

LAG-3: from molecular functions to clinical applications

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ABSTRACT

To prevent the destruction of tissues owing to excessive and/or inappropriate immune responses, immune cells are under strict check by various regulatory mechanisms at multiple points. Inhibitory coreceptors, including programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), serve as critical checkpoints in restricting immune responses against self-tissues and tumor cells. Immune checkpoint inhibitors that block PD-1 and CTLA-4 pathways significantly improved the outcomes of patients with diverse cancer types and have revolutionized cancer treatment. However, response rates to such therapies are rather limited, and immune-related adverse events are also observed in a substantial patient population, leading to the urgent need for novel therapeutics with higher efficacy and lower toxicity. In addition to PD-1 and CTLA-4, a variety of stimulatory and inhibitory coreceptors are involved in the regulation of T cell activation. Such coreceptors are listed as potential drug targets, and the competition to develop novel immunotherapies targeting these coreceptors has been very fierce. Among such coreceptors, lymphocyte activation gene-3 (LAG-3) is expected as the foremost target next to PD-1 in the development of cancer therapy, and multiple clinical trials testing the efficacy of LAG-3-targeted therapy are underway. LAG-3 is a type I transmembrane protein with structural similarities to CD4. Accumulating evidence indicates that LAG-3 is an inhibitory coreceptor and plays pivotal roles in autoimmunity, tumor immunity, and anti-infection immunity. In this review, we summarize the current understanding of LAG-3, ranging from its discovery to clinical application.

INTRODUCTION

Immune cells can rapidly activate powerful defense mechanisms when they encounter invading pathogens. However, excessive and/or undesirable immune responses can exert deleterious effects. Immune cells are regulated by various molecules and cells with suppressive functions at multiple checkpoints. Such checkpoints are critical to the development of self-tolerance as the immune cells learn not to attack host cells. However, such checkpoints can be hijacked by tumors and pathogens to escape from the immune system.

Cancer immunotherapies targeting inhibitory coreceptors programmed cell death 1

(PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) significantly improved the outcomes of patients with diverse cancer types, revolutionizing cancer treatment. The success of these therapies verified that inhibitory coreceptors serve as critical checkpoints for immune cells to not attack the tumor cells as well as self-tissues. However, response rates are typically lower and immune-related adverse events (irAEs) are also observed in patients administered with immune checkpoint inhibitors. This is indicative of the continued need to decipher the complex biology of inhibitory coreceptors to increase response rates and prevent such unwanted side effects in patients with cancer.^{1–5}

To date, many stimulatory and inhibitory coreceptors have been identified in addition to PD-1 and CTLA-4. These coreceptors are supposed to control the activation of lymphocytes by regulating the quality and quantity of the antigen receptor signaling to optimize beneficial immune responses while avoiding autoimmunity and excess immune responses. There is an intense competition among pharmaceutical companies to develop novel immunotherapies targeting these coreceptors. Among such coreceptors, lymphocyte activation gene-3 (LAG-3, CD223) is the foremost target next to PD-1, and multiple clinical trials to validate LAG-3-targeted therapies are ongoing.^{6,7}

MOLECULAR CHARACTERISTICS OF LAG-3

LAG-3 was identified in 1990 by Triebel and colleagues⁸ in a screening designed to isolate molecules that were selectively expressed in F₅ cells, a CD3-negative interleukin (IL)-2-dependent NK cell line. They screened the cDNA library of F₅ cells with a probe obtained by subtraction of the cDNA of F₅ cells with the mRNA of Jurkat (T cell leukemia), Laz 388 (Epstein-Barr virus-transformed B lymphoblastoid cell), K562 (erythro-myeloid leukemia), and U937 (cell of histiocytic origin) cells. The LAG-3 cDNA



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function of LAG-3 strongly correlates with its expression levels on the cell surface,²⁰ the regulation of LAG-3 expression is very critical. Continuous antigen exposure owing to the chronic infection with viruses,^{21–22} bacteria,²³ and parasites²⁴ leads to high and sustained expression of LAG-3 as well as other inhibitory coreceptors on CD4⁺ and CD8⁺ T cells. These T cells lose robust effector function and are termed exhausted T cells. LAG-3 blockade has been demonstrated to reinvigorate exhausted T cells and strengthen anti-infection immunity, although to a lesser extent compared with that by PD-1 blockade.^{22–24–30} Tumor-infiltrating T cells are also persistently exposed to tumor-associated antigens and express high levels of multiple inhibitory coreceptors including LAG-3, resulting in functional exhaustion.^{31–36} IL-2, IL-7, and IL-12, but not IL-4, IL-6, IL-10, tumor necrosis factor (TNF), and interferon (IFN)- γ , have been reported to augment LAG-3 expression on activated T cells.^{37–38}

LAG-3 expression is also detected on several subsets of CD4⁺ T cells with suppressive function. Foxp3⁺ regulatory T (T_{reg}) cells constitutively express LAG-3,³⁹ presumably due to the continuous activation of T cell receptor (TCR) signal by self-antigens, which is required for the differentiation, homeostasis, and suppressive functions of T_{reg} cells.⁴⁰ Zhang *et al*⁴¹ demonstrated that LAG-3 on T_{reg} cells limited their proliferation intrinsically. The role of LAG-3 in the effector function of T_{reg} cells is contentious. Huang *et al*³⁹ reported that T_{reg} cells from LAG-3-deficient mice inhibited the activation of effector T cells with lower efficiency whereas other studies demonstrated that the suppressive function of LAG-3-deficient T_{reg} cells is comparable.^{19–42–43} Further studies are required to elucidate the actual role of LAG-3 in association with the T_{reg} cells. LAG-3 is also expressed on CD4⁺ type 1 T regulatory (Tr1) cells. Although Tr1 cells demonstrate strong immunosuppressive activity by secreting high amount of IL-10, specific cell surface markers that define this population had not been detected until they were observed to express LAG-3 and CD49b.⁴⁴ In addition, LAG-3-expressing CD4⁺CD25⁺Foxp3[−] T cells that produce IL-10 and transforming growth factor (TGF)- β 3 have been proposed to exhibit regulatory function as a distinct cell population.^{45–46} Interestingly, IL-10-producing natural regulatory plasma cells have also been observed to express LAG-3.⁴⁷ Currently, the roles of LAG-3 in the cell-extrinsic inhibitory function of these non-classical regulatory cells remain unclear and require further investigation.

Multiple transcriptional regulators such as thymocyte selection-associated high mobility group box protein (TOX), nuclear factor of activated T cells (NFAT), nuclear receptor subfamily 4, group A (NR4A), interferon regulatory factor 4, and B lymphocyte-induced maturation protein-1 are known to engage in the generation of exhausted T cells.^{48–56} Among these, NFAT, NR4A, and TOX have been demonstrated to augment the expression levels of LAG-3, together with other inhibitory coreceptors, when overexpressed in T cells. Early growth response gene 2 (EGR2) is also reported to be

a key transcription factor in the induction of LAG-3 expression in CD4⁺CD25⁺Foxp3[−] regulatory T cells.⁴⁵ On the other hand, T-box expressed in T cells (T-bet) has been reported to repress the expression of LAG-3 and other inhibitory coreceptors and sustain antigen-specific response of CD8⁺ T cells during chronic infection.^{57–58} In addition to the transcriptional regulation, the cell surface expression level of LAG-3 is also regulated by subcellular trafficking and proteolytic cleavage.^{17–59–60}

LAG-3 is also expressed on CD3⁺CD4⁺CD8[−] T cells,⁶¹ TCR $\alpha\beta$ CD8 $\alpha\alpha$ intraepithelial lymphocytes,⁶² $\gamma\delta$ T cells,^{63–64} and NKT cells.⁶⁵ Besides, its expression on activated NK cells is reported to be involved in the cytotoxicity against MHCII-negative target cells in mice.^{8–66} Plasmacytoid dendritic cells⁶⁷ and activated B cells⁶⁸ also express LAG-3 on their cell surface. However, the functional roles of LAG-3 in these populations remain poorly understood. Furthermore, LAG-3 is reported to be expressed on neurons and acts as a receptor for α -synuclein fibrils.⁶⁹

LIGANDS OF LAG-3

LAG-3 has been proposed to bind to MHCII with higher affinity than CD4 and inhibit T cell activation by interfering with the association of CD4 with MHCII.^{12–64–70} However, LAG-3 has been demonstrated to inhibit T cell activation by a mechanism that is different from the competitive inhibition of CD4, and the discrepancy between the binding capacity of soluble LAG-3 protein and MHCII expression levels in cells has been recognized, making the actual ligand of LAG-3 elusive.^{19–71} Recently, MHCII transactivator (CIITA) has been identified as a critical regulator of LAG-3 ligand. CIITA induces the expression of not only MHCII but also MHCII accessory molecules, including CD74 (invariant chain, Ii) and H2-DM. The MHCII accessory molecules contribute to the formation and the cell surface sorting of peptide-MHCII complex (pMHCII) exhibiting stable structural conformation in the conventional pathway of antigen presentation.⁷² LAG-3 distinguishes the conformation of pMHCII and selectively binds to stable pMHCII. Accordingly, LAG-3 preferentially inhibits the activation of CD4⁺ T cells that recognize stable pMHCII (figure 2A).⁷¹ It has also been demonstrated that LAG-3 does not compete with CD4 for pMHCII binding.⁷¹ Instead, LAG-3 inhibits T cell activation by transducing inhibitory signals via the intracellular region, as described in the next section.

As mentioned, LAG-3 is expressed on exhausted CD8⁺ T cells in tumors and is a potent therapeutic target for cancer immunotherapy. However, the mechanism by which LAG-3 binding to pMHCII could impact the activation of CD8⁺ T cells has not been elucidated. Recently, the activation of CD8⁺ T cells has been demonstrated to be inhibited weakly when antigen-presenting cells express a substantial amount of stable pMHCII in addition to cognate pMHCI, indicating that LAG-3 can also directly suppress CD8⁺ T cells to a certain extent.⁷¹

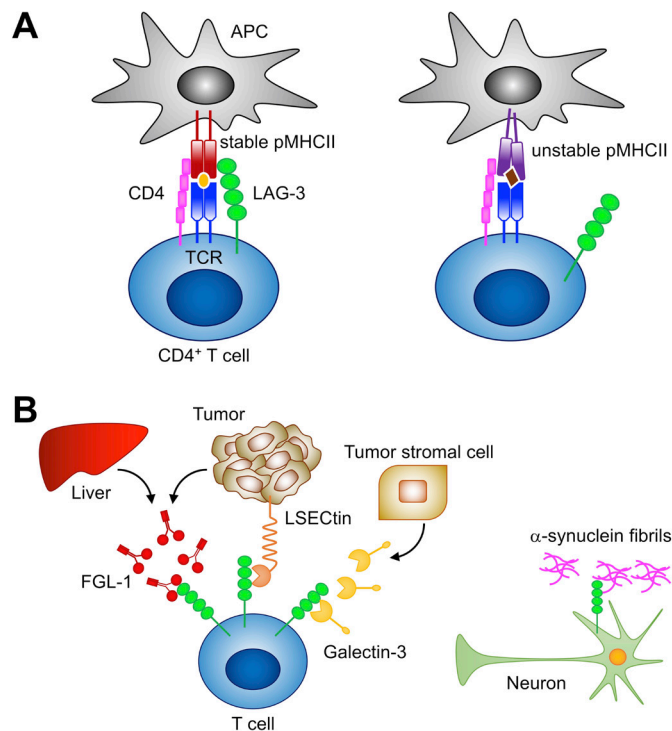


Figure 2 Ligands of LAG-3. (A) LAG-3 selectively binds to stable pMHCII and inhibits the activation of CD4⁺ T cells that recognize stable pMHCII. (B) Reported non-MHCII ligands. LAG-3 has been reported to associate with FGL1, LSEctin, galectin-3, and α -synuclein fibrils. FGL1, fibrinogen-like protein 1; LSEctin, liver sinusoidal endothelial cell lectin; MHCII, major histocompatibility complex class II; pMHCII, peptide-MHCII complex; APC, antigen presenting cell; TCR, T cell receptor.

To date, several molecules other than stable pMHCII have been reported as possible ligands for LAG-3 (figure 2B). As mentioned, galectin-3 and LSEctin have been indicated to interact with glycans on LAG-3. LSEctin is a member of the C-type lectin family and is expressed mainly in the liver.^{16 73} Xu *et al*¹⁶ reported that LAG-3 suppressed the IFN- γ production from T cells upon stimulation with anti-CD3 Ab in the presence of LSEctin-expressing melanoma cells. Galectin-3, belonging to the galectin family, is a soluble galactose-binding lectin secreted from various types of tumor cells and tumor stromal cells.⁷⁴ Kouo *et al*¹⁵ demonstrated that galectin-3 reduced the frequency of IFN- γ -producing CD8⁺ T cells upon stimulation with anti-CD3 and anti-CD28 Abs when CD8⁺ T cells from LAG-3-sufficient and not LAG-3-deficient mice were used. Recently, Wang *et al*⁷⁵ identified fibrinogen-like protein 1 (FGL1), a member of the fibrinogen family, as a potential ligand for LAG-3. FGL1 is secreted from hepatocytes in the liver under normal physiological conditions, whereas some tumor cells can also produce FGL1 at high levels. FGL1 has been demonstrated to reduce the secretion of IL-2 from 3A9 T hybridoma cells expressing LAG-3 upon stimulation with the cognate peptide. Further studies are required to elucidate whether and how these potential

ligands independently and/or cooperatively contribute to the inhibitory function of LAG-3.

In addition to the immunoinhibitory role, LAG-3 seems to have a distinct role in the nervous system as well. Mao *et al*⁶⁹ reported that LAG-3 can bind to α -synuclein fibrils, which are associated with the pathogenesis of Parkinson's disease. The association of α -synuclein fibrils with LAG-3 triggers endocytosis, cell-to-cell transmission, and neurotoxicity of α -synuclein fibrils. In addition to LAG-3, other molecules such as semaphorins and paired Ig-like receptor B are known to exhibit dual roles in immune and nervous systems.^{76 77} Further studies are expected to demonstrate the similarities and discrepancies in the roles of such coreceptors between immune and nervous systems.

INHIBITORY MECHANISMS OF LAG-3

The intracellular region of LAG-3 consisting of approximately 60 amino acid residues lacks a typical inhibitory motif, such as immunoreceptor tyrosine-based inhibitory motif. However, it contains several amino acid sequences that are well conserved over different species of LAG-3 but are not shared with other inhibitory coreceptors. Such sequences include FSAL in the juxtamembrane region, KIEELE in the central region, and 10–15 tandem repeats of glutamate, and favorably but not limited to proline (EX-repeat) in the C-terminal region (figure 1D). As the intracellular region is required for LAG-3 to inhibit T cell activation, it can transduce distinct yet undetermined inhibitory signals via such sequences.

Workman *et al*¹³ reported that the lysine residue of the KIEELE sequence is required for the LAG-3-mediated inhibition of the antigen-dependent activation of 3A9 hybridoma T cells. However, a contradictory report has been published and the mechanisms of the contribution of lysine residue to the LAG-3-mediated inhibition have not been elucidated yet.²⁰ Iouzalen *et al*⁷⁸ identified LAG-3-associated protein (LAP) bound to the EX-repeat of LAG-3 by employing yeast two-hybrid cloning experiment. To date, no follow-up study has been reported and the function of LAP remains elusive.

The inhibitory function of LAG-3 strongly correlates with its expression levels on the cell surface, and the amino acid substitutions and deletions substantially affect the expression levels of LAG-3.²⁰ When the potency of the inhibitory effects of mouse LAG-3 mutants was evaluated relative to their expression levels in the antigen-dependent activation of DO11.10 T hybridoma cells, the substitutions of phenylalanine and leucine in the FSAL sequence to alanine reduced the inhibitory capacity of LAG-3 significantly. In addition, when mutations in the FSAL sequence were combined with the deletion of the EX-repeat, the inhibitory capacity of LAG-3 was completely lost similar to the LAG-3 mutant lacking the entire intracellular region. Interestingly, the deletion of EX-repeat alone does not affect the inhibitory capacity of LAG-3. These results demonstrate that LAG-3 likely transduces

two independent inhibitory signals via the FxxL motif and EX-repeat, while the molecular mechanisms of these signals are still unknown.²⁰ Further analyses are expected to delineate the precise molecular mechanisms by which such motifs independently or cooperatively regulate the signaling pathways in T cell activation.

LAG-3 IN AUTOIMMUNITY

Inhibitory coreceptors play critical roles in the establishment and/or maintenance of immune tolerance to self as represented by the spontaneous development of autoimmune diseases in mice deficient for PD-1 and CTLA-4.^{79–81} In addition, immune checkpoint inhibitors targeting PD-1 and CTLA-4 activate not only tumor-specific T cells but also self-reactive T cells to induce tissue toxicities, termed irAEs.^{82–84} Unlike PD-1 and CTLA-4, LAG-3 deficiency itself does not cause autoimmunity in non-autoimmune-prone mouse strains. However, genetic deletion or blockade of LAG-3 exacerbates type 1 diabetes (T1D) in non-obese diabetic (NOD) mice, an animal model of T1D.^{19 85} LAG-3-deficient NOD mice demonstrate accelerated infiltration of autoreactive CD4⁺ and CD8⁺ T cells in islets compared with that in the age-matched LAG-3-sufficient NOD mice. In contrast, NOD mice lacking the cell surface expression of LAG-3 on T_{reg} cells exhibit delayed onset and decreased incidence of T1D, which is attributed to the enhanced proliferation and function of T_{reg} cells in the absence of LAG-3.⁴¹ Mice with compound deficiency of LAG-3 and PD-1 develop lethal autoimmune myocarditis on BALB/c, C57BL/6, and B10.D2 backgrounds, indicating that LAG-3 acts synergistically with PD-1 to prevent autoimmunity.^{19 86}

In addition, LAG-3 has been reported to mitigate the autoimmune symptoms in experimental autoimmune models. Jha *et al*⁸⁷ reported that LAG-3 deficiency or blockade increased the susceptibility to mercury (Hg)-induced autoimmunity by inhibiting the induction of tolerance to Hg in C57BL/6.H2^s mice. In myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) model, Kadowaki *et al*⁸⁸ demonstrated that LAG-3 blockade abrogated the anti-inflammatory effect of gut environment-induced intraepithelial MOG-specific CD4⁺ T cells. Kim *et al*⁸⁹ also reported that the ability of in vitro-generated induced T_{reg} (iT_{reg}) cells to rescue T_{reg}-depleted mice from lethal EAE was dependent on the expression of LAG-3 on the iT_{reg} cells.

Given the critical regulatory role of LAG-3 in autoimmunity, LAG-3 has been expected to be a promising therapeutic target in inflammatory and autoimmune diseases. A humanized anti-LAG-3 Ab with antibody-dependent cell cytotoxic activity (GSK2831781) has been developed to treat autoimmune diseases by eliminating LAG-3-expressing T cells that presumably include pathogenic autoreactive T cells.⁹⁰ In addition, agonistic anti-LAG-3 Ab (IMP761) has been reported to exert immunosuppressive

effects both in vitro and in vivo by eliciting the inhibitory function of LAG-3.⁹¹

LAG-3 IN ANTITUMOR IMMUNITY

As mentioned, LAG-3 is expressed on exhausted CD4⁺ and CD8⁺ tumor-infiltrating T cells that are defective in cytokine production.^{32–36} LAG-3 is also expressed on T_{reg} cells in the peripheral blood and tumor tissues of patients with melanoma, colorectal cancer, and non-small cell lung cancer.^{92 93} Such LAG-3-expressing T_{reg} cells produce high levels of immunoregulatory cytokines IL-10 and TGF- β and suppress tumor-specific T cells. Consistently, the levels of LAG-3 expression and infiltration of LAG-3⁺ cells in tumors have been reported to be associated with tumor progression, poor prognosis, and unfavorable clinical outcomes in various types of human tumors, such as colorectal cancer,⁹⁴ renal cell carcinoma,⁹⁵ follicular lymphoma,³⁶ head and neck squamous cell carcinoma (HNSCC),⁹⁶ non-small cell lung cancer,⁹⁷ breast cancer,⁹⁸ and diffuse large B cell lymphoma.⁹⁹ These results strongly indicate that LAG-3 contributes to immune escape mechanisms in tumors similar to PD-1. Therefore, LAG-3 has been proposed as a promising therapeutic target for cancer immunotherapy, which is also supported by studies using animal models. Tumor growth delayed by anti-LAG-3 Ab has been reported in the mouse models of HNSCC and fibrosarcoma.^{96 100} Grosso *et al*¹⁰¹ demonstrated that the combinatorial therapy incorporating anti-LAG-3 Ab and vaccination with tumor-associated antigen increased the number of activated CD8⁺ T cells in the tumor and disrupted the tumor parenchyma in the tumor-tolerance model of prostate cancer. However, no substantial difference in the grade of tumor was noted between mice administered with combination therapy or vaccination alone.

LAG-3 acts synergistically with PD-1 to suppress antitumor immunity as well as autoimmunity. In patients with epithelial ovarian cancer, Matsuzaki *et al*⁸⁴ observed that approximately 80% and 50% of LAG-3⁺ and LAG-3⁻ tumor-infiltrating CD8⁺ T cells expressed PD-1, respectively. They also reported that the co-blockade of LAG-3 and PD-1 augmented the proliferation and cytokine production of tumor-infiltrating CD8⁺ T cells upon ex vivo stimulation with the tumor-associated antigen NY-ESO-1. Coexpression of LAG-3 and PD-1 on tumor-infiltrating CD4⁺ and CD8⁺ T cells and the strong therapeutic effect of co-blockade or compound genetic deletion of LAG-3 and PD-1 have also been observed in various mouse tumor models, such as B16 melanoma, MC38 colon adenocarcinoma, Sa1N fibrosarcoma, ovalbumin-expressing mouse epithelial ovarian cancer cell (IE9mp1), chronic lymphocytic leukemia derived from Em-TCL1 mice, and recurrent melanoma.^{86 102–104} Although LAG-3 blockade is expected to activate tumor-specific CD4⁺ and/or CD8⁺ T cells in such studies, it may also increase the number of T_{reg} cells, since LAG-3 has been reported to limit the proliferation of T_{reg} cells. According to Goding *et al*,¹⁰² T_{reg} cells are rather reduced by the co-blockade of LAG-3 and PD-1 in the

**Table 1** Summary of LAG-3-targeted drugs under clinical trial

Name	Description	Target disease	Clinical trial*	Combination†
Relatlimab	Antagonistic Ab	Tumors	I (5), I/II (6), II (13)	PD-1
LAG525	Antagonistic Ab	Tumors	I (1), I/II (1), II (3)	PD-1, M-CSF, IL-1 β , A2AR
BI754111	Antagonistic Ab	Tumors	I (3), Ia/Ib (1), II (1)	PD-1
MK-4280	Antagonistic Ab	Tumors	I (1), I/II (1), II (1)	PD-1
Sym022	Antagonistic Ab	Tumors	I (2)	PD-1
TSR-033	Antagonistic Ab	Tumors	I (2)	PD-1, Tim3
REGN3767	Antagonistic Ab	Tumors	I (1), II (1)	PD-1
INCAGN2385-101	Antagonistic Ab	Tumors	I (1)	–
MGD013	Bispecific to LAG-3/PD-1	Tumors	I (2), I/II (1), II/III (1)	–
FS118	Bispecific to LAG-3/PD-L1	Tumors	I (1)	–
XmAb22841	Bispecific to LAG-3/CTLA-4	Tumors	I (1)	PD-1
GSK2831781	Depleting Ab	Autoimmune diseases	I (2), II (1)	–
IMP321	Soluble LAG-3-Ig	Tumors	I (8), II (2)	PD-1, PD-L1, vaccination

*The phase and the number of clinical trials listed on ClinicalTrials.gov are shown.

†Immune-related molecules targeted in combination therapies listed on ClinicalTrials.gov are shown.

A2AR, adenosine A2a receptor; CTLA-4, cytotoxic T lymphocyte antigen 4; IL-1 β , interleukin 1b; M-CSF, macrophage colony stimulating factor; PD-1, programmed cell death 1; PD-L1, PD-1 ligand 1; Tim3, T-cell immunoglobulin and mucin-domain containing-3.

model of recurrence melanoma. Further studies are needed to delineate the exact effect of LAG-3 on the number and function of T_{reg} cells as well as effector T cells.

In most of these studies, anti-LAG-3 Ab resulted in modest therapeutic effects when used as monotherapy but markedly augmented the therapeutic effect of anti-PD-1 Ab. Differences in the inhibitory mechanisms and/or expression profiles of these two molecules most likely explain their functional synergy. Further studies are needed to comprehend the exact mechanisms of the synergistic or additive action of LAG-3 with PD-1.

CLINICAL APPLICATION OF LAG-3

Based on preclinical observations including those mentioned, agents that block or stimulate LAG-3 functions are expected to provide therapeutic benefits in the treatment of cancer or autoimmune diseases, especially when combined with agents targeting PD-1. To date, at least 13 agents that target LAG-3 have been developed (table 1). Anti-LAG-3 blocking Abs (relatlimab (BMS-986016), Sym022, TSR-033, REGN3767, LAG525, INCAGN2385-101, MK-4280, and BI754111) and antagonistic bispecific Abs (MGD013 (anti-PD-1/LAG-3), FS118 (anti-LAG-3/PD-L1), and XmAb22841 (anti-CTLA-4/LAG-3)) are under clinical trials for various cancers either as monotherapy or in combination primarily with anti-PD-1 or anti-PD-L1 blocking Abs. According to the National Cancer Institute Drug Dictionary (<https://www.cancer.gov/publications/dictionaries/cancer-drug>), meeting abstracts,^{105–107} and a published report,¹⁰⁸ most of them are supposed to block the interaction between LAG-3 and MHCII, while their effects on the interaction between LAG-3 and reported ligands other than MHCII are not specified.

To date, only a few interim reports of combinatorial therapies targeting LAG-3 and PD-1 are available. Future reports are awaited to observe the actual therapeutic efficacy of anti-LAG-3 Abs as monotherapy or the exact additive effects of anti-LAG-3 Abs in the combination therapy targeting PD-1 and LAG-3. In phase I/II study evaluating the safety and efficacy of relatlimab in combination with anti-PD-1 Ab (nivolumab) in patients with advanced melanoma that had progressed during previous anti-PD-1 or anti-PD-L1 immunotherapy (NCT0198609), the combination of relatlimab and nivolumab was well tolerated and the objective response rate (ORR) was 11.5% in 61 patients. ORR was at least 3.5-fold higher in patients with LAG-3 expression in at least 1% of tumor-associated immune cells within the tumor margin (n=33) than that in the patients with less than 1% LAG-3 expression (n=22) (18% and 5%, respectively).¹⁰⁹ LAG525 in combination with anti-PD-1 Ab (spartalizumab) exhibited a durable response in 9.9% of patients (n=121) with a variety of solid tumors, including mesothelioma (two of eight patients) and triple-negative breast cancer (two of five patients) in phase I/II study (NCT02460224).¹¹⁰ The precise mechanism of the function of such anti-LAG-3 Abs remains to be investigated. Especially, the examination of target cells in anti-LAG-3 therapy and elucidation of the mechanisms of synergy between anti-LAG-3 and anti-PD-1 therapies have garnered interest and are required for the rational design of anti-LAG-3 therapy with maximum efficacy and minimal adverse effects.

Other LAG-3-targeting agents have also been tested for cancer treatment. IMP321, a soluble recombinant fusion protein comprising the extracellular region of LAG-3 and the Fc region of IgG, has been reported to activate antigen-presenting cells by transducing a reverse signal via MHCII,

resulting in the enhanced production of IL-12 and TNF and the upregulation of CD80 and CD86.^{111–112} IMP321 demonstrated only minimal or modest efficacy as monotherapy or in combination with other therapies in clinical trials conducted so far.^{113–116} The details of the reverse signal via MHCII remain unknown and require careful investigation.

The development of anti-LAG-3 depleting Ab (GSK2831781) and agonistic Ab (IMP761) as potential therapeutic agents in the treatment of autoimmune diseases has also been reported.^{90–91} Although these Abs aim to remove or suppress pathogenic T cells, they may also deplete or suppress T_{reg} cells. Further studies elucidating the functions of such Abs together with the biological properties of LAG-3 are expected to help advance their development by providing the rationale for their use.

CONCLUSIONS

As checkpoint immunotherapies targeting inhibitory coreceptors PD-1 and CTLA-4 revolutionized cancer treatment, LAG-3 is expected to be a highly promising target in cancer therapies. However, our understanding of LAG-3 is still very limited and many fundamental questions remain unanswered. The signaling mechanism of LAG-3 is unknown and the ligands of LAG-3 are perplexing. LAG-3 is expressed on a variety of cell types. However, the function of LAG-3 and the effect of LAG-3 blockade in each type of cells have not been elucidated. We also need to examine the functional differences, redundancies, and co-operations of LAG-3 and other coreceptors. By elucidating the functional properties of LAG-3 more in detail, we can rationally design LAG-3-targeted therapies for various diseases, such as cancer, autoimmunity, and infection.

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