

TIGIT as an emerging immune checkpoint

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Accepted for publication 4 December 2019

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Introduction

The immune system protects against cancer. However, malignant cells have evolved various ways to escape immune cell recognition and/or killing. Tumour cells may hide themselves by down-regulating their antigen presentation machinery or by inhibiting immune cell trafficking to the tumour bed [1,2]. Tumour cells can also create an immune suppressive microenvironment by secreting or promoting the secretion of immunosuppressive cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)- β , by recruiting regulatory cells including regulatory T cells (T_{regs}), myeloid-derived suppressive cells (MDSCs) and type 2 macrophages or by affecting immune cell metabolism [1–3]. However, another powerful mechanism utilized by tumour cells to evade immune surveillance is the activation of immune checkpoint pathways [4]. These pathways consist of receptor–ligand pairs which, following receptor–ligand interaction, suppress the effector functions of T cells and natural killer (NK) cells and thereby impair anti-tumour immunity.

Monoclonal antibodies (mAbs) targeting the inhibitory receptors cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) have

Summary

T cell immunoglobulin and ITIM domain (TIGIT) is an inhibitory receptor expressed on lymphocytes that was recently propelled under the spotlight as a major emerging target in cancer immunotherapy. TIGIT interacts with CD155 expressed on antigen-presenting cells or tumour cells to down-regulate T cell and natural killer (NK) cell functions. TIGIT has emerged as a key inhibitor of anti-tumour responses that can hinder multiple steps of the cancer immunity cycle. Pre-clinical studies indicated that TIGIT blockade may protect against various solid and haematological cancers. Several monoclonal antibodies (mAbs) that block the inhibitory activity of human TIGIT have been developed. Clinical trials are ongoing, investigating TIGIT blockade as a monotherapy or in combination with anti-PD1/PD-L1 mAbs for the treatment of patients with advanced solid malignancies. In this review, we cover our current knowledge on TIGIT, from its discovery in 2009 to its current status as a clinical target.

Keywords: cancer, checkpoint, immunotherapy, NK cells, T cells

shown clinical efficacy and durable responses in more than 15 types of human malignancy [5–7]. The importance of immune checkpoint blockade (ICB) in revolutionizing modern cancer therapy has been acknowledged by the Nobel Prize in Physiology or Medicine 2018 being awarded to James P. Allison and Tasuku Honjo for their discovery of cancer therapy by inhibition of CTLA-4 and PD-1, respectively [8]. However, despite the enormous success of ICB, a still substantial number of patients do not respond to currently available immunotherapies [9]. In addition, a significant number of patients treated with ICB developed treatment-related toxicities termed ‘immune-related adverse events’ (irAEs), which sometimes led to fatalities [7,10]. Thus, there is great interest in discovering new immune checkpoints that could be safely targeted with high anti-tumour efficacy across various malignancies.

Receptors for nectin and nectin-like (NECL) proteins have recently entered the spotlight as promising targets for cancer immunotherapy [11]. This group includes DNAX accessory molecule-1 (DNAM-1), CD226, PTA1, T lineage-specific activation antigen 1 (TLISA1), CD96 [Tactile (T cell activation, increased late expression)] and T cell immunoglobulin and ITIM domain [TIGIT, also

called Washington University cell adhesion molecule (WUCAM), V-set and transmembrane domain-containing protein 3 (Vstm3) and V-set and immunoglobulin domain-containing protein 9 (VSIG9)] [12] (Fig. 1). DNAM-1, TIGIT and CD96 are expressed on T cells and NK cells and share CD155 [polio virus receptors (PVR), NECL-5] as a ligand. DNAM-1 is a co-stimulatory molecule known to stimulate cytotoxic lymphocyte functions [13,14] and the protective role of DNAM-1 in cancer is well established [15,16]. By contrast, data obtained using *CD96*^{-/-} mice suggested that CD96 acts as an inhibitory receptor that promotes tumour escape from the immune system [17,18]. Similar to CD96, TIGIT is a negative regulator of cytotoxic lymphocytes [19,20]. TIGIT has emerged as a particularly attractive target for cancer therapy due to its seemingly central role in limiting anti-tumour responses. Moreover, experiments using *TIGIT*^{-/-} mice suggested that targeting TIGIT would be safe, and possibly trigger fewer irAEs than anti-PD-1 or anti-CTLA-4 mAbs [21]. Here, we review our current knowledge on TIGIT, from its discovery in 2009 to its current status as a clinical target.

TIGIT, an inhibitory receptor of the PVR-like family

TIGIT structure

TIGIT belongs to a constantly expanding family of PVR-like proteins [22]. It was independently discovered by three groups in 2009 through genome-wide analysis aiming to identify proteins containing domain structures typical for immunomodulatory receptors [22–24]. TIGIT consists of one extracellular immunoglobulin variable domain, a type I transmembrane domain and a short intracellular domain with one immunoreceptor tyrosine-based inhibitory motif (ITIM) and one immunoglobulin tyrosine tail (ITT)-like motif [22,23,25]. The immunoglobulin variable domain shares sequence homology with other members of the PVR-like family, including DNAM-1, CD96, CD155, CD111, CD112 [PVR-related 2 (PVRL2), nectin-2], CD113 [poliovirus receptor-related 3 (PVRL3), nectin-3] and PVRL4 [22]. Human TIGIT shares 58% sequence homology with mouse TIGIT [22,26] and the ITIM-containing sequence in TIGIT cytoplasmic tail is identical in mice and humans [26].

TIGIT expression

In both mice and humans, TIGIT is expressed on NK cells and T cells, including CD4⁺ T cells, CD8⁺ T cells and T_{regs} [22–25]. TIGIT expression is usually low in naive cells, but both T cells and NK cells have been shown to up-regulate TIGIT upon activation [22]. Consequently, in naive mice and healthy individuals, T_{regs}, memory and activated T cells and NK cells show the highest expression of TIGIT [22,25].

TIGIT's ligands

TIGIT has three ligands, CD155, CD112 and CD113, which all belong to a family of nectin and NECL molecules. This family regroups cell surface molecules that mediate cell adhesion, cell polarization and tissue organization, and several members also function as receptors for herpes- and poliovirus [19,27]. In both humans and mice, the main ligand for TIGIT is CD155 [22–25]. Based on crystal structure analysis, both TIGIT and CD155 form homodimers and, following ligand–receptor interaction, heterotetramers [28]. TIGIT binds CD112 and CD113 with lower affinity compared to CD155 [22,24,25]. CD155 is mainly expressed on dendritic cells (DCs), T cells, B cells and macrophages but also in non-haematopoietic tissues such as kidney, nervous system and intestines [23,29]. CD112 has a wide expression in both haematopoietic and non-haematopoietic tissues such as bone marrow, kidney, pancreas and lung [30,31], but the expression of CD113 is restricted to non-haematopoietic tissues, including placenta, testis, kidney, liver and lung [32,33]. Interestingly, CD155 and CD112 are over-expressed in many human malignancies [34–37]. Several factors including oncogene expression or cytokines such as interferon (IFN)- γ have been found to cause up-regulation of CD155 and CD112 on tumour cells [38,39].

Similar to TIGIT, DNAM-1 and CD96 bind to CD155, but with different affinities [40–42]. TIGIT binds CD155 with the highest affinity, followed by CD96 and then DNAM-1 [22]. Together, these receptors share a relationship analogous to the CTLA-4/CD28 pathway, where the inhibitory receptor with higher affinity and activating receptor with lower affinity compete for the same ligands, thereby fine-tuning immune responses [11]. However, the TIGIT/CD96/DNAM-1 pathway appears even more complex than the CTLA-4/CD28 pathway. Indeed, TIGIT and DNAM-1 also share CD112 as a ligand [40], and CD112R (PVRIG), a recently discovered immune checkpoint receptor expressed mainly on T cells and NK cells, competes with DNAM-1 and TIGIT for the binding of CD112 [43,44].

TIGIT mechanisms of action

Several mechanisms of action have been proposed for TIGIT-mediated inhibition of effector T cells and NK cells (Fig. 2). TIGIT may either act in a cell-extrinsic manner, as a ligand for CD155 [22] or in a cell-intrinsic manner by interfering with DNAM-1 co-stimulation [45,46] or by directly delivering inhibitory signals to the effector cell [24]. It is currently unclear whether all these mechanisms are at play in every TIGIT-expressing cell or whether TIGIT mechanism of action differs between CD4⁺ T cells, CD8⁺ T cells and NK cells. In addition, when expressed

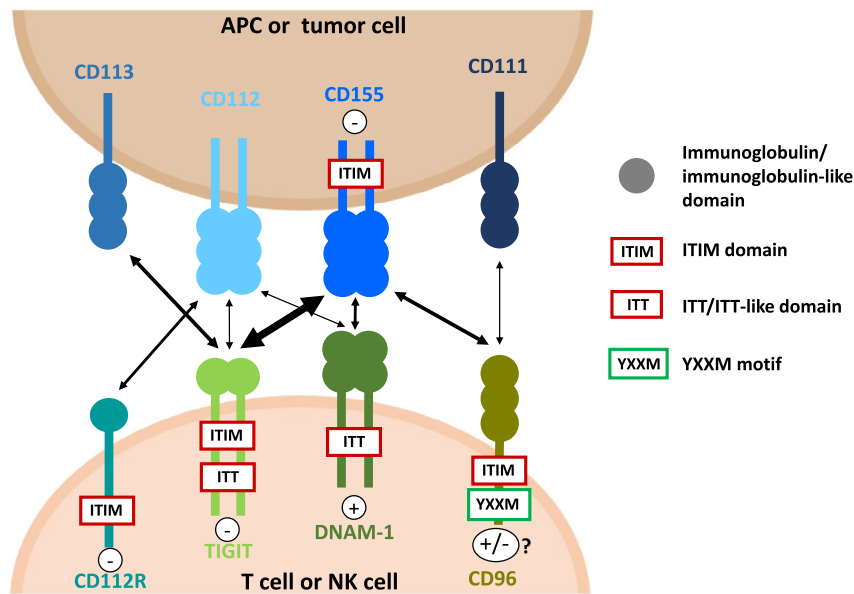


Fig. 1. T cell immunoglobulin and ITIM domain/DNAX accessory molecule-1 (TIGIT/DNAM-1) pathway. TIGIT, DNAM-1, CD96 and CD112R are expressed on T cells and natural killer (NK) cells. Their ligands, CD155, CD112, CD113 and CD111, are expressed on antigen-presenting cells (APCs) or tumour cells. TIGIT, CD112R and CD155 deliver inhibitory signals (-) to cells via their cytoplasmic tails while, despite containing one immunoglobulin tyrosine tail (ITT)-like domain, DNAM-1 delivers an activating (+) signal. Both human and mouse CD96 contain an ITIM domain, but human CD96 also contains a YXXM motif. CD96 has been shown to inhibit mouse T cells and NK cells, but the YXXM motif may cause differences in the signal CD96 delivers in human and mouse cells. The number of extracellular immunoglobulin/immunoglobulin-like domains and possible homodimerization of the receptor or ligand are also shown. Arrows are proportional to the reported affinities of the interactions.

on T_{regs} , TIGIT enhances T_{reg} suppressive functions and may thereby inhibit a wide range of immune cells [47,48].

Cell-extrinsic mechanism

Early studies have suggested a cell-extrinsic mechanism based on the observation that neither TIGIT-specific small interfering RNA (siRNA) nor anti-TIGIT mAbs affected human memory $CD4^+$ T cell responses to anti-CD3 stimulation [22]. However, when T cells were cultured with autologous $CD11c^+$ DCs, the addition of anti-TIGIT mAbs increased T cell proliferation and IFN- γ production. DCs are antigen-presenting cells (APCs) that are crucial for the priming of T cell responses [49]. The quality of the T cell response induced depends upon the type of DCs, as well as on their maturation level. Yu *et al.* demonstrated that TIGIT's interaction with CD155 modulated cytokine production by DCs [22]. Upon TIGIT ligation, CD155 signalling in human monocyte-derived DCs led to increased secretion of IL-10 and decreased secretion of proinflammatory cytokine IL-12. These data suggested that TIGIT-CD155 interactions promote tolerogenic DCs that down-regulate T cell responses. Moreover, the ability of TIGIT-Fc to relieve T cell-mediated delayed-type hypersensitivity symptoms in wild-type but not $Il10^{-/-}$ mice suggested that this mechanism of action is conserved across

species. The regulatory role of TIGIT as a ligand that promotes suppressive functions of CD155-expressing myeloid cells was confirmed by another group [50]. This second study indicated that, in mice, TIGIT promotes the polarization of CD155-expressing type 1 proinflammatory macrophages into IL-10-secreting type 2 macrophages.

Cell-intrinsic mechanisms

In addition to its role as a regulator of DCs and macrophages, TIGIT also suppresses T cell functions in a cell-intrinsic manner. Multiple studies have shown that agonistic anti-TIGIT mAbs inhibit anti-CD3/anti-CD28 mAb-mediated human and mouse T cell proliferation and cytokine production in the absence of APCs [25,46,51]. Further, a recent study demonstrated that melanoma cells expressing a truncated version of CD155 suppressed $CD8^+$ T cell IFN- γ production in a similar manner as cells expressing wild-type CD155 [52]. This indicated that TIGIT-CD155 interaction can inhibit T cell functions without downstream signalling via CD155. For cell-intrinsic mechanism of action it was hypothesized that, given the high affinity of TIGIT for CD155, TIGIT may inhibit T cells by out-competing DNAM-1 for the binding of CD155. This was first suggested by the observation that TIGIT knock-down in human $CD4^+$ T cells increased their

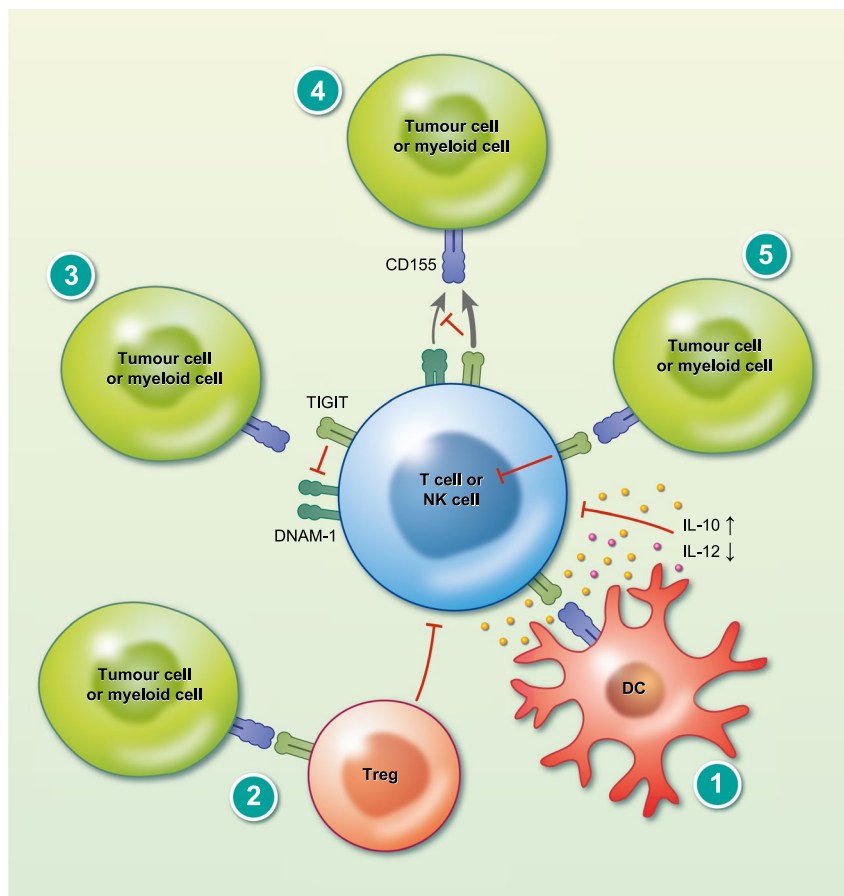


Fig. 2. T cell immunoglobulin and ITIM domain (TIGIT) mechanism of action. TIGIT has been reported to inhibit T cells and natural killer (NK) cells via several mechanisms. (1) Following TIGIT–CD155 interaction, CD155-expressing DCs may become tolerogenic. The acquisition of tolerogenic DC properties is characterized by decreased antigen presentation, low expression of co-stimulatory molecules and reduced production of proinflammatory cytokines [e.g. interleukin (IL)-12] associated with the secretion of anti-inflammatory cytokines (e.g. IL-10). Together, these alterations of dendritic cell (DC) functions result in impaired T cell activation. (2) TIGIT is often up-regulated in regulatory T cells (T_{reg}) and marks a highly suppressive T_{reg} phenotype. (3) TIGIT has been shown to disrupt DNAM-1 accessory molecule-1 (DNAM-1) cis-homodimerization on the cell surface and thus prevent DNAM-1 interaction with CD155. (4) TIGIT binds CD155 with higher affinity compared to DNAM-1 and may therefore out-compete DNAM-1 from interacting with CD155. (5) TIGIT can also deliver the inhibitory signal directly to T cells and NK cells via its cytoplasmic tail.

expression of T-bet and IFN- γ , and this could be overcome by DNAM-1 blockade [46]. Similarly, in another study, TIGIT was found to suppress mouse CD8⁺ T cell responses in a DNAM-1-dependent manner [45]. In addition, time-resolved fluorescence resonance energy transfer (TR-FRET) analysis revealed TIGIT's ability to interact with DNAM-1 on the surface of human T cells and to disrupt DNAM-1 cis-homodimerization, suggesting another mechanism by which TIGIT interferes with DNAM-1-mediated co-stimulation [45].

Besides preventing DNAM-1 signalling, TIGIT can also directly transmit inhibitory signals via its cytoplasmic tail. Most experiments investigating intracellular TIGIT signalling have been performed using the human NK cell line YTS, transfected with human or mouse TIGIT. Two

publications from the same group established that the ITIM motif is essential for human TIGIT signalling, whereas mouse TIGIT inhibition can be mediated by either the ITIM motif or the ITT motif alone [24,26]. Indeed, while human TIGIT with a mutated or truncated ITIM motif failed to inhibit NK cell cytotoxic activity [24], mouse TIGIT function was lost only when tyrosine residues in both the ITIM and ITT-like motifs were mutated [26]. Moreover, another group suggested an important role for the intracellular ITT-like motif in human TIGIT and highlighted two different signalling pathways interfering with NK cell cytotoxicity or IFN- γ production [53,54]. Following ligand binding, ITT-like motif becomes phosphorylated and binds cytosolic adapter growth factor receptor-bound protein 2 (Grb2), which then recruits

SH2-containing inositol phosphatase-1 (SHIP-1), leading to the inhibition of phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signalling cascade and the down-regulation of NK cell killing activity [53]. In addition, association of adaptor β -arrestin 2 with phosphorylated TIGIT mediates recruitment of SHIP-1 through the ITT-like motif and impairs TNF factor receptor (TNFR)-associated factor 6 (TRAF6) auto-ubiquitination to abolish nuclear factor kappa B (NF- κ B) activation, leading to inhibition of IFN- γ production [54]. Only one study investigated the role of direct TIGIT signalling in T cells. Whole-genome microarray analysis on mouse T cells showed that TIGIT engagement suppressed T cell activation via down-regulating T cell receptor (TCR) expression, together with several other molecules involved in TCR and CD28 signalling [51].

TIGIT in T_{regs}

In both humans and mice, TIGIT is highly expressed on a subset of natural T_{regs} and marks an activated T_{reg} phenotype [47,48]. Compared to TIGIT⁻ T_{regs}, TIGIT⁺ T_{regs} demonstrated to be superior in suppressing T cells; however, they seemed to specifically suppress T helper type 1 (Th1) and Th17 responses, but not Th2 cells [47]. *In vitro*, mouse T_{reg} stimulation with agonistic anti-TIGIT mAbs induced the up-regulation of several genes encoding transcription factors, chemokine receptors and T_{reg} effector molecules such as IL-10 or fibrinogen-like protein 2 [47,55]. Finally, the importance of TIGIT⁺ T_{regs} in suppressing T cell responses has been demonstrated *in vivo*. B16F10 tumour-bearing *Rag*^{-/-} mice which received *Tigit*^{-/-} T_{regs} together with wild-type CD4⁺ and CD8⁺ T effector cells showed suppressed tumour growth compared to the same mice receiving wild-type T_{regs} with wild-type CD4⁺ and CD8⁺ T effector cells [55].

TIGIT inhibits anti-cancer immune responses

TIGIT expression in the tumour microenvironment (TME)

Studies in both mice and humans reported increased TIGIT expression on tumour-infiltrating lymphocytes (TILs). TIGIT up-regulation has been observed in various malignancies, including melanoma, breast cancer, non-small-cell lung carcinoma (NSCLC), colon adenocarcinoma (COAD), gastric cancer, acute myeloid leukaemia (AML) and multiple myeloma (MM) [45,56–62]. Many studies reported up-regulated TIGIT expression on CD8⁺ T cells, but there are also descriptions of increased TIGIT levels on tumour-infiltrating T_{regs} and NK cells [55,63–65]. In mouse pre-clinical models and

in cancer patients, TIGIT expression on tumour-infiltrating CD8⁺ T cells often correlates with increased expression of other inhibitory receptors such as PD-1, lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), and with decreased expression of DNAM-1 [45,55,57,66,67]. As a result, TIGIT marks dysfunctional CD8⁺ T cells with decreased cytokine production and degranulation capacities [45,55,56,58]. In particular, TIGIT expression on CD8⁺ T cells in the peripheral blood of gastric cancer patients has been associated with decreased cellular metabolism which resulted in impaired proliferation, cytokine production and migration [62]. Similar to TIGIT⁺ CD8⁺ T cells, TIGIT⁺ NK cells infiltrating mouse subcutaneous tumours or human endometrial cancers were found to co-express other inhibitory receptors, such as LAG-3 and TIM-3 [64,65].

TIGIT correlates with dismal clinical outcomes in cancer

TIGIT expression on TILs of melanoma patients or on peripheral blood CD8⁺ T cells of gastric cancer patients has been associated with metastases development and poor survival [59,61,68]. Moreover, a strong correlation has been observed between TIGIT expression on peripheral blood CD8⁺ T cells and AML relapse post-transplantation [56]. In endometrial cancer, high levels of TIGIT on tumour-resident NK cells have been associated with disease severity [65]. Finally, a high TIGIT/DNAM-1 ratio on tumour-infiltrating T_{regs} was shown to correlate with poor clinical outcome following ICB targeting PD-1 and/or CTLA-4 [63].

TIGIT deficiency protects mice against tumour challenge

Compared to wild-type mice, *TIGIT*^{-/-} mice have shown reduced growth of B16F10 and MC38 subcutaneous tumours and increased survival upon challenge with VK*MYC myeloma cell lines [55,58]. Moreover, Zhang *et al.* reported that TIGIT deficiency protected mice against B16 experimental lung metastasis [64], but these results contrast with others who observed no difference in the number of lung metastases between wild-type and *TIGIT*^{-/-} mice in B16F10 and RM-1 experimental and EO771 spontaneous lung metastasis models [17,18]. Interestingly, however, following B16F10, RM-1 or EO771 challenge, anti-CD96 mAb-treated *TIGIT*^{-/-} mice exhibited reduced numbers of lung metastases compared to wild-type mice receiving identical treatment [18]. Reduction in lung metastases development upon challenge with B16F10 or RM-1 cell lines was also observed in TIGIT-deficient mice treated with anti-TIM-3 mAbs compared to wild-type mice receiving the same treatment [55].

Targeting TIGIT in cancer

A significant body of work has highlighted the great therapeutic potential of targeting TIGIT with antagonistic mAbs in a wide range of malignancies. Experiments have either been performed *in vivo*, using mouse pre-clinical models of disease or *in vitro*, using patient samples.

TIGIT blockade in mouse pre-clinical models

In two independent studies, anti-TIGIT mAbs used as single agents were found insufficient to suppress the growth of already established subcutaneous tumours in mice [45,69]. By contrast, Zhang *et al.* recently reported that early treatment with anti-TIGIT mAbs (i.e. started before the tumour being established) delayed the growth of CT26 subcutaneous tumours and methylcholanthrene (MCA)-induced fibrocarcinomas [64]. The same group showed that anti-TIGIT mAbs protected mice against 4T1 or B16 experimental metastasis. Interestingly, Zhang *et al.* proposed that NK cells were involved in the protection observed following TIGIT-blockade in all these different models. Similarly, we found that blocking TIGIT significantly decreased tumour burden in an aggressive mouse myeloma model (Vk12653) and increased survival in two additional mouse myeloma models, Vk12598 and 5TGM1 [58]. However, in opposition to Zhang *et al.*, we observed only minor TIGIT expression on NK cells in MM-bearing mice and we found that TIGIT blockade protected against MM in a CD8⁺ T cell-dependent manner. The promising potential of targeting TIGIT in MM was confirmed by another group, who established that anti-TIGIT mAbs protected mice against Vk12653 myeloma relapse following haematopoietic stem cell transplantation [66].

Several groups have shown that the limited efficacy of anti-TIGIT mAbs against established subcutaneous tumours could be overcome by combining TIGIT blockade with other therapies, notably with ICB of the PD-1/PD-L1 pathway [45,69]. In the MC38 model, co-blockade of TIGIT and PD-1 was associated with enhanced effector cell functions of both CD4⁺ and CD8⁺ T cells compared to either therapy alone; and TIGIT/PD-1 co-blockade led to a 100% cure rate [69]. In addition, combination of anti-TIGIT mAbs with PD-1 or PD-L1 blockade induced the regression of CT26 and EMT6 tumours, with most mice experiencing complete response; this phenomenon was dependent upon CD8⁺ T cells and DNAM-1 [45]. In this last study, even though single therapies increased CD8⁺ T cell IFN- γ production in the tumour-infiltrating lymph node, only TIGIT/PD-L1 co-blockade significantly increased CD8⁺ T cell IFN- γ production in the tumour. Further supporting the potent anti-tumour effect of TIGIT/PD-1 co-blockade, two groups have shown that this combination therapy protected mice against orthotopically

implanted GL261 glioblastoma [69,70]. Finally, co-blockade of TIGIT and PD-L1 in combination with radiotherapy resulted in 90% cure rates of mice bearing CT26 subcutaneous tumours [71].

TIGIT blockade of human T cells

There is convincing evidence that blocking TIGIT may restore T cell activity in cancer patients. In AML, siRNA knock-down of TIGIT expression reversed the dysfunctional phenotype of blood TIGIT⁺ CD8⁺ T cells, leading to increased IFN- γ and TNF- α production and decreased apoptosis [56]. Further supporting the application of TIGIT blockade in haematological cancers, we showed that human anti-TIGIT mAbs increased the proliferation and production of IFN- γ and TNF- α and degranulation of MM patients' bone marrow CD8⁺ T cells following stimulation with anti-CD3/anti-CD28/anti-CD2 microbeads [58]. In melanoma, anti-TIGIT mAbs were shown to enhance cytokine production and proliferation of peripheral blood CD8⁺ T cells stimulated *in vitro* with NY-ESO tumour peptide; this effect was enhanced upon TIGIT/PD-1 co-blockade [57]. In addition, when combined with anti-PD1 or anti-PD-L1 mAbs, TIGIT blockade could increase the activity of melanoma TILs. More precisely, TIGIT/PD-1 co-blockade increased the proliferation and degranulation of CD8⁺ TILs from metastatic melanoma patients following anti-CD3 mAb stimulation [57] and increased IFN- γ production from melanoma TILs co-cultured with autologous melanoma cells [52].

Translating TIGIT blockade into the clinics

Safety considerations

Because immune checkpoint pathways play a key role in maintaining immune homeostasis and preventing autoimmunity, targeting these pathways have the potential to induce irAEs, which are caused by increased cytokine release and immune effector cell infiltration into tissues [72]. The most common irAEs target the skin, gastrointestinal tract, the lung or the liver and can even occasionally cause ICB-related deaths. The majority of patients treated with ipilimumab (anti-CTLA-4 mAb) showed irAEs with any grade, while patients treated with PD-1 blockade showed fewer and less severe irAEs compared to CTLA-4 blockade [10]. Contrary to *CTLA4*^{-/-} mice or to *Pdcd1*^{-/-} mice, which develop severe autoimmune and lymphoproliferative syndromes [73–77], mice deficient for TIGIT show no signs of spontaneous autoimmunity nor defects in the development of haematopoietic cells [21,25]. However, when *TIGIT*^{-/-} mice are immunized or crossed with an autoimmune-prone strain, they show enhanced development of autoimmune disease [25,51]. Nevertheless, given that mice deficient for TIGIT

display a milder autoimmune phenotype compared to *CTLA-4^{-/-}* or *PD-1^{-/-}* mice, we anticipate TIGIT ICB to be relatively safe.

Ongoing clinical trials

Six human anti-TIGIT mAbs of the IgG1 isotype have entered clinical trials. Etigilimab (OMP-313M32) is an anti-TIGIT mAb developed by OncoMed Pharmaceuticals. In an abstract presented at the American Association for Cancer Research (AACR) Annual Meeting 2017, Park *et al.* reported that monotherapy with mouse anti-TIGIT mAb, 313R12, which reportedly functions in a similar fashion to etigilimab, suppressed the growth of syngeneic colon and kidney tumours in immune competent mice [78]. Further, 313R12 therapy was associated with increase in Th1-type T cell responses and increased the function of CD8⁺ T cells. Finally, etigilimab inhibited the growth of patient-derived melanoma in mice reconstituted with human haematopoietic stem cells [78]. Etigilimab was tested for its safety and pharmacokinetics in a Phase I, dose-escalation study (NCT031119428) as a single agent or in combination with nivolumab (anti-PD-1 mAb) to treat various advanced or metastatic solid malignancies [27,79]. In spite of the success of the Phase Ia trial and etigilimab being well tolerated at doses up to 20 mg/kg, the Phase Ib clinical trial was terminated due to sponsor decision. Five other human anti-TIGIT mAbs are currently being tested in Phase I/II clinical trials either as a monotherapy or in combination with PD-1/PD-L1 blockade or chemotherapies for the treatment of advanced solid cancers (Table 1). For some of these mAbs, the Fc portion on the IgG has been mutated to avoid binding to Fcγ receptors (FcγR), as FcR-dependent mechanisms were found to inhibit the anti-tumour activity of anti-PD1 mAbs [80].

Concluding remarks

Due to its broad expression on lymphocytes, TIGIT has emerged as an important immune checkpoint capable of inhibiting each step of the cancer immunity circle [19]. TIGIT may prevent tumour antigen release by NK cells, impair T cell priming by DCs or inhibit cancer cell killing by CD8⁺ T cells (Fig. 3). A significant number of pre-clinical studies have indicated that TIGIT would constitute a suitable target for cancer patients, and the results of six ongoing clinical trials are eagerly awaited. Of note, only solid tumours are currently targeted with anti-TIGIT mAbs in these trials, but TIGIT blockade may also prove beneficial for haematological cancer patients, including AML and MM [56,58]. Given that haematological cancers, with the exception of Hodgkin's lymphoma, seem poorly sensitive to anti-PD1 mAb monotherapy [81], there is a need to develop alternative ICB strategies for these patients. Interestingly, using

Table 1. Currently ongoing clinical trials involving human anti-TIGIT mAbs

Agent	Trial sponsor	Antibody type	Clinical trial identifier	Type of trial	Condition	Combination therapy	Number of patients
Tiragolumab (MTIG7192A; RG6058)	Genentech/Roche	Fully human IgG1 mAb	NCT03563716	Phase II	Locally advanced or metastatic NSCLC	Combination with atezolizumab (anti-PD-L1 mAb)	135
AB154	Arcus Bioscience	Fully humanized IgG1 mAb	NCT03628677	Phase I	Advanced solid cancers	Monotherapy or combination with AB122 (anti-PD-1 mAb)	242
MK-7684	Merck	IgG1 mAb	NCT02964013	Phase I	Solid cancers	Monotherapy or combination with pembrolizumab (anti-PD-1 mAb), with or without pemtrexed and carboplatin (chemotherapeutics)	392
BMS-986207	Bristol-Myers Squibb	IgG1 mAb (FcγR-null)	NCT02913313	Phase I/II	Advanced or metastatic solid cancers	Monotherapy or combination with nivolumab (anti-PD-1 mAb)	170
ASP8374	Astellas Pharma; Potenza Therapeutics	IgG1 mAb (FcγR-null)	NCT03260322	Phase I	Advanced or metastatic solid cancers	Monotherapy or combination with pembrolizumab (anti-PD-1 mAb)	363
ASP8374	Astellas Pharma; Potenza Therapeutics	IgG1 mAb (FcγR-null)	NCT03945253	Phase I	Advanced or metastatic solid cancers	Monotherapy	9

TIGIT = T cell immunoglobulin and ITIM domain; m.Abs = monoclonal antibodies; Ig = immunoglobulin; PD-1 = programmed cell death 1; PD-L1 = programmed cell death ligand 1.vvv

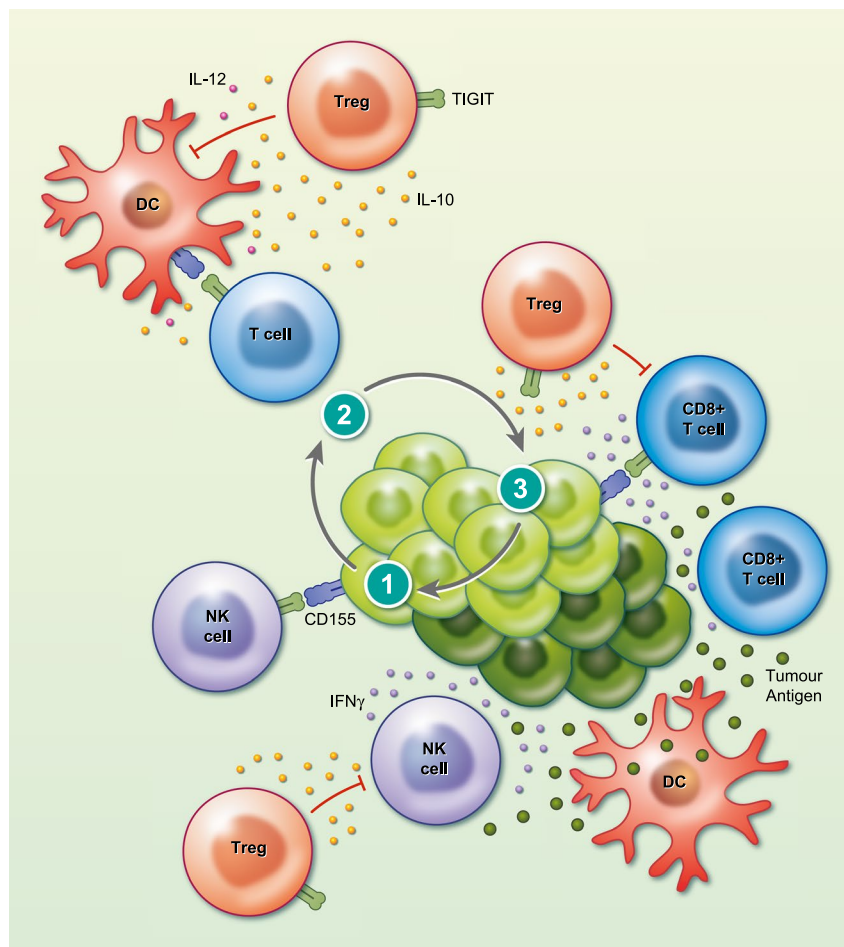


Fig. 3. T cell immunoglobulin and ITIM domain (TIGIT) in the cancer-immunity cycle. TIGIT can suppress anti-tumour immune responses in multiple steps of the cancer-immunity cycle. In step 1, TIGIT can inhibit natural killer (NK) cell function and therefore tumour cell killing. In the absence of inhibition, NK cells kill tumour cells and thus promote the release of tumour antigens which are taken up by dendritic cells (DCs) for presentation to T cells. In step 2, TIGIT can suppress DC functions by interacting with CD155 expressed on DCs leading to impaired T cell priming. In step 3, TIGIT can directly inhibit the effector functions of tumour-specific CD8⁺ T cells through cell-intrinsic mechanism. This prevents tumour cell killing, tumour antigen release and tumour antigen uptake by DCs. In addition, TIGIT expressing regulatory T cells (T_{regs}) are highly suppressive, and may suppress the function of T cells, NK cells and DCs in every step of the cycle.

Vk*MYC mouse myeloma cell lines, we did not observe any anti-tumour effect of anti-PD1 mAbs or CTLA-4 mAbs, whereas TIGIT blockade reduced MM growth and improved survival [16,58,82].

TIGIT targeting is likely to be most efficient when combined with other immunotherapies. In pre-clinical studies, targeting TIGIT together with the PD-1/PD-L1 pathway demonstrated superior tumour suppression compared to either monotherapy [45,69]. Five clinical trials are currently evaluating the safety of TIGIT/PD-1 co-blockade in cancer patients. Other promising combination targets for TIGIT include TIM-3 [55], CD112R [44,83] or CD96 [18,21]. Interestingly, while most efforts are focused on blocking TIGIT inhibitory activity through mAbs, a

recent study considered TIGIT in the context of T cell engineering [84]. Hoogi *et al.* designed a chimeric costimulatory switch receptor composed of the TIGIT exodomains fused to the signalling domain of CD28 that could enhance the functions of chimeric antigen receptor T cells.

In this review, we have focused on TIGIT's functions in cancer. However, TIGIT has a broader role in immunity and the development of TIGIT-targeting therapies may show benefits beyond cancer patients. Compared to PD-1 or CTLA-4, TIGIT plays an important role in NK cell biology as it has been involved in NK cell education [85], NK cell sensing of the microbiota [86] and down-regulation of TIGIT expression on adaptive NK cells confers resistance to MDSCs [87]. In agreement with TIGIT's role as

an immune checkpoint, enhancing TIGIT's function may protect against autoimmune or inflammatory diseases [25,69,88]. TIGIT has also been found to regulate antiviral responses [45] and perhaps impair immune control to human immunodeficiency virus [89].

In conclusion, only 10 years after its discovery, TIGIT has already entered clinical trials as an immunotherapy target. Our increasing understanding of TIGIT-mediated regulation of immune responses will facilitate the design of optimized combination strategies for TIGIT blockade in cancer patients, but will also help the development of TIGIT-targeting therapies to treat other chronic diseases.

Acknowledgements

C. G. was supported by a National Health and Medical Research Council (NHMRC) of Australia Early Career Fellowship (1107417) and a grant (1139048) awarded through the Priority-driven Collaborative Cancer Research Scheme and co-funded by Cancer Australia, Cure Cancer Australia and Can Too Foundation.

Disclosures

The authors have no conflict of interest to declare.

Author contributions

H. H. and C. G. wrote the manuscript. H. H. designed and drew the figures. C. G. edited the figures.

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