic hepatitis B virus infection, *PLoS One* 7 (2012), e47648.
- [117] J. Liu, S. Xiao, S. Huang, F. Pei, F. Lu, Upregulated Tim-3/galectin-9 expressions in acute lung injury in a murine malarial model, *Parasitol. Res.* 115 (2016) 587–595.
- [118] S. Xiao, J. Liu, S. Huang, F. Lu, Increased Gal-9 and Tim-3 expressions during liver damage in a murine malarial model, *Parasitol. Res.* 115 (2016) 663–672.
- [119] B.P. Dembele, H. Chagan-Yasutan, T. Niki, Y. Ashino, N. Tangpukdee, E. Shinichi, S. Krusood, S. Kano, T. Hattori, Plasma levels of Galectin-9 reflect disease severity in malaria infection, *Malar. J.* 15 (2016) 403.
- [120] N. Hou, Y. Zou, X. Piao, S. Liu, L. Wang, S. Li, Q. Chen, T-cell immunoglobulin- and mucin-domain-containing molecule 3 signaling blockade improves cell-mediated immunity against malaria, *J. Infect. Dis.* 214 (2016) 1547–1556.
- [121] Y. Wang, E. Zhao, Z. Zhang, G. Zhao, H. Cao, Association between Tim3 and Gal9 expression and gastric cancer prognosis, *Oncol. Rep.* 40 (2018) 2115–2126.
- [122] T. Liang, X. Wang, F. Wang, E. Feng, G. You, Galectin-9: a predictive biomarker negatively regulating immune response in glioma patients, *World Neurosurg* 132 (2019) e455–e462.
- [123] M. Esteller, M. Sanchez-Céspedes, R. Rosell, D. Sidransky, S.B. Baylin, J. G. Herman, Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients, *Canc. Res.* 59 (1999) 67–70.
- [124] I.H. Wong, Y.M. Lo, J. Zhang, C.T. Liew, M.H. Ng, N. Wong, P.B. Lai, W.Y. Lau, N. M. Hjelm, P.J. Johnson, Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients, *Canc. Res.* 59 (1999) 71–73.
- [125] V. Sasidharan Nair, H. El Salhat, R.Z. Taha, A. John, B.R. Ali, E. Elkord, DNA methylation and repressive H3K9 and H3K27 trimethylation in the promoter regions of PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, and PD-L1 genes in human primary breast cancer, *Clin. Epigenet.* 10 (2018) 78.
- [126] T.A.W. Holderried, L. de Vos, E.G. Bawden, T.J. Vogt, J. Dietrich, R. Zarbl, F. Bootz, G. Kristiansen, P. Brossart, J. Landsberg, D. Dietrich, Molecular and immune correlates of TIM-3 (HAVCR2) and galectin 9 (LGALS9) mRNA expression and DNA methylation in melanoma, *Clin. Epigenet.* 11 (2019) 161.
- [127] L.L. van den Hoogen, J.A.G. van Roon, J.S. Mertens, J. Wienke, A.P. Lopes, W. de Jager, M. Rossato, A. Pandit, C.G.K. Wichers, F. van Wijk, R.D.E. Fritsch-Stork, T. Radstake, Galectin-9 is an easy to measure biomarker for the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome, *Ann. Rheum. Dis.* 77 (2018) 1810–1814.
- [128] Y.M. Li, Y.Y. Shi, Y. Li, L. Yan, J.T. Tang, Y.J. Bai, X.J. Wu, B. Dai, Y.G. Zou, L. L. Wang, Soluble Tim-3 and Gal-9 are associated with renal allograft dysfunction in kidney transplant recipients: a cross-sectional study, *Int. Immunopharm.* 55 (2018) 330–335.
- [129] R. Brubel, A. Bokor, A. Pohl, G.K. Schilli, L. Szereday, R. Bacher-Szamel, J. Rigo Jr., B. Polgar, Serum galectin-9 as a noninvasive biomarker for the detection of endometriosis and pelvic pain or infertility-related gynecologic disorders, *Fertil. Steril.* 108 (2017) 1016–1025 e1012.
- [130] J.A. Mengshol, L. Golden-Mason, T. Arikawa, M. Smith, T. Niki, R. McWilliams, J. A. Randall, R. McMahan, M.A. Zimmerman, M. Rangachari, E. Dobrinskikh, P. Busson, S.J. Polyak, M. Hirashima, H.R. Rosen, A crucial role for Kupffer cell-derived galectin-9 in regulation of T cell immunity in hepatitis C infection, *PLoS One* 5 (2010), e9504.
- [131] M. Hirsch, J. Duffy, C.J. Davis, M. Nieves Plana, K.S. Khan, O. International, Collaboration to Harmonise, E. Measures for, Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic review and meta-analysis, *BJOG* 123 (2016) 1761–1768.