Update on lymphocyte-activation gene 3 (LAG-3) in cancers: from biological properties to clinical applications

Lishu Zhao[1,2], Hao Wang[1,2], Kandi Xu[1,2], Xinyue Liu[1,2], Yayi He[1,2]

[1]Department of Medical Oncology, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai 200433, China; [2]School of Medicine, Tongji University, Shanghai 200092, China.

Abstract

Immunotherapy that targets checkpoints, especially programmed cell death protein 1 and programmed cell death ligand 1, has revolutionized cancer therapy regimens. The overall response rate to mono-immunotherapy, however, is limited, emphasizing the need to potentiate the efficacy of these regimens. The functions of immune cells are modulated by multiple stimulatory and inhibitory molecules, including lymphocyte activation gene 3 (LAG-3). LAG-3 is co-expressed together with other inhibitory checkpoints and plays key roles in immune suppression. Increasing evidence, particularly in the last 5 years, has shown the potential of LAG-3 blockade in anti-tumor immunity. This review provides an update on the biological properties and clinical applications of LAG-3 in cancers.

Keywords: Lymphocyte-activation gene 3 (LAG-3); Immune checkpoint; Cancer; Immunotherapy

Introduction

Immune checkpoint inhibitors (ICIs) significantly improve survival in patients with multiple cancers, and representative targets are programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1).[1,2] However, the overall objective response rate (ORR) of mono-immunotherapy in cancers is unsatisfactory.[3,4] Activating immune cells and restoring anti-tumor immunity are the main action mechanisms of ICIs, but diverse co-stimulatory and co-inhibitory molecules regulate immune cells activity. Compared with inhibition of PD-1/PD-L1 pathway alone, the anti-tumor effect of immunotherapy can be potentiated by multiple combination immunotherapy protocols, such as combinations of immunotherapy with immunotherapy or chemotherapy or radiotherapy.

In the era of immunotherapy, targeting of novel immune checkpoints can also achieve a degree of anti-tumor immunity.[5,6] Lymphocyte-activation gene 3 (LAG-3; CD223) is a novel immune checkpoint receptor associated with CD4. Our previous review described the roles of LAG-3 in inflammatory and autoimmune diseases and cancers.[7] Many preclinical and clinical studies over the last 5 years, however, have demonstrated the anti-tumor effects of LAG-3-targeted agents.[8] The present review summarizes recent researches on the biological properties and clinical applications of LAG-3 in cancers.

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Structure and Ligands of LAG-3

LAG-3 is a transmembrane protein consisting of four immunoglobulin (Ig)-like extracellular domains (D1–D4) and a cytoplasmic domain,[9] as we show in Figure 1A. The extracellular region of LAG-3 is similar to that of CD4, with 20% amino acid identity, but the genomic regions encoding the intracellular regions vary, leading to different functions.[10] In addition, a connecting peptide located between D4 and the transmembrane domain makes LAG-3 more susceptible to cleavage by a disintegrin and metal-loproteinase domain-containing protein (ADAM), producing soluble LAG-3 (sLAG-3) that consists of the four extracellular domains.[11] The cytoplasmic domain is composed of three motifs: a serine-based motif, a "KIEELE" motif, and a glutamic acid and proline dipeptide repeat (EP) motif, with the "KIEELE" motif being mainly responsible for the inhibitory activity of this protein.[12]

One of the most principal ligands of LAG-3 was major histocompatibility complex class II (MHC class II),[13,14] and a proline-enriched loop in D1 mediates their

Correspondence to: Dr. Yayi He, Department of Medical Oncology, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai 200433, China
E-Mail: yayi.he@tongji.csu.edu.cn

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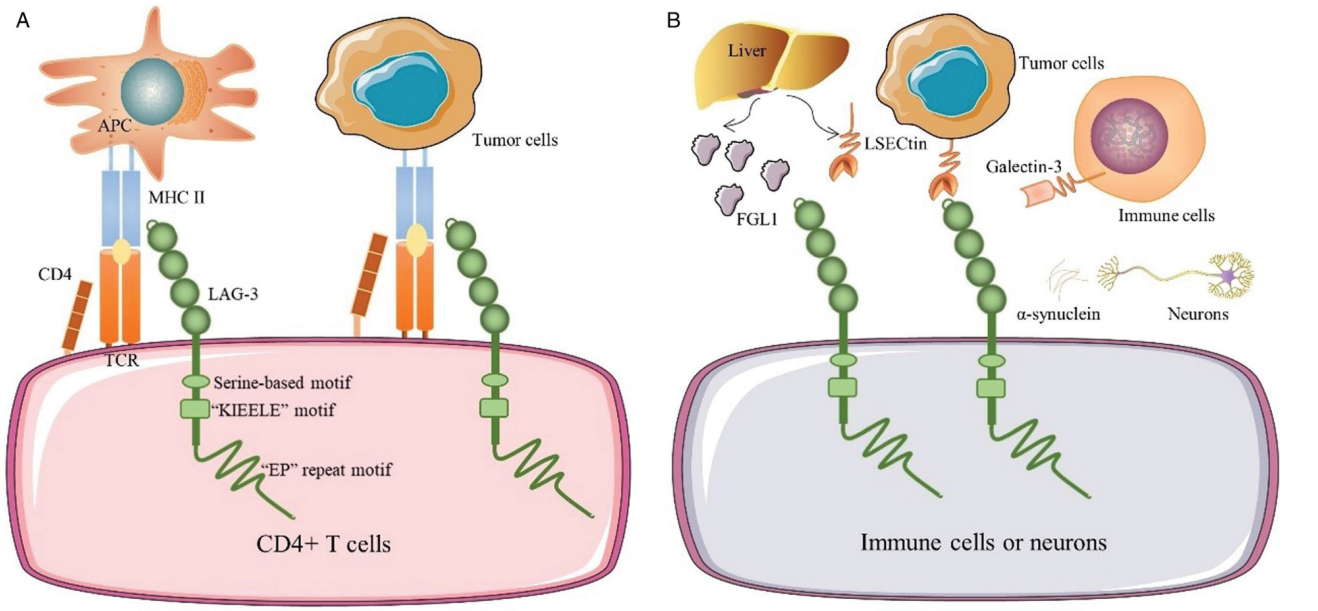

Figure 1: The structure and ligands of LAG-3. (A) The structure of LAG-3 and its main ligand MHC II; (B) Other ligands include FGL1, LSECtin, galectin-3, and α-synuclein. APC: Antigen-presenting cells; FGL1: Fibrinogen-like protein 1; LAG-3: Lymphocyte-activation gene 3; LSECtin: Liver sinusoidal endothelial cell lectin; MHC II: Major histocompatibility complex class II; TCR: T cell receptor.

interactions, as shown in Figure 1A. And plentiful studies have demonstrated that the interactions between LAG-3 and MHC class II modulate the proliferation, activation, apoptosis, and cytokine secretion of multiple immune cells.[7,15,16]

In the following years, multiple other ligands are found, and Figure 1B displays other reported ligands including galectin-3,[17] liver sinusoidal endothelial cell lectin (LSECtin),[18] fibrinogen-like protein 1 (FGL-1),[19,20] and α-synuclein preformed fibrils from neurons.[21] And LAG-3 binding to its ligands can hamper anti-tumor T cell immunity, leading to tumor immune evasion.[18,22]

Regulation of LAG-3 at the Epigenetic, Transcriptional, Post-transcriptional, and Post-translational Levels

The regulation mechanisms of LAG-3 at multiple levels are increasingly excavated, particularly in recent 5 years.[23] And they can be categorized into four types: epigenetic, transcriptional, post-transcriptional, and post-translational levels.

Epigenetic alterations have been reported to regulate LAG-3 expression in various cancers, such as renal cell cancer (RCC),[24] melanoma,[25] breast cancer,[26] and colorectal cancer.[27,28] In patients with RCC and melanoma, LAG-3 is broadly hypomethylated in tumor *vs.* normal tissues, and LAG-3 promoter methylation status correlated negatively with levels of LAG-3 messenger RNA (mRNA) expression.[24,25] LAG-3 promoter hypomethylation on T cells in peripheral blood and dysregulation of histone methylation of LAG-3 have been associated with high levels of LAG-3 expression.[27,29] Moreover, LAG-3 methylation status is associated with the infiltration of immune cells, such as CD4+/CD8+ T cells, interferon-γ (IFN-γ) signature, and survival.[24,25] These results suggest that LAG-3 methylation is a likely predictive and prognostic biomarker, as well as a therapeutic target in cancer patients.

At the transcriptional level, multiple transcription regulators have been found to potentiate the expression of LAG-3, such as thymocyte selection-associated high mobility group box protein (TOX),[30,31] a nuclear factor of activated T-cells family member,[32] nuclear receptor subfamily 4, group A,[33] and early growth response gene 2.[34,35] Moreover, glycogen synthase kinase-3 was found to reduce LAG-3 transcription by enhancing the expression of Tbet, which inhibits the transcription of LAG-3.[36] In head and neck squamous cell carcinoma (HNSCC), microRNA (miR)-7704, miR-21-5p in the extracellular vesicles (EVs) could increase the expression of LAG-3.[37] These transcription factors and miRNAs in EVs exert roles in LAG-3 expression and T cell exhaustion and may be potential targets for cancer immunotherapy.

N6-methyladenosine (m6A) modifications are also involved in the post-transcriptional regulation of LAG-3. For example, AlkB homolog 5 (ALKBH5) and YTH domain family, member 1 (YTHDF1), which act as RNA demethylase during m6A modifications, have been associated with the expression of 14 genes, particularly LAG-3.[38] In addition, checkpoint expression levels are higher in colon cancer patients with higher levels of ALKBH5 and lower levels of YTHDF1 expression.[38]

Post-translationally, LAG-3 is degraded in lysosomes in the absence of antigen stimulation but is translocated to the cell surface upon stimulation through protein kinase C signaling in an intracellular domain-dependent manner.[39] However, the specific mechanism of translation

remains to be determined. LAG-3 cleavage by ADAM10 and ADAM17 produces sLAG-3, which alleviates T cell inhibition;[11] positively regulates CD8+ T cells, interleukin (IL)-12, IFN-γ, and dendritic cells (DCs); and functions as a prognostic marker in multiple cancers.[15,40-43]

Collectively, these results show that LAG-3 expression is regulated through multiple mechanisms, including at the epigenetic, transcriptional, post-transcriptional, and post-translational levels. Agents targeting LAG-3 regulators may contribute to novel combination immunotherapy treatment strategies in cancers.

Expression of LAG-3 and Its Role in Immune Suppression and Anti-tumor Immunity

LAG-3 can be constitutively expressed or induced on multiple immune cells, including CD4/CD8+ T cells, natural killer (NK) cells, invariant NK T cells, plasmacytoid DCs (pDCs), and B cells.[44] Overexpression of LAG-3 has been detected in various cancers, where it participates in immune regulation and resistance to treatment, thus affecting patient survival.[45,46]

LAG-3 on CD4+ T cells

LAG-3 is rarely expressed on resting T cells but is expressed on CD4+ T cells following antigen stimulation, making it a biomarker of T cell exhaustion.[8] LAG-3 interacts with MHC class II to downregulate CD4+ T cell proliferation and cytokine secretion [Figure 2A].[47] Removal of the "KIEELE" motif of LAG-3 was found to lead to the complete abrogation of LAG-3 function, suggesting that the " KIEELE" motif is key to the activation of downstream signaling pathways in CD4+ T cells.[12,48] To date, however, the binding partner of the intracellular "KIEELE" motif has not been identified.

LAG-3 on CD8+ T cells

LAG-3 is expressed on CD8+ T cells within multiple tumors, such as non-small-cell lung cancer (NSCLC) particularly non-adenocarcinoma,[49,50] esophageal carcinoma,[51,52] RCC,[53] ovarian cancer,[54] and breast cancer.[55] LAG-3 inhibits effector T cells and inflammatory cytokine production.[17,54] In RCC, CD8+ tumor-infiltrating lymphocytes (TILs) expressing the co-inhibitory molecule LAG-3 were accompanied by low-density mature DCs and predicted a higher risk of disease progression.[53] The effector functions of CD8+LAG-3+ TILs are hampered in ovarian cancers, manifesting as reduced production of IFN-γ and tumor necrosis factor-a. Inhibition of LAG-3 alone or together with PD-1 inhibition augments the activation and immune responses of T cells.[56]

The inhibitory mechanism of LAG-3 on CD4+ T cells is dependent on LAG-3 binding to MHC class II molecules. In contrast, MHC class I-restricted CD8+ T cells have different and more sophisticated LAG-3-mediated inhibitory mechanisms [Figure 2B]. Many LAG-3 ligands have been reported to be involved in the effects of LAG-3 on CD8+ T cell functions. Galectin-3, one of the ligands of LAG-3, suppresses the anti-tumor response of CD8+ TILs in mice, with this activity restored by the depletion of galectin-3.[17] Moreover, LSECtin was found to promote melanoma tumor growth, mainly by weakening antitumor T cell responses.[18] Mechanistically, LSECtin could markedly inhibit the proliferation of CD8+ rather than CD4+ T cells by preventing CD8+ cells from entering cell cycles, manifesting as decreased cyclin-dependent kinases (CDK) 2, CDK4, and CDK6 expression.[18] FGL-1 was found to inhibit anti-tumor immunity dependent on CD8+ T cells.[19] FGL-1 expression, in turn, was downregulated by oxysophocarpine through the IL-6-mediated Janus kinase/signal transducer and activator of transcription (STAT) pathway, sensitizing the LAG-3 immunotherapy effect on CD8-positive T cells.[57] Moreover, a type of C25 peptide was reported to inhibit LAG-3/MHC class II interactions, stimulating the activation of $CD8_+$ T cells and inhibiting tumor growth.[58] The intrinsic mechanism, however, remains poorly understood. LAG-3 expression correlated positively with the expression of nearly all MHC-associated genes in various cancers,[59] indicating that LAG-3 may not just cooperate with MHC class II. Additional studies are needed to determine whether MHC class I molecules participate in LAG-3-mediated inhibitory function. Together, these findings show that the mechanisms of action of LAG-3 on CD8+ T cells involve multiple ligand interactions, and that blockade of LAG-3 enhances CD8+ T cell functions and promotes anti-tumor immunity.

LAG-3 on CD4+ T regulatory (Treg) cells

LAG-3 is also expressed on CD4+CD25+ Treg cells, binding to MHC class II molecules on immature DCs. This binding suppresses DC maturation and immunostimulatory activities through an immunoreceptor tyrosine-based activation motif-induced repressive signaling, recruiting Src homology region 2 domain-containing phosphatase 1 mediated by Fc gamma receptor and extracellular signal-regulated kinase [Figure 2C].[60] Although LAG-3 is only slightly expressed on CD4+CD25+ forkhead box P3 (FOXP3)+ Treg cells, it is specifically detected on T cells negative for CD25 and Foxp3, which secrete massive amounts of IL-10.[61] These cells, also known as LAG-3+ Treg cells, produce high levels of transforming growth factor-β3, which suppresses B cell responses.[62] Moreover, co-expression of LAG-3 and CD49b is specific to CD4+ type 1 T regulatory (Tr1) cells,[63,64] which have strong immunosuppressive activity through secreting massive IL-10 [Figure 2C].[65]

LAG-3 on other immune cells including NK, natural killer T (NKT), pDCs, and B cells

LAG-3 can be detected on NK cells, but does not participate in natural killing activities.[66] Moreover, blockade of LAG-3 does not affect the natural killing by NK cells of various target cells.[67] IFN-γ production is impaired in LAG-3-positive NKT cells.[68] pDCs are a unique subgroup of DCs that produce massive amounts of type I IFNs upon pathogen stimulation.[69] LAG-3

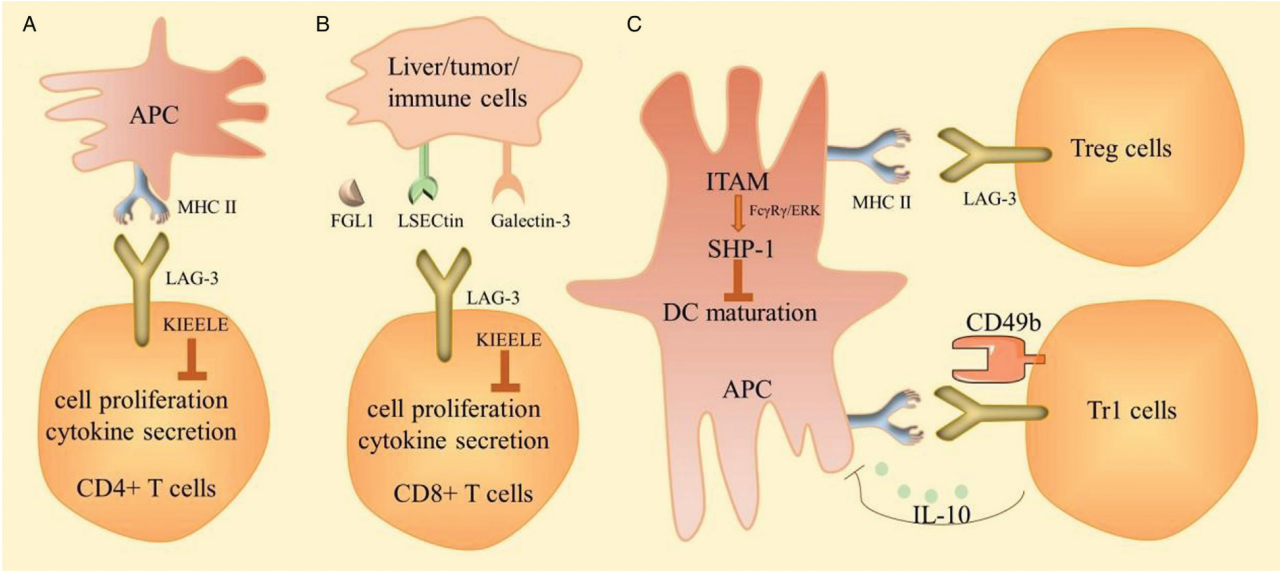

Figure 2: Mechanisms of LAG-3 in immune suppression. The inhibitory mechanisms of LAG-3 on $CD4_+$T cells (A), CD8+ T cells (B), Treg cells, and Tr1 cells (C). APC: Antigen-presenting cells; DC: Dendritic cells; ERK: Extracellular signal-regulated kinase; FcγRγ: Fc gamma receptor gamma; FGL1: Fibrinogen-like protein 1; IL-10: Interleukin 10; ITAM: Immunoreceptor tyrosine-based activation motif; LAG-3: Lymphocyte-activation gene 3; LSECtin: Liver sinusoidal endothelial cell lectin; MHC II: Major histocompatibility complex class II; SHP-1: Src homology region 2 domain-containing phosphatase 1; Treg: T regulatory cells; Tr1 cells: Type 1 T regulatory cells.

expression is ten-fold higher on activated pDCs than on T effector or Treg cells, and sLAG-3 secretion by activated pDCs is five-fold higher than secretion by activated T cells.[70] LAG-3 expressed on pDCs intrinsically regulates the homeostasis of pDCs and extrinsically modulates the homeostasis of T cells.[70] Moreover, a natural plasma cell subset of B cells with unique transcriptomic and epigenomic characteristics also expresses LAG-3 and suppresses memory T cell formation through IL-10 production.[71]

Taken together, these results show that LAG-3 is expressed on various immune cells and is closely associated with immune escape. Recent studies have focused primarily on the inhibitory mechanisms of LAG-3 on these cells. Additional studies are required to determine the mechanisms by which downstream inhibitory signals are transmitted in each type of immune cell and the dependence of these signals on specific ligands.

Associations of LAG-3 with Immunoregulatory Factors in Cancers

Increasing evidence has shown that LAG-3 acts together with other inhibitory modulators, including immune checkpoints and immune cells.[72] Our previous review summarized the co-expression patterns of LAG-3 with the immune checkpoints PD-1, PD-L1, and cytotoxic T lymphocyte antigen 4 (CTLA-4) in autoimmune and infectious diseases.[7] LAG-3 is also co-expressed with PD-1/PD-L1 in various types of cancer, including NSCLC[49] breast cancer,[55,73,74] RCC,[75] ovarian cancer,[76] gastric cancer,[77] and colorectal cancer.[78] Evaluation of the expression patterns of LAG-3 and other checkpoints in 33 types of cancer using samples from a public database showed multiple high expression patterns in various cancers, including LAG-3 and PD-1 in urogenital tumors; LAG-3 and CTLA-4 in HNSCC; and LAG-3, PD-1, and CTLA-4 in skin melanoma.[59] The most common co-expression patterns of checkpoints varied among different cancer types, such as LAG-3 and PD-1 in RCC,[75] LAG-3 and CTLA-4 in triple-negative breast cancer,[79] and PD-1 and T cell Ig and mucin domain-containing protein 3 in ovarian cancer.[74] Evaluation of five checkpoints in TILs isolated from RCC patients showed that the most frequent co-expression pattern was PD-1 and LAG-3 on CD4/CD8+ TILs.[75] Dual positive expression of LAG-3 and PD-1 was found to be associated with the inflamed immunotype in breast cancer,[80] and inhibition of PD-1 and LAG-3 resulted in the release of IFN-γ.[81] ICIs enhanced the expression of multiple immune checkpoints, including LAG-3, both in *vitro* and *in* vivo.[82,83] Co-blockade of PD-1/PD-L1 in breast cancer cell lines enhanced the expression of LAG-3 on CD25-positive T cells and FOXP3-positive Treg cells in a tumor dependent manner, suggesting that this may be a resistance mechanism.[84] In $Kras^{G12D}$ mice models of lung cancer, LAG-3 and CTLA-4 significantly increased on CD8+ T cells in tumors resistant to PD-1 inhibitors.[85] These findings indicate that the co-expression patterns of checkpoints and compensatory inhibitory mechanisms of immunotherapy in various tumor types should be considered to optimize the effectiveness of combination immunotherapy and reverse tumor resistance to immunotherapy.

LAG-3 was also shown to be associated with other immunoregulatory factors, including immune cells and inflammatory factors. An investigation of the relationship of LAG-3 gene expression with abundance of TILs and chemokines in 30 kinds of cancer using samples from a public database showed that LAG-3 expression was correlated with activated CD8+ T cells, Treg cells, myeloid-derived suppressor cells, and certain chemokines and their receptors.[59] And in RCC and glioblastoma, LAG-3 had a more robust association with CD8A than

checkpoints.[86] In HNSCC, LAG-3 had a positive correlation with C-X-C motif chemokine ligand 9/10/11.[87] In melanoma patients, LAG-3 showed a positive correlation with CD163, a biomarker of M2-type tumor-associated macrophages, with high co-expression of LAG-3 and CD163 being related to poor pathological indicators and worse prognosis.[88] In HNSCC, LAG-3 expression was closely correlated with the infiltration of pDCs.[89] These results indicate that LAG-3 has negative effects on the tumor microenvironment and patient outcomes.

Correlation between LAG-3 and Efficacy and Prognosis in Cancers

LAG-3 expression has been shown to be a predictive biomarker of the efficacy of immunotherapy in cancers. For example, high LAG-3 expression on CD4+/CD8+ TILs before and after the first cycle of nivolumab predicted a longer progression-free survival (PFS) in patients with gastric cancer.[90] Moreover, a gene signature based on CD274, CD8A, LAG-3, and STAT1 was found to be associated with greater efficacy of nivolumab or nivolumab combined with ipilimumab in gastric cancer[91] and nivolumab in hepatic cancer.[92] Conflicting results, however, have been observed in other cancers. For example, increased LAG-3 in patients with advanced NSCLC being treated with PD-1 inhibitors was significantly associated with poorer PFS and greater resistance to PD-1 inhibitors.[93] Elevated LAG-3 in tissues was found to be a biomarker of resistance to ICIs in breast cancers and mouse cancer models.[94,95] Moreover, high baseline sLAG-3 was associated with poorer PFS in HNSCC patients receiving ICIs or chemotherapy.[41] A mutation in the LAG-3 gene, preventing cleavage by ADAM-10/17, led to elevated levels of LAG-3, which may be a mechanism of resistance to ICIs.[96]

Higher LAG-3 expression in tumor tissues is related to an adverse prognosis in most types of cancer, including pancreatic cancer,[97] high-grade soft-tissue sarcoma,[98] salivary gland carcinomas,[99] clear cell renal cell carcinoma,[100] HNSCC,[101] esophageal squamous cell carcino-ma,[52] and oral squamous cell carcinoma.[102] In contrast, higher LAG-3 expression correlates with longer survival in other types of cancer, such as high-grade serous ovarian cancer,[103] blood cancer,[104] esophageal adenocarcinoma,[105] and advanced gastric cancer.[90] Studies on the association of LAG-3 with prognosis in patients with NSCLC, colorectal cancer, and breast cancer have yielded contradictory results. For example, one study reported that LAG-3 positivity on TILs in patients with NSCLC was predictive of shorter recurrence-free survival,[49] whereas another study found that LAG-3 on TILs in the intraepithelial and stromal parts of tumors and metastases predicted better disease-specific survival in 553 patients with stage I-IIIB NSCLC.[106] A study in 773 patients with stage I-III colorectal cancer found that high tumor expression of LAG-3 was related to a shorter survival, whereas high LAG-3 on immune cells in the stroma correlated with better survival.[107] Another study found that LAG-3 on TILs was a good prognostic factor in 89 patients who underwent resection for microsatellite instability-high colon cancers,[108] whereas LAG-3+ CD49b+ Tr1 cells were predictive of a poor prognosis and higher LAG-3 mRNA levels predicted advanced stages in colorectal cancer.[109,110] An evaluation of the levels of expression of LAG-3 on stromal and intra-epithelial TILs (sTILs or iTILs) in breast cancers found that patients with LAG-3+iTILs had a better prognosis.[111] Flow cytometry analysis of dynamic changes of multiple checkpoints in the peripheral blood of HNSCC patients from before to after treatment showed that LAG-3 expression increased at the time of tumor recurrence.

Collectively, these findings indicate that LAG-3 expression is closely associated with the efficacy of ICIs and with patient prognosis. Tumor origin, tumor stage (early *vs.* advanced), tumor location (stromal *vs.* intraepithelial; primary tumor *vs.* metastasis; tissue *vs.* blood), and different cut-off values for positivity may partly account for the differences among cancers in the correlation of LAG-3 expression with treatment efficacy and patient prognosis. Dynamic quantification of LAG-3 in tumor tissues and peripheral blood may help predict the efficacy of ICIs and guide treatment options, including whether and when to block LAG-3 pathways.

Clinical Application of Targeting LAG-3 in Cancers

Preclinical studies and preliminary clinical trials have shown that LAG-3 is involved in immune suppression and anti-tumor immunity. Drugs targeting LAG-3 have been developed to treat cancer patients, particularly during the past 5 years. At least 21 kinds of LAG-3-targeting agents are being tested in clinical trials in various types of cancer (https://clinicaltrials.gov/). These agents can be divided into three types: anti-LAG-3 monoclonal antibodies, sLAG-3, and bispecific antibodies targeting LAG-3 and PD-1/PD-L1/CTLA-4 [Table 1].

Anti-LAG-3 monoclonal antibodies are the first class of LAG-3 targeting agents to be developed. These agents include relatlimab, SHR-1802, Sym-022, ieramilimab (LAG525), MK-4280, INCAGN-2385, TSR-033, LBL-007, DNV-3, IBI-110, BI754111, and REGN3767 (fianlimab). Most of them have been or are being tested in phase one or two clinical trials, showing that they possess acceptable safety and a certain efficacy.[112-116] For instance, relatlimab plus nivolumab (PD-1 inhibitor) obtained an ORR of 11.5% in melanoma patients resistant to PD-1/PD-L1 inhibitors. Moreover, ORR was higher in patients with ≥1% than with <1% LAG-3 expression (18% *vs.* 5%).[112] The 2021 American Society of Clinical Oncology annual meeting reported positive results of a phase 2/3 trial (RELATIVITY-0477, NCT03470922) of relatlimab in melanoma patients.[117] In that study, 714 patients with melanoma were treated with nivolumab alone or nivolumab plus relatlimab as first line therapy. PFS was significantly longer in patients treated with nivolumab plus relatlimab than in those treated with nivolumab alone (10.1 *vs.* 4.6 months; $P = 0.0055$); although the rate of grade three or higher adverse events associated with therapy was also higher in the combination therapy group, it was acceptable (18.9% *vs.* 9.7%).

Table 1: Summary of LAG3-targeted agents in cancers under clinical trial.

Drug name	Company	Drug type	Phase	Combination
Relatlimab (BMS-986016)	BMS	Anti-LAG3	1/2/3	PD-1
SHR-1802	Hengrui	Anti-LAG3	1	–
Sym-022	Symphogen	Anti-LAG3	1	PD-1
Ieramilimab (LAG525)	Immutep	Anti-LAG3	1/2	PD-1
MK-4280	MSD	Anti-LAG3	1/2	PD-1
INCAGN-2385	Incyte	Anti-LAG3	1/2	PD-1/TIM-3
TSR-033	Tesaro Inc	Anti-LAG3	1	PD-1/TIM-3
LBL-007	Nanjing Leads Biolabs	Anti-LAG3	1	PD-1
DNV-3	Zhejiang Shimai	Anti-LAG3	1	PD-1
IBI-110	Innovent Biologics	Anti-LAG3	1/2	PD-1
BI754111	Boehringer Ingelheim	Anti-LAG3	1/2	PD-1
REGN3767 (Fianlimab)	Regeneron	Anti-LAG3	1	PD-1
89Zr-DFO-REGN3767	MSKCC	Anti-LAG3 labeled with 89Zr	1/2	PD-1
IMP321	Immutep	Soluble LAG3	1/2	PD-1/PD-L1/vaccine
MK-4280A	MSD	Bispecific anti-LAG-3/PD-1	1/2	PD-1
MGD013 (Tepotelimab)	Macrogenics	Bispecific anti-LAG-3/PD-1	1/2/3	B7-H3
EMB-02	Anmai	Bispecific anti-LAG-3/PD-1	1/2	–
RO-7247669	Roche	Bispecific anti-LAG-3/PD-1	1/2	–
FS 118	F-Star	Bispecific anti-LAG-3/PD-L1	1/2	–
IBI-323	Innovent Biologics	Bispecific anti-LAG-3/PD-L1	1	–
XmAb-22841	Xencor Inc	Bispecific anti-LAG-3/CTLA-4	1	PD-1

CTLA-4: Cytotoxic T lymphocyte antigen 4; LAG-3: Lymphocyte-activation gene 3; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death ligand 1; TIM3: T cell immunoglobulin domain and mucin domain-3; B7-H3: B7 homolog 3 protein; –: Not available.

IMP321, a soluble recombinant protein of LAG-3 consisting of the four extracellular domains, has been shown to activate antigen presenting cells by binding to MHC II, while not inducing inhibitory signals due to the absence of an intracellular domain.[118] Twelve trials have tested IMP321 alone or with PD-1/PD-L1 inhibitors or IMP321 vaccine in the treatment of multiple solid tumors (NCT00324623, NCT00349934, NCT00351949, NCT00365937, NCT00732082, NCT02614833, NCT02 676869, NCT03252938, NCT03625323, NCT042 52768, NCT01308294, and NCT04811027). Results to date have shown that vaccination with IMP321 induces durable cellular antitumor immune responses.[119,120] Patients vaccinated with IMP321 plus melanoma antigen recognized by T cells 1 (MART-1) peptide had significantly higher numbers of antigen-specific CD8+ T cells and lower numbers of exhausted T cells and Treg cells.[120] The TACTI-002 study (NCT03625323), which enrolled NSCLC/HNSCC patients naïve to ICIs, found that IMP321 plus pembrolizumab was safe, achieving an ORR of 47% as first line treatment in patients with NSCLC and an ORR of 40% (6/15) as second line treatment in patients with HNSCC.[121] Similarly, this combination achieved an ORR of 50% in PD-1 naïve patients with melanoma.[122] sLAG-3 has shown encouraging antitumor activity, but additional studies are needed to determine the role of ligand-receptor interactions in reversing inhibitory signaling.

Given that co-expression of LAG-3 and other checkpoints often occurs in cancers and cooperatively mediates immune escape, multiple bispecific antibodies that synchronously target LAG-3 and PD-1 or PD-L1 or CTLA-4 have been developed, including MK-4280A, MGD013 (Tepotelimab), EMB-02, RO-7247669, FS 118, IBI-323, and XmAb-22841 [Table 1]. Preclinical studies have shown that IBI-323, a bispecific antibody synchronously targeting LAG-3 and PD-L1, exhibited a greater immune stimulatory effect than each parent antibody.[123] Interestingly, bispecific antibodies of LAG-3 and PD-L1 decreased LAG-3 expression, whereas LAG-3 inhibitors combined with PD-L1 inhibitors increased LAG-3 expression.[124] The mechanism of action of bispecific antibodies in antitumor immunity remains to be determined.

Collectively, LAG-3-targeting drugs have exhibited great potential in cancer immunotherapy, especially when combined with PD-1/PD-L1 inhibitors. Dynamic quantification of LAG-3 expression may help to predict the efficacy of LAG-3 inhibitors and optimize the treatment strategy. Additional studies are needed to evaluate the ability of LAG-3 inhibitors to block ligand-receptor interactions and the specific action mechanisms of bispecific antibodies and each parent antibody inhibiting both PD-1/PD-L1 and LAG-3 signaling.

Conclusions

The synergistic effects of LAG-3 and PD-1/PD-L1 inhibitors in preclinical and clinical studies suggest that blockade of LAG-3 can potentiate the efficacy of immunotherapy in cancer and expand the numbers of patients who may benefit from immunotherapy. Although LAG-3 is expressed on multiple immune cells and plays key roles in immune escape by interacting with its ligands, the mechanism responsible for the transmission of downstream inhibitory signals and the ligand responsible

for activation of each immune cell in response to LAG-3 blockade remains to be determined. In addition, further studies are needed to assess the mechanisms of action of dual blockade of LAG-3 and other checkpoints, and whether the molecules that regulate the expression of LAG-3 act synergistically with ICIs. In conclusion, this review summarizes recent research on LAG-3, including its biological properties and clinical applications in cancers. These findings may result in a more comprehensive understanding of LAG-3 signaling and may direct future studies.

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Conflicts of interest

None.

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