Clinical Insights Into Novel Immune Checkpoint Inhibitors

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The success of immune checkpoint inhibitors (ICIs), notably anti-cytotoxic T lymphocyte associated antigen-4 (CTLA-4) as well as inhibitors of CTLA-4, programmed death 1 (PD-1), and programmed death ligand-1 (PD-L1), has revolutionized treatment options for solid tumors. However, the lack of response to treatment, in terms of de novo or acquired resistance, and immune related adverse events (IRAE) remain as hurdles. One mechanisms to overcome the limitations of ICIs is to target other immune checkpoints associated with tumor microenvironment. Immune checkpoints such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and ITIM domain (TIGIT), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B7 homolog 3 protein (B7-H3), inducible T cell costimulatory (ICOS), and B and T lymphocyte attenuator (BTLA) are feasible and promising options for treating solid tumors, and clinical trials are currently under active investigation. This review aims to summarize the clinical aspects of the immune checkpoints and introduce novel agents targeting these checkpoints.

Keywords: immune checkpoint, LAG-3, TIGIT, TIM-3, B7-H3, VISTA, ICOS, BTLA

BACKGROUND

Cancer cells have characteristics that allow diversification and sustenance of their neoplastic state (Hanahan and Weinberg, 2011). One of the hallmarks of cancer is immune evasion; cancer cells hamper immune activation by limiting T cell activation and expressing immune checkpoint proteins on T cells (Vinay et al., 2015). Blocking cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and the interaction between programmed death 1 (PD-1) and programmed death ligand-1 (PD-L1) elicit activation of the host immune system through T cell responses (Pardoll, 2012). These findings have led to the development of immune checkpoint inhibitors (ICIs) to control one of the key mechanisms utilized by cancer cells (Pardoll, 2012). In 2011, ipilimumab, the first anti-CTLA-4 monoclonal antibody (mAb), was approved for treating metastatic melanoma (Cameron et al., 2011). Thereafter, anti-PD-1 mAbs such as pembrolizumab, nivolumab, cemiplimab and as well as anti-PD-L1 mAbs such as atezolizumab, avelumab, durvalumab, have been used to treat patients with cancer, especially

Abbreviations: adhesion molecule 1; BTLA, B and T-lymphocyte attenuator; HVEM, herpes-virus entry mediator; ICOS, Inducible T cell costimulator; ICOSL, Inducible T cell costimulatory ligand; LAG-3, lymphocyte-associated gene 3; mAb, monoclonal antibody; PtdSer, phosphatidyl serine; TIGIT, T cell immunoglobulin and ITIM domain; TIM-3, T-cell immunoglobulin and mucin domain-3; VISTA, V-domain immunoglobulin suppressor of T cell activation; VSIG-3, V-Set and Immunoglobulin domain containing 3.
in locally advanced and metastatic settings (Qin et al., 2019; Vaddepally et al., 2020). Besides PD-L1 expression, several emerging biomarkers have gained wide attention (Darvin et al., 2018). Pembrolizumab was approved in solid tumors harboring microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR), and high tumor mutation burden (TMB-H) defined as ≥10 mutations/megabase based on FoundationOneCDx assay (Foundation Medicine, Inc.) (Marcus et al., 2019; Marabelle et al., 2020).

Despite the feasibility and anti-tumor activity of ICIs, there remain several hurdles in immunotherapy for cancer. Only a subset of patients respond to treatment, and the majority of patients who have durable responses eventually experience disease progression (Trebeschi et al., 2019). Furthermore, patients experience IRAE, some of which are highly toxic (Boutros et al., 2016; Wang et al., 2018). To overcome these impediments, treatment strategies such as combination with chemotherapy, targeted agents, or radiotherapy have been implemented (Gandhi et al., 2018; Wang et al., 2018; Rini et al., 2019). Notably, treatment with a combination of different ICIs has resulted in increased clinical responses, as observed with the combination of nivolumab and ipilimumab in melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC) (Rizvi et al., 2016; Hellmann et al., 2018; Motzer et al., 2018).

Promising results from the combination of anti-CTLA-4 and PD-L1 mAbs have resulted in the launch of several other ICI combinations with non-overlapping mechanisms of action that may increase efficacy and minimize toxicity (Barbari et al., 2020). Currently, approximately 2/3 of all oncology trials are dedicated to T cell-targeting immunomodulators, and there are more than 3,000 ongoing clinical trials (Xin Yu et al., 2019).

Resistance to immunotherapy is associated with loss of immunogenic neoantigens, increase of immunosuppressive cells, and upregulation of alternate immune checkpoint receptors (Sharma et al., 2017). This review provides an overview of the mechanisms and ongoing clinical trials specifically on novel emerging immunopeptides, including lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B7 homolog 3 protein (B7-H3), inducible T cell costimulator (ICOS), and B and T lymphocyte attenuator (BTLA) (Chapoval et al., 2001; Monney et al., 2002; Yu et al., 2009; Paulos and June, 2010; Wang et al., 2011; Andrews et al., 2017; Marinelli et al., 2018).

LAG-3

LAG-3 is a protein comprising four parts—the hydrophobic, extracellular, transmembrane, and cytoplasmic domains. LAG-3 shares structural similarity with CD4 in having four extracellular regions (Triebel et al., 1990; Huard et al., 1997). It is expressed mainly on activated CD4+ and CD8+ T cells, regulatory T cells (Tregs), and natural killer (NK) cells, as well as on B cells and plasmacytoid dendritic cells (DCs) (Table 1) (Huard et al., 1995; Andreae et al., 2002; Huang et al., 2004; Kisielow et al., 2005). LAG-3 binds its canonical ligand, major histocompatibility complex class II (MHC-II), as well as other ligands, including galectin-3, LSECtin, α-synuclein, and fibrinogen-like protein 1 (FGL1), thereby inducing exhaustion of immune cells and decreased cytokine secretion (Baixeras et al., 1992; Huard et al., 1994; Kouo et al., 2015; Anderson et al., 2016; Baumeister et al., 2016; Mao et al., 2016; Wang et al., 2019).

LAG-3 was found to be simultaneously co-expressed with other targets, such as PD-L1, TIGIT, and TIM-3, in preclinical settings (Woo et al., 2012; Baumeister et al., 2016). Blocking LAG-3 alone did not restore T cell exhaustion; however, the combination of LAG-3/PD-1 blockade resulted in reduced tumor volume (Woo et al., 2012). These findings were consistent across in vivo studies using murine models of other tumors, including melanoma, ovarian cancer, and lymphoma (Goding et al., 2013; Huang et al., 2015).

In humans, LAG-3 is expressed on CD8+ tumor-infiltrating lymphocytes (TILs) and peripheral Tregs (Camisaschi et al., 2010; Matsuoka et al., 2010; Li et al., 2013; Llosa et al., 2015; Taube et al., 2015). CD8+ TILs isolated from tumors such as hepatocellular carcinoma (HCC), melanoma, ovarian cancer, and microsatellite instability high (MSI) colorectal cancer (CRC), have high levels of both PD-1 and LAG-3 (Matsuoka et al., 2010; Li et al., 2013; Llosa et al., 2015; Taube et al., 2015). Peripheral Tregs have been observed in melanoma and CRC (Camisaschi et al., 2010). In patients with hormone receptor-positive breast cancer, treated with immunotherapy, soluble LAG-3 (sLAG-3) detected in the serum was correlated with better prognosis in terms of disease-free survival (DFS) and overall survival (OS) (Triebel et al., 2006). However, the mechanism of sLAG-3 has yet to be identified (Li et al., 2007).

Clinical Trials on LAG-3

Co-expression of LAG-3 with immune checkpoints, such as PD-1, and robust clinical data on the efficacy of LAG-3 and PD-1 dual blockade have prompted trials focusing on this combination as well as other immune checkpoint inhibitors. Currently, there are 17 agents targeting LAG-3 (Table 2), with multiple combinations of treatments across various tumors (Table 3). Eight of these agents have interim or final clinical results, and nine of the investigational agents are ongoing clinical trials.

A phase 1 study of eftilagimod alpha (IMP321), an antigen-presenting cell (APC) activator for LAG-3, in combination with pembrolizumab was conducted in 24 patients with metastatic melanoma (NCT02676869) (Atkinson et al., 2020). The primary endpoints were the recommended phase 2 dose (RP2D), safety, and tolerability of the combined agents. The study included cohort A of dose escalation and cohort B of extension, and the patients received subcutaneous pembrolizumab and eftilagimod alpha bi-weekly at doses of 1, 6, or 30 mg for up to 6 and 12 months for Cohorts A and B, respectively. There was no dose-limiting toxicity (DLT) and the treatment was well tolerated, with the injection site as the most common adverse event (AE). The response to treatment was encouraging, with an
Table 1: Overview of novel immune checkpoints.

| Immune checkpoints | LAG-3 | TIGIT | TIM-3 | B7-H3 | VISTA | ICOS | BTLA |
|--------------------|-------|-------|-------|-------|-------|------|------|
| Other names        | CD223 | Vstm3, Vaig9, WUCAM | HAVCR2 | CD278 | Dies1, DD1a, G24, B7-H5, PO-1H | CD278 | CD272 |
| Function           | Co-inhibition | Co-inhibition | Co-inhibition or co-stimulation | Co-inhibition or co-stimulation | Co-inhibition | Co-inhibition or co-stimulation | Co-inhibition or co-stimulation |
| Cells that express the immune checkpoints | NK cells, DC, activated T cells, Tregs, B cells, NK cells, T cells | NK cells, DCs, activated T cells, Tregs, B cells, monocytes, cancer cells | NK cells, DCs, activated T cells, monocytes, cancer cells | T cells, myeloid cells | Activated T cells | Mature T cells, Tregs, B cells, macrophages |
| Ligands or receptors | MHC-II, galectin-3, LSECtin, a-synuclein, FGL1 | CD155, CD112 | HMGB-1, galectin-9, ceacam-1, PtdSer | Unknown | VSIG-3 | ICOSL | HVEM, LIGHT, lymphotxin-α |
| Immune checkpoint agents | APC activator, anti-LAG3 mAb, LAG3 and PD1 DART protein, LAG3 fusion protein, bispecific Ab to both LAG3 and PD-L1 | Anti-TIGIT mAb | Anti-TIM-3 mAb, anti-PD-1/TIM3 bispecific Ab | Anti-B7-H3 mAb, B7-H3-targeting ADC, radiolabeled anti-B7-H3 mAb, CAR T-cell therapy | Anti-VISTA mAb, small molecule VISTA | Anti-ICOS agonist, anti-ICOS antagonist |
| No. of investigational agents | 17 | 10 | 8 | 11 | 3 | 4 | 4 |
| Clinical trials | | | | | | | |
| Phase 1 | Completed (eftilagimod alpha, BI754111, Symo22, INCAN02385), ongoing | Completed (Symo23), ongoing | Completed (enoblituzumab), ongoing | Completed (CA-170), ongoing | Ongoing | Completed (JTX-2011), ongoing |
| Phase 2 | Completed (eftilagimod alpha, LAGS25), ongoing | Ongoing | Ongoing | Ongoing | NA | NA |
| Phase 3 | Ongoing (MGDO13) | Ongoing (tiragolumab) | Ongoing (sabatolimab) | Ongoing | NA | NA |
| Combination treatment | Yes | Yes | Yes | Yes | No | Yes | Yes |
| Other immune checkpoint inhibitors | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Targeted agents | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Chemotherapy | Yes | Yes | Yes | Yes | Yes | Yes | No |
| Radiotherapy | No | No | No | No | No | No | No |

Abbreviations: APC, antigen-presenting cell; BTLA, B and T lymphocyte attenuator; CAR-T, chimeric antigen receptor T cell; DART, dual-affinity re-targeting proteins; DCs, dendritic cells; Des1, differentiation of embryonic stem cells; 1; HAVCR2, hepatitis A virus cellular receptor 2; HVEM, herpes-virus entry mediator; mAb, monoclonal antibody; ICOS, Inducible T cell costimulator; ICOSL, Inducible T cell costimulatory ligand; LAG-3, lymphocyte-associated gene 3; NK cells, natural killer cells; PD-1, programmed death-1; PtdSer, phosphatidyl serine; Tregs, regulatory T cells; TIGIT, T cell immunoglobulin and ITIM domain; TIM-3, T-cell immunoglobulin and mucin domain-3; VISTA, V-domain immunoglobulin suppressor of T cell activation; VSIG-3, V-Set and Immunoglobulin domain containing 3; WUCAM, Washington University cell adhesion molecule.

Overall response rate (ORR) of 33 and 50% for pembrolizumab-refractory cohort A and PD-1 naive cohort B patients, respectively.

Similarly, the combination of eftilagimod alpha and pembrolizumab has been investigated in NSCLC and head and neck squamous cell carcinoma (HNSCC) (NCT03625323) (Peguero et al., 2019). The AIPAC study, a placebo-controlled randomized phase IIb study on eftilagimod alpha (or placebo) with paclitaxel as the first-line treatment in patients with metastatic breast cancer (MBC), is also under investigation (NCT02614833) (Dirix and Triebel, 2019). Preliminary results show that the agent could elicit durable immune responses. Clinical data, including progression-free survival (PFS), ORR, OS, and safety, are all awaiting results.

Relatlimab (BMS-986016), an IgG4 mAb targeting LAG-3, has been investigated in various settings and agents, notably with well-established immune checkpoint inhibitors such as nivolumab and ipilimumab and other novel agents such as indoleamine 2,3-dioxygenase-1 (IDO1) inhibitors, CCR2/5 dual antagonist, and anti-TIGIT. Notably, clinical trials are ongoing for phase II/III in previously untreated metastatic melanoma, in combination with or without nivolumab (NCT03470922), phase II of nivolumab and oxaliplatin-based chemotherapy with or without relatlimab in GC or gastroesophageal junction (GEJ) cancer (NCT03662569), and phase II of relatlimab with nivolumab and ipilimumab and other novel agents such as indoleamine 2,3-dioxygenase-1 (IDO1) inhibitors, CCR2/5 dual antagonist, and anti-TIGIT. Notably, clinical trials are ongoing for phase II/III in previously untreated metastatic melanoma, in combination with or without nivolumab (NCT03470922), phase II of nivolumab and oxaliplatin-based chemotherapy with or without relatlimab in GC or gastroesophageal junction (GEJ) cancer (NCT03662569), and phase II of relatlimab with nivolumab in mismatch repair deficient (dMMR) cancers resistant to prior PD-1/PD-L1 inhibition (Lipson et al., 2018; Feeney et al., 2019; Bever et al.,...
| Target | Name of agent | Company | Mechanism |
|--------|--------------|---------|-----------|
| LAG-3  | Eftilagimod alpha (IMP321) | Immutep | APC activator |
|        | Relatlimab (BMS-986018) | Bristol-Myers Squibb | IgG4 mAb |
|        | Cemiplimab (REGN3767) | Regeneron | mAb |
|        | BI 754111 | Boehringer Ingelheim | mAb |
|        | Sym022 | Symphogen | Fc-inert mAb |
|        | MG0013 | MacroGenics | LAG-3 and PD1 DART protein |
|        | Mavezlimab (MK-4280) | Merck | IgG4 mAb |
|        | TSR-033 | Tesaro | IgG4 mAb |
|        | INCAGN02385 | Incyte | Fc engineered IgG1k antibody |
|        | EOC202 | EddingPharm Oncology | LAG-3 fusion protein |
|        | 89Zr-DFO-REGN3767 | Memorial Sloan Kettering Cancer Center | Anti-LAG-3 antibody labeled with 89Zr |
|        | XmAb®22,841 | Xencor | Bispecific antibody to both LAG3 and CTLA-4 |
|        | LBL-007 | NanJing Leads Biolabs Co | AlphaLAG3-3 mAb |
|        | FS118 | Hoffmann-La Roche | Bispecific antibody to both LAG3 and PD-L1 |
|        | RO7247669 | Shanghai EpimAb Biotherapeutics | Bispecific antibody to both LAG3 and PD-L1 |
|        | EMB-02 | Genentech | Anti-TIGIT mAb |
|        | Tiragolumab (MTIG7192A/RG-6058) | Merck | Anti-TIGIT mAb |
|        | Vibostimab (MK-7684) | OncoMed | Anti-TIGIT mAb |
|        | Eliglumab (OMP-313M02) | Bristol-Myers Squibb | Anti-TIGIT mAb |
|        | BMW-986207 | Arcus Biosciences | Anti-TIGIT mAb |
|        | Domnalimab (AB-154) | Potenza | Anti-TIGIT mAb |
|        | ASP-8374 | Innovent Biologics | Anti-TIGIT mAb |
|        | BGB-A1217 | BelGene | Anti-TIGIT mAb |
|        | COM902 | Compugen | Anti-TIGIT mAb |
|        | M223 | EMD Serono | Anti-TIGIT mAb |
| TIM-3  | Sym023 | Symphogen | Anti-TIM-3 mAb |
|        | L3Y3231367 | Eli Lilly and Company | Anti-TIM-3 mAb |
|        | Cobolimab (TSR-022) | Tesaro | Anti-TIM-3 mAb |
|        | Sabatolimab (MBG453) | Novartis | Anti-TIM-3 mAb |
|        | INCAGN2390 | Incyte | Anti-TIM-3 mAb |
|        | BMS-986258 | Bristol-Myers Squibb | Anti-TIM-3 mAb |
|        | SHR-1702 | Jiangsu HengRui | Anti-TIM-3 mAb |
|        | RO7121661 | Roche | Anti-TIM-3 mAb |
|        | 4SCAR-276 | Shenzhen Geno-Immune Medical Institute | CAR T-cell therapy |
|        | SCR-CARB7H3 | BiYuan RunSheng Pharma | CAR T-cell therapy |
|        | B7-H3 CAR-T | UNC Lineberger Comprehensive Cancer Center | CAR T-cell therapy |
|        | CAR.B7-H3 | Seattle Children’s Hospital | CAR T-cell therapy |
|        | Second-generation 4-1BB ζ B7-H3-EGFRt-DHFR | Seattle Children’s Hospital | CAR T-cell therapy |
| B7-H3  | Enotibuzumab (MGA271) | MacroGenetics | Anti-B7-H3 mAb |
|        | DS-7300a | Daiichi Sankyo | B7-H3-targeting ADC |
|        | Oritmab (MGGD09) | MacroGenetics | B7-H3 and CD3 DART protein |
|        | 1311-Omburtamab | Y-mAbs Therapeutics | Radiolabeled anti-B7-H3 mAb |
|        | 124-omburtamab | Y-mAbs Therapeutics | Radiolabeled anti-B7-H3 mAb |
|        | 177Lu-DTPA-Omburtamab | Y-mAbs Therapeutics | Radiolabeled anti-B7-H3 mAb |
|        | 4SCAR-276 | Shenzhen Geno-Immune Medical Institute | CAR T-cell therapy |
|        | SCR-CAR-B7H3 | BiYuan RunSheng Pharma | CAR T-cell therapy |
|        | B7-H3 CAR-T | UNC Lineberger Comprehensive Cancer Center | CAR T-cell therapy |
|        | CAR.B7-H3 | Seattle Children’s Hospital | CAR T-cell therapy |
|        | Second-generation 4-1BB ζ B7-H3-EGFRt-DHFR | Seattle Children’s Hospital | CAR T-cell therapy |
| VISTA  | JNJ-61610588 | Johnson & Johnson | Anti-VISTA mAb |
|        | CI-8993 | Curis | Anti-VISTA mAb |
|        | CA-170 | Curis | Small molecule targeting VISTA and PD-L1 |
| ICOS   | GSK3359609 | GlaxoSmithKine | Anti-ICOS agonist |
|        | JTX-2011 | Jounce Therapeutics | Anti-ICOS agonist |
|        | MEDI-570 | National Cancer Institute | Anti-ICOS antagonist |
|        | KY1044 | Kymab Limited | Anti-ICOS antagonist |
| BTLA   | INBRX-106 | Inhibrix | HexavalentOX40 agonist Ab |
|        | PF-05188600 | Pfizer | OX40 agonist |
|        | Cudarolimab (IBI101) | Innovative Biologics | Anti-OX40 mAb |
|        | TAB004 (JS004) | Shanghai Junshi Bioscience | Anti-BTLA mAb |

Abbreviations: ADC, antibody-drug conjugate; APC, antigen-presenting cell; BTLA, B and T-lymphocyte attenuator; CAR, chimeric antigen receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DART, dual-affinity re-targeting proteins; ICOS, inducible T-cell costimulator; LAG3, lymphocyte-associated gene 3; mAb, monoclonal antibody; PD-L1, programmed death-ligand 1; TIGIT, T cell immunoglobulin and ITIM domain; TIM, T-cell immunoglobulin and mucin domain-3; VISTA, V-domain immunoglobulin suppressor of T cell activation.
2020). Relatlimab is being tested in a wide range of tumor types and settings as front- or second-line treatment, in resectable status, and in stage II/III.

An open label, phase 2 study including 72 patients treated with LAG-525, which is an IgG4 mAb for LAG-3, and spartalizumab (PDR001), an anti-PD-1, for advanced solid tumors and hematologic malignancies showed promising activity, especially in neuroendocrine tumors, small cell lung cancer (SCLC), and diffuse large B-cell lymphoma (DLBCL), with a clinical benefit rate at 24 weeks (CBR24) of 0.86, 0.27, and 0.804, respectively, meeting its primary endpoint (NCT03365791) (Uboha et al., 2019). In GEJ cancer, the CBR24 was 0.071, and enrollment was stopped for these subsets of patients. Other tumors such as triple-negative breast cancer (TNBC) (NCT03742349 and NCT03499899) and melanoma (NCT03484923) are ongoing trials in advanced and metastatic settings.

The preliminary results of a phase 1 study on cemiplimab (REGN3767), an mAb for LAG-3, as monotherapy (n = 27), and in combination with PD-1 mAb (n = 42) was conducted in advanced malignancies (NCT03005782) (Papadopoulos et al., 2019). No DLT was observed with in the monotherapy group, whereas the combination group, during treatment with R3767 3 mg/kg every 3 weeks (Q3W) + cemiplimab 3 mg/kg Q3W, experienced grade 4 elevated creatine phosphokinase levels in addition to grade 3 myasthenia gravis. Overall, both treatments were deemed tolerable; cemiplimab 20 mg/kg or 1600 mg as a fixed dose of Q3W is ongoing further evaluation as monotherapy and as a combination.

Similarly, BI 754111, an mAb for LAG-3, was also tested with BI 754091 (anti-PD-1) in treatment-refractory solid tumors, in a dose escalation phase 1 study, followed by an expansion phase in microsatellite stable (MSS) CRC and anti-PD1-PD-L1 refractory tumors including NSCLC (NCT03156114) (Johnson et al., 2020). The primary endpoints for dose escalation and dose expansion phase were DLT and the maximum tolerated dose (MTD) and ORR, respectively. Biomarker analysis was performed in MSS CRC refractory to immunotherapy; the patients who responded to these agents with a partial response (PR) or stable disease (SD) had increased treatment-associated IFN-γ gene signature scores (Bendell et al., 2020). Furthermore, patients with high PD-L1 gene expression in pre-treatment biopsy samples responded better to the baseline. Baseline immunohistochemistry of LAG-3 was not a predictive factor for this subset of patients.

Sym022 (anti-LAG-3) was evaluated as a single agent or in combination with sym021 (anti-PD-1) in phase 1 trials for solid tumors or lymphomas (NCT03311412, NCT03489369, and NCT03489343) (Lakhani et al., 2020). Interim analysis showed that 15 patients who were administered monotherapy and 20 patients under combination treatment, had one unconfirmed PR. Both treatment arms had tolerable safety profiles, with the combination treatment showing one grade 3–4 immune-related hypophysitis. Further assessments of the pharmacokinetic (PK) and pharmacodynamic (PD) markers and the anti-tumor activity of the monotherapy and combination are awaiting results.

MGD013 is a LAG-3 and PD-1 dual-affinity re-targeting (DART) protein; its safety, tolerability, DLT, MTD, PK/PD, and antitumor activity were analyzed in patients with unresectable and metastatic tumors in a phase 1 study (NCT03219268) (Luke et al., 2020). Fifty patients in the dose-escalation phase and 157 patients in the dose-expansion phase, with 46 and 32% of patients with prior exposure to immunotherapy, respectively, were enrolled. No MTD was reached, and the most common treatment-related adverse events (TRAE), which were fatigue and nausea, were well tolerated. Despite exposure to previous immunotherapy, both cohorts included patients with objective responses. More mature clinical data are awaiting results, and biomarker analysis of LAG-3 and PD-L1 is ongoing.

Other agents that are undergoing clinical trials are: 1) mavezelimab (MK-4280), an IgG4 mAb targeting LAG-3 (NCT03598608, NCT02720068, and NCT03516981); 2) TSR-033, an IgG4 mAb targeting LAG-3 (NCT03250832); 3) INCAGN02385, a Fc engineered IgG1k antibody for LAG-3 (NCT03538028, NCT04370704, and NCT03311412); 4) EOC202, a LAG-3 fusion protein (NCT03600090); 5) 89Zr-DFO-REGN3767, an anti-LAG-3 antibody labeled with 89Zr (NCT04566978); 6) XmAb®22841, a bispecific antibody to both LAG-3 and CTLA-4 (NCT03849469); 7) LBL-007, an alphaLAG-3 mAb (NCT04640545), and 8) bispecific antibody to both LAG-3 and PD-L1, which includes agents FS118 (NCT03440437), RO7247669 (NCT04140500), and EMB-02 (NCT04618393) treated as monotherapy or in combination for patients with treatment refractory solid and/or hematologic malignancies.

### TIGIT

TIGIT, previously known as Vstm3, VSIG9, or Washington University cell adhesion molecule (WUCAM), is a protein comprising an extracellular IgV domain and an intracellular domain with a canonical ITIM and an immunoglobulin tyrosine tail (ITT) motif (Table 1) (Yu et al., 2009; Levin et al., 2011). TIGIT expression is tightly restricted to lymphocytes and is mainly observed in NK cells and T cell subsets, including effector and regulatory CD4+ T cells, follicular helper CD4+ T cells, and effector CD8+ T cells (Boles et al., 2009; Yu et al., 2009; Lozano et al., 2012; Stengel et al., 2012; Johnston et al., 2014; Joller et al., 2014). Three ligands bind to TIGIT: 1) poliovirus receptor (PVR), also known as CD155, Ncl5, and Tag4; 2) CD112, also called poliovirus receptor ligand2/nectin2 (PVRL2/nectin 2); and 3) PVRL3. PVR has a high affinity for TIGIT, whereas CD112 and PVRL3 bind to a lesser extent (Yu et al., 2009).

TIGIT plays multiple roles in the inhibition of cancer immunity. TIGIT inhibits NK cell-mediated tumor killing, induces immunosuppressive DCS, suppresses CD8 T cell priming and differentiation, and prevents CD8 T cell-mediated killing (Ruisson and Triebel, 2005; Li et al., 2014; Fuhrman et al., 2015; Kurtulus et al., 2015; Liu et al., 2015; Kourepini et al., 2016). The interaction of TIGIT with other constituents of the tumor microenvironments (TMEs), such as cancer-associated fibroblasts and angiogenesis, remains to be elucidated (Manieri et al., 2017).
| Target | Drug | Clinical trial no. | Phase | Settings | Tumor types | Treatment arms | Status |
|--------|------|---------------------|-------|----------|-------------|----------------|--------|
| LAG-3  | Eftilagimod alpha (IMP321) | NCT03252938 | 1 Advanced/metastatic | Solid tumors | Eftilagimod alpha | Active, not recruiting |
|        |      | NCT00351949 | 1 Advanced/metastatic | RCC | Eftilagimod alpha | Completed |
|        |      | NCT00349934 | 1 First line | Breast cancer | Eftilagimod alpha | Completed |
|        |      | NCT02614833 | 2 Advanced/metastatic | Breast cancer | Eftilagimod alpha | Active, not recruiting |
|        |      | NCT00324623 | 1 Advanced/metastatic | Melanoma | Cyclophosphamide, fludarabine followed by melan-A VLP vaccine and eftilagimod alpha | Completed |
|        |      | NCT00365937 | 1,2 Adjuvant | Melanoma | Eftilagimod alpha+HLA-A2 peptides | Terminated |
|        |      | NCT01308294 | 1,2 Stage II-IV | Melanoma | Eftilagimod alpha+tumor antigenic peptides+monatide | Terminated |
|        |      | NCT00732082 | 1 Advanced/metastatic | Pancreatic cancer | Eftilagimod alpha+gemcitabine | Terminated |
|        |      | NCT02676869 | 1 Stage III-IV | Melanoma | Eftilagimod alpha+pembrolizumab | Completed |
|        |      | NCT03625323 | 2 Advanced/metastatic | NSCLC and HNSCC | Eftilagimod alpha+pembrolizumab | Recruiting |
|        |      | NCT02366548 | 1 Advanced/metastatic | Solid tumors | Relatlimab±nivolumab | Recruiting |
|        |      | NCT01968109 | 1,2 First, second line | Solid tumors | Relatlimab±nivolumab | Recruiting |
|        |      | NCT03623554 | 2 Advanced/metastatic | Chordoma | Relatlimab±nivolumab | Recruiting |
|        |      | NCT03743766 | 2 Advanced/metastatic | Melanoma | Relatlimab±nivolumab | Recruiting |
|        |      | NCT03470922 | 2,3 Advanced/metastatic | Melanoma | Relatlimab±nivolumab | Recruiting |
|        |      | NCT03642067 | 2 Advanced/metastatic | MSS CRC | Relatlimab+nivolumab | Recruiting |
|        |      | NCT04658147 | 1 Recsectable | HCC | Relatlimab±nivolumab | Not yet recruiting |
|        |      | NCT02061761 | 1,2 Advanced/metastatic | Hematologic malignancies | Relatlimab±nivolumab | Active, not recruiting |
|        |      | NCT04567615 | 2 Advanced/metastatic | HCC | Relatlimab±nivolumab | Not yet recruiting |
|        |      | NCT03607890 | 2 Advanced, prior PD-(L)1 inhibitor | MSI-H solid tumors | Relatlimab±nivolumab | Recruiting |
|        |      | NCT04326257 | 2 Advanced, prior PD-(L)1 inhibitor | HNSCC | Relatlimab+nivolumab or ipilimumab | Recruiting |
|        |      | NCT03493932 | 1 Recurrent | Glioblastoma | Relatlimab±nivolumab | Recruiting |
|        |      | NCT02656881 | 1 Recurrent | Glioblastoma | Relatlimab±nivolumab or urelumab (anti-CD137) | Active, not recruiting |
|        |      | NCT02610711 | 1,2 Advanced/metastatic | GC, GEJ cancer | Relatlimab±nivolumab | Recruiting |
|        |      | NCT03044613 | 1 Stage II/III | GC, GEJ cancer | Nivolumab, carboplatin, paclitaxel, radiation±relatlimab | Recruiting |
|        |      | NCT03626269 | 2 Advanced/metastatic | GC, GEJ cancer | Relatlimab or nivolumab±investigator’s choice of chemotherapy | Active, not recruiting |
|        |      | NCT0333550 | 1,2 Advanced/metastatic | Solid tumors | Relatlimab±nivolumab or cabiralizumab or ipilimumab or IDO1 inhibitor or radiation therapy | Recruiting |
|        |      | NCT04611126 | 1,2 Advanced/metastatic | Ovarian cancer | Relatlimab, nivolumab, cyclophosphamide, fludarabine phosphate, tumor infiltrating lymphocytes infusion ± ipilimumab | Not yet recruiting |
|        |      | NCT02488759 | 1,2 Neoadjuvant and metastatic | Virus-associated tumors | Nivolumab±relatlimab or ipilimumab or daratumumab | Active, not recruiting |
|        |      | NCT02519322 | 2 Neoadjuvant and metastatic | Melanoma | Nivolumab±relatlimab or ipilimumab | Recruiting |
|        |      | NCT03499222 | 2 Advanced/metastatic | Solid tumors | Relatlimab, nivolumab±ipilimumab | Recruiting |
|        |      | NCT03996110 | 2 Advanced/metastatic | RCC | Nivolumab±ipilimumab or BMS-986205 (IDO1) or BMS-813160 (CCR2/C5 dual antagonist) | Recruiting |
|        |      | NCT02935634 | 2 Advanced/metastatic | GC, GEJ cancer | Nivolumab±relatlimab or ipilimumab or rucaparib or BMS-986205; ipilimumab±rucaparib | Recruiting |

(Continued on following page)
| Target          | Drug                          | Clinical trial no. | Phase | Settings            | Tumor types                                                                 | Treatment arms                                                                                     | Status                  |
|-----------------|-------------------------------|--------------------|-------|---------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-------------------------|
| NCT02750514     | 2 Advanced/metastatic         | NSCLC              | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Active, not recruiting                                                                          |
| NCT02060188     | 2 Advanced/metastatic         | CRC                | 2     | Advanced/metastatic | Nivolumab; relatlimab or daratumumab or ipilimumab and cobimetinib           | Active, not recruiting                                                                          |
| NCT04150965     | 1,2 Advanced/metastatic       | Multiple myeloma   | 1,2   | Advanced/metastatic | Relatlimab; pomalidromide and dexamethasone; BMS-986207 (anti-TIGIT); pomalidromide and dexamethasone; elotuzumab | Recruiting                                                                                       |
| NCT02460224     | 1,2 Advanced/metastatic       | Solid tumors       | 1,2   | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Active, not recruiting                                                                          |
| NCT03365791     | 2 Advanced/metastatic         | Solid or hematologic malignancy | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Active, not recruiting                                                                          |
| NCT03742349     | 1 Advanced/metastatic         | TNBC               | 1     | Advanced/metastatic | LAG525; spartalizumab                                                       | Recruiting                                                                                       |
| NCT03489899     | 2 Advanced/metastatic         | TNBC               | 2     | Advanced/metastatic | LAG525; spartalizumab                                                       | Recruiting                                                                                       |
| NCT03480423     | 2 Advanced/metastatic         | Melanoma           | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Active, not recruiting                                                                          |
| NCT03005782     | 1 Advanced/metastatic         | Solid tumors or lymphomas | 1     | Advanced/metastatic | LAG525; spartalizumab; Nivolumab; or capmatinib or lacnotuzumab               | Recruiting                                                                                       |
| NCT03433898     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | LAG525; spartalizumab; Nivolumab; or capmatinib or lacnotuzumab               | Recruiting                                                                                       |
| NCT03156131     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | LAG525; spartalizumab; Nivolumab; or capmatinib or lacnotuzumab               | Recruiting                                                                                       |
| NCT03780725     | 1 Advanced/metastatic         | NSCLC and HNSCC    | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03697304     | 2 Advanced/metastatic         | Solid tumors       | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03982303     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03499999     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | LAG525; spartalizumab; Nivolumab; or capmatinib or lacnotuzumab               | Recruiting                                                                                       |
| NCT03480898     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | LAG525; spartalizumab; Nivolumab; or capmatinib or lacnotuzumab               | Recruiting                                                                                       |
| NCT03219288     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | LAG525; spartalizumab; Nivolumab; or capmatinib or lacnotuzumab               | Recruiting                                                                                       |
| NCT030708725    | 1 Advanced/metastatic         | NSCLC and HNSCC    | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03697304     | 2 Advanced/metastatic         | Solid tumors       | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03962333     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03493829     | 1 Advanced/metastatic         | Solid tumors or lymphomas | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03219288     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT04082364     | 2 Advanced/metastatic         | GC, GEJ cancer     | 2,3   | Advanced/metastatic | Mereceptumab; INCMGA00012; margetuximab; MGD013 or INCMGA00012; trastuzumab; chemotherapy (XEOLOX or mFOLFOX-6) | Recruiting                                                                                       |
| Mavezelimab (MK-4280) | NCT03598608     | Measurable disease | 1,2   | Advanced/metastatic | Hematologic malignancies                                                    | MK-4280; pembrolizumab                                                                          | Recruiting              |
| NCT03598608     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT02720068     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03697304     | 2 Advanced/metastatic         | Solid tumors       | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03555284     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03697304     | 2 Advanced/metastatic         | Solid tumors       | 2     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT04370704     | 1,2 Advanced/metastatic       | Solid tumors       | 1,2   | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03311412     | 1 Advanced/metastatic         | Solid tumors or lymphomas | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT03690090     | 1 Advanced/metastatic         | Breast cancer      | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| NCT04566978     | 1 Advanced/metastatic         | Measurable disease by Lugano criteria | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| XmAe®22,841     | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| LBL-007         | 1 Advanced/metastatic         | Melanoma           | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| FS118           | 1 Advanced/metastatic         | Solid or hematologic malignancy | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| RO7247669       | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |
| EMB-02          | 1 Advanced/metastatic         | Solid tumors       | 1     | Advanced/metastatic | Nivolumab; relatlimab or ipilimumab or BMS-986205 or dasatinib               | Recruiting                                                                                       |

(Continued on following page)
| Target | Drug | Clinical trial no. | Phase | Settings | Tumor types | Treatment arms | Status |
|--------|------|-------------------|-------|----------|-------------|----------------|--------|
| **TIGIT** | Tiragolumab | NCT02794571 | 1 | Locally advanced or metastatic | Solid tumors | Tiragolumab±atezolizumab±chemotherapy | Recruiting |
| | | NCT03563716 | 2 | Locally advanced or metastatic | NSCLC | Atezolizumab±tiragolumab | Active, not recruiting |
| | | NCT04294810 | 3 | Locally advanced or metastatic | NSCLC | Atezolizumab±tiragolumab | Recruiting |
| | | NCT04256421 | 3 | First line, extensive stage | SCLC | Atezolizumab+tiragolumab; atezolizumab+carboplatin+etoposide+tiragolumab | Recruiting |
| | | NCT03281369 | 1,2 | Advanced/metastatic | Esophageal cancer | Atezolizumab+tiragolumab; atezolizumab+cisplatin/5-FU+tiragolumab; cisplatin/5-FU | Recruiting |
| | Vibostolimab (MK-7684) | NCT02964013 | 1 | Advanced/metastatic | Solid tumors | Vibostolimab+pembrolizumab+metetrexed/cisplatin/carboplatin; carboplatin+cisplatin+etoposide | Recruiting |
| | | NCT04305054 | 1,2 | Advanced/metastatic | Melanoma | Pembrolizumab+vibostolimab or quavonlimab (MK-1308)+lenvatinib | Recruiting |
| | | NCT03303169 | 1,2 | Stage III-IV | Melanoma | Pembrolizumab+quavonlimab+ vibrationlimab or lenvatinib | Recruiting |
| | | NCT03119428 | 1 | Locally advanced or metastatic | Solid tumors | Pembrolizumab+vibostolimab or V937 (oncolytic virus) | Recruiting |
| | Etiqilimab (OMP-313M32) | NCT02913313 | 1,2 | Advanced/metastatic | Solid tumors | BMS-986207±nivolumab | Active, not recruiting |
| | | NCT02913313 | 1,2 | Advanced/metastatic | Solid tumors | Nivolumab+BMS-986207 with COM701 (anti-PVRG Ab) | Recruiting |
| | | NCT04305041 | 1,2 | Stage III-IV | Melanoma | Pembrolizumab+dombvanalimab+etrumadenant | Recruiting |
| | | NCT03260322 | 1 | Advanced/metastatic | Solid tumors | ASP-8374±pembrolizumab | Completed |
| | | NCT04353830 | 1 | Advanced/metastatic | Solid tumors | IBI939±sintilimab (anti-PD-1) | Recruiting |
| | | NCT04672369 | 1 | Advanced/metastatic | NSCLC | IBI939±sintilimab | Not yet recruiting |
| | | NCT04672356 | 1 | Advanced/metastatic | NSCLC and SCLC | IBI939±sintilimab | Not yet recruiting |
| | BGB-A1217 | NCT04047862 | 1 | Advanced/metastatic | Solid tumors | BGB-A1217+tigelizumab±chemotherapy | Recruiting |
| | | NCT04354248 | 1 | Advanced/metastatic | Solid tumors | Dostarlimab±TSR-042+chemotherapy | Recruiting |
| | | NCT04465778 | 1 | Advanced/metastatic | Solid tumors | Milv223±binrafusp alfa (M7824) | Recruiting |
| | TIM-3 | NCT03489343 | 1 | Advanced/metastatic | Solid tumors or lymphomas | LY3300054 (anti-PD-1)+LY3321367 | Completed |
| | SYM023 | NCT03099010 | 1 | Advanced/metastatic | Solid tumors | LY3300054 (LY3321367+or abermacicil or ramucirumab or merestinib) | Active, not recruiting |
| | | NCT02791334 | 1 | Advanced/metastatic | Solid tumors | LY3300054 (LY3321367) | Active, not recruiting |
| | Cobolimab (TSR-022) | NCT02817633 | 1 | Advanced/metastatic | Solid tumors | Cobolimab±nivolumab or TSR-042±TSR-033±docetaxel | Recruiting |
| | | NCT03307785 | 1 | Advanced/metastatic | Solid tumors | Dostarlimab±TSR-042±chemotherapy | Active, not recruiting |
| | | NCT03680508 | 2 | BCLC stage B or C | HCC | Cobolimab+bevacizumab or niraparib + chemotherapy | Recruiting |
| | | NCT04149902 | 2 | Neoadjuvant | Melanoma | Cobolimab+dostarlimab | Recruiting |
| | Sabatolimab (MBG453) | NCT02608268 | 1,2 | Advanced/metastatic | Solid tumors | Sabatolimab±spartalizumab; decitabine | Active, not recruiting |

(Continued on following page)
| Target | Drug | Clinical trial no. | Phase | Settings | Tumor types | Treatment arms | Status |
|--------|------|-------------------|-------|----------|-------------|----------------|--------|
| GBM    |     | NCT03961971      | 1     | Advanced/metastatic | GBM        | Sabatolimab+spartalizumab | Recruiting |
|       |     | NCT04623216      | 1,2   | Received one prior aHSCT | AML        | Sabatolimab+azacitidine | Not yet recruiting |
|       |     | NCT03066648      | 1     | Relapse/refractory | AML or high risk MDS | Sabatolimab+spartalizumab±decitabine | Recruiting |
|       |     | NCT03940352      | 1     | Relapse/refractory | AML or high risk MDS | HDM201 (p53-DM2 inhibitor)+sabatolimab or venetoclax | Recruiting |
|       |     | NCT0946670       | 2     | IPSS-R-intermediate, high, or very high risk | MDS | Hypomethylating agents+sabatolimab | Active, not recruiting |
|       |     | NCT04266301      | 3     | IPSS-R-intermediate, high, or very high risk for MDS | MDS or CML | Sabatolimab+azacitidine | Recruiting |
|       |      | INCAGN2390        | 1     | Advanced/metastatic | Solid tumors | | Active, not recruiting |
|       |      | NCT03446040      | 1,2   | Advanced/metastatic | Solid tumors | | BMS-986258+nivolumab or rHuPH20 | Recruiting |
|       |      | NCT03871855      | 1     | Advanced/metastatic | Solid tumors | | SHR-1702.scamrelizumab | Unknown |
|       |      | NCT03708328      | 1     | Advanced/metastatic | Solid tumors | | | Recruiting |
|       |      | NCT0191143       | 1     | Advanced/metastatic | Solid tumors | | | Completed |
|       |      | NCT02982941      | 1     | Advanced/metastatic | Pediatric solid tumors | | | Recruiting |
|       |      | NCT02923180      | 2     | Localized intermediate and high-risk | Prostate cancer | | | Active, not recruiting |
|       |      | NCT04634825      | 2     | Advanced/metastatic | HNSCC       | Enoblituzumab+retifanlimab (anti-PD-1 antibody) or tebotelimab (PD-1 and LAG-3 bispecific DART molecule) | Not yet recruiting |
|       |      | NCT02381314      | 1     | Advanced/metastatic | Solid tumors | Enoblituzumab+ipilimumab | Completed |
|       |      | NCT02475213      | 1     | Advanced/metastatic | Solid tumors | Enoblituzumab+pembrolizumab or retifanlimab | Active, not recruiting |
|       |      | NCT04129320      | 2,3   | Advanced/metastatic | HNSCC       | Enoblituzumab+retifanlimab or tebotelimab | Withdrawn |
|       |      | NCT04145622      | 1,2   | Advanced/metastatic | Solid tumors | Enoblituzumab+retifanlimab | Recruiting |
|       |      | NCT02628535      | 1     | Advanced/metastatic | Solid tumors | Enoblituzumab+retifanlimab | Terminated |
|       |      | NCT03406949      | 1     | Advanced/metastatic | Solid tumors | Orlotamab+retifanlimab | Active, not recruiting |
|       |      | NCT01099644      | 1     | Peritoneal involvement | DSRCT | | Active, not recruiting |
|       |      | NCT00089245      | 1     | Advanced/metastatic | CNS or leptomeningeal cancer | | Active, not recruiting |
|       |      | NCT03275402      | 2,3   | Recurrent | Neuroblastoma, CNS, or leptomeningeal metastases | | | Recruiting |
|       |      | NCT01502917      | 1     | Prior external beam radiotherapy | Gliomas | 124I-Omburtamab+external beam radiotherapy (prior to study entry) | Recruiting |
|       |      | NCT04167618      | 1,2   | Recurrent | Medulloblastoma | | | Not yet recruiting |
|       |      | NCT04315246      | 1,2   | Advanced/metastatic | Leptomeningeal metastasis from solid tumors | | | Not yet recruiting |
|       |      | NCT04432649      | 1     | Advanced/metastatic | Solid tumors | | | Recruiting |
|       |      | NCT04185038      | 1     | Advanced/metastatic | Pediatric CNS tumors | | | Recruiting |
|       |      | NCT04077766      | 1,2   | Recurrent | GBM | | | Recruiting |
|       |      | NCT04700682      | 1     | Advanced/metastatic | Epithelial ovarian cancer | | | Recruiting |
|       |      | NCT04483778      | 1     | Recurrent | Non-primary CNS solid tumors | Second generation 4-1BBx B7-H3-EGFRt-DHFR, second generation 4-1BBx CD19-Her2tG | Recruiting |

(Continued on following page)
| Target | Drug | Clinical trial no. | Phase | Settings | Tumor types | Treatment arms | Status |
|--------|------|--------------------|-------|----------|-------------|----------------|--------|
| VISTA  | JNJ-61610588 | NCT02671955 | 1 | Advanced/metastatic | Solid tumors | Terminated |
|        | CI-8993 | NCT04475523 | 1 | Advanced/metastatic | Solid tumors | Recruiting |
|        | CA-170 | NCT02812875 | 1 | Advanced/metastatic | Solid tumors or lymphomas | Completed |
| ICOS   | GSK3359609 | NCT04428333 | 1,2 | Advanced/metastatic | HNSCC | Recruiting |
|        |        | NCT04128696 | 3 | Advanced/metastatic | HNSCC | Recruiting |
|        |        | NCT03693612 | 2 | Advanced/metastatic | Solid tumors | Recruiting |
|        |        | NCT02090226 | 1,2 | Advanced/metastatic | Solid tumors | Recruiting |
|        |        | NCT02520791 | 1 | Advanced/metastatic | Lymphomas | Recruiting |
|        | KY1044 | NCT03829501 | 1,2 | Advanced/metastatic | Solid tumors | Recruiting |
|        |        | NCT039198766 | 1 | Locally advanced or metastatic | Solid tumors | Recruiting |
|        | Cudarolimab (iBi101) | NCT03758001 | 1 | Advanced/metastatic | Solid tumors | Recruiting |
|        | PF-04518600 | NCT02315066 | 1 | Advanced/metastatic | Solid tumors | Recruiting |
|        | TAB004 (JS004) | NCT04137900 | 1 | Advanced/metastatic | Solid tumors or lymphomas | Recruiting |
|        |        | NCT04278859 | 1 | Advanced/metastatic | Solid tumors | Recruiting |
|        |        | NCT04477772 | 1 | Advanced/metastatic | Lymphomas | Recruiting |

Abbreviations: AML, acute myeloid leukemia; anti-PD-1, anti-programmed death-1; BCLC, Barcelona Clinic Liver Stage; BTLA, B and T-lymphocyte attenuator; CML, chronic myelogenous leukemia; CNS, central nervous system; CRC, colorectal cancer; DART, dual-affinity re-targeting proteins; DLBCL, diffuse large B cell lymphoma; DSRCT, desmoplastic small round cell tumor; GBM, glioblastoma multiforme; GC, gastric cancer; GEJ, gastroesophageal junction cancer; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ICOS, Inducible T cell costimulator; IDO1i, indoleamine 2,3-dioxygenase-1 inhibitor; IPSS-R, revised international prognostic scoring system; LAG3, lymphocyte-associated gene 3; MDM2, mouse double minute 2 homolog; MDS, myelodysplastic syndrome; MSB-H, microsatellite instability-high; MSS, microsatellite stable; NSCLC, non-small cell lung cancer; PEGPH20, pegylated recombinant human hyaluronidase; RCC, Renal cell carcinoma; rHuPH20, recombinant human hyaluronidase PH20 enzyme; SCLC, small cell lung cancer; TIGIT, T cell immunoglobulin and ITIM domain; TIM, T-cell immunoglobulin and mucin domain-3; TNBC, triple negative breast cancer; TNFRSF9, tumor necrosis factor receptor superfamily member 9; VEGF, vascular endothelial growth factor; VISTA, V-domain immunoglobulin suppressor of T cell activation.

Regimens: mFOLFOX, oxaliplatin 85 mg/m² intravenous (IV), leucovorin 400 mg/m² IV, and fluorouracil 2400 mg/m² IV over 46–48 h every 2 weeks (Q2W) FOLFIRI, irinotecan 180 mg/m² IV, leucovorin 400 mg/m² IV, and 5-FU 2400 mg/m² IV over 46–48 h (Q2W).

Chemotherapy: carboplatin/pemetrexed, carboplatin/nab-paclitaxel, or carboplatin/paclitaxel.
Recently, several studies have highlighted that TIGIT is co-expressed and associated with PD-1 expression (Johnston et al., 2014; Chauvin et al., 2015). Dual blockade of TIGIT and PD-1 resulted in the restoration of T-cell immunity in preclinical settings and provided a rationale for combination with these agents as a feasible anti-cancer therapeutic strategy (Johnston et al., 2014; Kurtulus et al., 2011; Anderson, 2012). Four ligands bind to TIM-3: two soluble ligands, high-mobility group protein B1 (HMGB1) and galectin-9, and two surface ligands, including carcinoembryonic antigen cell adhesion molecule 1 (ceacam-1) and phosphatidylinositol serine (PtdSer) (Zhu et al., 2005; Nakayama et al., 2009; Chiba et al., 2012; Huang et al., 2015; Kang et al., 2015). Interaction of TIM-3 with its ligands has been shown to induce T cell inhibition. TIM-3 is unique compared to other immune checkpoints in that its upregulation is initiated only by CD4+ and CD8+ cells that produce IFN-γ (Sakuishi et al., 2010; Gao et al., 2012).

Similar to PD-L1, TIM-3 is expressed in TILs is associated with disease progression in certain cancers (Ngiow et al., 2011). Meta-analysis of TIM-3 overexpression in solid tumors has shown that higher TIM-3 expression is associated with worse OS and may potentially be a prognostic marker (Zhang et al., 2017). Blocking TIM-3 expression results in T cell proliferation and cytokine production, thereby eliciting immune activation (Gao et al., 2012). In addition, targeting TIM-3 with PD-1 in preclinical settings has shown a synergistic effect by reinvigorating T cell function and increasing anti-tumor immunity (Sakuishi et al., 2010; Koyama et al., 2016). Thus, the dual blockade of PD-1 and TIM-3 is a feasible and promising therapeutic option.

Clinical Trials on TIM-3

There are seven anti-TIM-3 mAbs and one anti-PD-1 and TIM-3 bispecific Ab (RO7121661) undergoing clinical trials (Table 2). Sym021 (anti-PD-1), sym022 (anti-LAG-3), and sym023 (anti-TIM-3) were evaluated as single agents or combinations in phase 1 trials for solid tumors or lymphomas (NCT03114142, NCT03489369, and NCT03489343) (Lakhan et al., 2020). Sym023 monotherapy (n = 24) and in combination with Sym021 (n = 17) was administered; however, Sym023 and its combination did not reach their MTD. One patient in the monotherapy group had grade 3–4 immune-mediated arthritis. Overall, monotherapy and combination therapy were well tolerated, with two PRs observed in the combination group.

LY3321367 is also an anti-TIM-3 mAb; an interim analysis of a phase 1a/1b, dose-escalation and -expansion study showed that intravenous infusion of 3–1200 mg LY3321367 Q2W monotherapy (Arm A, 23 patients) or 70–1200 mg LY3321367
progression in RCC (Li et al., 2014; Jin et al., 2015). Patients associated with poor prognosis, and co-expression of B7-H3 disease progression (Li et al., 2014; Jin et al., 2015; Benzon NSCLC, RCC, CRC, and prostate cancer are correlated with advanced stage, and poor outcomes in prostate cancer levels of B7-H3 are associated with higher Gleason grade, with CRC, harboring B7-H3 and CD133 expression, have (Janakiram et al., 2017). In addition, B7-H3 is expressed in activated immune cells such as antigen-presenting cells (APCs), NK cells, T cells, and monocytes (Janakiram et al., 2017). In addition, B7-H3 is expressed in several tumors. Notably, high levels of B7-H3 expression in NSCLC, RCC, CRC, and prostate cancer are correlated with disease progression (Li et al., 2014; Jin et al., 2015; Benzon et al., 2017; Mao et al., 2017). In NSCLC, B7-H3 with Tregs was associated with poor prognosis, and co-expression of B7-H3 and CD14 was found to play a role in angiogenesis and tumor progression in RCC (Li et al., 2014; Jin et al., 2015). Patients with CRC, harboring B7-H3 and CD133 expression, have shorter survival (Castellanos et al., 2017). Similarly, high levels of B7-H3 are associated with higher Gleason grade, advanced stage, and poor outcomes in prostate cancer (Benzon et al., 2017).

Recently, the co-inhibitory function of B7-H3 in CD4+ and CD8+ T cells was discovered (Suh et al., 2003; Prasad et al., 2004). Studies are ongoing to identify the receptor for B7-H3, and the contradictory roles of B7-H3 in immune activity are yet to be fully elucidated (Yang et al., 2020). In addition to the immunological aspects of B7-H3, other signaling pathways, including PI3K/AKT/mTOR, JAK2/STAT3, and TLR4/NF-κB signaling, can activate B7-H3 expression (Kang et al., 2015; Zhang et al., 2015; Xie et al., 2016; Fan et al., 2017; Zhang et al., 2017). Other studies have highlighted that B7-H3 is associated with resistance to chemotherapy and targeted agents (Liu et al., 2011; Jiang et al., 2016; Flem-Karlsen et al., 2017; Flem-Karlsen et al., 2019).

Clinical Trials on B7-H3

Eleven agents targeting B7-H3 are currently under investigation in clinical trials (Table 2). Generally, patients harboring B7-H3 are enrolled in clinical trials. Enoblituzumab (MG271), an anti-B7-H3 mAb with antibody-dependent cellular toxicity (ADCC) function, has been investigated in multiple solid tumors, including pediatric tumors. Interim analysis of enoblituzumab in refractory solid tumors revealed that it was well tolerated up to 15 mg/kg, with no DLT and MTD (Powderly et al., 2015). Although TRAEs, such as fatigue (30%) and infusion-related reactions (26%), occurred in 71% of the patients, most of these AEIs were tolerated with adequate supportive care (NCT01391143). Enoblituzumab is currently being used as a monotherapy or in combination with anti-PD-1 antibody (retifanlimab or pembrolizumab), tebotelimab, a PD-1 and LAG-3 bispecific DART, or ipilimumab, as shown in Table 3.

DS-7300a is a B7-H3-targeting antibody drug conjugate (ADC) with DXd, a payload that is an exatecan derivative, which inhibits topoisomerase I (Bendell et al., 2020). The phase I/II study is ongoing with patients enrolled in the dose-escalation part (NCT04145622). Orlotamab (MGD009) is a B7-H3 and CD3 DART protein, and its monotherapy (NCT02628535) and combination with retifanlimab (NCT03406949) are under investigation in heavily treated solid tumors. Orlotamab with radioactive labeling such as 131I-Omburtamab (NCT01099644, NCT00089245, and NCT03275402), 124I-Omburtamab (NCT01502917), and 177Lu-DTPA-Omburtamab (NCT04167618 and NCT04315246) are also ongoing trials. In patients with desmoplastic small round cell tumor (DSRCT), treatment with 131I-Omburtamab via intraperitoneal administration followed by external beam intensity-modulated whole-abdominopelvic radiotherapy (WAP-IMRT) to 3,000 cGy was tolerable with a satisfactory safety profile, and appeared to demonstrate micrometastatic activity in a phase 1 trial (Modak et al., 2018). The biodistribution, organ, and whole-body exposure were measured with 124I-89H9-directed radioimmuno-PET, and the RP2D for 131I-Omburtamab was set at 80 mCi/m2.

Other investigational agents include chimeric antigen receptor (CAR) T cell therapy targeting B7-H3: 4SCAR-276 in solid tumors (NCT04432649), SCRIB-CARB7H3 in pediatric CNS tumors (NCT04185038), B7-H3 chimeric antigen receptor T cells (CAR-T) treated alone (NCT04385173) or with temozolamide (NCT04077866) in glioblastoma, CAR-B7-H3 with other agents in epithelial ovarian cancer (NCT04670068), and second-generation 4-1BB, B7-H3-EGFRt-DHFR in non-primary CNS solid tumors (NCT04483778).

VISTA

VISTA has several names such as differentiation of embryonic stem cells 1 (Dies1), DD1 α, Gi24, and B7H5 (Table 1). (Ceeraz
Notably, it is also named PD-1 homologue (PD-1H), as its extracellular domain shows structural similarity to PD-1; however, it is different, as it lacks the classical ITIM or ITSM motif in the cytoplasmic domain (Fliess et al., 2011). Furthermore, VISTA differs from PD-1, which functions in the effector stage, as VISTA is expressed on resting T cells, indicating its regulatory role in earlier stages (Kondo et al., 2016). Compared to that in peripheral lymph nodes, VISTA is more abundant in myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment (TME) (Le Mercier et al., 2014).

High levels of VISTA are expressed by mature APCs with CD11b, whereas relatively low expression is found on Tregs, CD8+, CD4+, and TILs (Lines et al., 2014). Although the counter structures for VISTA have not been comprehensively elucidated, recent *in vitro* findings on V-set and immunoglobulin domain containing 3 (Vsig-3) have shown that VISTA also acts as a co-inhibitory ligand on tumor cells (Wang et al., 2019). VISTA promotes Treg maturation and prevents T cell activation independent of PD-1 expression (Yoon et al., 2015; Torphy et al., 2017; Popovic et al., 2018). The non-overlapping mechanisms of VISTA and PD-L1 make their combination an ideal treatment strategy to overcome immune suppression. In mouse models, dual blockade of VISTA and PD-1, using monoclonal antibodies specific for these immune checkpoints, led to synergistic activity against T-cells with anti-tumor responses (Liu et al., 2015).

A wide array of tumors has been studied to determine the prognostic and predictive roles of VISTA. High-grade serous ovarian cancer patients with tumor cells expressing VISTA showed longer PFS and OS (Zong et al., 2020). Furthermore, VISTA expression on TILs in pT1/2 esophageal adenocarcinoma was associated with improved OS compared to the TILs negative for VISTA (Loeser et al., 2019). Similarly, VISTA+ and CD8+ TIL subtypes are associated with better OS in HCC (Zhang et al., 2018). Contrary to these findings, VISTA+ and CD8+ TIL subtypes were associated with worse prognosis in oral squamous cell carcinoma and cutaneous melanoma with VISTA expression, whereas VISTA had no correlation with survival outcome in GC expressing VISTA (Böger et al., 2017; Wu et al., 2017; Kuklinski et al., 2018).

**Clinical Trials on VISTA**

Ongoing clinical trials on VISTA include two anti-VISTA mAbs and one small-molecule antagonist of VISTA (Table 2). JNJ-61610588 (NCT02671955) and CI-8993 (NCT04475523) are anti-VISTA mAbs, currently under investigation in phase 1 trials for the treatment of refractory solid tumors. CA-170 is a small molecule that targets both VISTA and PD-L1 (Musielak et al., 2019). A phase 1 study in patients with advanced solid tumors or lymphomas showed no DLT during dose escalation in 19 patients treated across six dose levels (50–800 mg) (NCT02812875) (Powderly et al., 2017). Exploratory analysis showed an increased proportion of both circulating CD8+ and CD4+ cells after oral dosing with CA-170. Further data on dose escalation, the recommended phase 2 dose, and anti-tumor responses are awaiting results.

**ICOS**

ICOS, also known as cluster of differentiation 278 (CD278) in T cells, is a member of the CD28 coreceptor family, which includes costimulatory CD28 and coinhibitory receptor CTLA-4 (Table 1) (Hutloff et al., 1999). The ICOS ligand (ICOSL) is expressed in APCs such as macrophages, DCs, and B cells (Yoshinaga et al., 1999). In contrast to the expression of CD28 in both naive and memory T cells, the majority of ICOS is expressed only after the activation of memory T cells, with only small fractions expressed in resting memory T cells. Further, unlike CD28 and CTLA-4 ligands, which are expressed primarily on lymphoid tissues, ICOSL is expressed in non-lymphoid cells, such as endothelial cells, epithelial cells, mesenchymal cells, and fibroblasts, via the activation of tumor necrosis factor-a (Swallow et al., 1999; Khayyamian et al., 2002; Martin-Orozco et al., 2010).

Activation of the ICOS pathway induces the production of cytokines, such as IL-4, IL-10, and IL-21, by CD4+ T cells, CD4+ forkhead box P3 (FoxP3+) Tregs, and CD8+ cytotoxic T lymphocytes (CTL) (Hutloff et al., 1999; Giguex et al., 2009; Solinas et al., 2020). ICOS interacts with its ligand (ICOSL) to increase anti-tumor effects via the regulation of memory and effector T cell development and humoral immune responses (Marinelli et al., 2018). The rationale for targeting the ICOS/ICOSL axis with agonists and antagonists is its capacity to trigger both anti-tumor T cell responses by Th1 and other effector T cells, as well as its protumor responses via Tregs (Solinas et al., 2020).

In preclinical studies, ICOS expression on FoxP3+ Tregs and other Th subsets has been identified in multiple arrays of solid tumors, including melanoma, gastric, colorectal, and breast cancers (Strauss et al., 2008; Zhang et al., 2016; Gu-Trantien et al., 2017; Nagase et al., 2017). ICOS+ Treg TILs have been found to be associated with worse survival in GC, whereas high levels of ICOS in Th1 TILs in colorectal cancer indicated better survival outcomes (Zhang et al., 2016; Nagase et al., 2017). Dual blockade of ICOS with anti-CTLA-4 has been effective in eliciting anti-tumor responses in ICOS knockout mice that were unresponsive to anti-CTLA-4 monotherapy (Fu et al., 2011; Fan et al., 2014). More importantly, the utilization of ICOS-targeted agents is gaining attention in hematological malignancies owing to the enhancement of co-stimulatory receptor 4-1BB in CD4+ CAR T cells by ICOS (Guedan et al., 2018).

**Clinical Trials on ICOS**

Currently, both anti-ICOS agonists and anti-ICOS antagonists are under clinical investigation (Table 2). The phase 1 trial of GSK3359609 (INDUCE-1), a humanized anti-ICOS agonist monoclonal antibody, comprised two treatment groups: part 1 patients were treated with a monotherapy of GSK3359609, and part 2 patients were
administered a combination with pembrolizumab or other immunotherapy in the treatment of advanced solid tumors. The study is ongoing, with no dose-limiting toxicities from the first three dose-limiting cohorts (Angevin et al., 2017). In head and neck cancer, the efficacy of GSK3359609 and pembrolizumab with or without platinum-based chemotherapy is currently under investigation (NCT04428333 and NCT04128696).

Another investigational anti-ICOS agonist monoclonal antibody is JTX-2011, used in combination with either anti-PD1 (pembrolizumab or nivolumab) or anti-CTLA-4 (ipilimumab) in advanced solid tumors (NCT02904226) (Yap et al., 2018). In phase 1/II of the trial, anti-tumor activity was observed with JTX-2011 monotherapy and in combination with nivolumab, in heavily treated GC and TNBC with manageable toxicity profiles. Exploratory analysis showed that the peripheral blood CD4 ICOShigh T cell subsets may be a potential biomarker for the response.

Further, agonistic antibodies such as MEDI-570 alone and KY1044 with atezolizumab are under investigation in phases 1 and phase 1/II, respectively (NCT02520791 and NCT03829501).

### BTLA

BTLA (CD272) is also a member of the CD28 coreceptor family (Table 1) (Ceeraz et al., 2013). It is a co-inhibitory molecule with a structure and function similar to those of PD-1 and CTLA-4 (Paulos and June, 2010). When expressed on mature lymphocytes, such as B cells and T cells, macrophages, and DCS, BTLA binds to herpes virus entry mediator (HVEM), a member of the tumor necrosis factor receptor superfamily (TNFRSF), as well as to LIGHT and lymphotixin-a, two members of the tumor necrosis factor (TNF) superfamily (Han et al., 2004; Sedy et al., 2005; Steinberg et al., 2011). Binding of BTLA to HVEM via CD160 transmits inhibitory signals to T cells, which are necessary for proliferation and cytokine production, whereas binding to LIGHT induces co-stimulatory signals (Sedy et al., 2005; Murphy et al., 2006; Cai et al., 2008). Thus, the complexity of the BTLA receptor and ligand activity poses a challenge for BTLA blockade treatment.

Recently, the possibility of BTLA as a potential therapeutic target in cancer immunotherapy has been established in vivo, wherein human melanoma tumor antigen-specific effector CD8+ T cells expressing high levels of BTLA were downregulated with a vaccine formulated using CpG oligodeoxynucleotides, a toll-like receptor 9 (TLR9) agonist that triggers innate immunity, thereby proving that inhibition of BTLA may partially reverse the function of human CD8+ cancer-specific T cells (Derré et al., 2010; Paulos and June, 2010).

### Clinical Trials on BTLA

There are four agents targeting BTLA (Table 2): 1) INBRX-106, a hexavalent OX40 agonist Ab (NCT04198766), 2) PF-04518600 (NCT02315066), an OX40 agonist; 3) cudarolimab (IBI101) (NCT03758001), an anti-OX40 mAb, and 4) TAB004 (JS004) (NCT04278859), an anti-BTLA mAb. These agents target the OX40 receptor, also known as CD134 and tumor necrosis factor receptor superfamily member 4 (TNFRSF4), thereby preventing its interaction with BTLA (Croft et al., 2009). These phase 1 clinical trials are ongoing as monotherapy for patients with advanced/metastatic solid tumors and are awaiting results. TAB004 is also under investigation for the treatment of refractory lymphomas (NCT04137900 and NCT04477772).

### CONCLUSION

Cancer immunotherapy is one of the major pillars in the field of medical oncology, especially for the treatment of unresectable, metastatic, and recurrent cancers. The success of ICIs, such as anti-CTLA-4 and anti-PD-1/PD-L1, in combination with chemotherapy, immunotherapy, and targeted agents, has changed the paradigm of cancer treatment. Nonetheless, the limited efficacy and IRAEs of ICIs have paved way for the discovery of novel checkpoints. Among the immune checkpoint inhibitors, anti-LAG-3 and anti-TIGIT are promising targets, and their efficacy in combination with anti-PD-1/PD-L1 may help overcome the limitations seen in prior treatments. More robust data are yet to follow on agents targeting TIM-3, B7-H3, VISTA, ICOS, and BTLA.

### AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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