TIGIT: An Emerging Immune Checkpoint Target

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Immune checkpoints play a central role in regulating the magnitude and duration of the body's immune response to infection or malignancy, while also preventing harm to the host from an excessive activation. Dysregulation of these immune checkpoints by malignant cells can promote the growth and expansion of solid tumors and hematological malignancies. For example, cancer cells can mitigate an immune attack through upregulation of programmed cell death ligand-1(PD-L1) expression on their surface. PD-L1 engages programmed cell death receptor-1 (PD-1) on T cells and functions as a stop sign, suppressing the function of these immune system cells.[1]

Recognizing the ability of cancer cells to use immune checkpoints to their advantage, checkpoint blockade is an anti-cancer strategy designed to unleash the immune system against malignant cells.[2] Cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), PD-1 and PD-L1 are among the most widely studied checkpoints to date.[3] Immunotherapeutic approach towards cancer is highly effective but data involving its limitations have shown that < 13% of U.S.-based patients respond to checkpoint inhibitor drugs.[4] Furthermore, even when response is achieved, development of resistance to these agents is common.[5] Given the immense potential of checkpoint blockade as well as the inherent challenges, there is considerable interest in developing novel immune checkpoint therapies.[6]

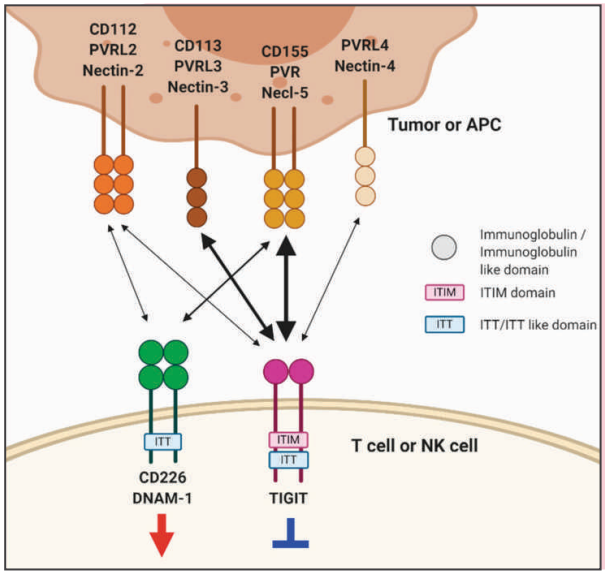
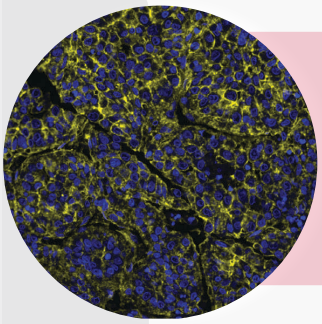


Figure 1. Interactions of T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains (TIGIT) and CD226 with nectin and nectin-like molecules. TIGIT and CD226 are mainly expressed on T and natural killer (NK) cells. TIGIT has multiple ligands, including poliovirus receptor (PVR), nectin-2, nectin-3, and nectin-4. TIGIT binds to nectin-2 and nectin-3 with lower affinity than PVR. Upon engagement, TIGIT transmits inhibitory signals through ITIM and immunoglobulin tyrosine tail (ITT)-like motifs in its cytoplasmic domain. CD226 interacts with PVR and nectin-2 to deliver a positive signal. TIGIT binds to PVR with higher affinity than CD226. The integrated signals formed by their complex interactions regulate immune-cell functions, which is important for immunity and inflammatory responses. Interactions between receptors and ligands are depicted by two-sided arrows. The arrows are proportional to the reported affinities of the interactions except nectin-4. Source: PMID: 33670993[6] used under Creative Commons CC BY 4.

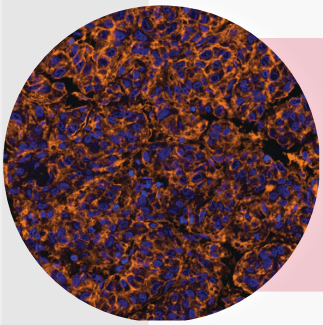
T cell immunoglobulin and ITIM domain (TIGIT), is an inhibitory immune checkpoint receptor which is expressed on memory, regulatory and cytotoxic T cells, and natural killer (NK) cells. TIGIT is emerging as a key target in cancer immunotherapy for several reasons.[7,16] Expression of TIGIT is weak on naïve

cells but is rapidly induced by antigenic challenge or inflammatory stimuli, with high expression on tumor infiltrating lymphocytes (TILs).[8] TIGIT expression is associated with T cell exhaustion, direct immunosuppression of NK cells, release of the immunoregulatory cytokines and tumor progression.[9] [10] Immune activation of TIGIT-expressing cytotoxic T cells and NK cells is suppressed when TIGIT interacts with one of its ligands, CD155 (polio virus receptor, PVR) or CD112 (PVR-related 2, PVRL2; also known as Nectin 2), which are widely expressed on tumor cells.[11]

Figure 2. Ligands CD 155 & CD 112



Panel A: Detection of human Nectin-2/CD112 in FFPE hepatic carcinoma by IHC-IF. Antibody: Rabbit anti-Nectin-2/CD112 recombinant monoclonal [BLR071G] (A700-071). Secondary: HRP-conjugated goat anti-rabbit IgG (A120-501P). Substrate: Opal™. Counterstain: DAPI (blue).



Panel B: Detection of human PVR/CD155 in FFPE hepatic carcinoma by IHC-IF. Antibody: Rabbit anti-PVR/CD155 recombinant monoclonal [BLR074G] (A700-074). Secondary: HRP-conjugated goat anti-rabbit IgG (A120-501P). Substrate: Opal™. Counterstain: DAPI (blue).

Preclinical studies have demonstrated that a dual targeting of TIGIT and PD-1 produces synergistic immune activation.[12] This synergy may be at least partially explained by the understanding that TIGIT inhibits immune responses meditated by both T cells and NK cells, in contrast to CTLA-4, PD-1 and PD-L1. The differential expression and action of the various immune checkpoints highlights their non-redundant, independent functions. Importantly, another feature of TIGIT which makes it an attractive target for immune checkpoint therapy is its high expression on TILs, but low expression in the periphery of the tumor.[11]

Targeting TIGIT has the potential to focus the immune response directly toward the cancer cells in a tumor while limiting systemic autoimmune activity. Greater understanding of TIGIT's function will also allow for design of complementary or synergistic combination therapies. TIGIT may offer a new strategy for addressing the challenges of immune-associated toxicity, treatment resistance and the limited clinical utility of approved cancer immunotherapies. A number of clinical trials investigating the use of anti-TIGIT agents are currently underway.[13]

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Table 1: TIGIT signaling axis related key immune checkpoints and cell types involved in the immune-tumor microenvironment.

Marker	Significance	Bethyl Catalog #
TIGIT	Immune checkpoint [14-16]	A700-047
DNAM-1/CD226	TIGIT ligand, T-cell cytotoxic activation [16-17]	A700-063
NECTIN2/CD112	TIGIT ligand, modulation of T cell signaling [14 17]	A700-071
PVR/CD155	TIGIT ligand, T-cell cytotoxic repression [14 17]	A700-074
CD96	TIGIT regulation analogous to CD28/CTLA-4 mechanism [18]	A700-065
TIM-3	Influencing and alternate checkpoint [19]	A700-033
CEACAM1/5	Cytotoxic activating ligand for TIM-3 [19]	A700-032
PD-1	Influencing and alternate checkpoint [14 13 21]	A700-076
PDL-1	Influencing and alternate checkpoint [14, 20-22]	A700-020
VISTA	Influencing and alternate checkpoint [14]	A700-035
LAG3	Influencing and alternate checkpoint [14]	A700-027
FOXP3	Regulator of TIGIT expression and activity [13, 23]	A700-034
Granzyme	Mediator of T-cell apoptosis [24]	A700-022
Marker	**Cell Type**	**Bethyl Catalog #**
CD3	T-cell [25 26]	A700-016
CD4+	T-cell [25 26]	A700-015
CD8+	T-cell [25 26]	A700-044
AHR	Treg [25 26]	A700-118
CD56	NK-cell [25 26]	A700-152
CD19	B-Cell [25 26]	A700-137
CD20	B-Cell [25 26]	A700-017A
CD68, CD11b	Macrophage [25 26]	A500-018A, A700-107

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A better understanding of the localization, mechanism of action and role of TIGIT in the cancer immunity cycle will inform development of these anti-TIGIT immunotherapies. These studies are enabled by the availability of a rabbit anti-TIGIT recombinant monoclonal antibody from Bethyl.

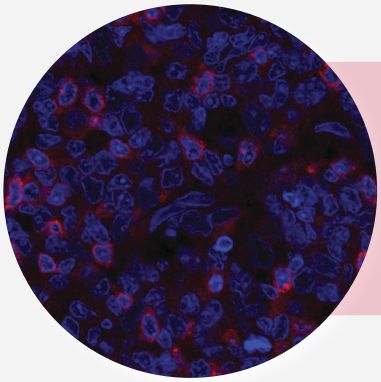
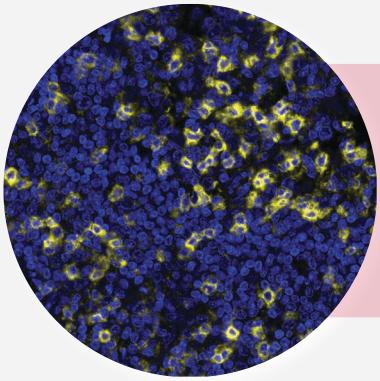
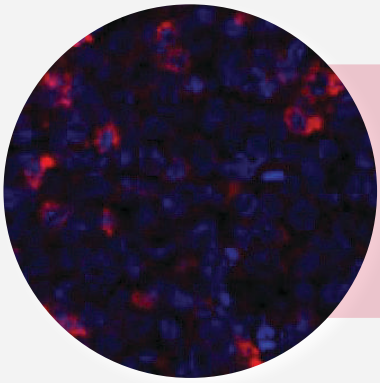


Figure 3. TIGIT Antibodies

Panel A: Detection of human TIGIT (red) in FFPE human tonsil. Antibody: Rabbit anti-TIGIT recombinant monoclonal [BLR047F] (A700-047). Secondary: Dylight 594 conjugated goat-anti-rabbit IgG (A120-101D4). Counterstain: DAPI (blue).



Panel B: Detection of human TIGIT (yellow) in FFPE human tonsil. Antibody: Rabbit anti-TIGIT recombinant monoclonal [BLR047F] (A700-047). Secondary: HRP-conjugated goat-anti-rabbit IgG (A120-501P). Substrate: Opal. Counterstain: DAPI (blue).



Panel C: Detection of human TIGIT (red) in FFPE tonsil by IHC-IF. Antibody: Rabbit anti-TIGIT recombinant monoclonal [BLR047F] (A700-047). Secondary: HRP-conjugated goat anti-rabbit IgG (A120-501P). Substrate: Opal. Counterstain: DAPI (blue).

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