Poliovirus receptor (PVR)-like protein cosignaling network: new opportunities for cancer immunotherapy

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Abstract

Immune checkpoint molecules, also known as cosignaling molecules, are pivotal cell-surface molecules that control immune cell responses by either promoting (costimulatory molecules) or inhibiting (coinhibitory molecules) a signal. These molecules have been studied for many years. The application of immune checkpoint drugs in the clinic provides hope for cancer patients. Recently, the poliovirus receptor (PVR)-like protein cosignaling network, which involves several immune checkpoint receptors, i.e., DNAM-1 (DNAX accessory molecule-1, CD226), TIGIT (T-cell immunoglobulin (Ig) and immunoreceptor tyrosine-based inhibitory motif (ITIM)), CD96 (T cell activation, increased late expression (TACLILE)), and CD112R (PVRIG), which interact with their ligands CD155 (PVR/Necl-5), CD112 (PVR/L2/nectin-2), CD111 (PVR/L1/nectin-1), CD113 (PVR/L3/nectin-3), and Nectin4, was discovered. As important components of the immune system, natural killer (NK) and T cells play a vital role in eliminating and killing foreign pathogens and abnormal cells in the body. Recently, increasing evidence has suggested that this novel cosignaling network axis costimulates and coinhibits NK and T cell activation to eliminate cancer cells after engaging with ligands, and this activity may be effectively targeted for cancer immunotherapy. In this article, we review recent advances in research on this novel cosignaling network. We also briefly outline the structure of this cosignaling network, the signaling cascades and mechanisms involved after receptors engage with ligands, and how this novel cosignaling network costimulates and coinhibits NK cell and T cell activation for cancer immunotherapy. Additionally, this review comprehensively summarizes the application of this new network in preclinical trials and clinical trials. This review provides a new immunotherapeutic strategy for cancer treatment.

Keywords: PVR, Cosignaling network, Receptor, Ligand, Cancer immunotherapy

Background

Immune checkpoint molecules, also known as cosignaling molecules, are pivotal cell-surface molecules controlling immune cell responses that either stimulate a signal (costimulatory molecules) or inhibit a signal (coinhibitory molecules) [1]. Checkpoint molecules mainly comprise members of the immunoglobulin superfamily and tumor necrosis factor/receptor superfamily [2]. Costimulatory molecules enhance the immune response by promoting a signal, whereas coinhibitory molecules attenuate the immune response by inhibiting a signal [3]. Natural killer (NK) cells and T cells are important components of the immune system, and their surface checkpoint molecules have been intensively studied. To date, several signaling pathways mediated by immune checkpoint molecules in immune cells have been reported to modulate the immune response while protecting against and killing virus-infected cells or malignant
neoplastic cells. CTLA-4, as an immune checkpoint molecule, has been intensively investigated since its discovery [3–7]. Subsequently, PD-1, another immune checkpoint molecule, inhibits T cell cytotoxicity after binding with its ligands PD-L1/PD-L2 [8]. However, the immune checkpoint molecules mentioned above are secondary signals in the immune system that involve costimulatory or coinhibitory T cell cytotoxicity but not NK cell cytotoxicity. Similar to T cells, stimulatory and inhibitory receptors exist on the NK cell surface and guarantee that NK cells efficiently kill invading pathogens and infected or transformed cells but not normal cells by exerting cytotoxic effects after binding to their ligands [9].

Currently, researchers have developed drugs targeting checkpoint molecules for the clinical treatment of cancer. Ipilimumab is a monoclonal antibody (mAb) that enhances T cell activity by specifically targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and was the first drug to be used in the clinic to significantly prolong survival in patients with advanced melanoma [3, 10]. Additionally, after this mAb drug was developed, two additional mAb drugs, pembrolizumab and nivolumab, were developed to treat cancers by selectively inhibiting the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitory pathway. The cancer types that are responsive to PD-1 therapy with an objective clinical response include advanced melanoma, nonsmall-cell lung cancer, and kidney cancer [11]. The emergence of these mAbs is a milestone in cancer immunotherapy. However, clinical results indicate that many types of cancer, including pancreatic ductal adenocarcinoma (PDAC), colon cancer, prostate cancer, and ovarian cancer, do not respond or respond poorly to PD-1 blockade therapy [11].

Recently, poliovirus receptor (PVR)-like proteins, which are expressed on most immune cells, including NK cells and T cells, were identified. These proteins include DNAX accessory molecule-1 (DNAM-1, CD226); T-cell immunoglobulin and mucin domain protein-3 (Tim3); and lymphocyte activation gene-3-like protein-1 (LAG-3). PVR-like proteins belong to a newly emerging immunoglobulin superfamily of proteins containing a PVR signature motif in the Ig variable-like (IgV) domain. This group of proteins initially attracted attention as adhesion proteins that mediate epithelial cell-cell contacts. The two main ligands, CD155 (PVR/Necl-5) and CD112 (PVRIL2/nectin-2), can interact with these four receptors. In addition, other ligands, such as CD111 (PVRL1/nectin-1), CD113 (PVRL3/nectin-3), and Nectin4 (PVRL4), also interact with these receptors. In this review, we describe a new cosignaling network comprising four receptors and their ligands. This novel cosignaling pathway critically modulates the immune cell response and acts as a valuable checkpoint for cancer immunotherapy by regulating NK cells and T cells. However, the molecular and functional relationships among the components of the cosignaling pathway are unclear and need to be further researched.

**PVR like protein receptors**

**DNAM-1**

DNAM1 is a 65-kD immunoglobulin-like transmembrane glycoprotein consisting of an extracellular region with two IgV-like domains, a transmembrane region, a cytoplasmic region with an Ig tail-tyrosine (ITT), and four putative tyrosine residues and one serine residue that are phosphorylated [12, 13]. DNAM-1 is mainly expressed on the majority of monocytes, T cells, NK cells and platelets and on small subsets of B cells [12]. DNAM-1 is a costimulatory receptor that promotes intercellular adhesion, lymphocyte signaling, and lymphokine secretion and enhances NK cell and CTL cytotoxicity [12]. CD112/nectin2 and CD155/PVR have been identified as ligands of the DNAM1 receptor [14, 15], and the interaction between these ligands and receptors ensures NK or T cell-mediated lysis of tumor cells.

Although some studies have investigated the cell-intrinsic signaling cascade, synergy with other NK receptors and cell-extrinsic mechanisms after DNAM-1 interacts with its ligands are described in the following section. However, further research is needed.

**Intrinsic signaling cascades and synergy with other NK receptors**

In NK cells, after DNAM-1 engages with its ligands CD112 and CD155 (Fig. 1), the Ser^322 residue of DNAM-1 is phosphorylated by protein kinase C (PKC). Phosphorylated DNAM-1 subsequently crosslinks with leucocyte function-associated antigen-1 (LFA-1), resulting in lipid raft relocation and engagement with the cytoskeleton due to cooperation with the MAGUK homolog, large human discs, and the actin-binding protein 4.1G [16–18]. Then, the phosphorylation of the Y^322 residue of DNAM-1 by Fyn src kinase is induced by LFA-1 [19]. It is now clear that DNAM-1 alone is not sufficient to trigger NK activation. Studies have shown that DNAM-1 synergizes with 2B4 to costimulate NK cells by phosphorylating the Y^128 and Y^113 residues of SLP-76 (SH2 domain-containing leukocyte phosphoprotein of 76 kDa). The dual phosphorylated form of SLP-76 binds to two Vav1 molecules, which activates phospholipase Cy2 (PLC-γ2), resulting in degranulation, cytokine secretion, and Ca^{2+} flux [20, 21]. Additionally, DNAM-1 synergizes with 2B4 to overcome the inhibition of Vav1 by the E3 ubiquitin ligase c-cbl [20].
Recently, a study showed that upon recognition of its ligand, the asparagine residue at position 321 (N\textsubscript{321}) of DNAM-1 cooperates with the phosphorylated Y\textsubscript{319} residue to recruit adaptor growth factor receptor-bound protein 2 (Grb2), leading to the activation of Vav-1, phosphatidylinositol 3 kinase (PI3K), and phospholipase C\textgamma\textsubscript{1} (PLC\textgamma\textsubscript{1}) [13]. Furthermore, DNAM-1 promotes ERK and AKT activation. Activated AKT phosphorylates the downstream transcription factor FOXO1, a negative regulator of NK cell homing, late-stage maturation, and effector functions [22], and phosphorylated FOXO1 translocates from the nucleus to the cytoplasm, where it regulates natural killer cell antitumor responses [23].

Cell-extrinsic signaling cascades
In addition to being involved in intrinsic signaling cascades and synergizing with other NK receptors, DNAM-1 exerts a costimulatory effect by disrupting Tregs (Fig. 2), thus exerting an immunosuppressive effect on the microenvironment in humans. In melanoma, a low TIGIT/DNAM-1 ratio in Tregs can attenuate their suppressive function and stability, resulting in a decreased number of Tregs in tumors [24]. Additionally, DNAM-1+ TIGIT- Tregs are associated with a reduced Treg number and weak suppressive capacity after in vitro expansion while significantly increasing cytokine interleukin (IL)-10 production [25].

Preclinical and clinical trials of DNAM-1-related strategies
Induction of the expression of DNAM-1 and its ligand represents a promising therapeutic strategy for cancer. An increasing number of studies have already reported that targeting DNAM-1 is a novel anticancer therapeutic strategy. Several preclinical (Table 1) and clinical trials (Table 2) of promising immune checkpoint-targeting strategies for cancer have been reported.

Effect of DNAM-1 on NK cells in preclinical trials
DNAM-1 can enhance NK cell cytotoxicity, which is involved in cancer cell recognition, regulation, and death. DNAM-1-induced NK cytotoxicity relies on the interaction of DNAM-1 with its ligands CD155 and CD112, which are highly expressed in cancer cells [51]. In vivo evidence has shown that DNAM-1 controls tumor metastasis, as recently demonstrated in mice lacking DNAM-1 [52, 53]. In coordination with the antitumor response, the overexpression of DNAM-1 ligands (DNAM-1Ls) on the cancer cell surface is induced to
identify and eliminate NK cells [26, 54–60]. A previous study showed that antibody-mediated masking of NK cell-activating receptors and costimulatory molecules, such as DNAM-1, natural cytotoxicity receptors (NCRs), and NK cell activating receptor natural-killer group 2, member D (NKG2D), frequently induces melanoma cell lysis after receptor-ligand engagement [61]. As mentioned above, PVR (CD155) and nectin2 (CD112) are ligands for DNAM-1. PVR-expressing neuroblastoma cells are efficiently killed by NK cells when engaging with DNAM-1. Blocking either DNAM-1 or PVR with an anti-DNAM-1 or anti-PVR antibody results in the significant inhibition of NK-mediated tumor cell lysis [26]. Similarly, NK-mediated lysis of tumor cells is enhanced after DNAM-1 (in NK cells) interacts with PVR or Nectin-2 (in target cells), whereas this effect is inhibited by treatment with a mAb targeting DNAM-1 or its receptor [15]. Similarly, soluble DNAM-1 (sDNAM-1) also inhibits the growth of cancer cell lines (K562 and HeLa cells) after binding CD155 or CD112 by enhancing NK cell cytotoxicity, and the inhibitory effect of DNAM-1 is significantly attenuated after DNAM-1 mAb blockade [62]. DNAM-1-mediated NK cell activation is altered by the microRNA miR-30c-1*, which enhances NK cell cytotoxicity in human hepatoma cells by targeting the inhibitory transcription factor HMBOX1 and increases the expression of transmembrane tumor necrosis factor-alpha (mTNF-α) [27]. After treatment with the anti-DNAM-1 mAb LeoA1, miR-30c-1* expression alters NK cell killing capacity [27]. The abovementioned studies demonstrated that anti-DNAM-1 mAbs alter NK cell cytotoxicity and are thus additional novel potential immunotherapeutic agents that inhibit tumors through DNAM-1 agonism. A few studies have demonstrated that DNAM-1 agonists increase DNAM-1 expression on the NK cell surface. Increased DNAM-1 improves NK cell cytotoxicity against melanoma cells and inhibits CNS autoimmunity in experimental autoimmune encephalomyelitis by interacting with the ligand CD155 in dendritic cells [28].

**Effect of DNAM-1 on T cells in preclinical trials**

DNAM-1 is expressed on T cells, and several recent preclinical studies have focused on cancer immunotherapy involving targeting DNAM-1 receptors on the T cell surface. DNAM-1 expression affects Treg function, and one study showed that DNAM-1- Tregs are more stable than DNAM-1+ Tregs during in vitro expansion [29]. Blocking the DNAM-1-CD155 interaction promotes Treg expansion and in vitro Treg production. Furthermore, an anti-DNAM-1 mAb prolongs skin allograft survival in a skin allograft model.
Table 1: Preclinical trials in promising cancer target of PVR like receptors

| PVR like protein | Treatment | Immune cells | Tumor | Results | Reference |
|-----------------|-----------|--------------|-------|---------|-----------|
| DNAM-1          | Anti-DMAN-1 or anti-PVR mAbs | NK | Neuroblastoma | monoclonal antibody-mediated masking of either DNAM-1 (on NK cells) or PVR (on neuroblasts) resulted in strong inhibition of tumor cell lysis | [26] |
| DNAM-1          | Anti-DMAN-1 or anti-PVR mAbs | NK | Tumor cell lines | The ability of NK-mediated lysis of tumor cells mediated by DNAM-1 engages with its ligands that was downregulated by mAb-mediated masking of the receptor or its ligands | [15] |
| DNAM-1          | anti-CD226 mAb LeoA1 | NK | Hepatoma | Crosslinking CD226 with the anti-CD226 mAb LeoA1 regulate miR-30c-1* expression, which promoted NK cell cytotoxicity against hepatoma cells by targeting HMBOX1 | [27] |
| DNAM-1 agonist  | NK | Melanoma, experimental autoimmune encephalomyelitis | DNAM-1 agonist could activate DNAM-1 modifies the bidirectional crosstalk of NK cells with CD155 DC, which can suppress CNS autoimmunity and strengthen tumor surveillance | [28] |
| DNAM-1          | Anti-CD226 mAb | Tregs | Allogeneic skin transplant | CD226 mAb promoted Treg expansion, reduced inflammation and prolonged allogeneic graft survival | [29] |
| DNAM-1          | Anti-CD226 mAb | yST | Hepatocellular carcinoma | Anti-DNAM-1 mAb-mediated masking experiments that yST cells cytotoxicity against HCC cells as well as IFN-γ production were decreased | [30] |
| DNAM-1 agonist antibody | CD8+ T | pancreatic ductal adenocarcinoma | CD226 agonist antibody-mediated activation of CD226 augments the effect of TIGIT or PD-1 blockade on CD8 T-cell responses | [31] |
| TIGIT           | Anti-TIGIT | NK | Colon cancer | Blockade of TIGIT prevented NK cell exhaustion and promoted NK cell-dependent tumor immunity, enhanced therapy with antibody to the PD-1 ligand PD-L1 | [32] |
| TIGIT           | Anti-TIGIT | NK | Ovarian cancer | Blockade of TIGIT enhanced degranulation and interferon gamma (IFN-γ) production of NK cells in response to OC tumor cells | [33] |
| TIGIT           | Anti-TIGIT | CD8+ T | Melanoma | TIGIT and PD-1 blockade should be further explored to elicit potent antitumor CD8+ T cell responses | [34] |
| TIGIT           | Anti-TIGIT | CD8+ T | Gastric cancer | Blockade TIGIT enhanced CD8 T cell activation and improved survival in tumor bearing mice | [35] |
| TIGIT           | Anti-TIGIT | CD8+ T | Multiple myeloma | Blockade TIGIT by mAb increased the effector function of MM patient CD8+ T cells and suppressed MM development | [36] |
| TIGIT           | Anti-TIGIT | CD8+ T | Myeloma | Immune checkpoint blockade using mAb against TIGIT significantly restored CD8+ T exhaustion and prolonged myeloma control after stem cell transplantation | [37] |
| TIGIT           | Anti-TIGIT | CD8+ T, Tregs | Head and neck squamous cell carcinoma | Anti-TIGIT treatment significantly reverse T-cell exhaustion and reduce the population of Tregs in vitro and in vivo | [38] |
| TIGIT           | Anti-TIGIT | CD4+ T, CD8+ T, Tregs | Multiple myeloma | Anti-TIGIT mAb depleted FoxP3+ Tregs, increased proliferation of IFN-γ-producing CD4+ T cells, and overcome the inhibition effect of CD8+ T cell signaling and cell proliferation by PVR ligation | [39] |
| TIGIT           | Anti-TIGIT | αβT, yST, Tregs | Hematologic malignancies | Anti-TIGIT mAbs could restore αβT-cell function, prevent CD155 mediated inhibition of yST T cells, depletion of Tregs, and direct killing of tumor cells | [40] |
| TIGIT           | Anti-TIGIT | Effector T, Tregs | Glioblastoma | TIGIT a checkpoint blockade increased effector T cell function and downregulation of suppressive Tregs and TIDCs to enhance antitumor immunity and survival in glioblastoma | [41] |
| TIGIT           | Anti-TIGIT | CD4+ Tregs | Ovarian cancer | Anti-TIGIT treatment reduced the proportion of CD4+ Tregs | [42] |
| CD96            | Anti-CD96 | NK | Melanoma lung metastases | Anti-CD96 enhances the NK cell IFN-γ-dependent effector function, which significantly reduced experimental and spontaneous lung metastases | [43] |
| CD96            | Anti-CD96 | NK | Hepatocellular carcinoma | Anti-CD96 antibody of blocking CD96 and its ligand CD155 interaction, the human NK cell lines cytotoxicity was restored and enhanced | [44] |
| CD96            | Anti-CD96 | NK | Tumor metastases | CD96 targeted antibodies promote NK cell anti-tumor activity | [45] |
| CD96            | Anti-CD96 | CD8+ T | Anti-tumor | Ab blockade on CD8+ T cells could eliminate IFN-γ and/or TNF-α production, which associated with CD8+ T cell activation | [46] |
exhibiting novel therapeutic potential for allogeneic transplantation [29]. In addition to Tregs, DNAM-1-expressing γδ T cells promote cancer cell lysis by engaging ligand nectin-like-5 (CD155) on hepatocellular carcinoma (HCC) cells. Combined mAb-mediated blockade shows that DNAM-1 and NKG2D synergistically affect the cytolytic activity of γδ T cells [30]. The abovementioned studies demonstrated that anti-DNAM-1 mAbs alter T cell cytotoxicity and represent novel potential immunotherapeutic agents against tumors. DNAM-1 + CD8+ tumor-infiltrating T cells possess greater self-renewal ability and cytotoxicity, and DNAM-1 agonist antibody-mediated activation of DNAM-1 augments the effect of TIGIT blockade on CD8 T-cell responses and increases the number of DNAM-1 CD8 T cells, which improves responses to anti-TIGIT therapy for cancer immunotherapy [31].

Clinical trials
A phase aI/bI clinical trial of the DNAM-1 agonist LY3435151 alone or combined with pembrolizumab (anti-PD-1 mAb) is ongoing in patients with solid tumors, triple-negative breast cancer, gastric adenocarcinoma, head and neck squamous cell carcinoma, cervical carcinoma, high-grade serous ovarian carcinoma, undifferentiated pleomorphic sarcoma, and leiomyosarcoma (NCT04099277).

TIGIT
T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (TIGIT) is a member of the immunoglobulin superfamily. It was first identified in 2009 as a coinhibitory receptor on immune cells [63]. TIGIT is a surface protein containing an extracellular IgV-like domain, a type 1 transmembrane domain, and an intracellular domain that contains an ITIM and an ITT-like motif [63]. As highlighted, ITT-like motifs play a crucial role in inhibiting signals. An increasing number of studies have demonstrated that TIGIT is expressed on NK cells, T cells, follicular helper T cells, and follicular regulatory T cells [63–67].

The ligands for TIGIT identified to date include CD112/nectin2 and CD155/PVR, which exhibits the highest affinity. As one of the members of the CD28 family [65], TIGIT shares the ligand CD155 with DNAM-1. The binding of CD155 to TIGIT suppresses T cell activation [35, 63]. In addition to binding to CD155, TIGIT can bind to CD112, and both receptor-ligand interactions inhibit NK cytotoxicity directly through the ITIM motif, which inhibits the NK-mediated killing of tumor cells [64]. CD113 (Nectin-3 or PVRL3) was recently identified as a ligand for TIGIT, which suppresses T cell activity [63]. Nectin4 was originally described as belonging to the nectin family and mediates various cell functions, such as proliferation, differentiation, migration, and invasion [68, 69]. A recent study revealed that Nectin4 is a novel ligand for TIGIT [70]. TIGIT suppresses immune responses mainly through three different mechanisms: direct signaling in cis, induction of ligand signaling in trans, and indirect signaling in competition with costimulatory receptors (Fig. 2).

Direct signaling in cis
To date, TIGIT direct signaling has been mainly investigated in NK cells (Fig. 3). The phosphorylated Y225 residue in the ITT-like motif of TIGIT binds to cytosolic adapter Grb2, which recruits SH2-containing inositol phosphatase-1 (SHIP1), subsequently resulting in the inhibition of the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways [71]. Moreover, the phosphorylation of the two downstream signaling factors AKT and ERK is inhibited, which decreases NK cell cytotoxic activity. In addition, phosphorylated Y225 in the ITT-like motif of TIGIT binds to adaptor β-arrestin 2, which is involved in the modulation of immunity through the recruitment of SHIP1. Furthermore, phosphorylated TIGIT inhibits IFN-γ production by impairing tumor necrosis factor

| PVR like protein | Treatment | Immune cells | Tumor | Results | Reference |
|-----------------|-----------|--------------|-------|---------|-----------|
| CD96            | Anti-CD96 | CD8+ T       | Melanoma | Anti-CD96 therapy is effective to enhance CD8+ T activity and limit tumor growth | [47] |
| CD96            | Anti-CD96 | Th19         | Inflammatory diseases | Blockade of CD96 significantly restored the expansion and inflammatory properties of CD96+ Th9 cells | [48] |
| CD112R          | Anti-CD112R | NK | Breast cancer | Blockage CD112R could improve trastuzumab therapy for breast cancer by enhancing NK cells activity | [49] |
| CD112R          | Anti-CD112R | CD8+ T       | Melanoma, pancreatic cancer | Blockade of PVRIG increased CD8+ T-cell function, an effect enhanced by combination with TIGIT or PD-1 blockade | [50] |

mAb monoclonal antibody, CNS central nervous system, HCC hepatocellular carcinoma, PD-1 programmed cell death 1, PD-L1 programmed cell death-ligand 1, TIDCs tumor-infiltrating dendritic cells
Table 2 Clinical trials in promising cancer target of PVR like receptors

| NCT Number | Target | Agent | Estimated Enrollment | Phase | Condition | Recruitment Status | Estimated Study Completion Date |
|------------|--------|-------|----------------------|-------|-----------|-------------------|---------------------------------|
| NCT04099277 DNAM-1  | LY3435151 | 2 | al/b1 | Solid Tumor, Triple-negative Breast Cancer, Gastric Adenocarcinoma, Head and Neck Squamous Cell Carcinoma, Cervical Carcinoma, High Grade Serous Ovarian Carcinoma, Undifferentiated Pleomorphic Sarcoma, Leiomyosarcoma | Terminated | May 5, 2020 |
| NCT04656535 TIGIT | AB154 | 46 | 0/I | Glioblastoma | Not yet recruiting | Jul, 2023 |
| NCT03628677 TIGIT | AB154 | 66 | I | Solid Tumor, Unspecified, Adult | Recruiting | Nov 10, 2021 |
| NCT04262856 TIGIT | AB154 | 150 | II | Non Small Cell Lung Cancer, Nonsquamous Non Small Cell Lung Cancer, Squamous Non Small Cell Lung Cancer, Lung Cancer | Recruiting | Jun 23, 2022 |
| NCT04736173 TIGIT | AB154 | 625 | III | Non Small Cell Lung Cancer, Nonsquamous Non Small Cell Lung Cancer, Squamous Non-small-cell Lung Cancer, Lung Cancer | Recruiting | Jun 30, 2026 |
| NCT03945253 TIGIT | ASP8374 | 6 | I | Advanced Solid Tumors | Completed | Jun 12, 2020 |
| NCT03260322 TIGIT | ASP8374 | 169 | I | Advanced Solid Tumors | Active, not recruiting | Mar, 2022 |
| NCT04693234 TIGIT | BGB-A1217 | 167 | II | Cervical Cancer | Not yet recruiting | Mar 31, 2023 |
| NCT04732494 TIGIT | BGB-A1217 | 280 | II | Esophageal Squamous Cell Carcinoma | Recruiting | Mar, 2024 |
| NCT04746924 TIGIT | BGB-A1217 | 605 | III | Non-small Cell Lung Cancer | Recruiting | Aug, 2023 |
| NCT04150965 TIGIT | BMS-986207 | 104 | I/II | Multiple Myeloma, Relapsed Refractory Multiple Myeloma | Recruiting | Mar 30, 2022 |
| NCT04354246 TIGIT | COM902 | 45 | I | Advanced Cancer, Ovarian Cancer; Lung Cancer, Colon Cancer; Plasma Cell Neoplasm, Breast Cancer | Recruiting | Sep 30, 2022 |
| NCT04353830 TIGIT | IB939 | 270 | la/ib | Advanced Malignancies | Recruiting | Dec 31, 2023 |
| NCT04672369 TIGIT | IB939 | 36 | I | Advanced Lung Cancer | Not yet recruiting | Oct 9, 2024 |
| NCT04672356 TIGIT | IB939 | 20 | I | Advanced Lung Cancer | Recruiting | Nov 9, 2024 |
| NCT04457778 TIGIT | M6223 | 35 | I | Metastatic Solid Tumors | Recruiting | Sep 14, 2022 |
| NCT04305054 TIGIT | MK-7684 | 315 | I/II | Melanoma | Recruiting | Apr 3, 2030 |
| NCT04305041 TIGIT | MK-7684 | 200 | I/II | Melanoma | Recruiting | Apr 3, 2030 |
| NCT04303169 TIGIT | MK-7684 | 65 | I/II | Melanoma | Recruiting | Apr 3, 2030 |
| NCT04761198 TIGIT | MPH313 | 125 | I/II | Solid Tumor, Adult Advanced Solid Tumor, Metastatic Solid Tumor | Recruiting | Jun 30, 2023 |
| NCT03543617 TIGIT | MTI7912A | 750 | III | Esophageal Squamous Cell Carcinoma | Recruiting | Dec 26, 2025 |
| NCT04256421 TIGIT | MTI7912A | 470 | III | Small Cell Lung Cancer | Recruiting | Sep 29, 2023 |
| NCT04294810 TIGIT | MTI7912A | 560 | III | Non-Small Cell Lung Cancer | Recruiting | Feb 21, 2025 |
| NCT03702224 TIGIT | MTI7912A | 55 | II | Cancer, Carcinoma, Squamous Cell Carcinoma, Head and Neck Cancer | Recruiting | Nov 30, 2025 |
| NCT03563716 TIGIT | MTI7912A | 135 | II | Non-small Cell Lung Cancer | Active, not recruiting | Oct 30, 2021 |
| NCT03281369 TIGIT | MTI7912A | 410 | I/II | Gastric Adenocarcinoma or Gastroesophageal Junction Adenocarcinoma or Esophageal Carcinoma | Recruiting | Feb 11, 2023 |
| NCT03119428 TIGIT | OMP-31 M32 | 33 | I | Locally Advanced Cancer, Metastatic Cancer | Terminated | May 15, 2019 |
(TNF) factor receptor (TNFR)-associated factor 6 (TRAF6) autoubiquitination and nuclear factor kappa B (NF-κB) activation [72]. Similarly, TIGIT exerts immunosuppressive effects on CD8+ T cells by regulating signaling pathways, significantly decreasing the p-AKT/AKT and p-ERK/ERK ratios and increasing p-IκBα levels in HCC [73]. This action eventually results in decreased secretion of interferon-γ (INF-γ), TNF-α, and IL-17A and increased IL-10 secretion [73].

**Induction of ligand signaling in trans**

TIGIT exerts an immunosuppressive effect by inducing ligand signaling in trans (Fig. 2). TIGIT binds to its ligands expressed on dendritic cells (DCs). The TIGIT-Fc fusion protein inhibits T cell activation by enhancing the production of IL-10 and diminishing the production of IL-12p40 in DCs, creating an immunosuppressive microenvironment [63]. As mentioned previously, TIGIT is expressed on Treg cells, which are superior in suppressing

### Table 2 Clinical trials in promising cancer target of PVR like receptors (Continued)

| NCT Number | Target | Agent | Estimated Enrollment | Phase | Condition | Recruitment Status | Estimated Study Completion Date |
|------------|--------|-------|----------------------|-------|-----------|-------------------|---------------------------------|
| NCT04254107 | TIGIT | SGN-TGT | 231 | I | Non-small Cell Lung Cancer, Gastric Carcinoma, Gastroesophageal Junction Carcinoma, Classical Hodgkin Lymphoma, Diffuse Large B-cell Lymphoma, Peripheral T-cell Lymphoma, Cutaneous Melanoma, Head and Neck Squamous Cell Carcinoma; Bladder Cancer, Ovarian Cancer, Triple Negative Breast Cancer | Recruiting | Mar 31, 2023 |
| NCT04570839 | CD112R | COM701 | 100 | I/II | Endometrial Neoplasms, Ovarian Cancer, solid Tumor | Recruiting | Dec, 2023 |
| NCT03667716 | CD112R | COM701 | 140 | I | Advanced Cancer, Ovarian Cancer, Breast Cancer, Lung Cancer, Endometrial Cancer, Ovarian Neoplasm, Triple Negative Breast Cancer, Lung Neoplasm, Neoplasm Malignant, Colo-rectal Cancer | Recruiting | Dec, 2023 |

**Fig. 3** Created with BioRender.com. The bidirectional arrow represents the interaction between TIGIT and its ligands. The thickness of the arrow represents affinity between TIGIT and its common ligands. TIGIT exerts a coinhibitory effect after engagement with its ligands CD155, CD112, CD113, and Nectin4. The phosphorylated Y225 of TIGIT binds to cytosolic adapter Grb2 and β-arrestin 2 after engaging with its ligands, leading to recruitment of SHIP1, which eventually influences cytokine secretion by regulating several signaling pathways, such as PI3K, MAPK, TRAF6, and NF-κB.
T cell activation. TIGIT+ Tregs exhibit better suppressive capacity than TIGIT- Tregs because they augment Treg suppression and stability [24, 25], which may be associated with high expression of IL-10, perforin, and TGF-β. Additionally, a study found that deletion of TIGIT in Tregs is effective in inhibiting tumor growth and enhancing CD8+ TIL cytotoxicity [74]. Thus, TIGIT may play a more dominant role in suppressing antitumor immunity via Tregs. However, Foxp3+ Treg cells increase the expression of the effector molecule fibrinogen-like protein 2 (Fgl2), which suppresses proinflammatory T helper 1 (Th1) and Th17 cell responses but not Th2 cell responses [75]. IL-4-expressing Th2 cells promote the differentiation of type 2 tumor-associated macrophages (TAM2), which inhibits T-cell responses by secreting IL-10 and TGF-β and producing arginase-1, 2,3-dioxygenase (IDO) [76]. These cytokines and enzymes consume nutrients within the tumor microenvironment (TME), which regulates T cell activity.

Indirect competition with costimulatory receptors
PVR-like protein receptors exhibit different affinities for binding to different ligands. TIGIT has the highest affinity for CD155 followed by CD96 and DNAM-1 [63, 77]. Hence, TIGIT exerts an immunosuppressive effect on immune cells by abolishing DNAM-1-mediated costimulation. One study showed that TIGIT indirectly inhibits T cell activation by directly competing with DNAM-1 for binding to CD155 [78]. Additionally, TIGIT can disrupt DNAM-1 cis-homodimerization in human T cells [79]. Both studies suggest that TIGIT inhibits T cell activity by indirectly competing with costimulatory receptors (Fig. 2).

Preclinical and clinical trials
Evidence from preclinical (Table 1) and clinical trials (Table 2) supports the use of TIGIT-targeted immuno-therapies. TIGIT has been shown to be a negative regulator of NK cell or T cell activity in preclinical trials. The number of recruiting, not yet recruiting, active but not recruiting and completed clinical trials assessing the safety and efficacy of a human anti-TIGIT monoclonal antibody for tumor treatment continues to increase.

Effect of TIGIT on NK cells in preclinical trials
Given the cytotoxicity of NK cells, increasing evidence has shown that TIGIT+ NK cell cytotoxicity is inhibited and that this inhibition is accompanied by decreased cytokine production. The cytotoxicity of human YTS NK cells transfected with TIGIT is inhibited [64]. The redirected killing ability of both human [64] and mouse primary NK cells [80] is decreased after TIGIT engages with its ligand CD155. TIGIT expression levels are related to the NK cell phenotype and functional heterogeneity [81]. Compared to NK cells with high expression of TIGIT, NK cells with low TIGIT expression exhibit an increased cytokine secretion capacity, degranulation activity and cytotoxicity [81]. In addition, MDSC-induced NK cell inhibition is associated with high expression of TIGIT and the TIGIT/CD155 interaction [82]. These data consistently show that high expression of the PVR receptor TIGIT exerts a coinhibitory effect on NK cells. Therefore, blockade of TIGIT or the TIGIT-ligand interaction represents a potentially promising cancer therapy. Studies have shown that blockade of TIGIT restores NK cell exhaustion and promotes NK cell-dependent tumor immunity, enhancing degranulation and IFN-γ production in healthy donor CD56dim NK cells. This phenomenon is more apparent in combination with other checkpoint receptors [32, 33].

Effect of TIGIT on T cells in preclinical trials
Aberrant TIGIT expression results in tumor immune escape in the TME. Several groups have consistently reported that TIGIT is highly expressed on CD8+ TILs in many cancers, such as nonsmall cell lung cancer, colon cancer, melanoma [34, 79], and acute myelogenous leukemia [83]. Both melanoma and AML patients have an immunosuppressed microenvironment, largely due to low production of cytokines caused by high TIGIT expression [34, 83]. TIGIT expression is upregulated and DNAM-1 expression in CD8+ TILs is decreased in melanoma and AML patients, indicating the role of a TIGIT/DNAM-1 imbalance in tumor progression. These data consistently demonstrate that TIGIT negatively regulates T-cell function. Knockdown of TIGIT in CD8+ T cells from AML patients reverses cytotoxic and proliferative capacity defects [83]. Moreover, blockade of TIGIT or an anti-TIGIT antibody enhances T cell activation and restores CD8+ T exhaustion, enhancing antitumor activity in gastric cancer, colon cancer, myeloma, and head and neck squamous cell carcinoma [34–40]. In addition, TIGIT regulates Treg function. As mentioned above, Tregs are superior in suppressing T cell activation, and anti-TIGIT treatment reduces the proportion of CD4+ Tregs and inhibits the suppressive ability of Tregs [39–42], suggesting another pathway for cancer immunotherapy.

Clinical trials
Evidence from clinical trials supports the use of TIGIT-mediated immunotherapies. To date, twelve human anti-TIGIT monoclonal antibodies have been developed, i.e., AB154 (NCT04656535, NCT03628677, NCT04262856, NCT04736173), ASP8374 (NCT03945253, NCT03260322), BGB-A1217 (NCT04693234, NCT04732494, NCT04047862, NCT04746924), BMS-
CD96

CD96, which was identified as TACTILE (T cell activation, increased late expression), is also a member of the Ig superfamily [84]. Human CD96 comprises one single-pass transmembrane region, three extracellular Ig-like domains/loops, and one cytoplasmic domain. These structural characteristics of CD96 enhance the complexity of its ligand interactions. Short cytoplasmic domains contain multiple binding sites with signal transduction capabilities, but the characteristics of intracellular signal transduction are less understood. The cytoplasmic domain of CD96 in both mice and humans contains a single putative ITIM motif, which is associated with its inhibitory function [85]. Moreover, the human CD96 cytoplasmic domain consists of a YXXM motif [86], which may activate receptors in certain contexts.

CD96 is expressed primarily on conventional αβ and γδ T cells, NK cells, and a proportion of hematopoietic stem cells in humans [84, 87]. In mice, CD96 is expressed on αβ and γδ T cells, NK cells, and NKT cells [52, 88]. However, CD96 is not expressed on B cells, DCs, resident or inflammatory monocytes, neutrophils, or granulocytes [52, 88–90]. Two ligands of CD96 have been identified: CD155 and CD111 (nectin-1). Similar to TIGIT and DNAM-1, CD96 interacts with CD155 to regulate NK cell and T cell functions. CD96 also interacts with CD111 (nectin-1) to regulate NK cell and T cell functions [88, 89, 91]. The costimulatory and coinhibitory effects of NK cells and T cells after CD96 engages with its ligands are complex and are described in the following section.

**CD96 signaling pathway**

CD96 regulates T cell function by inducing signaling pathways (Fig. 4). A region of the mouse CD96 intracellular domain binds with Grb2 and the SH2 and SH3 domains, which transduce signals [92, 93]. The mechanisms underlying the regulation of T cells by the...
ERK signaling pathway have been investigated [94]. Beads coated with CD3/CD96 induce marked ERK phosphorylation. ERK is rapidly and transiently phosphorylated, and a dramatic reduction in signaling and differentiation is subsequently observed [46]. This finding indicates that CD96 functions as a costimulatory receptor in mouse CD8+ T cells by regulating the ERK signaling pathway. Human CD96 is similar to mouse CD96, and it is worth noting that human CD96 contains a YXXM motif. CD3/CD96 stimulation also enhances the phosphorylation of ERK in human CD8+ T cells [46]. Additionally, the YXXM motif binds the p85 subunit of PI3K [95], which phosphorylates and activates the downstream effector AKT. These data demonstrate that CD96 functions as a costimulatory receptor in human CD8+ T cells by regulating the PI3K/AKT signaling pathway [46].

**Preclinical and clinical trials of CD96-targeted therapies**

Current preclinical data reveal that CD96 may have a coactivating or coinhibitory effect on human and/or mouse NK cells or T cells (Table 1) as described below. This apparent difference can be explained by the inherent differences in CD96 signaling between mice and humans as described previously; however, this effect has not been confirmed to date. Alternatively, these inconsistent results have been confirmed by several studies in mice and humans.

**Effect of CD96 on NK cells in preclinical trials**

CD96 negatively regulates human and mouse NK cell activity. CD96 attenuates NK cell cytotoxicity by competing with DNAM-1 for CD155 binding [52]. CD96−/− mice display hyperinflammatory responses to the bacterial product lipopolysaccharide (LPS) and show resistance to carcinogenesis and experimental lung metastases. These results indicate that CD96 negatively controls cytokine responses by NK cells [52]. Hence, blocking CD96 is a potential cancer immunotherapy. An increasing number of preclinical trials have confirmed this hypothesis. Blocking CD96 or administering an anti-CD96 antibody alone enhances NK cell function, which significantly reduces experimental and spontaneous lung metastases in mice. This effect is more apparent when these strategies are used in combination with anti-CTLA4 antibodies, anti-PD-1/PD-L1 antibodies, and doxorubicin for cancer immunotherapy [44, 89]. In human HCC samples, the number of CD96+ NK cells is significantly increased, and these cells are functionally exhausted and exhibit impaired IFN-γ and TNF-α production, high gene expression of IL-10 and TGF-β1, and low gene expression of T-bet, IL-15, perforin, and granzyme B [44]. In contrast, human NK cell cytotoxicity is restored and enhanced in the presence of an anti-CD96 antibody that blocks CD96 and its interaction with CD155 [44, 89]. Two anti-CD96 antibodies block the CD96-CD155 interaction (3.3 and 6A6), and one antibody (8B10) does not. Consistent with its inability to block CD96-CD155 interactions, 8B10 retains antitumor activity in CD155-deficient mice, whereas 3.3 and 6A6 lose potency in CD155-deficient mice, suggesting that CD96-targeted antibodies promote NK cell antitumor activity without blocking the CD96-CD155 interaction [45].

**Effect of CD96 on T cells in preclinical trials**

CD96 regulates T cell activity rather than NK cell activity. However, the potential coactivating and coinhibitory effects of CD96 on human and/or mouse T cells are complicated. CD96 promotes human CD8+ T cell cytokotoxic activity through the MEK-ERK pathway. Therefore, the absence of CD96 or Ab-mediated CD96 blockade on CD8+ T cells abolishes IFN-γ and/or TNF-α production, which is associated with CD8+ T cell activation in vivo models [46]. However, CD96−/−CD8+ T cells in mice promote tumor growth more than CD96-sufficient CD8+ T cells. In addition, anti-CD96 therapy is effective in enhancing CD8+ T activity and limiting tumor growth and is more effective when administered in combination with blockade of a number of immune checkpoints, including PD-1, PD-L1, TIGIT, and CTLA-4 [47]. CD96low Th9 cells in (Rag1−/−) mice can cause severe weight loss, intestinal and colonic inflammation, and destruction of allogeneic skin grafts, showing expansion and tissue accumulation. In contrast, CD96high Th9 cells do not cause colitis and exhibit reduced expansion and migratory potential [48]. Interestingly, blockade of CD96 significantly restores the expansion and inflammatory properties of CD96high Th9 cells [48]. These results that CD96 plays an inhibitory role in suppressing IL-9-expressing Th9 cell activity, providing novel opportunities for the treatment of IL-9-associated inflammation, such as inflammatory bowel disease (IBD).

**Clinical trials**

Although the abovementioned preclinical trials have suggested that CD96 on NK cells and T cells may be potential targets for cancer immunotherapy, the expression levels of this immune checkpoint receptor differ on mouse and/or NK cells and T cells. In addition, CD96-associated studies and clinical trials in human cancer patients are lacking. Thus, further studies of the potential of CD96 as a target molecule for cancer immunotherapy are needed.

**CD112R**

CD112R, previously named poliovirus receptor-related immunoglobulin domain-containing protein (PVRIG), is
a novel member of the PVR-like cosignaling network. CD112R is a 36-kD transmembrane monomer composed of a single extracellular IgV domain, one transmembrane domain, and a long intracellular domain [97]. The human CD112R intracellular domain comprises two tyrosine residues, one of which is part of an ITIM-like motif and a potential site for phosphatases [98]. The extracellular domain sequences of human and mouse CD112R exhibit ~65.3% homology. In addition, phylogenetic tree analysis of the first IgV of the PVR family revealed that CD112R is closely related to PVR-like proteins [97].

CD112R is expressed at low and variable levels on the surface of freshly isolated T-cells and NK cells (predominantly on CD8+ T-cells, which are mainly memory/effector but not naïve cells) and on both CD16+ and CD16- NK cells in humans. CD112R is not detected in B-cells (CD19+), naïve (CD45RA+ CCR7+) or helper (CD4+) T-cells, monocytes (CD14+), or neutrophils (CD66b+) at the protein level. Moreover, CD112R is not detected on DCs in humans. CD112R expression on the surface of T cells is further upregulated by treatment with anti-CD3 and anti-CD28 antibodies [97].

**CD112R signaling pathway**

The Y233 residue of the ITIM-like motif in the CD112R intracellular domain is phosphorylated by tyrosine phosphatases to mediate signal transduction [97] (Fig. 5). This finding was confirmed in the CD112Rhigh leukemia T cell line Molt4. The results showed that SHIP is strongly associated with CD112R in untreated Molt4 cells, and this interaction further increases upon pervanadate treatment. In addition, SHP-1 and SHP-2 are weakly associated with CD112R in untreated Molt4 cells, but pervanadate treatment further enhances these associations [97]. All these results suggest that CD112R transduces signals by recruiting tyrosine phosphatases. Additionally, this study suggested that CD112R

![Fig. 5](https://www.biorender.com/images/education/pathways/2021-02-03/00/041.png) - Created with BioRender.com. The bidirectional arrow represents the interaction between CD112R and its ligand. CD112R engages with its ligand, and the phosphorylated Y233 residue of the ITIM-like motif recruits SHIP-1 and SHP1/2 to mediate signal transduction, leading to a decrease in T cell cytotoxicity. Additionally, CD112R potentially represents a new coinhibitory receptor that suppresses T cell receptor-mediated NFAT activation.
potentially represents a new coinhibitory receptor that suppresses T cell receptor-mediated NFAT activation.

Effect of CD112R on NK cells in preclinical trials
Few studies on cancer immunotherapies have focused on targeting CD112R PVR-like checkpoint proteins. CD112R is expressed on human NK cells, although its function in this cell type is unclear. The number of human NK cells expressing the PVR receptor CD112R or TIGIT (CD56b+) decreases with decreased IFN-γ production in CD112-positive breast cancer, indicating that CD112R engagement with the ligand CD112 suppresses NK cell cytotoxicity [49]. Xu and coworkers showed that blockade of TIGIT and CD112R separately or together improves the effect of trastuzumab on breast cancer by enhancing NK cell activity [49].

Effect of CD112R on T cells in preclinical trials
Interestingly, instead of mediating NK cells, CD112R plays a vital role in T-cell-mediated cancer immunity. CD112R is a distinct inhibitory signaling molecule on human CD8+ T cells that decreases IFN-γ production [50]. COM701 is a humanized anti-CD112R hinge-stabilized IgG4 that binds to human CD112R and disrupts the CD112R–CD112 interaction, which enhances T-cell function. This effect is enhanced by TIGIT or PD-1 blockade in Mel-624 cells and Panc.05.04 cells [50]. Furthermore, COM701 + nivolumab (anti-PD-1) or COM701 alone result in better outcomes in patients with advanced solid tumors in a phase I clinical trial [50]. CD112R is a novel member of the PVR-like protein cosignaling network. Research on the interactions of CD112R with other poliovirus-like ligands is lacking, and these interactions need to be studied further.

Clinical trials
One human anti-CD112R monoclonal antibody, COM701, has entered clinical trials. When combined with blockade of CD112R and TIGIT, PD-1 exerts a more powerful antitumor effect in preclinical models. Thus, two clinical trials are being performed to evaluate the safety and efficacy of combination anti-TIGIT (BMS-986207) and anti-PD-1 (Opdivo, Nivolumab) therapy as well as anti-CD112R monotherapy. These trials are ongoing in patients with endometrial neoplasms, ovarian cancer, solid tumors (NCT04570839), advanced cancer, ovarian cancer, breast cancer, lung cancer, endometrial cancer, ovarian neoplasm, triple-negative breast cancer, and colorectal cancer (NCT03667716).

Conclusion
In recent years, immunotherapy has represented one of the most promising therapeutic methods for cancer therapy and has attracted a considerable amount of attention. Immunotherapy utilizes the body’s immune system to kill and eliminate infected and transformed cells by enhancing NK and T cell activities and is safe and highly effective. Although the costimulatory and coinhibitory mechanisms of NK and T cells after receptors in the PVR-like protein cosignaling network engage with their ligand have been studied, the detailed intracellular signaling mechanisms are unclear and need to be elucidated. Recently, anti-PD-1 mAbs were approved by the Food and Drug Administration (FDA) for the treatment of many cancers. The challenge in the future will be to develop mAbs targeting molecules in this cosignaling network to treat cancers. Based on the abovementioned data, we know that the efficacy of strategies targeting these cosignaling network receptors is increased when used in combination with other existing immune checkpoint blockade therapies, such as anti-CTLA-4 or anti-PD-1 mAbs, to treat cancer. The next challenge we face is the development of combination therapies involving mAbs targeting molecules in this novel cosignaling network and other unknown immune checkpoints for cancer treatment. More importantly, clinical results indicate that many cancers, such as kidney, gallbladder, or bile duct malignant tumors, do not respond or respond poorly to immune checkpoint blockade therapy. How numerous innate receptors regulate NK cell and T cell responsiveness spatially and temporally is unclear and therefore should be investigated to identify other receptors and their ligands that share the same structure.

Abbreviations
Abbreviations
PVR: Poliovirus receptor; DNAM-1: DNAX accessory molecule-1; TIGIT: T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif; NK: Natural killer; CTLA-4: Cytotoxic T lymphocyte antigen-4; PD-1: Programmed cell death 1; PD-L1: Programmed cell death-ligand 1; Ig: Immunoglobulin; ITIM: Immunoreceptor tyrosine-based inhibitory motif; IgV: Ig variable; mAb: Monoclonal antibody; CNS: Central nervous system; HCC: Hepatocellular carcinoma; ITT: intention to treat; PKC: Protein kinase C; LFA-1: Leucocyte function-associated antigen-1; SLP-76: SH2 domain-containing leukocyte phosphoprotein of 76 kDa; Grb2: Growth factor receptor-bound protein 2; PLC-γ: phospholipase C-γ; PKC: Protein kinase C; DNAM-1Ls: DNAX accessory molecule-1 ligands; NKG2D: NK cell activating receptor natural-killer group 2, member D; SHIP1: SH2-containing inositol phosphatase-1; MAPK: Mitogen-activated protein kinase; INF-γ: Interferon-γ; TNF-α: Tumor necrosis factor-alpha; TGF-β: Transforming growth factor-beta

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Authors’ contributions
BKW, CLZ, QL, ZYL, YZZ, and ZZ drafted the manuscript; YY and HMZ completed the figures and tables; YT and FX managed the article design, reviewed the manuscript; YT provided funding support. All authors have read and approved the final manuscript.

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