Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4

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Abstract

The emergence of immune checkpoint inhibitors (ICIs), mainly including anti-programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) and anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) monoclonal antibodies (mAbs), has shaped therapeutic landscape of some type of cancers. Despite some ICIs have manifested compelling clinical effectiveness in certain tumor types, the majority of patients still showed de novo or adaptive resistance. At present, the overall efficiency of immune checkpoint therapy remains unsatisfactory. Exploring additional immune checkpoint molecules is a hot research topic. Recent studies have identified several new immune checkpoint targets, like lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), T cell immunoglobulin and ITIM domain (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), and so on. The investigations about these molecules have generated promising results in preclinical studies and/or clinical trials. In this review, we discussed the structure and expression of these newly-characterized immune checkpoints molecules, presented the current progress and understanding of them. Moreover, we summarized the clinical data pertinent to these recent immune checkpoint molecules as well as their application prospects.

Keywords: Immunotherapy, Immune checkpoint, LAG-3, TIM-3, TIGIT, VISTA, B7-H3, BTLA

Background

The past decade has witnessed the rapid development of immunotherapy. Now, it has been recognized as a key strategy to control the progression of malignant tumors. Among these immunotherapies, immune checkpoint inhibitors (ICIs) [1], chimeric antigen receptor T cell [2] and bispecific antibodies [3] are the most promising immunotherapy strategies. Encouragingly, the 2018 Nobel Prize in Physiology and Medicine was awarded to Drs. James Allison and Tasuku Honjo, who discovered programmed cell death protein-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), to honor their outstanding work for the development of immunotherapy [4]. In addition, Dr. Lieping Chen did seminal contributions to the discovery of PD-L1 (CD274, B7-H1) [5]. Now, these immune checkpoint targets have realized the transformation from the laboratory to the clinical application.

CTLA-4 is a cell-surface receptor related to CD28, binding to the ligands CD80 (B7–1) and CD86 (B7–2) [6]. The binding of CTLA-4 to CD80/CD86 delivers a negative signal to T cells activation by making CD80/CD86 less available to CD28 [7]. In the early 1996, Leach and his colleagues found that injecting anti-CTLA-4 antibody in mice with pre-established tumors significantly reduced tumor growth [8]. Ipilimumab, as the first anti-CTLA-4 monoclonal antibody (mAb) reaching the clinic [9], garnered the approval for the treatment of patients with malignant melanoma in 2011 [10]. PD-1, also known as CD279, belongs to CD28 superfamily [11]. The binding of PD-1 to its ligands PD-L1 and PD-L2 (CD273, B7-DC) serves as a “rheostat” of immunological regulation, suppressing the activation and function of T cells to downregulate the immune response [12, 13]. Pembrolizumab, the first humanized mAb against PD-1, gained its first global approval for patients with unresectable or metastatic melanoma by United States Food and Drug Administration (FDA) in 2014 [14]. Subsequently, its indication was expended to head and neck squamous cell carcinoma...
(HNSCC) [15], non-small cell lung cancer (NSCLC) [16], metastatic urothelial carcinoma [17], cervical carcinoma [18] among others in a list that continues to grow. By the end of 2018, as many as 7 types of ICIs have been approved by FDA for the treatment of cancers and all of them were immune checkpoint blockers against PD-1/PD-L1 or CTLA-4 [19].

Nevertheless, the response rate of anti-PD-1/PD-L1 mAb and anti-CTLA-4 mAb in overall patients is far from satisfactory. Most patients show primary or acquired resistance to these ICIs [9]. Thus, intensive researches aimed at finding novel immune checkpoint targets have been ongoing. The next generation immune checkpoints such as lymphocyte activation gene-3 (LAG-3) [20], T cell immunoglobulin and mucin-domain containing-3 (TIM-3) [21], T cell immunoglobulin and ITIM domain (TIGIT) [22], V-domain Ig suppressor of T cell activation (VISTA) [23], B7 homolog 3 protein (B7-H3) [24] and B and T cell lymphocyte attenuator (BTLA) [25] demonstrate as promising therapeutic targets with the chance to realize clinical application. In this review, we will emphasize these newly-characterized immune checkpoint molecules and their clinical studies that suggest the promising future for the clinical application.

**Immune checkpoints**

The full activation of T cell dependents on two different signals, signal one is derived from the interaction between antigenic peptide/major histocompatibility complex (MHC) on the surface of APCs and the T cell receptor (TCR), and signal two requires an antigen-independent co-signaling molecules [26]. Note worthwhile, T cell activation is tightly regulated by co-stimulators or co-inhibitors known as immune checkpoints [7]. If antigen/MHC and TCR binding is accompanied by the engagement of costimulatory receptors, such as CD28, it allows T cell to proliferate and to migrate toward specific antigen. On the contrary, if antigen/MHC and TCR binding is accompanied by the engagement of co-inhibitory receptors, such as CTLA-4, it will suppress T cell activation [27, 28]. CTLA-4 is not detectable in naïve T cell but is rapidly induced upon T cell activation and it primarily regulates the amplitude of T cell during the early priming phase in lymphoid organs [29, 30]. The binding of CTLA-4 to B7 proteins competes CD28 costimulatory signals and eventually acts to impede excessive immunity [31]. The aim of this co-inhibitor is to minimize damage to normal tissues and prevent unwanted autoimmunity [31, 32]. In contrast to CTLA-4, PD-1 plays a major role in the maintenance of peripheral tolerance [33]. The engagement of PD-1 by its ligands results in the recruitment of Src homology 2 (SH2) domain containing phosphatases 1/2 (SHP1/2) and then inhibits T cell proliferation and cytokine secretion mediated by TCR [34]. Some cancer cells possess the ability to generate inhibitory ligands which can bind co-inhibitory receptor molecules. This engagement limits normal anti-tumor immune responses thus assisting in immune escape [35]. Therefore, the blockades of these immune checkpoints are capable to invoke patient’s own anti-tumor immune response [32]. Immune checkpoint therapies do not kill cancer cells directly, instead they harness the power of the host’s immune system to re-enhance endogenous anti-tumor activity [36].

**Newly emerging immune checkpoints**

Apart from CTLA-4 and PD-1, novel immune checkpoint molecules on T cells have been discovered continuously. So far, all these emerging immune checkpoints targets are either in the clinical trial or under active development. Those delineated below are the most promising immune checkpoint targets for which blocking antibodies are available in clinical trials (Table 1). A substantial body of evidence accumulates to indicate the synergistic effect of combinatorial blockade among these new immune checkpoints and anti-PD-1/L1 and/or anti-CTLA-4 mAbs. Huang and his colleague utilized a murine model to explore the effect of combinatorial blockade of LAG-3 and PD-1 pathways in ovarian cancer [37]. Their results showed that dual blockade of LAG-3 and PD-1 synergistically enhanced anti-tumor immunity and suppressed tumor growth by enhancing CD8+ tumor infiltrating T cells (TILs) and decreasing regulatory T cells (Tregs) in the tumor microenvironment (TME) [37]. The same group further detected the level of other inhibitory receptors when PD-1 or LAG-3 was blocked. When the mice were treated with anti-PD-1 mAb, the level of LAG-3 and CTLA-4 were increased. Interesting, treatment with anti-LAG-3 mAb upregulated the level of PD-1 [38]. Their experiments indicated that the blockade of a single immune checkpoint targets may lead to compensatory upregulation of other checkpoint receptors in TME. The similar compensatory mechanism between TIM-3 and PD-1 was observed in lung cancer [39] and melanoma [40]. It seems that the compensatory mechanism is common across different types of cancer. These preclinical results pave the way for the combinatorial blockade strategies in clinical trials.

**Lymphocyte activation Gene-3 (LAG-3)**

**The biology of LAG-3**

LAG-3 (CD223) was discovered by Triebel and colleagues as early as 1990 [41]. The LAG-3 gene encompass 8 exons and the corresponding cDNA can encode a 498-amino acid type I membrane protein [41]. LAG-3 gene is located adjacent to CD4 gene on chromosome 12, and further analysis of amino acid sequence reveals
| Target Drugs (company) | Combination agents | Phase | Tumor types | Clinical Trial NO. | State |
|-----------------------|--------------------|-------|-------------|-------------------|-------|
| LAG-3 IMP321/Eftilagimod alpha (Immutep) | Paclitaxel | I | Metastatic RCC | NCT00351949 | Completed |
| | Cyclophosphamide, fludarabine, Melan-A VLP vaccine | I | Metastatic melanoma | NCT00324623 | Completed |
| | HLA-A2 peptides | I/II | Disease-free melanoma | NCT00365937 | Terminated |
| | Gemcitabine | I | Advanced pancreas cancer | NCT00732082 | Terminated |
| | Tumor antigenic peptides, montanide | I/II | Advanced melanoma | NCT01308294 | Terminated |
| | Paclitaxel | II | Metastatic breast cancer | NCT02614833 | Active, not recruiting |
| | Pembrolizumab | I | Metastatic melanoma | NCT02676869 | Active, not recruiting |
| | Relatlimab /BMS-986016 (BMS) | Pembrolizumab | I | Advanced solid tumors | NCT02966548 | Recruiting |
| | Nivolumab | I | Advanced solid tumors | NCT02658981 | Recruiting |
| | Nivolumab and Urelumab | I | Recurrent glioblastoma | NCT03493932 | Recruiting |
| | Nivolumab | I | Recurrent glioblastoma | NCT03743766 | Recruiting |
| | Nivolumab, Carboplatin, Paclitaxel, Radiation | I | Gastro/esophageal cancer | NCT03044613 | Recruiting |
| | Nivolumab, Cabiralizumab, Ipilimumab, anti-GITR, IDO1 Inhibitor, Lirilumab, Radiation | I | Advanced solid tumors | NCT03335540 | Recruiting |
| | Nivolumab, Ipilimumab | I/II | Virus-associated tumors | NCT02488759 | Recruiting |
| | Nivolumab | I/II | Advanced hematologic malignancies | NCT02061761 | Recruiting |
| | Nivolumab, Ipilimumab, BMS-986205 | I/II | Advanced solid tumors | NCT03459222 | Recruiting |
| | Nivolumab | II | Advanced chordoma | NCT03623854 | Recruiting |
| | Nivolumab | II | Metastatic melanoma | NCT03743766 | Recruiting |
| | Nivolumab | II | MSS advanced CRC | NCT03642067 | Recruiting |
| | Nivolumab | II | MSI-H solid tumors | NCT03607890 | Recruiting |
| | Nivolumab, Ipilimumab, BMS-986205, BMS-813160 | II | Advanced RCC | NCT02966548 | Recruiting |
| | Nivolumab, Ipilimumab, BMS-986205 | II | Advanced GC | NCT02935634 | Recruiting |
| | Nivolumab, Dasatinib, Ipilimumab, BMS-986205 | II | Advanced NSCLC | NCT02750514 | Active, not recruiting |
| | Ipilimumab, Nivolumab, Cobimetinib, Daratumumab, anti-LAG-3 antibody | II | Advanced CRC | NCT02060188 | Active, not recruiting |
| | Nivolumab, Ipilimumab | II | Melanoma | NCT02519322 | Recruiting |
| | LAG525 (Novartis) | PDR001 | I/II | Advanced solid tumors | NCT02460224 | Active, not recruiting |
| | | PDR001, NIR178, capmatinib, MCS110, canakinumab | I | TNBC | NCT03742349 | Recruiting |
| | | PDR001 | II | Advanced solid and hematologic malignancies | NCT03365791 | Active, not recruiting |
| | | PDR001, carboplatin | II | Advanced TNBC | NCT03499899 | Recruiting |
| | | PDR001, capmatinib, canakinumab, ribociclib | II | Advanced melanoma | NCT03484923 | Recruiting |
| | MK-4280 (Merck) | Pembrolizumab, Oxaliplatin, Irinotecan, | I | Advanced solid tumors | NCT02720068 | Recruiting |
| Target | Drugs (company) | Combination agents | Phase | Tumor types | Clinical Trial NO. | State |
|--------|----------------|-------------------|-------|-------------|--------------------|-------|
|        |                | Leucovorin, 5-FU, MK-4280A | I/II  | Hematological malignancies | NCT03598608 | Recruiting |
|        |                | Pembrolizumab, Lenvatinib, MK-1308 | II    | Advanced NSCLC | NCT03516981 | Recruiting |
|        | REGN3767 (Regeneron) | REGN2810 | I | Advanced Cancers | NCT03005782 | Recruiting |
|        | TSR-033 (Tesar) | Anti-PD-1 | I | Advanced solid tumors | NCT03250832 | Recruiting |
| Bi754111 (Bohringer Ingelheim) | Bi754091 | Early I | Neoplasms | NCT03433898 | Recruiting |
|        | Bi754091 | I | Advanced cancers | NCT03156114 | Recruiting |
|        | Bi754091 | I | Advanced NSCLC and HNSCC | NCT03780725 | Recruiting |
|        | Bi754091, Bi907828 | I | Advanced solid tumors. | NCT03697304 | Recruiting |
| Sym022 (Symphogen) | – | I | Advanced solid tumor or lymphomas | NCT03489369 | Recruiting |
|        | Sym021, Sym023 | I | Advanced solid tumor or lymphomas | NCT03311412 | Recruiting |
|        | FS118 <sup>a</sup> (F-star) | – | I | Advanced malignancies | NCT03440437 | Recruiting |
|        | MGD013 <sup>b</sup> (MacroGenics) | – | I | Advanced cancers | NCT03219268 | Recruiting |
| TIM-3 TSR-022 (Tesar) | TSR-042, TSR-033 | I | Advanced solid tumors | NCT02817633 | Recruiting |
|        | Niraparib, TSR-042, Bevacizumab, Platinum-Based chemotherapy | I | Advanced solid tumors | NCT03307785 | Recruiting |
|        | TSR-042 | II | Liver Cancer | NCT03680508 | Not yet recruiting |
|        | M8G453 (Novartis) | PDR001 | I/II | Advanced malignancies. | NCT02608268 | Recruiting |
|        | Decitabine, PDR001 | I | AML or high risk MDS | NCT03066648 | Recruiting |
|        | HD-M201, Venetoclax | I | AML or high risk MDS | NCT03940352 | Recruiting |
|        | Spartializumab | I | GBM | NCT03961971 | Not yet recruiting |
| Sym023 (Symphogen) | – | I | Advanced solid tumor or lymphomas | NCT03489343 | Recruiting |
|        | Sym021, Sym022 | I | Advanced solid tumor or lymphomas | NCT03311412 | Recruiting |
| INCAGN2390 (Incyte) | – | I | Advanced malignancies | NCT03652077 | Recruiting |
| L33213167 (Eli Lilly and Company) | LY3300054 | I | Advanced solid tumor | NCT03099109 | Recruiting |
|        | LY3300054, Ramucirumab, Abemaciclib, Merestinib | I | Advanced solid tumor | NCT02791334 | Recruiting |
|       | BMS-986258 (BMS) | Nivolumab, rHuPH20 | I/II | Advanced solid tumor | NCT03446040 | Recruiting |
|       | SHR-1702 (Jiangsu HengRui) | Camrelizumab | I | Advanced solid tumor | NCT03871855 | Not yet recruiting |
|       | RO7121661 <sup>c</sup> (Roche) | – | I | Advanced solid tumor | NCT03708328 | Recruiting |
| TIGIT | MK-7684 (Merck) | Pembrolizumab | I | Advanced solid tumor | NCT02964013 | Recruiting |
|       | Etigilimab /OMP-313 M32 (OncoMed) | Nivolumab | I | Advanced solid tumor | NCT03119428 | Active, not recruiting |
|       | Tiragolumab/MTIG7192A/RG-6058 (Genentech) | Atezolizumab | I | Advanced solid tumor | NCT02794571 | Active, not recruiting |
|       | Atezolizumab | II | Advanced NSCLC | NCT03563716 | Active, not recruiting |
|       | BMS-986207 (BMS) | Nivolumab | I/II | Advanced solid tumor | NCT02913313 | Recruiting |
Mature LAG-3 protein includes four parts, hydrophobic leader, extracellular region, transmembrane region, and cytoplasmic region (Fig. 1). The extracellular region is consisted of four immunoglobulin (Ig) superfamily-like domains (D1-D4) [42]. The membrane-distal D1 domain contains a unique short amino acid sequence, the so-called “extra loop” [43]. The cytoplasmic domain of LAG-3 has three conserved regions: a serine-phosphorylation site, a KIEELE motif, and a glutamic acid-proline repeats, of which the KIEELE motif is essential for LAG-3 to exert inhibitory function [44]. Metalloproteases can cleave LAG-3 within the connecting peptide between the D4 transmembrane domain and the transmembrane domain, generating a soluble LAG-3 (sLAG-3) [45]. Some researches demonstrated that sLAG-3 could limit the magnitude of the T cell immune responses [46]. LAG-3 is usually expressed on activated CD4+ and CD8+ T cells [41], Tregs [47], a subpopulation natural killer (NK) cells [48], B cells [49], plasmacytoid dendritic cells (pDCs) as well [50]. Ample of evidence have indicated that LAG-3 signaling play a negative regulatory role in T helper 1 (Th1) cell activation, proliferation and cytokine secretion [51–53]. During tumorigenesis and cancer progression, tumor cells exploit this pathway to escape from immune surveillance.

Based on the structural similarity between LAG-3 and CD4, MHC-II is reasonable considered as ligand for LAG-3. In fact, the binding affinity between LAG-3 and MHC-II is 100-fold higher than CD4 [20]. Now, MHC-II, galectin-3 [55], LSECtin [56], and α-synuclein [57] have been described to interact with LAG-3, with the MHC-II as a canonical ligand (Fig. 2). More recently, Wang and his co-workers elucidated that fibrinogen-like protein 1 (FGL1) was a novel high-affinity ligand for LAG-3 independent from MHC-II [58].

### Table 1
The clinical trials of novel immune checkpoint inhibitors in cancer immunotherapy (Continued)

| Target | Drugs (company) | Combination agents | Phase | Tumor types | Clinical Trial NO. | State          |
|--------|----------------|--------------------|--------|-------------|--------------------|----------------|
| AB-154 (Arcus Biosciences) | AB122 | I | Advanced malignancies | NCT03628677 | Recruiting |
| ASP-8374 (Potenza) | Pembrolizumab | – | Advanced solid tumors | NCT03260322 | Recruiting |
| VISTA | JNJ-61610588 (Johnson & Johnson) | – | Advanced solid tumor | NCT02671955 | Terminated |
| CA-1702 (Curis) | – | I | Advanced solid tumors and lymphomas | NCT02812875 | Active, not recruiting |
| B7-H3 | Enoblituzumab/MGA271 (MacroGenics) | Ipilimumab | I | Advanced solid tumors | NCT02381314 | Completed |
| | | Pembrolizumab | I | Advanced solid tumors | NCT02475213 | Active, not recruiting |
| | | – | I | Children with B7-H3-expressing solid tumors | NCT02982941 | Completed |
| | | – | II | Prostate cancer | NCT02923180 | Recruiting |
| | | MGA012 | I | Advanced solid tumors | NCT03406949 | Recruiting |
| | | – | I | B7-H3-expressing tumors | NCT02628535 | Recruiting |
| | | – | I | DSRCT | NCT01099644 | Recruiting |
| | | – | I | Advanced CNS or leptomeningeal cancer | NCT00089245 | Recruiting |
| | | – | II/III | Neuroblastoma central nervous system/leptomeningeal metastases | NCT03275402 | Recruiting |
| | 124I-8H9 /omburtamab (Y-mAbs) | – | I | Gliomas | NCT01502917 | Recruiting |

**Abbreviation:** *, a bispecific anti-LAG-3/PD-L1 antagonistic mAb; †, a bispecific anti-LAG-3/PD-1 antagonistic mAb; ‡, a bispecific anti-TIM-3/PD-1 antagonistic mAb; §, an oral inhibitor targeted PD-L1 and VISTA; ¶, a bispecific mAb designed to bind CD3 on T cells and B7-H3 on tumor; BMS Bristol-Myers Squibb, RCC Renal cell carcinoma, MBC Metastatic breast cancer, NSCLC Non-small cell lung cancer, HNSCC Squamous cell carcinoma of the head and neck, CRC Colorectal cancer, TMB Triple Negative Breast Cancer, AML Acute Myeloid Leukemia, MDS Myelodysplastic, MSS Microsatellite stable, MSH Microsatellite instability high, GC Gastric Cancer, DSRCT Desmoplastic Small Round Cell Tumors, CNS Central nervous system, GBM Glioblastoma multiforme
Clinical trials on LAG-3

Based on the experimental results that administration of recombinant sLAG-3 molecule with irradiated tumor cells can dampen the growth of established tumors, early clinical work centered on developing a sLAG-3-Ig [59]. IMP321 (Eftilagimod alpha) was initially developed as a vaccine immunological adjuvant [60]. It is a soluble recombinant protein by fusing the four extracellular Ig domains of LAG-3 to the Fc portion of human IgG1 [61]. The first-in-man phase I trial was conducted in patients with metastatic renal cell carcinoma (mRCC) (NCT00351949) [62]. The results showed that adverse events (AEs) related to IMP321 were minimal and IMP321 increased the subset of circulating activated CD8+ T cells which was correlated to tumor growth reduction. Although no objective response was observed in this trial, 7 of 8 patients experienced stable disease in higher IMP321 dose group (> 6 mg) while only 3 of 11 in the lower dose group [62]. IMP321 monotherapy showed a modest efficacy in cancer therapy, thus it was rational to combine the agent with other chemotherapeutic drugs. Subsequently, another two clinical trials were lunched in metastatic breast cancer (MBC) (NCT00351949) [63] and advanced pancreatic cancer (NCT00732082) [64]. Ultimately, the former phase I/II trial showed a 50% objective response rate (ORR) at the end of treatment point, which compared favorably to a historical response rate of approximately 25% [63]. Furthermore, the authors found an absolute and proportional increase in MHC class II-expressing APCs, NK cells and CD8+ T cell populations and these subsets were known to connect with antitumor activity [63]. This encouraging result has prompted a further phase Ib multicenter clinical trial that is currently recruiting patients with MBC (NCT02614833) [65]. Unfortunately, the later phase I clinical trial intended to assess the role of IMP321 and gemcitabine as a front-line therapy in patients with pancreatic cancer showed no meaningful objective response [64]. The role of IMP321 in combination with other immunotherapies (e.g., anti-PD-1 mAb, NCT03625323) or as an adjuvant for cancer vaccines are being explored (NCT00324623, NCT01308294) [66, 67].

Relatlimab, also named BMS-986016, is the first commercially mAb directed against LAG-3 [68]. Many pre-clinical mouse models have showed that PD-1/PD-L1 blockade upregulated LAG-3 or other immune checkpoints as a compensatory mechanism [37, 38, 69]. These data evoked the further exploration of combination therapy strategies. For example, the first phase I clinical trial about Relatlimab was opened in 2013 (NCT01968109) [38]. The aim of this clinical trial was to evaluate the efficacy of Relatlimab as a monotherapy or in combination with Nivolumab (anti-PD-1 antibody) in patients with various advanced malignancies including melanoma, NSCLC, and RCC [20]. At ESMO 2017, researcher announced the updated efficacy and safety results in a cohort of 68 melanoma patients who had received prior immunotherapy [70]. The ORR was 11.5% in 61 patients who were able to assess efficacy, including one patient achieved complete response and 6 were partial response (PR). Noticeably, the ORR was higher in patients with the expression of LAG-3 ≥ 1% and the AEs were tolerable [70]. As of July 15, 2019, at least 18 clinical trials on

| Receptor | LAG-3 | TIM-3 | TIGIT | VISTA | B7-H3 | BTLA |
|----------|-------|-------|-------|-------|-------|------|
| Alternate name | CD223 | HAVCR2 |       |       |       |      |
| Chromosomal location | 12p13.32 | 5q33.2 | 3q13.31 | 10q22.1 | 15q24.1 | 3q13.2 |
| Function of ligand-receptor interaction | Co-inhibition | Co-inhibition | Co-inhibition | Co-inhibition | Co-inhibition | Co-inhibition |
| Binding Partner | MHC-II, galectin-3, LSECtin, a-synuclein, FGL1 | Galectin-9, Ceacam-1, HMGB-1, PtdSer | CD155, CD112 | Vsig3 | Unknown | HVEM |
| Number of amino acids | 498 amino acids | 302 amino acids | 244 amino acids | 311 amino acids | 316 amino acids | 289 amino acids |
| Signaling motif | KIEELE motif | Tyrosine residues | ITT and ITIM | Unknown | Unknown | ITIM and ITSM |
| Receptor Expression | Activated T cells, B cells, Tregs, NK cells, DCs | Activated T cells, B cells, Tregs, DCs, NK cells, monocytes | T cells, NK cells | Myeloid cells, T cells | Activated T cells, NK cells, DCs, monocytes, tumor tissue | Mature B cells, T cells, Tregs, macrophages, DCs |

Table 2 Comparison of coinhibitory immune checkpoint receptors mentioned in manuscript

Based on the experimental results that administration of recombinant sLAG-3 molecule with irradiated tumor cells can dampen the growth of established tumors, early clinical work centered on developing a sLAG-3-Ig [59]. IMP321 (Eftilagimod alpha) was initially developed as a vaccine immunological adjuvant [60]. It is a soluble recombinant protein by fusing the four extracellular Ig domains of LAG-3 to the Fc portion of human IgG1 [61]. The first-in-man phase I trial was conducted in patients with metastatic renal cell carcinoma (mRCC) (NCT00351949) [62]. The results showed that adverse events (AEs) related to IMP321 were minimal and IMP321 increased the subset of circulating activated CD8+ T cells which was correlated to tumor growth reduction. Although no objective response was observed in this trial, 7 of 8 patients experienced stable disease in higher IMP321 dose group (> 6 mg) while only 3 of 11 in the lower dose group [62]. IMP321 monotherapy showed a modest efficacy in cancer therapy, thus it was rational to combine the agent with other chemotherapeutic drugs. Subsequently, another two clinical trials were lunched in metastatic breast cancer (MBC) (NCT00351949) [63] and advanced pancreatic cancer (NCT00732082) [64]. Ultimately, the former phase I/II trial showed a 50% objective response rate (ORR) at the end of treatment point, which compared favorably to a historical response rate of approximately 25% [63]. Furthermore, the authors found an absolute and proportional increase in MHC class II-expressing APCs, NK cells and CD8+ T cell populations and these subsets were known to connect with antitumor activity [63]. This encouraging result has prompted a further phase Ib multicenter clinical trial that is currently recruiting patients with MBC (NCT02614833) [65]. Unfortunately, the later phase I clinical trial intended to assess the role of IMP321 and gemcitabine as a front-line therapy in patients with pancreatic cancer showed no meaningful objective response [64]. The role of IMP321 in combination with other immunotherapies (e.g., anti-PD-1 mAb, NCT03625323) or as an adjuvant for cancer vaccines are being explored (NCT00324623, NCT01308294) [66, 67].

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Relatlimab had been registered on the ClinicalTrials.gov. All of them were phase I or II but none of them are completed.

LAG525 is another humanized anti-LAG-3 mAb. It is a high-affinity IgG4 antibody which blocks the binding of MHC-II to LAG-3. Currently, LAG525 is undergoing a series of phase I or II testing in combination with anti-PD-1 antibody for patients with advanced cancers. For example, LAG525’s first clinical trial was launched in 2015 (NCT02460224). It was a phase I trial to determine the efficacy and safety of LAG525 plus PDR001 (anti-PD-1 mAb) in advanced malignancies [36]. Other LAG-3 inhibitory antibodies MK-4280, REGN3767 [71], TSR-033 [72], BI754111 [73], and Sym022 [74] have also been investigated at various stages of clinical development. To capitalize on synergistic effects of co-blockade PD-1 and LAG-3 pathways in preclinical models [75], some bispecific anti-LAG-3/PD-(L)1 antagonistic mAbs have also been developed, such as FS118 [76] and MGD013 [77]. To date, at least 10 kinds of LAG-3 blockade agents have been developed and studied in clinical trials, yet their results are not available now.

T cell immunoglobulin and mucin-domain containing-3 (TIM-3)

The biology of TIM-3

TIM-3, also called hepatitis A virus cellular receptor 2 (HAVCR2), presents several unique features making it another intriguing immune checkpoint [78]. It was first identified as a protein selectively expressed on CD4+ Th1 and CD8+ T cytotoxic 1 (Tc1) cells as early as 2002 [21]. But now it is commonly classified as immune checkpoint molecule similar to CTLA-4 and PD-1. The genomic analysis shows that the TIM gene family is composed of three genes, namely TIM-1, TIM-3, TIM-4, located on human chromosome 5q33.2 [79]. Human TIM-3 protein
comprises of 302 amino acids, while mouse homolog includes 281 amino acids residues with 63% identity to human TIM-3 [21]. It belongs to Ig superfamily (IgSF) with an N-terminal Ig variable region (IgV)-like domain, a membrane-proximal mucin-like domain containing sites for O-linked glycosylation (glycosylated mucin domain), a single transmembrane region and a C-terminal cytoplasmic tail. There are also sites for N-linked glycosylation between the mucin and transmembrane [79]. The TIM-3 cytoplasmic tail does not have the classical inhibitory signaling motif, like immune receptor tyrosine based inhibitory motif (ITIM) or immune receptor tyrosine-based switch motif (ITSM), but contains five conserved tyrosine residues, two of which (Y265 and 272) can be phosphorylated by Src kinases or interleukin inducible T cell kinase and are crucial for downstream signaling [35, 80]. The expression of TIM-3 was not limited on T cell, it was known to express on different types of immune cells, including B cells, Tregs, NK cells, DCs, monocytes, and macrophages [81]. Lately, the expression of TIM-3 has been identified in leukemic stem cells and tumor-associated endothelium [82, 83].

Hitherto, four distinct ligands have been reported to bind to the IgV domain of TIM-3, including galectin-9, high-mobility group protein B1 (HMGB1), carcinoembryonic antigen cell adhesion molecule 1 (Ceacam-1), and phosphatidyl serine (PtdSer) [84]. It is noteworthy that galectin-9 and HMGB1 are soluble ligands, while Ceacam-1 and PtdSer belong to surface ligands. The engagement of TIM-3 with galectin-9 triggered intracellular calcium flux of Th1 cells, inducing cell death [85]. Furthermore, a study by Kang et al. showed that galectin-9 also induced apoptosis of TIM-3 +CD8+T cell in colon cancer [86]. The interaction between HMGB1 and TIM-3 mainly had an impact on innate immune response. In tumor, TIM-3 was highly expressed on tumor infiltrating DCs and can compete with nucleic acid binding to HMGB1, therefore dampening anti-tumor immunity mediated by nucleic acids [87]. Ceacam-1 was a molecule involved in T cell inhibition. Huang and his colleague elucidated that TIM-3 and Ceacam-1 can form a heterodimer in both cis and trans which acts as a negative regulator of T cell responses [88]. The interaction of PtdSer with TIM-3 has been showed to connect with the clearance of apoptotic bodies and also improve the antigen cross-presentation [89]. More importantly, higher expression of TIM-3 was associated with a poor prognosis in solid malignant [90] and accumulating preclinical
models have verified the therapeutic benefit of TIM-3 blockade by regulating TME and restricting tumor growth especially in combination with PD-1 blockade [91].

Clinical trials on TIM-3
To date, at least eight TIM-3 antagonistic mAbs have been registered on ClinicalTrials.gov. TSR-022 (Cobolimab), a novel IgG4 anti-TIM-3 mAb developed by Tesaro entered the first phase I clinical trial in 2016 (NCT02817633) [92]. This multicenter, open-label study intended to evaluate the safety and efficacy of TSR-022 as a monotherapy or in combination with TSR-042 (anti-PD-1 mAb) in patients with advanced solid tumor. The results have been released in 2018 Annual Meeting of the Society for Immunotherapy of Cancer (SITC) Conference [93]. Clinical benefits have been observed in the combination group, especially at a high dose of TSR-022 (300 mg) with a 15% ORR (3/20) and 40% stable disease (8/20) [93]. Another two clinical trials including TSR-022 are still recruiting patients with no clinical results available (NCT03307785, NCT03680508). MBG453 is another anti-TIM-3 mAb produced by Novartis. Similar to TSR-022, the first clinical trial aimed to assess the safety and efficacy of MBG453 as single agent or in combination with PDR001 (an anti-PD-1 mAb) in advanced malignancies patients (NCT02608268). Another clinical trial was conducted in patients with acute myelocytic leukemia or high-risk myelodysplastic syndromes (NCT03066648). Sym023, is a recombinant, fully human antibody that bound TIM-3 [94]. A phase I trial evaluating the safety, tolerability, and dose-limiting toxicities of sym023 is recruiting at present (NCT03489343). Other TIM-3 inhibitors INCAGN2390, LY3321367, BMS-986258 and SHR1702 are also being tested in phase I trial alone (INCAGN02390 NCT03652077) or in combination with anti-PD-1/PD-L1 mAb (LY3321367 NCT03099109; BMS-986258 NCT03446040; SHR1702 NCT03871855) in the metastatic setting [84, 95]. RO7121661 is a bispecific antibody targeting PD-1 and TIM-3 simultaneously. It was developed by Roche and a phase I dose escalation and expansion study has been ongoing on advanced solid tumors (NCT03708328).

T cell immunoglobulin and ITIM domain (TIGIT)
The biology of TIGIT
TIGIT was first identified by Yu and his colleagues as an immune checkpoint rheostat that suppress the activation of T cells in 2009 [22]. Subsequently, it was described by other groups with each group giving a different name including WUCAM [96], Vstn3 [97], and Vsig9 [98]. TIGIT gene is located on human chromosome 3q13.31 and encodes a 244-amino acid transmembrane glycoprotein. The protein includes an extracellular IgV region, a transmembrane domain, and a cytoplasmic tail that harbors a canonical ITIM and an immunoglobulin tail tyrosine (ITT)-like phosphorylation motif [22]. The expression of TIGIT was demonstrated to be tightly restricted to lymphocytes, mainly on T cell subsets (including Tregs and memory T cells) and NK cells [22, 99]. TIGIT binds two ligands, namely CD155 (PVR or Nectin-5) and CD112 (nectin-2, also known as PRR2 or PVRL2) with different affinity. Whether nectin-3 is another ligand for TIGIT is still in question [99]. TIGIT exerts its immunosuppressive effects by competing with other counterparts, CD266 (DNAM-1) or CD96 [100]. CD226 delivered a positive co-stimulatory signal, while TIGIT delivered inhibitory signals. This group of interacting proteins formed a co-stimulatory axis that are similar to the CTLA-4/B7/CD28 pathway [101].

As TIGIT was initially identified by a genomic search for structures shared a conserved ITIM motif, its immunosuppressive effects were delineated as expected. The initial research believed that TIGIT suppressed T cell activation in an indirect way. Specifically, the engagement of TIGIT with CD155 on DCs induced phosphorylation of CD155 and Erk, increased the secretion of IL-10, thus inhibiting T cell responses indirectly [22]. Subsequent studies demonstrated that TIGIT could also directly suppress T cell function by competing with CD226 [97, 102]. The role of TIGIT molecule in NK cells has been well studied. Stanietzky et al. indicated that ligation of TIGIT could lead to the inhibition of NK cells cytotoxicity through its cytoplasmic ITIM domain both in human and mouse [99, 103]. Furthermore, the major role of ITT-like motif in negatively modulating NK cells has been proved by two independent studies [104, 105]. Work from the Kurtulus group showed that the expression of TIGIT on Tregs was critically involved in Treg suppressive function [106]. Interestingly, Gur and his co-workers discovered that TIGIT could directly bind to the Fap2 protein derived from Fusobacterium nucleatum, triggering a negative signal to suppress the activities of NK cells and T cells, and hence mediating a tumor-immune evasion mechanism [107]. Many groups generated agonistic anti-TIGIT mAb to verify the effect of TIGIT, indeed, they consistently reported a direct inhibitory effect on T cell proliferation [97, 102, 108]. Recently, some groups have reported that co-blockade of TIGIT with other checkpoint receptors, such as anti-PD-1 and TIM-3, can exert synergistic effects in regulating antitumor responses [106, 109, 110].

Clinical trials on TIGIT
Based on the promising preclinical results, targeting TIGIT as a strategy for cancer treatment attracts the attention of many pharmaceutical companies, especially combined with anti-PD-1/PD-L1 mAb. There are at least six major agents targeting TIGIT now, focusing on three
products. MK-7684, a candidate anti-TIGIT drug developed by Merck entered into a phase I clinical trial to analysis the safety, efficacy, and pharmacokinetics of MK-7684 as monotherapy and in combination with pembrolizumab in metastatic solid tumors (NCT02964013) [111]. The early phase I data was announced at the SITC’s 3rd Annual Meeting in 2018. Sixty eight individuals were enrolled with 34 patients in the monotherapy and 34 patients in combination arms. Finally, one PR and eight PR were observed in these two groups, and the disease control rates were 35 and 47%, respectively [112]. Etigilimab (OMP-313 M32) is a humanized mAb that developed to block TIGIT from binding CD155. It was developed by OncoMed/Celgene and entered the first phase I clinical trial in April 2017 (NCT03119428). This open-label research was designed to evaluate the safety and tolerability of Etigilimab as a single agent or in combination with an anti-PD-1 mAb in patients with advanced malignancies. At the 2017 American Association for Cancer Research Annual Meeting, OncoMed presented some positive results from several preclinical trials, thus its clinical performance was worth pursuing [111]. Another anti-TIGIT candidate drugs made by Genentech was named Tiragolumab (MTIG7192A, RG-6058). It’s also a fully human mAb designed to engage to TIGIT and hinder its interaction with CD155. There were two clinical trials about Tiragolumab registered on ClinicalTrials.gov (NCT02794571, NCT03563716). Other drugs, such as BMS-968207 made by Bristol-Myers Squibb, AB-154 made by Arcus biosciences and ASP-8374 made by Potenza also have initiated their phase I clinical trial with no clinical results reported [111].

VISTA, B7-H3, BTLA, and Siglec-15

Apart from these three new immune checkpoints mentioned above, many other immune checkpoint co-inhibitors are also attractive targets, with a few drugs step into clinical trials. VISTA, is also known as PD-1 homolog (PD-1H), DD1α, Gi24, differentiation of embryonic stem cells 1 (Dies1), and B7-H5 [113]. It was first described as an IgSF ligand which can negatively regulate T cells, and a proline rich motif that may function as docking sites, suggesting that VISTA has the potential function as both a receptor and a ligand [115]. Up to now the counter structures for VISTA has not been well identified [116], and VSIG-3 was reported as a novel ligand for VISTA a short time ago [117]. VISTA was highly expressed on myeloid cells and a lesser extent on T cells, but not on tumor cells within the TME [118]. The preclinical studies on multiple murine models showed that VISTA blockade improved the infiltration, proliferation, and effector function of tumor-infiltrating T cells within the TME, thus altered the suppressive character of the TME [118]. JNJ-61610588 is a fully human IgG1 anti-VISTA mAb made by Johnson & Johnson. The phase I clinical trial was intended to evaluate safety and pharmacokinetics of JNJ-61610588 in patients with advanced cancers (NCT02671955) [119]. Another candidate, CA-170, is an oral inhibitor which can selectively target both PD-L1 and VISTA. The results from preclinical models showed remarkable antitumor effects with well-tolerance and the phase I clinical trial in patients with advanced solid tumor and lymphomas is currently recruiting (NCT02812875) [119]. Based on the newest result published by Blando et al., VISTA was regard as a promising target for patients with pancreatic cancer [120] and the HMBD-002, a novel anti-VISTA antibody developed by Hummingbird Bioscience, have received a financial assistance from Cancer Prevention and Research Institute of Texas (CPRIT) with the plan to initiate clinical trials in 2020.

B7-H3, also named CD276, is a type I transmembrane glycoprotein that is encoded on human chromosome 15 [24]. It was discovered as early as 2001 [24]. The initial study described it as a positive co-stimulator for it can stimulate the T cell response and IFN-γ production [24]. But recently studies reported that B7-H3 was involved in the inhibition of T cells [121, 122]. The receptor for B7-H3 has not yet been identified and it may explain the intricate immunomodulatory activity of B7-H3 for it may have more than one binding partner with distinct function [123]. The expression of B7-H3 protein can be detected on activated immune cells such as T cells, NK cells, and APCs. More importantly, it was overexpressed on a wide spectrum of tumor tissue and linked to disease states and prognosis [124]. Recently, Enoblituzumab (MGA271), an engineered Fc humanized IgG1 mAb against B7-H3, has been developed and brought to clinical trials [125]. Among the five clinical trials about Enoblituzumab, one of which had been completed but did not reported the final results. Another agents MGD009, is a bispecific mAb designed to bind both CD3 on T cells and B7-H3 on tumor cells [36]. It is being studied on two phase I clinical studies in patients with B7-H3 expression (NCT02628535, NCT03406949) [36]. Furthermore, 8H9 (omburtamab) is an antibody specific to B7-H3 [126]. It has showed a positive clinical efficacy as an antibody drug conjugate after it was labeled with
radioactive iodine-131 ($^{131}$I) and administrated to patients with metastatic central nervous system (CNS) neuroblastoma [127]. Currently, clinical trials with radiolabeled 8H9 have been evaluated on peritoneal cancers, gliomas, and CNS (NCT01099644, NCT01502917, NCT00089245 et al.) The newest result of NCT01502917 supported the further study in expanded cohort [128].

BTLA (CD272) is identified as another inhibitory receptor that belongs to CD28 superfamily [113]. It is located on human chromosome 3 in q13.2 and encodes a 289-amino acid type I glycosylated transmembrane protein [25]. Similar to PD-1 and CTLA-4, the protein structure of BTLA includes a single extracellular region, a transmembrane domain and cytoplasmic domain. The ITIM and ITSM within the cytoplasmic tail mediate a negative signaling to T cells by recruiting the SHP-1 and SHP-2 [129]. BTLA was expressed on mature lymphocytes (such as B cells, T cells, and Tregs), macrophages, and mature bone marrow-derived DCs [130]. Herpesvirus entry mediator (HVEM), a member of the tumor necrosis factor receptor superfamily (TNFRSF), was identified as the unique BTLA ligand in 2005 [131]. But BTLA was not the unique binding partner for HVEM, it competed with other two TNF family members, LIGHT and lymphotixin-α, as well as IgSF member CD160 for binding to HVEM [132]. CD160 is another negative regulator of T cell while LIGHT is a costimulatory molecule [133, 134]. The ligation of BTLA with HVEM triggered the inhibition of T cell proliferation and cytokine production [131]. At present, there are no clinical trials opened for BTLA. But, in the past April, Junshi Biosciences announced that the world’s first anti-BTLA mAb, TAB004/JS004, have been approved for clinical trial by FDA [135].

A recent publication reported a new immune suppressor, sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) [136]. The team of Dr. Lieping Chen elegantly demonstrated the expression of Siglect-15 (mainly on cancer cells, macrophages, and myeloid cells) and the inhibitory role of Siglect-15 in regulation of T cell responses. More importantly, they revealed that both genetic ablation and antagonize antibody of Siglec-15 suppressed the growth of tumor in murine models [136]. Right now, a clinical trial lead by Chen’s group is recruiting to test the efficacy of NC318 (an anti-Siglec-15 mAb) in solid tumors (NCT03665285) [136, 137].

**Conclusion**

The success of CTLA-4 or PD-1/PD-L1 blockade catalyzed the enthusiasm for a new class of antibody that block negative immune checkpoint regulators for cancer therapy. The past two decades have witnessed the significant progress in identifying alternative targets and developing novel specific agents in treating cancer. As we described above, considerable immune checkpoints have been explored with some been chosen as novel therapeutic targets by pharmaceutical companies. Although the number of clinical trials about these emerging immune modulators, such as anti-LAG-3 antibody and anti-TIM-3 antibody, registered on ClinicalTrial.gov has grown exponentially, no drugs entered the clinic up to date. There are still some puzzles to be solved, like identification of ligand for VISTA and B7-H3 which may be the key to fully understand their therapeutic potential. In addition, while the results of monotherapy treatments are compelling, more attempts should be made to design rational combinations of immune-therapeutics that target non-redundant pathways to achieve synergistic effects in inhibiting tumor growth. We are still in the early stage of understanding these new immune systems with the aim that more candidates’ agents can transform to clinical fields and achieve even greater success than that initially observed with CTLA-4 or PD-1 blockade.

**Abbreviations**

AEs: Adverse events; B7-H3: B7 homolog 3 protein; BTLA: B and T cell lymphocyte attenuator; Ceacam-1: Carcinoembryonic antigen cell adhesion molecule 1; CNS: Central nervous system; CTLA-4: Cytotoxic T lymphocyte-associated antigen-4; DC: Dendritic cells; FDA: United States Food and Drug Administration; HVG-B1: High-mobility group protein B1; HVEM: Herpesvirus entry mediator; ICi: Immune checkpoint inhibitor; IgSF: Ig superfamily; IgV: Ig variable region; ITIM: Immune receptor tyrosine based inhibitory motif; ITSM: Immune receptor tyrosine-based switch motif; ITT: Immunoglobulin tail tyrosine; LAG-3: Lymphocyte activation gene-3; mAb: Monoclonal antibody; MBC: Metastatic breast cancer; MHC: Major histocompatibility complex; NK: Natural killer; NSCLC: Non-small cell lung cancer; ORR: Objective response rate; PD-1: Programmed cell death protein-1; PD-L1: Programmed cell death ligand 1; PR: Partial response; PdtSer: Phosphatidyl serine; RCC: Renal cell carcinoma; SHP1/2: Src homology 2 domain containing phosphatases 1/2; Siglec-15: Sialic acid-binding immunoglobulin-like lectin 15; SITC: Society for Immunotherapy of Cancer; TCR: T cell receptor; TIGIT: T cell immunoglobulin and mucin-domain containing-3; TME: Tumor microenvironment; Tregs: Regulatory T cells; VISTA: V-domain Ig suppressor of T cell activation

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**Authors’ contributions**

SQ performed the selection of literature, drafted the manuscript, and prepared the Figs. LX, MY, and SY helped to collect literatures, participate in the discussion and revise the manuscript. SL and KW carried out the design of this review and revised the manuscript. All authors contributed to this manuscript. All authors read and approved the final manuscript.

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References
1. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. Mol Cancer. 2018;17:129.
2. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Chimeric antigen receptor T cells: a novel therapy for solid tumors. J Hematol Oncol. 2017;10:278.
3. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Recent advances of bispecific antibodies in solid tumors. J Hematol Oncol. 2017;10:155.
4. Sharpe AH. Mechanisms of costimulation. Immunol Rev. 2009;229:5–47.
5. Wang J, Yuan R, Song W, Sun J, Liu D, Li Z. PD-1, PD-L1 (B7-H1) and tumor-site immune modulation therapy: the historical perspective. J Hematol Oncol. 2017;10:34.
6. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182:459–65.
7. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol. 2004;4:436–47.
8. Leach D, Krummel M, Allison J. Enhancement of antitumor immunity by CTLA-4 blockade. Science (New York, NY). 1996;271:1734–6.
9. Yi J, Yuan R, Song W, Sun J, Liu Q, Xu H, Zhao W, et al. Gut microbiome modulates efficacy of immune checkpoint inhibitors. J Hematol Oncol. 2018;11:47.
10. Cameron F, Whiteside G, Perry C. Ipilimumab: first global approval. Drugs. 2011;71:1093–104.
11. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol. 2007;19:813–24.
12. Zhu B, Tang L, Chen S, Yin C, Peng S, Li X, et al. Targeting the upstream surface protein TIGIT suppresses T cell activation by promoting the specific cell surface protein Tim-3 regulates macrophage activation and signal transduction. Immunol Rev. 2009;229:12–26.
13. Sharma P, Wagner K, Wolchok JD, Allison JP. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. Nat Rev Cancer. 2011;11:805–12.
14. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.
15. Boosiosis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. N Engl J Med. 2016;375:1767–78.
16. Toor SM, Saidifarhan Nair V, Decock J, Elkord E. Immune checkpoints in the tumor microenvironment. Semin Cancer Biol. 2019. https://doi.org/10.1016/j.semcancer.2019.06.021.
17. DAS M, ZHU C, KUCHCOO VK. TIM-3 and its role in regulating anti-tumor immunity. Immunol Rev. 2017;276:67–91.
18. Marin-Acevedo JA, Dholaria B, Soyaner AE, Knutson KL, Chumski S, LOU Y. Next generation of immune checkpoint therapy in cancer: new developments and challenges. J Hematol Oncol. 2018;11:39.
19. Huang RY, Eppolito C, Lele S, Shinkat P, Matsuzaki J, Odusoni K. LAG3 and CD1 co-inhibitory molecules collaborate to limit CD8+ T cell signaling and dampen antitumor immunity in a murine ovarian cancer model. Oncotarget. 2015;6:27359–77.
20. Huang RY, Francois A, McGray AR, Milotito A, Odusoni K. Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. Oncol Immunol. 2017;6:1249561.
21. Koyama A, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun. 2016;7:10501.
22. Fourcade J, sun z, Benalloua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med. 2010;207:2175–86.
23. Triebel F, Jitsukawa S, Baierova E, Roman-Roman S, Genevee C, Vegas-Pequignot E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. J Exp Med. 1999;190:139–405.
24. Huard B, Mastrangelo R, Prigent P, Bruniquel D, El-Tayar N, et al. Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. Proc Natl Acad Sci U S A. 1997;94:5744–9.
25. Goldberg MV, Drake CG. LAG-3 in cancer immunotherapy. Curr Top Microbiol Immunol. 2011;344:269–78.
26. Workman CJ, Vignali DA. The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. Eur J Immunol. 2003;33:970–9.
27. Triebel F, Jitsukawa S, Baierova E, Roman-Roman S, Genevee C, Vegas-Pequignot E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. J Exp Med. 1999;190:139–405.
28. Huard B, Triebel F, Vignali DA. LAG-3 does not define a specific mode of natural killing in human. Immunol Lett. 1998;61:109–12.
29. Kisielow M, Kisielow J, Capoferri-Sollami G, Karjalainen K. Expression of CD80, CD86, and HLA class II molecules on LAG-3 protein. Proc Natl Acad Sci U S A. 1997;94:5744–9.
30. Koyama A, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun. 2016;7:10501.
31. Fourcade J, sun z, Benalloua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med. 2010;207:2175–86.
32. Triebel F, Jitsukawa S, Baierova E, Roman-Roman S, Genevee C, Vegas-Pequignot E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. J Exp Med. 1999;190:139–405.
33. Huard B, Mastrangelo R, Prigent P, Bruniquel D, El-Tayar N, et al. Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. Proc Natl Acad Sci U S A. 1997;94:5744–9.
34. Goldberg MV, Drake CG. LAG-3 in cancer immunotherapy. Curr Top Microbiol Immunol. 2011;344:269–78.
35. Workman CJ, Vignali DA. The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. Eur J Immunol. 2003;33:970–9.
36. Huard B, Tournier M, Triebel F. LAG-3 does not define a specific mode of natural killing in human. Immunol Lett. 1998;61:109–12.
37. Kisielow M, Kisielow J, Capoferri-Sollami G, Karjalainen K. Expression of lymphocyte activation gene 3 (LAG-3) on B cells is induced by T cells. Eur J Immunol. 2005;35:2081–9.
38. Andreade S, Piras F, Bordin N, Triebel F. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). J Immunol. 2002;168:3874–80.
39. Huard B, Prigent P, Tournier M, Bruniquel D, Triebel F. CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. Eur J Immunol. 1995;25:2718–21.
52. Macon-Lemaire T, Triebel F. The negative regulatory function of the lymphocyte-activation gene-3 co-receptor (CD223) on human T cells. Immunology. 2005;115:170–8.

53. Workman CJ, Dugger KJ, Vignali DA. Cutting edge: molecular analysis of the negative regulatory function of lymphocyte activation gene-3. J Immunol. 2002;169:592–5.

54. Baieras E, Huard B, Miossec C, Jitsukawa S, Martin M, Hercend T, et al. Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. J Exp Med. 1992;173:327–37.

55. Kouo T, Huang L, Pucek AB, Cao M, Soft S, Armstrong T, et al. Galectin-3 shapes antitumor immune responses by suppressing CD8+ T cells via LAG-3 and inhibiting expansion of Plasmacytoid dendritic cells. Cancer Immunol Res. 2015;3:412–23.

56. Xu F, Liu J, Liu D, Liu B, Wang M, Hu Z, et al. LECCTin expressed on melanoma cells promotes tumor progression by inhibiting antitumor T-cell responses. Cancer Res. 2014;74:3418–28.

57. Mao X, Ou MT, Karuppugounder SS, Kann T, Yin X, Xiong Y, et al. Pathological alpha-synuclein transmission initiated by binding lymphocyte-activation gene-3. Science (New York, NY). 2016;353:aa33374.

58. Wang J, Sannamed MF, Datar I, Su TT, Li L, Sun J, et al. Fibrinogen-like protein 1 is a major immune inhibitory ligand of LAG-3. Cell. 2015;167:334–347.e2.

59. Pignet P, El Mir S, Deano M, Triebel F. Lymphocyte activation gene-3 induces tumor regression and antitumor immune responses. Eur J Immunol. 1999;29:8962–70.

60. Fougery S, Brignone C, Triebel F. A soluble LAG-3 protein as an immunomodulator for therapeutic vaccines: preclinical evaluation of IMP321. Vaccine. 2006;24:5426–33.

61. Huard B, Pignet P, Pages F, Brunquel D, Triebel F. T-cell major histocompatibility complex class II molecules down-regulate CD4+ T cell clone responses following LAG-3 binding. Eur J Immunol. 1996;26:1180–6.

62. Brignone C, Escudier B, Grygar C, Marcu M, Triebel F. A phase I trial of IMP321 in patients with advanced renal cell carcinoma. Clin Cancer Res. 2009;15:6225–31.

63. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, Cvitkovic F, et al. First-line chemotherapy plus IMP321 (LAG-3Ig) added to weekly paclitaxel in patients with metastatic breast cancer. Future Oncol. 2019;15:1963–76.

64. Wang-Gillam A, Plambeck-Suess S, Goedegebuure P, Simon PO, Mitchem JB, et al. Prospective separation of normal and leukemic stem cells based on differential expression of TIM-3, a human acute myeloid leukemia stem cell marker. Proc Natl Acad Sci U S A. 2011;108:5009–14.

65. Huang X, Bai X, Cao Y, Wu J, Huang M, Tang D, et al. Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. J Exp Med. 2010;207:505–20.

66. Liu F, Liu Y, Chen Z. Tim-3 expression and its role in hepatocellular carcinoma. J Hematol Oncol. 2018;11:126.

67. Zhu C, Anderson AC, Schubart A, Xiong H, Imotila J, Khoury SJ, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6:1245–52.

68. Kang CW, Dutta A, Chang LY, Mahalingam J, Lin YC, Chiang JM, et al. APo-1 ligand TIM-3 suppresses the autocrine growth of melanoma cells. Sci Rep. 2015;5:15659.

69. Chiba S, Baghdadi M, Akiba H, Yoshihama H, Kinoshita I, Dosaka-Akita H, et al. TIM-3 ligand galectin-9 negatively regulates T helper type 1 immunity. J Exp Med. 2010;207:505–20.

70. Liu F, Liu Y, Chen Z. Tim3 expression and its role in hepatocellular carcinoma. J Hematol Oncol. 2018;11:126.

71. La Motte-Mohs R, Shah K, Brown JG, Smith D, Gorlatov S, Ciccarone V, et al. Preclinical characterization of MGD013, a PD-1 x LAG-3 bispecific DART (R) molecule. J Immunother Cancer. 2017;5(Suppl 2):P337.

72. He Y, Cao J, Zhao C, Li X, Zhou C, Hirsch FR. TIM-3: a promising target for cancer immunotherapy. OncoTargets Ther. 2018;11:7005–9.

73. Meyers JH, Sabatos CA, Chakravarti S, Kuchoo VK. The TIM gene family regulates autoimmunity and allergic diseases. Trends Mol Med. 2005;11:362–9.

74. van de Weyer PS, Muehlfeit M, Klose C, Bonventre JV, Walz G, Kuehn EW. A highly conserved tyrosine of Tim-3 is phosphorylated upon stimulation by its galectin-9. Biochem Biophys Res Commun. 2006;335:571–76.

75. Anderson AC. Tim-3, a negative regulator of anti-tumor immunity. Curr Opin Immunol. 2012;24:213–6.

76. Jan M, Chao MP, Cha AC, Alizadeh AA, Gentles AJ, Weissman L, et al. Prospective separation of normal and leukemic stem cells based on differential expression of TIM-3, a human acute myeloid leukemia stem cell marker. Proc Natl Acad Sci U S A. 2011;108:5009–14.

77. Huang X, Bai X, Cao Y, Wu J, Huang M, Tang D, et al. Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. J Exp Med. 2010;207:505–20.

78. Liu F, Liu Y, Chen Z. Tim-3 expression and its role in hepatocellular carcinoma. J Hematol Oncol. 2018;11:126.

79. Zhu C, Anderson AC, Schubart A, Xiong H, Imotila J, Khoury SJ, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6:1245–52.

80. Kang CW, Dutta A, Chang LY, Mahalingam J, Lin YC, Chiang JM, et al. APo-1 ligand TIM-3 suppresses the autocrine growth of melanoma cells. Sci Rep. 2015;5:15659.

81. Chiba S, Baghdadi M, Akiba H, Yoshihama H, Kinoshita I, Dosaka-Akita H, et al. TIM-3 ligand galectin-9 negatively regulates T helper type 1 immunity. J Exp Med. 2010;207:505–20.

82. Liu F, Liu Y, Chen Z. Tim3 expression and its role in hepatocellular carcinoma. J Hematol Oncol. 2018;11:126.

83. Huang X, Bai X, Cao Y, Wu J, Huang M, Tang D, et al. Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. J Exp Med. 2010;207:505–20.

84. Liu F, Liu Y, Chen Z. Tim-3 expression and its role in hepatocellular carcinoma. J Hematol Oncol. 2018;11:126.

85. Zhu C, Anderson AC, Schubart A, Xiong H, Imotila J, Khoury SJ, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6:1245–52.

86. Kang CW, Dutta A, Chang LY, Mahalingam J, Lin YC, Chiang JM, et al. APo-1 ligand TIM-3 suppresses the autocrine growth of melanoma cells. Sci Rep. 2015;5:15659.
111. Solomon BL, Garrido-Laguna I. TIGIT: a novel immunotherapy target moving forward.

117. Wang JH, Wu GP, Manick B, Hernandez V, O’Connell S, et al. VISTA is an immune checkpoint receptor specific for the programmed death-1 homolog prevents graft-versus-host disease in mouse models. J Immunol. 2011;187:1537–42.

118. Le Mercier I, Chen W, Lines JL, Day M, Li J, Sergent P, et al. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. Nat Immunol. 2003;4:899–906.

119. Janevski M, Shah UA, Liu W, Zhao A, Schoenberg MP, Zang X. The third group of the B7-CD28 immune checkpoint family: HHLA2, TMIGD2, B7x, and B7-H3. Immunol Rev. 2017;276:26–39.

123. Kramer K, Kushner BH, Modak S, Pandit-Taskar N, Smith-Jones P, Zanuncio P, et al. Compartmental intrathelial radioimmunotherapy: results for treatment for metastatic CNS neuroblastoma. J Neurooncol. 2013;110:409–18.

124. Souweidane MM, Kramer K, Pandit-Taskar N, Zhou Z, Haque S, Zanuncio P, et al. Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-Centre, dose-escalation, phase 1 trial. Lancet Oncol. 2018;19:1040–50.

125. Gavrieli M, Watanabe N, Loftin SK, Murphy TL, Murphy KM. Characterization of phosphotyrosine binding motifs in the cytoplasmic domain of B and T lymphocyte attenuator required for association with protein tyrosine phosphatases SHP-1 and SHP-2. Biochem Biophys Res Commun. 2003;312:1236–43.

126. Ahmed M, Cheng M, Zhao Q, Goldgur Y, Cheal SM, Guo HF, et al. Humanized affinity-matured monoclonal antibody B8H9 has potent antitumor activity and binds to FG loop of tumor antigen B7-H3. J Biol Chem. 2015;290:3018–29.

127. Kramer K, Kushner BH, Modak S, Pandit-Taskar N, Zhou Z, Haque S, Zanuncio P, et al. Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-Centre, dose-escalation, phase 1 trial. Lancet Oncol. 2018;19:1040–50.

128. Souweidane MM, Kramer K, Pandit-Taskar N, Zhou Z, Haque S, Zanuncio P, et al. Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-Centre, dose-escalation, phase 1 trial. Lancet Oncol. 2018;19:1040–50.

129. Gavrieli M, Watanabe N, Loftin SK, Murphy TL, Murphy KM. Characterization of phosphotyrosine binding motifs in the cytoplasmic domain of B and T lymphocyte attenuator required for association with protein tyrosine phosphatases SHP-1 and SHP-2. Biochem Biophys Res Commun. 2003;312:1236–43.

130. Cao G, Xiao Z, Yin Z. Normalization cancer immunotherapy: blocking Siglec-G with anti-Siglec-G antibodies. Trends Cancer. 2019;5:37–44.

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