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Cancer Immunotherapy and the Nectin Family

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Abstract

It is increasingly clear that the nectin family and its immunoreceptors shape the immune response to cancer through several pathways. Yet, even as antibodies against TIGIT, CD96, and CD112R advance into clinical development, biological and therapeutic questions remain unanswered. Here, we review recent progress, prospects, and challenges to understanding and tapping this family in cancer immunotherapy.

THE NECTIN FAMILY TREE

The nectin family consists of four nectins [Nectin-1–4, also known as PVRL1–4 (poliovirus receptor–related 1–4), or CD111, CD112, CD113, and PRR4] and five nectin-like molecules (Necls) [Necl1–5, also known as SynCAM1–4 and PVR (poliovirus receptor)/CD155]. They are type I integral membrane proteins and have ectodomains composed of three immunoglobulin (Ig) domains, which can form homophilic and heterophilic interactions resulting in homodimeric or heterodimeric complexes (Harrison et al. 2012, Liu et al. 2019, Mandai et al. 2015, Samanta et al. 2012, Satoh-Horikawa et al. 2000, Yu et al. 2009). Although primarily regulators of cell adhesion, motility, and proliferation (Takai et al. 2008), several nectins are also immunomodulatory. Among them, CD155 (Necl5/PVR) and CD112 (Nectin-2/PVRL2) are ligands that interact with the costimulatory receptor CD226, also known as DNAX accessory molecule 1 (DNAM-1) (Bottino et al. 2003, Reymond et al. 2004, Tahara-Hanaoka et al. 2004), as well as with the coinhibitory receptor TIGIT [T cell immunoreceptor with Ig and ITIM (immunoreceptor tyrosine-based inhibitory motif) domains] (Deuss et al. 2017, Yu et al. 2009). The coinhibitory receptors CD96 and CD112R have also been identified to bind CD155 and CD112, respectively (Fuchs et al. 2004, Zhu et al. 2016), making ligand competition a prominent feature of the family (Dougall et al. 2017). CD111 has also been shown to bind CD96 (Holmes et al. 2019), although its interactions to the other receptors have not been determined (**Figure 1**). The natural killer (NK) cell receptor KIR2DL5 has also been identified as a likely ligand for CD155 in a recent study utilizing a novel method for ligand-receptor proteomics, although the significance of this interaction is not yet understood (Husain et al. 2019).

Structural characterization of ligands and receptor-ligand complexes have revealed the recognition modes of these molecules. The nectin molecules can assemble into homodimers through their membrane-distal D1 domain (Harrison et al. 2012, Liu et al. 2012, Samanta et al. 2012). Similarly, the receptor-ligand complexes form a 1:1 heterodimeric binding stoichiometry in which the D1 domain of the receptor binds to that of the ligand (Deuss et al. 2017, 2019a,b; Stengel et al. 2012). Based on the structures determined so far, the D1 region of the ligands involved in ligand homodimerization is also responsible for receptor interaction, making ligand-dimer and ligand-receptor complexes mutually exclusive (**Figure 2**).

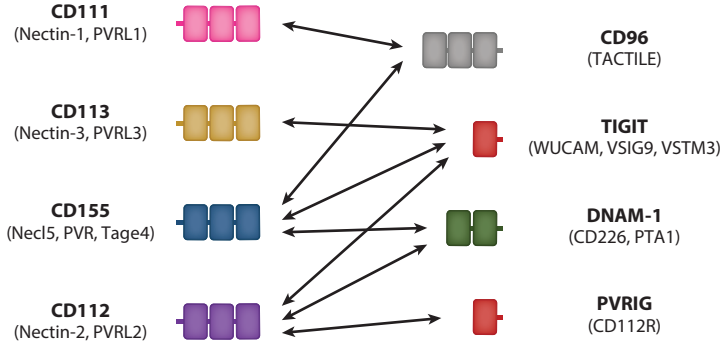

Figure 1

The nectin family. For each immunoreceptor, reported ligands are indicated by arrows and reported functions are indicated by color (*green*, costimulatory; *red*, coinhibitory). DNAM-1 (CD226, PTA1; *green*) is a costimulatory receptor for CD155 (Necl5, PVR, Tage4) and CD112 (Nectin-2, PVRL2). TIGIT (WUCAM, VSIG9, VSTM3; *upper red*) is a coinhibitory receptor for CD155, CD112, and CD113 (Nectin-3, PVRL3). PVRIG (CD112R; *lower red*) is a coinhibitory receptor for CD112. CD96 (TACTILE; *gray*) is a receptor of CD155 and CD111. CD96 has been described as both a coinhibitory and a costimulatory receptor.

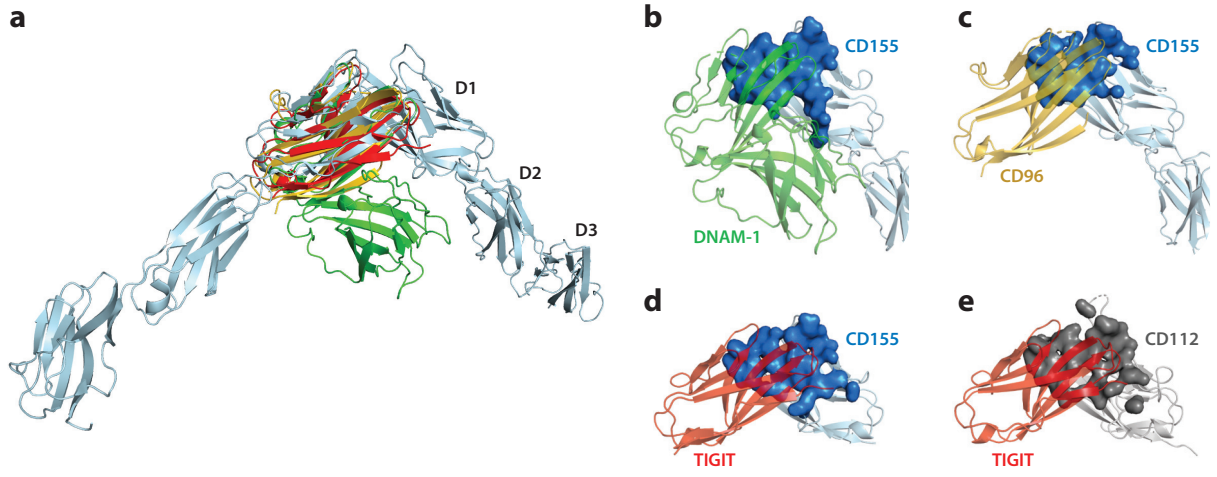

Figure 2

Nectin family receptor-ligand binding. Conserved binding mode to nectin ligands. (*a*) Overlay of costimulatory and coinhibitory receptors bound to domain D1 of CD155. The CD155 homodimer is shown in light blue [Protein Data Bank (PDB) identifier (ID) 4FQP]. (*b–d*) Interactions to CD155 by (*b*) DNAM-1 (~830 Å^2 buried; PDB ID 6O3O), (*c*) CD96 (~650 Å^2 buried; PDB ID 6ARQ), and (*d*) TIGIT (~780 Å^2 buried; PDB ID 3UDW) are represented by a blue surface. (*e*) Interactions with CD112 by TIGIT (~790 Å^2 buried; PDB ID 5V52) are represented by a gray surface. CD155 and CD112 are in the same orientation and illustrate recognition of a similar interface. The epitopes were calculated by AREAIMOL using CCP4 (Winn et al. 2011).

In general, these interactions are facilitated by conserved lock-and-key binding motifs that function as molecular latches, where an aromatic residue packs into a hydrophobic pocket of its neighboring binding partner, and the binding interfaces of the ligand homodimers and receptor-ligand complexes overlay well. Uniquely, TIGIT-ligand complexes assemble into a 2:2 binding stoichiometry, in which TIGIT forms a homodimer on the opposite side of the lock-and-key interface and disruption of the TIGIT homodimer inhibits CD155 signaling (Deuss et al. 2017, Stengel et al. 2012). In addition, TIGIT can disrupt DNAM-1 homodimers expressed on cells (Johnston et al. 2014). While the assembly of a subset of these receptor-ligand pairs has been characterized, a complete biophysical and functional understanding of the interaction network remains to be fully elucidated.

To profile the binding strength of receptor-ligand interactions, researchers have measured their binding affinities. While the D1 domains of the receptors and ligands share a low sequence identity (<28% between DNAM-1, TIGIT, CD96, and CD112R; <49% between CD155 and CD112), the receptor-ligand interactions have been reported to have single- to triple-digit nanomolar affinity (Stengel et al. 2012, Tahara-Hanaoka et al. 2004, Yu et al. 2009, Zhu et al. 2016). These measurements, however, used Fc-fusion constructs, resulting in avidity and enhanced apparent affinity. In contrast, SPR (surface plasmon resonance) measurements of the monovalent receptor-ligand interactions have affinities in the single-digit micromolar range (Deuss et al. 2017, 2019a,b; Liu et al. 2012). While the avidity of these interactions in the context of a cell membrane can overcome the weak affinities, the relative and temporal expression levels of the receptors and ligands need to be considered for a deeper understanding of the hierarchical ranking of this family.

CD155

CD155 (PVR/Necl5/Tage4) was originally defined as a receptor facilitating poliovirus entry (Kučan Brlić et al. 2019, Mendelsohn et al. 1989). CD155 is commonly overexpressed by tumor

cells and upregulated by tumor-associated myeloid cells in both membrane-bound and soluble forms (Kučan Brlić et al. 2019, Li et al. 2018). Elevated CD155 expression has been reported in melanoma (Bevelacqua et al. 2012), many other solid cancers (Carlsten et al. 2009, Gromeier et al. 2000, Masson et al. 2001, Nakai et al. 2010, Nishiwada et al. 2015), and blood cancers (Pende et al. 2005). High expression has been associated with poor clinical measures including tumor histological grade, primary tumor size, lymph node metastasis, reduction in tumor-infiltrating lymphocytes, and poor prognosis in retrospective studies across various solid cancer types (Huang et al. 2017, Nishiwada et al. 2015, Yong et al. 2019). To better understand the relationship between CD155 and clinical correlates, researchers are conducting several clinical trials that are attempting to measure CD155 expression in tumor tissue prior to, or following, the initiation of various treatments in several cancers (**https://clinicaltrials.gov** identifiers NCT03667716, NCT03712358, NCT03789682, NCT03342417, NCT03071328). Elevated soluble CD155 has also been associated with increased tumor burden and has been proposed as a potential biomarker of disease progression (Iguchi-Manaka et al. 2016, Koike et al. 1990).

The tumor cell–intrinsic roles of CD155 include promoting cell-to-cell adhesion (Lange et al. 2001), cell motility (Lange et al. 2001, Oda et al. 2004, Reymond et al. 2004, Sullivan et al. 2013), contact inhibition (Takai et al. 2008), proliferation, and survival (Kakunaga et al. 2004). All of these features have implicated CD155 in tumor progression. CD155 overexpression observed in cancers has been associated with the cellular stress responses to reactive oxygen species and with constitutive expression of the *MYC* oncogene with induction of the ATM-ATR DNA damage repair pathway in human tumor cell lines (Ardolino et al. 2011, Croxford et al. 2013, Fionda et al. 2015, Soriani et al. 2009, Vassena et al. 2013). When expressed, CD155 staining tends to be homogeneous throughout tumor tissue, independent of immune cell localization (Sloan et al. 2004), and $CD155^{low}$ cancers seem to be an exception. This observation lends itself to a genetic rather than an external immunological cause. One possible mechanism is amplification of the *PVR* gene itself, which appears to be most common in uterine carcinoma (~7% of cases) and is associated with a relatively high expression of CD155. In addition to amplification of the *PVR* gene, mutations in other genes that result in enhanced Ras-Raf-MEK-ERK activation, elevated fibroblast growth factor signaling (Hirota et al. 2005, Ikeda et al. 2003), or aberrant activation of the hedgehog signaling pathway have been shown to result in CD155 overexpression in vitro (Solecki et al. 2002). An understanding of the mechanisms responsible for CD155 overexpression by tumor cells may allow for the development of therapies to decrease its expression and increase sensitivity to cancer immunotherapies.

The extracellular region of CD155 contains IgV (variable) domains, a C1-like domain, and a C2 domain (Takai et al. 2008). There are four splice isoforms, designated α, β, δ, and γ. The α and δ isoforms contain transmembrane domains, with the α isoform containing a longer C terminus and an ITIM that is necessary for CD155 signaling and its tumor cell–intrinsic biology. By contrast, the β and γ isoforms lack a transmembrane domain and are secreted. However, the biological function of these isoforms has not been clarified (Koike et al. 1990), only that enforced expression of soluble CD155 in mouse tumors renders the tumors resistant to NK cell–mediated control (Okumura et al. 2020). The CD155α ITIM domain recruits SHP-2 (SH2-containing tyrosine phosphatase 2) to initiate signal transduction (Oda et al. 2004, Yusa et al. 2002). The initiation of signaling by CD155 is usually preceded by its interaction with either other nectins, growth factor receptors, or integrins (e.g., $\alpha_V\beta_3$) (Kakunaga et al. 2004, Kinugasa et al. 2012, Mueller & Wimmer 2003). Collectively, these signaling inputs enable the cell-intrinsic roles of CD155 in promoting cellular proliferation, migration/metastatic spread (Ikeda et al. 2003, Sloan et al. 2004), contract inhibition, and survival, thereby implicating tumor cell–intrinsic CD155 in promoting tumor progression, invasion, and metastasis. CD155 signaling appears to be important for tumor

cell proliferation, where CD155 deletion has demonstrated defects in proliferation and cell cycle arrest (Kono et al. 2008). Subsequently, observations made using mouse tumor models support this conclusion, where CD155-expressing tumor cells have been shown to have greater metastatic potential than tumor cells in which CD155 has been gene-deleted by CRISPR (Li et al. 2018).

CD155 signaling to immune cells has been demonstrated by interactions with the costimulatory receptor DNAM-1 and the inhibitory receptors TIGIT and CD96 on T cells and NK cells. CD155 overexpression is associated with diminished activity of tumor-infiltrating lymphocytes and worse prognosis in several cancer types (Gao et al. 2017, Nishiwada et al. 2015, Triki et al. 2019), suggesting that the overall effect of CD155 in the tumor microenvironment (TME) is often immunosuppressive. This effect is likely driven by an imbalance favoring the coinhibitory CD155 receptors, reminiscent of the process by which the CTLA4 checkpoint receptor competes with the costimulatory receptor CD28 for binding to B7 molecules expressed on antigen-presenting cells (APCs) (Dougall et al. 2017, Husain et al. 2019). One recent study in advanced malignant melanoma has shown that tumor CD155 supports an increase in the fraction of PD-1$^+$CD8$^+$ T cells in anti-PD-1 refractory melanoma tumors, suggesting that targeting the CD155 pathway might improve response to anti-PD-1 therapy for metastatic melanoma patients (Lepletier et al. 2020).

Under physiological conditions, CD155 is also widely expressed at low levels on immune cells, where it has been proposed to play various roles such as MHC-independent positive selection of T cells in the thymus (Georgiev et al. 2016) and costimulation of CD4$^+$ T cells (Yamashita-Kanemaru et al. 2015). CD155 expression on immune cells can be detected among tumor-infiltrating leukocytes in the primary tumor and tumor-draining lymph nodes in mice; however, it appears to be mostly restricted to myeloid cells (Li et al. 2018). Primary tumor growth and experimental metastasis are significantly delayed in CD155-deficient mice and in mice reconstituted with CD155-deficient hematopoietic cells (Li et al. 2018). In human melanoma samples, CD155 expression has been observed on CD14$^+$CD11c$^-$ macrophages, CD14$^+$CD11c$^+$ myeloid cells, and CD14$^-$CD11c$^+$ dendritic cells. Colocalization of CD155-expressing myeloid cells with an M2-like immunosuppressive phenotype and T cells in these tumors has suggested a potentially immunosuppressive role for CD155 (Li et al. 2018). The causes of upregulation of CD155 on tumor-infiltrating myeloid cells are currently unclear, but primarily TLR and NF-κB are implicated (Escalante et al. 2011, Kamran et al. 2013, Pende et al. 2006). CD155 may also be expressed on endothelial cells, where it has been shown to attenuate CD8$^+$ T cell responses (Escalante et al. 2011).

CD112

CD112 (Nectin-2, PVRL2) was first described as an adhesion receptor involved in the formation of cell junctions (Lopez et al. 1998). It is regulated in tumorigenesis, being overexpressed in different types of cancers such as acute myeloid leukemia, multiple myeloma, and epithelial cancers (Sanchez-Correa et al. 2012, Casado et al. 2009, El-Sherbiny et al. 2007, Mastaglio et al. 2018). CD112 expression is associated with aggressiveness and poor prognosis of gallbladder cancer (Miao et al. 2013), but other studies in hepatocellular carcinoma (HCC) suggest that patients with low CD112 expression on their tumors do worse (Huang et al. 2014).

The relationship between CD155 and CD112 remains to be determined, but one study reported the overexpression of CD112 in carcinogen-induced tumors lacking CD155 (Nagumo et al. 2014).

Like CD155, CD112 is a ligand for DNAM-1, and its interaction along with other NK cell receptors triggers human NK cell–mediated cytotoxicity (Pende et al. 2006, Tahara-Hanaoka

et al. 2004). Loss of CD112 and CD155 in acute myeloid leukemia renders them resistant to NK cell–mediated killing (Kearney et al. 2016). CD112 is mainly expressed in cytoplasmic pools, where ubiquitination regulates its degradation and intracellular retention. Inhibition of the ubiquitin pathway results in increased CD112 surface expression and enhances tumor cell susceptibility to NK cell–mediated cytotoxicity (Molfetta et al. 2019). PVRIG (PVR-related Ig domain–containing), also termed as CD112R, was identified in 2016 (Zhu et al. 2016) as a new inhibitory receptor of CD112. It has also been reported that TIGIT recognizes CD112, leading to inhibition of NK cell–mediated cytotoxicity (Stanietsky et al. 2009, Zhu et al. 2016). For TIGIT, however, CD155 still seems to be the predominant ligand in this ligand-receptor network because the interaction between CD112 and TIGIT is very weak (Yu et al. 2009).

DNAM-1 (CD226)

DNAM-1 has long been understood to support NK cell cytotoxicity and T effector cell competency (Castriconi et al. 2004, Pende et al. 2005). Mice genetically deficient in DNAM-1 have demonstrated significantly less antitumor activity against CD155-positive tumor cells than wild-type mice, with a higher incidence of carcinogen-induced tumors, an accelerated tumor growth rate, and increased experimental metastasis (Iguchi-Manaka et al. 2008). Signaling occurs via an intracellular immunoreceptor tyrosine tail (ITT)-like motif and the adaptor proteins Grb2 (Zhang et al. 2015) and VAV1 (Gaud et al. 2018). As is the case for other costimulatory molecules including CD28, DNAM-1 downregulation has been seen on T cells in the setting of chronic infection (Cella et al. 2010, Vallejo et al. 1999), as well as on NK cells in advanced cancers, and has been associated with T cell exhaustion and diminished NK cell–mediated cytotoxicity (Carlsten et al. 2009, Castriconi et al. 2004, Pende et al. 2005, Sanchez-Correa et al. 2012). More recent work has linked tumor CD155 expression directly to loss of CD226 (Braun et al. 2020). These findings might suggest that loss of costimulation is a key feature of lymphocyte dysfunction; however, the mechanisms underlying this effect are poorly understood (Seth et al. 2011).

TIGIT (WUCAM, VSTM3, VSIG9)

In contrast to DNAM-1, TIGIT is highly upregulated on both $CD8^+$ T cells and tumor-infiltrating regulatory T cells and has also been reported on tumor-infiltrating NK cells (Zhang et al. 2018). Single-cell RNA sequencing of human cancers has revealed coexpression of TIGIT among tumor-infiltrating $CD8^+$ T cells expressing other checkpoint receptors such as TIM-3, LAG-3, CTLA-4, and PD-1 (Chevrier et al. 2017, Chung et al. 2017). This was also shown in a recent series of articles defining the transcriptional regulation of $CD8^+$ T cell exhaustion in both chronic viral infection and cancer (Alfei et al. 2019, Khan et al. 2019, Scott et al. 2019). Its intracellular ITT and ITIM motifs are likely responsible for mediating inhibitory signaling (Li et al. 2014). Improved $CD8^+$ T cell function has been seen following blockade of TIGIT alone or in combination with other immunotherapies (Chauvin et al. 2015, Guillerey et al. 2018, Johnston et al. 2014). Additionally, Tregs in $TIGIT^{-/-}$ mice have reduced suppressive activity, and TIGIT-expressing Tregs are more efficient suppressors of Th1 (T helper type 1 cell) and Th17 immune responses than their non-TIGIT-expressing counterparts (Joller et al. 2014, Kurtulus et al. 2015).

TIGIT has been aggressively targeted for immunotherapeutic development, nearly always in combination with PD-(L)1 blockade (**Table 1**). The two most advanced candidates, Genentech's tiragolumab and Merck's vibostolimab, have reported promising results in non-small-cell lung cancer and are advancing into phase III trials (Niu et al. 2020, Rodriguez-Abreau et al. 2020). TIGIT antagonism is expected to reverse coinhibitory signaling downstream of TIGIT (Johnston et al. 2014; Liu et al. 2012, 2013; Lozano et al. 2012) and indirectly enhance

Table 1 TIGIT antibody development landscape

Developer	Isotype	Status	Trials
Seattle Genetics	IgG1 (afucosylated)	Preclinical	NCT04389632 (advanced malignancies)
Astellas	IgG4	Phase I	NCT03260322 (advanced malignancies, phase I)
Beigene	IgG1	Phase I	NCT04047862 (advanced malignancies)
Compugen	IgG4	Phase I	NCT04354246 (advanced malignancies)
EMD Serono	Not disclosed	Phase 1	NCT04457778 (advanced malignancies)
Innovent Biologics	Not disclosed	Phase I	NCT04353830 (advanced malignancies)
iTeos	IgG1	Phase 1	NCT04335253 (advanced malignancies)
OncoMed/Mereo	IgG1	Phase I	NCT03119428 (advanced malignancies)
Arcus, Gilead	IgG1 (Fc-inert)	Phase I/II	NCT03628677 (advanced malignancies, phase I)
			NCT04262856 (NSCLC, phase II)
Bristol Myers Squibb	IgG1 (Fc-inert)	Phase I/II	NCT04150965 (myeloma, phase I)
			NCT02913313 (advanced malignancies, phase I/II)
Merck	IgG1	Phase I/II	NCT01295827 (advanced malignancies, phase I)
			NCT04305041/0430505/04303169 (melanoma, phase I/II)
Genentech/Roche	IgG1	Phase III	NCT04294810 (NSCLC, phase III)
			NCT04256421 (SCLC, phase III)

Abbreviations: Ig, immunoglobulin; NA, not applicable; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

costimulatory signaling downstream of DNAM-1 (Fourcade et al. 2018, Jin et al. 2020, Johnston et al. 2014, Lozano et al. 2012, Zhang et al. 2018). Preclinically, TIGIT-blocking antibodies have demonstrated activity in multiple mouse models and on effector T cells, NK cells, and regulatory T cells (Dixon et al. 2018, Chauvin et al. 2020, Guillerey et al. 2018, Hung et al. 2018, Johnston et al. 2014, Chiu et al. 2020, Minnie et al. 2018, Waight et al. 2018, Wu et al. 2019, Zhang et al. 2018). Similar effects have also been reported in several in vitro assays with human lymphocytes (Chauvin et al. 2015, Chew et al. 2016, Fourcade et al. 2018, Inozume et al. 2016, Jin et al. 2020, Johnston et al. 2014, Waight et al. 2018).

There is an emerging consensus that Fc effector function also plays a role in TIGIT antibody activity. Many studies have utilized TIGIT antibody isotypes that efficiently coengage activating Fc gamma receptors (FcγR), such as mouse IgG2a and human IgG1. Unlike anti-PD-1, anti-TIGIT activity appears to be reduced or even lost in the absence of FcγR engagement (Waight et al. 2018, Williams et al. 2020). Although evidence of clinical relevance remains tenuous (Ha et al. 2019, Romano et al. 2015, Sharma et al. 2019), it is clear that antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) can mediate depletion of intratumor Tregs and enhance the efficacy of antibodies against Treg-expressed targets such as CTLA-4, GITR, OX40, and 4-1BB in mouse models (Bulliard et al. 2013, 2014; Freeman et al. 2020; Selby et al. 2013; Simpson et al. 2013). Depletion of TIGIT-expressing Treg cells is a possible explanation of anti-TIGIT Fc effector function, although evidence of depletion remains elusive (Johnston et al. 2014, Waight et al. 2018). An intriguing alternative put forward by Wilson and colleagues suggests that coengagement of activating FcγR on APCs supports TIGIT (and CTLA-4) antibody activity through modulation of the T cell synapse or APCs, although this mechanism and its potential relevance also need to be better understood (Waight et al. 2018). FcγR coengagement can also be a double-edged sword. Treg cells are not uniquely sensitive to ADCC and ADCP; the same mechanisms result in effector cell depletion and reduced treatment efficacy with anti-PD-1 (Dahan et al. 2015). Additionally, TIGIT agonist antibodies have been identified and reported to

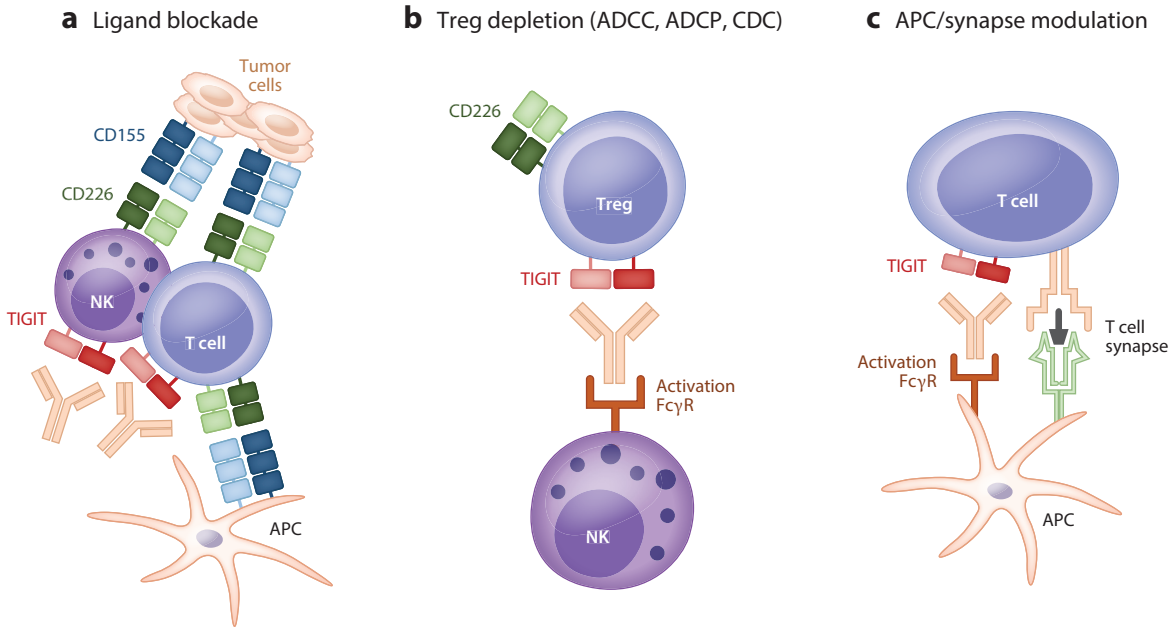

Figure 3

Anti-TIGIT mechanisms of action. Multiple TIGIT antibody mechanisms of action have been proposed: (*a*) ligand blockade; (*b*) intratumoral Treg cell depletion via ADCC, ADCP, or CDC; and (*c*) modulation of the T cell synapse and APCs. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; APC, antigen-presenting cell; CDC, complement-dependent cytotoxicity; FcγR, Fc gamma receptor; NK, natural killer; Treg, regulatory T cell.

suppress T cell responses upon cross-linking (Dixon et al. 2018, Foks et al. 2013, Joller et al. 2014, Levin et al. 2011, Lozano et al. 2012, Lucca et al. 2019).

A comprehensive understanding of how FcγR coengagement can support—or subvert—TIGIT antagonist antibody function will likely have important implications in the clinic. The TIGIT antibodies currently in development span an exceptionally broad range of isotypes, including IgG1, IgG4, and variants engineered to artificially enhance or minimize FcγR coengagement. The most advanced candidates possess an IgG1 isotype, placing them near the middle in terms of potential for FcγR-driven mechanisms of action. Evidence of anti-TIGIT-mediated Treg cell depletion could push the field towards nonfucosylated IgG1 antibodies or other strategies for greater coengagement of activating FcγRs. Conversely, evidence of effector cell depletion or TIGIT agonism could drive a shift to reduced or eliminated FcγR coengagement (**Figure 3**).

CD96 (TACTILE)

Similar to TIGIT, upregulation of CD96 expression on intratumor NK cells and T cells, particularly $CD8^+$ T cells, has been shown in several solid cancer types (Blake et al. 2016, Mittal et al. 2019, Zhang et al. 2018). In human colorectal carcinoma, expression of PD-1 and CD96 appears to be selectively upregulated by $CD8^+$ T cells infiltrating tumor tissue, but not by those in tumor-associated stroma, suggesting that CD96 expression is regulated by antigen exposure or other factors in the TME (Mittal et al. 2019). The percentage, number, and mean fluorescence intensity of $CD96^+$ NK cells were significantly increased in the intratumor tissues of human HCC (Sun et al. 2019). $CD96^+$ NK cells were functionally exhausted with impaired IFNγ and TNF-α production, as well as low gene expression of T-bet, IL-15, perforin, and granzyme B.

HCC patients with a high level of within-tumor CD96 or CD155 expression were strongly associated with shorter disease-free survival and overall survival times.

Evidence of CD96's function is somewhat mixed. Initial studies suggested that CD96 mediated human NK cell adhesion to CD155-expressing target cells (Fuchs et al. 2004). However, NK cells in CD96$^{-/-}$ mice were subsequently found to produce greater IFNγ in response to IL-12, IL-18, or lipopolysaccharide stimulation (Chan et al. 2014), providing the first evidence of coinhibitory activity. CD96$^{-/-}$ mice are resistant to methylcholanthrene carcinogen–induced tumor development (Chan et al. 2014) and to experimental lung metastases in B16F10, B16/BL6, and other models (Chan et al. 2014, Okumura et al. 2020). CD96 has also been shown to reduce the activity of CD8^{+} T cells and promote their acquisition of a dysfunctional phenotype in mouse tumor models (Chan et al. 2014, Lepletier et al. 2019, Mittal et al. 2019).

A recent report of CD96 costimulatory rather than coinhibitory function has raised interesting new questions (Chiang et al. 2020). Grogan and colleagues found that anti-CD96, like anti-CD226 and anti-CD28, enhanced in vitro T cell proliferation, cytotoxicity, and MEK-ERK signaling when cocaptured with anti-CD3 on beads. Additionally, CD96-knockout mice or mice treated with a mouse CD96–blocking antibody exhibited modest impairments in effector T cell differentiation in vivo. The implications of CD96 costimulatory function would be significant. Tumor-infiltrating T cells often upregulate CD96 expression even while downregulating CD226 expression, enabling a potential role for CD96 in sustaining antitumor responses and raising the prospect of a CD96-agonist therapeutic strategy. Future studies will be needed to reconcile these findings with previous ones, and to reconcile the notion of CD96 as costimulatory receptor with that of CD96 as CD226 ligand competitor. Species differences are also possible; the human and mouse CD96 intracellular domains share an ITIM, but only human CD96 possesses an additional YXXM motif (Georgiev et al. 2018).

Evidence of anti-CD96 efficacy in mouse models is less robust than for anti-TIGIT efficacy, but still substantial. CD96 blockade has been found potent in metastatic tumor models sensitive to NK cells and IFNγ (Barrow et al. 2018, Blake et al. 2016, Brooks et al. 2018, Chan et al. 2014, Mittal et al. 2019, Roman Aguilera et al. 2018). Combination activity has been reported with anti-PD-1, anti-CTLA-4, and anti-TIGIT therapies in mice (Blake et al. 2016, Mittal et al. 2019). Similar to TIGIT antibodies, activity has been largely dependent on DNAM-1 (Blake et al. 2016, Mittal et al. 2019), making coblockade of CD96 and TIGIT conceptually appealing as a strategy to maximally enable DNAM-1 engagement of CD155. However, substantial questions of mechanism of action remain. As noted above, studies have disagreed on CD96's role in stimulating or inhibiting T cell responses. Additionally, as with TIGIT, most studies have utilized CD96 antibodies with isotypes that are competent to engage FcγR, leaving the possibility of a role for FcγR engagement.

CD112R (PVRIG)

CD112R (PVRIG) avoids the melee for CD155 and instead recognizes CD112 as its sole ligand. Like TIGIT and CD96, CD112R's intracellular domain contains an ITIM domain that is thought to suppress TCR-mediated signaling (Zhu et al. 2016). Only recently identified (Zhu et al. 2016), CD112R still has a somewhat limited characterization. Surface CD112R appears to be constitutively expressed on NK and NK T cells (Murter et al. 2019, Zhu et al. 2016). It is induced on activated and antigen-experienced T cells, particularly CD8^{+} T cells, and is coexpressed with PD-1 and TIGIT in tumors (Murter et al. 2019, Whelan et al. 2019, Zhu et al. 2016). Like TIGIT, CD112R blockade and deficiency have been shown to potentiate mouse and human T and NK cell responses (Murter et al. 2019, Whelan et al. 2019, Zhu et al. 2016). TIGIT and CD112R antibody

combination activity has also been reported (Whelan et al. 2019, Xu et al. 2017). Joint enablement of DNAM-1 ligand engagement and costimulation is an obvious and promising mechanism of functional synergy, although evidence thus far is mixed (Whelan et al. 2019, Xu et al. 2017). A single CD112R antagonist (Compugen's COM701) is in early clinical trials (NCT03667716). Combination studies with both anti-PD-1 and anti-TIGIT have already been announced.

PATIENT SELECTION AND BIOMARKER STRATEGIES

TIGIT antibody trials have thus far utilized established PD-L1 assays for patient stratification and selection, but biomarkers more proximal to the nectin family may be on the horizon. A recent study reported a wide range of CD155 expression levels in metastatic melanoma despite also finding far lower intratumor hetereogeneity than is seen with PD-L1 (Lepletier et al. 2020). High CD155 expression was correlated with increased PD-1 expression by tumor-infiltrating $CD8^+$ T cells and with reduced responsiveness to immune checkpoint blockade, particularly in tumors with low-PD-L1 expression. These findings and previous reports (Bevelacqua et al. 2012, Li et al. 2018) suggest that patients with $CD155^{high}$ tumors may particularly benefit from TIGIT or CD96 antibody treatments and from the resulting exposure of tumor CD155 to DNAM-1. Tumor CD112 expression might similarly predict responsiveness to CD112R and TIGIT antibody treatments. Still, it is important to note that even in cases where tumor cells themselves express little or no CD155 and CD112, these ligands will still be expressed by tumor-infiltrating myeloid cells and APCs in draining lymph nodes. Further study is needed to understand which contexts, and thus which patients, will be most responsive to TIGIT, CD96, and CD112R blockades.

A related question concerns the propensity of chronically stimulated T cells and NK cells to downregulate DNAM-1. It is becoming increasingly clear that DNAM-1^{low} effector cells are functionally impaired and likely to be less responsive to antibodies against TIGIT, CD96, and CD112R (Guillamon et al. 2019; Jin et al. 2020; Wang et al. 2018, 2019). It will be important to determine if baseline DNAM-1 expression on infiltrating effector cells is needed for optimal responsiveness to antibodies or other therapeutics targeting nectin receptors. The mechanisms behind DNAM-1 loss on intratumor $CD8^+$ T cells will also be important to discern.

CONCLUSIONS

While early signals of TIGIT antibody activity are promising, the nectin family's complexity and the limited success targeting coinhibitory receptors beyond PD-1 and CTLA-4 present reasons for caution. To more fully understand and leverage the therapeutic potential of these targets, we propose three lines of questioning and investigation.

First, to what extent do CD155 and other nectins and Necls shape cancer progression and response to treatment by tumor-intrinsic and by immune-intrinsic functions? Tumor CD155 expression appears to be detrimental to patient outcome, but its activity in both areas needs to be more fully understood to ensure proper interpretation of biomarker data and selection of optimal treatment strategies. The role of CD112 in relation to CD155 also needs better definition. Second, what is DNAM-1's role? While not at the top of the costimulatory receptor functional hierarchy, DNAM-1 ligands are likely the most plentiful in many tumor beds. The degree to which its function is impaired by downregulation and ligand competition is also nebulous. Third, what are the actual and optimal mechanisms of action for anti-TIGIT and other nectin immunoreceptor antibodies? TIGIT antibodies may possess several functions, including inhibition of TIGIT signaling, potentiation of CD226 costimulation, intratumoral Treg depletion, and modulation of

APCs and T cell synapses. The TIGIT antibody Fc strategies now in development make it clear that the field lacks consensus on which of these functions are key.

DISCLOSURE STATEMENT

M.J.S. has scientific research agreements with Bristol Myers Squibb and Tizona Therapeutics and is on the scientific advisory boards of Tizona Therapeutics and Compass Therapeutics. P.S.L. and P.S. are employees of Bristol Myers Squibb. R.J.J. is a past employee of Bristol Myers Squibb and a current employee of Genentech.

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