European Review for Medical and Pharmacological Sciences 2023; 27: 248-255

Research progress of immune checkpoint LAG-3 in gastric cancer: a narrative review

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Abstract. – In recent years, the immunotherapy of gastric cancer has made a breakthrough. With the emergence of immune checkpoint inhibitors, blocking the inhibitory molecules in the body can reactivate the immune system to resist tumors, which dramatically improves the survival rate of gastric cancer patients. Lymphocyte activation gene-3 (LAG-3), also known as CD223, is a kind of immune checkpoint receptor protein, mainly expressed in activated immune cells, and it has the functions of maintaining internal environment stability and immunological regulation and is closely related to the occurrence and development of tumor. Therefore, LAG-3 can be used as a new target for tumor immunotherapy. In this narrative review, the structure, immunological function, and research progress of immune checkpoint LAG-3 in gastric cancer is explored to provide a reference for further research and immunotherapy of gastric cancer.

Key Words:
Lymphocyte activation gene 3 (LAG-3), Immune checkpoint, Gastric cancer, Immunotherapy.

Introduction

According to the latest global cancer statistics, gastric cancer (GC) is one of the most malignant cancers in human beings, with the highest morbidity and mortality among the world's five malignant tumors[1]. East Asia has the highest incidence of stomach cancer. According to the latest global cancer data released by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), there were about 480,000 new cases of gastric cancer in China in 2020, accounting for about 44% of the recent cases of gastric cancer in the world[2]. In fact, gastric cancer is already the third most common malignancy in China[3]. Studies[4] have reported a 5-year survival rate of over 90% for early gastric cancer. However, most gastric cancer patients are already beyond the early stages when first diagnosed because the disease is stealth-onset and progresses rapidly. Even with perioperative and adjuvant chemotherapy or chemoradiotherapy, 5-year disease survival in patients beyond stage II decreased significantly, from 61-63% in stage IIIa to 30%-35% in stage IIIc[5].

With the progress of research on the pathological features and molecular classification of GC, the treatment method has gradually changed from an extensive mode to a more accurate individualized treatment mode based on traditional chemotherapy. At present, a variety of new treatment methods such as targeted therapy and immunotherapy bring hope for improving the prognosis of patients, but it is far less optimistic than one might expect[6].The Cancer Genome Atlas (TCGA) proposes a new classification of four subtypes based on molecular classification, namely EBV-positive, microsatellite instability (MSI), genome stability and chromosomal instability, emphasizing the role of PD-1 and its receptor PD-L1 in tumor immune evasion[7]. In subsequent studies, targeted therapy, as an important treatment method in the treatment of malignant tumors, has significantly improved the overall survival rate of patients[8]. In addition, immune checkpoint LAG-3 and LAG-3 are found in a stable internal environment and play an important role in immune regulation function and changes in cancer conditions It is expected to become the successor of programmed cell death 1/programmed cell death 1 ligand, Pd-1 /PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), followed by another novel antitumor target. This paper aims to summarize the structure, immunological function, and research progress of immune checkpoint LAG-3 in gastric cancer, expecting to further study LAG-3 and new ideas of immunotherapy for gastric cancer.

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The Structure of LAG-3

The Genomic Location of LAG-3

In 1990, Triebel et al[9] identified LAG-3 lymphocyte activating gene 3(LAG3, CD223) as an immunosuppressive checkpoint, located on human chromosome 12 (20P13.3) and on chromosome 6 in mice, a screening of selectively expressed molecules isolated from F5 cells. It is described on the surface of lymphocytes, such as CD4+T cells, CD8+T cells, natural killer (NK) cells, natural killer T (NKT) cells, and regulatory T (Treg) cells and stored in lysosomes, which appear more rapidly when T cells are activated[10-12].

Protein Structure of LAG-3

LAG-3 is a type I transmembrane protein, with a molecular weight of 70,000 and composed of 489 amino acids, which is divided into a cytoplasmic region, extracellular region and transmembrane region[13]. It has structural homology with CD4. The extracellular domain consists of four immunoglobulin superfamily (Ig SF) domains, namely, D1, D2, D3, and D4, consisting of eight cysteine residues and four N-linked glycosylation sites. The transmembrane region is a long-linked peptide linked to D4, encoded by exon VII. The cytoplasmic region has three conserved domains: the first region is a serine phosphorylation site, the second region contains the unique KIEELE motif, which has been shown to be critical for the inhibitory function of LAG-3 in effector CD4 + T cells, preventing T cells from entering the S-phase of the cell cycle and thus inhibiting T cell amplification[14]. The third is glutamate-proline (EP) duplication, which binds to the LAG-3 associated protein (LAP) and thus helps locate LAG-3. Although LAG-3 is structurally similar to CD4, only about 20% of the aminoacid sequences in the two molecules are identical[15]. Similar to CD4, LAG-3 binds to the major histocompatibility complex II (MHC-II) on antigen-presenting cells (APC) but has a stronger affinity. In addition, Li et al[16] conducted an in-depth study and they found that after T cells were stimulated by antigen, the molecule LAG-3 on the membrane surface would break and split into two membrane-related fragments P54 and P16 on the membrane. P54 molecular weight of 54 kDa, containing D1, D2, and D3 domains, was released in a soluble form, namely, soluble LAG-3(sLAG-3). P16 is a transmembrane intracellular part with a molecular weight of about 16 kDa. The essence of the molecular rupture is the cleavage of the linking peptide between the D4 domain at the proximal end of the LAG-3 membrane and the transmembrane region, which is mediated by matrix metalloproteinases ADAM10 and ADAM17[17]. Therefore, the molecules of LAG-3 generally exist in two forms *in vivo*: membrane LAG-3 (mLAG-3 or LAG-3) and sLAG-3. The two forms are not only very different in structure, but also have diametrically opposite immunological functions.

Ligand of LAG-3

As the classical ligand of LAG-3, MHC-II has a higher affinity for MHC-II than FOR CD4, and inhibits T cell activation by interfering with THE binding of CD4 to MHC-II[18]. However, it has been confirmed that anti-LAG-3 antibodies without blocking MHC-II binding can still stimulate T cell activation and antitumor activity. In view of these results, other ligands may exist in LAG-3[19].

Hepatic sinusoidal endothelial cell lectin (LSECtin) is a member of the c-type lectin family and is mainly expressed in the liver[20]. Xu et al[21] adopted surface plasmon resonance (SPR) technology and cell staining method and found that LAG-3 inhibited the production of IFN-γ by anti-CD3 antibody in Lsectin-expressing melanoma cells. It was confirmed that LSECtin was one of the ligands of LAG-3.

Galectin-3, a member of the galectin family, is a soluble galactose-binding lectin secreted by various types of tumor cells and tumor stromal cells[22]. Kouo et al[23] used immunoprecipitation to find that galectin-3 interacts with LAG-3 and inhibits IFN-γ secretion by CD8 + T cells *in vitro*, proving that galectin-3 is also a ligand of LAG-3.

α -synuclein fibrils (α -syn fibrils) is a protein aggregate found in the enormous brain substantia nigra in patients with tremor paralysis and is one of the members of the Synucleus egg white family[24,25]. Mao et al[26] research found that pathogenic α -Syn fibrils can be transmitted between cells by binding to LAG-3 and blocking the binding of the two LAG-3 antibodies can significantly reduce the toxicity of pathological α-Syn fibrils and their transmission between cells, suggesting that α-Syn fibrils are ligands of LAG-3.

Recently, Wang et al[27] found that fibrin original protein 1 (FGL1), a member of the fibrinogen family, is a potential ligand of LAG-3. FGL1 is secreted by hepatocytes in the liver under normal physiological conditions, and some tumor cells can also produce FGL1 at high levels. The inter-

action sites of LAG3 and FGL1 are D1 and D2 of LAG3 and FD of FGL1. The interaction of LAG3 and FGL1 may lead to changes in the tumor immune microenvironment, such as reduced IL-2 levels[28]. However, further studies are needed to clarify whether and how each of these potential ligands independently and or synergistically contribute to the function of LAG-3.

Immunological Function of LAG-3

LAG-3 and T Cells

Activation of initial T cells requires the combined stimulation of 2 different extracellular signals (dual signal activation hypothesis): the first signal comes from the antigen. The interaction and binding of HMC- antigen peptide complex on the surface of antigen presenting cells (APC) with T cell receptor (TCR) is introduced into cells by CD3. The second signal is the microbial product or the response molecule of innate immunity to the microorganism, i.e., the costimulatory molecule[29]. LAG-3 was expressed in T H1 cells, but not in T H2 cells. Il-12 had the most tremendous potential to stimulate the expression of LAG-3[30]. LAG-3 negatively regulates T cell expansion and controls the memory T cell pool[31]. This negative regulatory function is associated with LAG-3: the binding of MHC-II molecules is inseparable and requires signal transduction through the cytoplasmic regional structure, especially the highly conserved KIEELE sequence. LAG-3 binds MHC-II and conducts negative regulatory signals through the TCR-CD3 complex. Antibody cross-linking of human T cells has shown that LAG-3 binds to CD3 in the T cell receptor (TCR) complex, resulting in T cell proliferation and reduced cytokine production[32]. This regulatory function is not competitive with CD4 molecules binding MHC class II molecules. Thus, LAG-3 is an independent negative regulatory molecule[33].

LAG -3 can regulate signal transduction in Treg and sensitivity to Treg suppression by limiting STAT5 signal transduction, and LAG-3 signal transduction can also increase the differentiation of Foxp3+Treg. When LAG-3 is blocked, the induction of Foxp3+Treg is reduced, resulting in reduced inhibition and increased CD4+T cell amplification[34] The LAG -3 expression makes it more susceptible to Treg-based inhibition and modulates Th1 cellular response. Huang et al[35] had proven that Treg cells based on LAG-3 deficient mice inhibited the activation of effector T cells with low efficiency, demonstrating that LAG-3 may be directly involved in the selective up-regulation of Treg function and is necessary to induce the maximum inhibitory activity of Treg.

Regulation of LAG-3 On APC Cells

Dendritic cells (DCSS) include myeloid dendritic cells (mDC) and plasmoid dendritic cells (pDC), which have the ability of antigen presentation and activate lymphocytes to participate in specific immune responses. PDC is highly expressed with LAG-3[36]. LAG-3 expressed in Treg cells can bind to MHC class II molecules on APCS, especially dendritic cells (DCS), which inhibit THE maturation of DCSS through cytoplasmic signal transduction and induce the formation of tolerance DCS. In turn, it regulates the activation and proliferation of T cells, which requires the involvement of immune receptor tyrosine activation motifs. Meanwhile, LAG-3 may synergistically enhance the inhibitory activity of Treg cells with other inhibitory molecules (PD-1, CTLA-4, etc.), leading to APC-induced immune tolerance[37]. Studies[38] using human DCS: Treg co-culture showed that antibodies blocking LAG-3 could block Treg-mediated DC inhibition.

LAG-3 and Natural Killer Cells

Lag-3 has been confirmed to be expressed in activated NK cells, but its direct effect and mechanism are still not fully understood. Sun et al[39] studied the interaction between cytokines and NK cells and found that IL-12 was the most effective inducer of LAG-3 and transforming growth factor-β was the most potent inhibitor of PD-1. At the same time, LAG-3 down-regulated the proliferation of NKT cells expressing NK and T cell receptors. Soluble recombinant LAG-3-IG fusion white (IMP321) induced the production of cytokines (IFN-γ and, or TNF-α) in NK cells in healthy individuals (52 of 60 donors) and 21 patients with untreated metastatic cancer for a short time[40]. IMP321 can be used as monotherapy to induce NK cell activation in dose-escalation studies in patients with metastatic renal carcinoma[41]. Therefore, LAG-3 has the potential to activate NK cells, but the function and potential mechanism of LAG-3 on NK cells need to be further studied.

Research Progress of LAG-3 in Gastric Cancer

LAG-3 is a checkpoint molecule expressed by T lymphocytes (CD4+ and CD8+) and acts as a negative regulator of T cell function when interact-

ing with its ligand[42]. The expression of LAG-3 on tumor-infiltrating lymphocytes or chronic virus-infected T cells is associated with immune dysfunction and is characterized by T cell depletion[43]. T cell failure is characterized by a gradual loss of effector function, particularly the production of pro-inflammatory cytokines such as IL-2, tumor necrosis factor -α, and interferon -γ, as well as the continuous expression of the inhibitory receptor programmed cell death receptor-1 (PD-1), cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), lymphocyte-activating gene-3 (LAG-3) and T cell immunoglobulin (TIM-3) that inhibit T cell activity[44,45]. LAG-3 has been expressed in Tumor-infiltrating lymphocyte (until) in various solid tumors, including esophageal cancer, melanoma, lymphoma and hepatocellular carcinoma, and co-expressed with other immunosuppressive molecules[46-48]. Antitumor efficacy was decreased by inhibiting TIL activity. Scholars[47] confirmed that the analysis of 34 gastric cancer specimens showed that 88% of the specimens had LAG-3 positive immune infiltration. Recent studies[49] have shown that the higher proportion of LAG3+CD4+/CD4+T cells and LAG3+CD8+/CD8+T cells in advanced gastric cancer, the better the prognosis is, and the higher LAG3 expression is associated with better prognosis. FGL1, a newly developed ligand of LAG-3, was found to be positively correlated with gastric cancer stage, lymph node metastasis and overall survival[50]. In addition, both MHC II and LSECtin are ligands of LAG3, and their expression indicates good survival of gastric cancer. Li et al[51] found in the study that sLAG3 acts as a soluble form of LAG-3. sLAG3 positively regulates CD8+T cells, IL-12, and interferon -γ in the peripheral blood of gastric cancer patients, and the high expression of sLAG3 is associated with a better prognosis. *In vivo* experiments showed that SLAG3 inhibited tumor growth and promoted the secretion of CD8+T cells, IL-12 and interferon -γ. SLAG3 also prolonged the overall survival time and improved the survival rate of tumor-bearing mice. These results suggest that sLAG3 may be a potential treatment for GC associated with tumor immunity. However, more statistics are needed to provide some more reliable results, and more specific mechanisms by which sLAG3 affects the frequency of CD8+T cells in GC are yet to be discovered.

The current anti-PD-1 monoclonal antibody, navurliumab, has been shown to be effective against advanced gastric cancer (AGC). Ohmura et al[52] used flow cytometry to systematically analyze the proportion of peripheral immune cell subsets and serum cytokine concentrations in 30 AGC patients treated with navurliumab before the first and second treatment and during disease progression. The expression level of LAG-3 on T cells was closely related to the efficacy of navurliumab treatment. Mimura et al[53] tested 365 gastric cancer samples by immunohistochemical method and found that LAG-3 could be used as a potential biomarker for anti-PD-1 treatment. Cen et al[54] using gene expression profiles in TCGA and GEO datasets, found that high expression of HER2 was significantly associated with low expression at multiple immune checkpoints, including LAG-3, in the TCGA dataset, and similar results were found in the GSE84437 dataset. Furthermore, the actual situation of tumor tissues was further studied. Immunohistochemistry was performed on the samples of gastric cancer patients. It was also found that the expression level of LAG-3 in gastric cancer tissues with high HER2 expression was significantly lower than that in the group with low HER2 expression.

However, Lv et al[55] found that LAG-3 expression is a poor prognostic factor in EBV-positive gastric cancer, which may be related to the immune escape environment characterized by the reduction of interferon -γ+ cells and perforin -1+ cells and the increase of Tregs and M2 macrophages. Thus, we found that the expression of LAG-3 has different biological significance in various types of gastric cancer. The Cancer Genome Atlas Project has identified four major genomic subtypes found in GC adenocarcinoma: Epstein-barr virus (EBV) positive microsatellite instability (MSI), genomic stability and chromosomal instability[56,57]. Further clarifies the biological significance of LAG-3 in different types of gastric cancer, which is conducive to better elucidate the role of LAG-3 in the occurrence and development of gastric cancer.

Currently, researchers consider LAG-3 as an emerging immune checkpoint and a very promising therapeutic target, and multiple approaches involving LAG-3 targeted immunotherapy are in clinical trials[58]. The first is IMP321, a soluble LAG-3IG fusion protein, which has shown moderate success in clinical trials[59] in renal cell carcinoma, metastatic breast cancer, and melanoma, where IMP321 was found to enhance DCs proliferation and reduce the immunosuppressive effect of Tregs. The second type is antagonistic LAG-3 antibodies, such as BMS-986016, TSR-033, LAG525 and REGN3767, which have the ability to release the anti-tumor immune response. Numerous clinical trials[60] are underway to evaluate the efficacy of LAG-3 antibody monotherapy or in combination with PD-1 antibody. The third category is

first-class bispecific proteins that bind PD-1 and LAG-3, such as MGD013 and FS118, which are currently undergoing phase I clinical trials[61]. Phase I/II clinical trials are currently being recruited using anti-LAG-3 monotherapy in combination with anti-LAG-3 and anti-PD-1 in solid tumors such as gastric cancer. Phase II clinical trials of LAG525 in combination with PDR001 in gastric cancer and other solid tumors are also ongoing[62]. Up to now, clinical trials of immunotherapy of immune checkpoint LAG-3 in gastric cancer and common gastrointestinal tumors are shown in Table I.

Conclusions

Currently, gastric cancer has become the most common malignant tumor with the highest morbidity and mortality in the world. Most gastric cancers are already in the advanced stage when diagnosed. Although perioperative adjuvant chemotherapy was performed, its survival is still not satisfactory. With the advent of immune checkpoint inhibitors, immunotherapy has become one of the breakthroughs in the treatment of gastric cancer in recent years and has become an effective treatment after surgery, chemotherapy, and radiotherapy. As checkpoint immunotherapy targeting inhibitory co-receptors PD-1 and CTLA-4 has revolutionized gastric cancer therapy, LAG-3 is expected to become a very promising target for gastric cancer therapy. However, our understanding of LAG-3 in gastric cancer is still very limited, and many fundamental questions remain unanswered. The signal transduction mechanism of LAG-3 in gastric cancer is still unclear, and its ligand and signal transduction mechanism, as well as the unknown association between ligands, are also puzzling. Therefore, solving these critical problems is helpful in optimizing the targeted treatment strategy of LAG-3 and further improve the targeted treatment effect of LAG-3 in gastric cancer.

Table I. Clinical trials of lag-3 immunotherapy in gastric cancer and common gastrointestinal tumors (Available at: https://www.ClinicalTrials.gov).

Drug name	Drug form	Clinical approval date	Clinical trial registration number	Clinical disease
IMP321	Synthetic protein	2009-02	NCT00732082	Pancreas cancer
Sym022	Monoclonal antibody	2018-05	NCT03489369	Metastatic cancer; solid tumor;
RO7247669	Bispecific antibody	2019-11	NCT04140500	Esophageal squamous cell carcinoma
BMS-986213	Monoclonal antibody	2018-10	NCT03662659	Gastric cancer; cancer of the stomach; esophagogastric junction
LAG525	Monoclonal antibody	2018-01	NCT03365791	Gastric adenocarcinoma; esophageal adenocarcinoma;
GSK2831781 RO7121661	Monoclonal antibody	2019-05	NCT03893565	Colitis; ulcerative
RO7247669	Bispecific antibody	2021-06	NCT04785820	Advanced or metastatic esophageal squamous cell carcinoma
Relatlimab	Monoclonal antibody	2019-02	NCT03642067	Microsatellite stable (mss) colorectal adenocarcinomas; colorectal adenocarcinoma
Relatlimab	Monoclonal antibody	2021-05	NCT04658147	Hepatocellular carcinoma
XmAb®22841	Monoclonal bispecific antibody	2019-05	NCT03849469	Pancreatic carcinoma; hepatocellular carcinoma; gastric or gastroesophageal Junction adenocarcinoma; advanced or metastatic solid Tumors; intrahepatic cholangiocarcinoma; squamous cell anal cancer colorectal carcinoma;
INCAGN02385	IgG1-Fc	2018-09	NCT03538028	Gastric cancer
MGD013	Bispecific antibody	2020-02	NCT04178460	Gastric cancer

Conflict of Interest
The Authors declare that they have no conflict of interests.

Funding
This paper relies on the 2020 Qinghai Provincial Health System Guidance Plan (2021-wjzdx-01).

Informed Consent
Not applicable.

Ethics Approval
Not required.

References

1) The global, regional, and national burden of stomach cancer in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease study 2017. Lancet Gastroenterol Hepatol 2020; 5: 42-54.
2) Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249.
3) Du L, Zhao Z, Zheng R, Li H, Zhang S, Li R, Wei W, He J. Epidemiology of Thyroid Cancer: Incidence and Mortality in China, 2015. Front Oncol 2020; 10: 1702.
4) Green PH, O'Toole KM, Slonim D, Wang T, Weg A. Increasing incidence and excellent survival of patients with early gastric cancer: experience in a United States medical center. Am J Med 1988; 85: 658-661.
5) Li P, Huang CM, Zheng CH, Russo A, Kasbekar P, Brennan MF, Coit DG, Strong VE. Comparison of gastric cancer survival after R0 resection in the US and China. J Surg Oncol 2018; 118: 975-982.
6) Benson AB. Advanced gastric cancer: an update and future directions. Gastrointest Cancer Res 2008; 2: 47-53.
7) Chia NY, Tan P. Molecular classification of gastric cancer. Ann Oncol 2016; 27: 763-769.
8) Hsu PC, Jablons DM, Yang CT, You L. Epidermal Growth Factor Receptor (EGFR) Pathway, Yes-Associated Protein (YAP) and the Regulation of Programmed Death-Ligand 1 (PD-L1) in Non-Small Cell Lung Cancer (NSCLC). Int J Mol Sci 2019; 20: 3821.
9) Yu L, Sun M, Zhang Q, Zhou Q, Wang Y. Harnessing the immune system by targeting immune checkpoints: Providing new hope for Oncotherapy. Front Immunol 2022; 13: 982026.
10) Andrews LP, Marciscano AE, Drake CG, Vignali DA. LAG3 (CD223) as a cancer immunotherapy target. Immunol Rev 2017; 276: 80-96.
11) Solinas C, Migliori E, De Silva P, Willard-Gallo K. LAG3: The Biological Processes That Motivate Targeting This Immune Checkpoint Molecule in Human Cancer. Cancers (Basel) 2019; 11.
12) Ruffo E, Wu RC, Bruno TC, Workman CJ, Vignali DAA. Lymphocyte-activation gene 3 (LAG3): The next immune checkpoint receptor. Semin Immunol 2019; 42: 101305.
13) Wang M, Du Q, Jin J, Wei Y, Lu Y, Li Q. LAG3 and its emerging role in cancer immunotherapy. Clin Transl Med 2021; 11: e365.
14) Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation. Immunity 2016; 44: 989-1004.
15) Li N, Wang Y, Forbes K, Vignali KM, Heale BS, Saftig P, Hartmann D, Black RA, Rossi JJ, Blobel CP, Dempsey PJ, Workman CJ, Vignali DA. Metalloproteases regulate T-cell proliferation and effector function via LAG-3. Embo j 2007; 26: 494-504.
16) Graydon CG, Mohideen S, Fowke KR. LAG3's Enigmatic Mechanism of Action. Front Immunol 2020; 11: 615317.
17) Orme JJ, Jazieh KA, Xie T, Harrington S, Liu X, Ball M, Madden B, Charlesworth MC, Azam TU, Lucien F, Wootla B, Li Y, Villasboas JC, Mansfield AS, Dronca RS, Dong H. ADAM10 and ADAM17 cleave PD-L1 to mediate PD-(L)1 inhibitor resistance. Oncoimmunology 2020; 9: 1744980.
18) Workman CJ, Rice DS, Dugger KJ, Kurschner C, Vignali DA. Phenotypic analysis of the murine CD4-related glycoprotein, CD223 (LAG-3). Eur J Immunol 2002; 32: 2255-2263.
19) Chen X, Song X, Li K, Zhang T. FcγR-Binding Is an Important Functional Attribute for Immune Checkpoint Antibodies in Cancer Immunotherapy. Front Immunol 2019; 10: 292.
20) Liu W, Tang L, Zhang G, Wei H, Cui Y, Guo L, Gou Z, Chen X, Jiang D, Zhu Y, Kang G, He F. Characterization of a novel C-type lectin-like gene, LSECtin: demonstration of carbohydrate binding and expression in sinusoidal endothelial cells of liver and lymph node. J Biol Chem 2004; 279: 18748-18758.
21) Xu F, Liu J, Liu D, Liu B, Wang M, Hu Z, Du X, Tang L, He F. LSECtin expressed on melanoma cells promotes tumor progression by inhibiting antitumor T-cell responses. Cancer Res 2014; 74: 3418-3428.
22) Ruvolo PP. Galectin 3 as a guardian of the tumor microenvironment. Biochim Biophys Acta 2016; 1863: 427-437.
23) Kouo T, Huang L, Pucsek AB, Cao M, Solt S, Armstrong T, Jaffee E. 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: 412-423.

24) Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. Nat Rev Neurol 2013; 9: 13-24.

25) Goedert M, Jakes R, Spillantini MG. The Synucleinopathies: Twenty Years On. J Parkinsons Dis 2017; 7: 51-69.

26) Mao X, Ou MT, Karuppagounder SS, Kam TI, Yin X, Xiong Y, Ge P, Umanah GE, Brahmachari S, Shin JH, Kang HC, Zhang J, Xu J, Chen R, Park H, Andrabi SA, Kang SU, Gonçalves RA, Liang Y, Zhang S, Qi C, Lam S, Keiler JA, Tyson J, Kim D, Panicker N, Yun SP, Workman CJ, Vignali DA, Dawson VL, Ko HS, Dawson TM. Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. Science 2016; 353.

27) Wang J, Sanmamed MF, Datar I, Su TT, Ji L, Sun J, Chen L, Chen Y, Zhu G, Yin W, Zheng L, Zhou T, Badri T, Yao S, Zhu S, Boto A, Sznol M, Melero I, Vignali DAA, Schalper K, Chen L. Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3. Cell 2019; 176: 334-347.

28) Lecocq Q, Keyaerts M, Devoogdt N, Breckpot K. The Next-Generation Immune Checkpoint LAG-3 and Its Therapeutic Potential in Oncology: Third Time's a Charm. Int J Mol Sci 2020; 22.

29) Joller N, Kuchroo VK. Tim-3, Lag-3, and TIGIT. Curr Top Microbiol Immunol 2017; 410: 127-156.

30) Andersson PO, Stockelberg D, Jacobsson S, Wadenvik H. A transforming growth factor-beta1-mediated bystander immune suppression could be associated with remission of chronic idiopathic thrombocytopenic purpura. Ann Hematol 2000; 79: 507-513.

31) Workman CJ, Cauley LS, Kim IJ, Blackman MA, Woodland DL, Vignali DA. Lymphocyte activation gene-3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo. J Immunol 2004; 172: 5450-5455.

32) Batista-Duharte A, Hassouneh F, Alvarez-Heredia P, Pera A, Solana R. Immune Checkpoint Inhibitors for Vaccine Improvements: Current Status and New Approaches. Pharmaceutics 2022; 14.

33) Goldberg MV, Drake CG. LAG-3 in Cancer Immunotherapy. Curr Top Microbiol Immunol 2011; 344: 269-278.

34) Durham NM, Nirschl CJ, Jackson CM, Elias J, Kochel CM, Anders RA, Drake CG. Lymphocyte Activation Gene 3 (LAG-3) modulates the ability of CD4 T-cells to be suppressed in vivo. PLoS One 2014; 9: e109080.

35) Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HI, Powell JD, Pardoll DM, Drake CG, Vignali DA. Role of LAG-3 in regulatory T cells. Immunity 2004; 21: 503-513.

36) Workman CJ, Wang Y, El Kasmi KC, Pardoll DM, Murray PJ, Drake CG, Vignali DA. LAG-3 regulates plasmacytoid dendritic cell homeostasis. J Immunol 2009; 182: 1885-1891.

37) Liang B, Workman C, Lee J, Chew C, Dale BM, Colonna L, Flores M, Li N, Schweighoffer E, Greenberg S, Tybulewicz V, Vignali D, Clynes R. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. J Immunol 2008; 180: 5916-5926.

38) Bayry J, Triebel F, Kaveri SV, Tough DF. Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with CD4+CD25+ regulatory T cells. J Immunol 2007; 178: 4184-4193.

39) Sun H, Sun C, Xiao W. Expression regulation of co-inhibitory molecules on human natural killer cells in response to cytokine stimulations. Cytokine 2014; 65: 33-41.

40) Brignone C, Grygar C, Marcu M, Schäkel K, Triebel F. A soluble form of lymphocyte activation gene-3 (IMP321) induces activation of a large range of human effector cytotoxic cells. J Immunol 2007; 179: 4202-4211.

41) Brignone C, Escudier B, Grygar C, Marcu M, Triebel F. A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. Clin Cancer Res 2009; 15: 6225-6231.

42) Passariello M, Yoshioka A, Takahashi K, Hashimoto SI, Rapuano Lembo R, Manna L, Nakamura K, De Lorenzo C. Novel Bi-Specific Immuno-Modulatory Tribodies Potentiate T Cell Activation and Increase Anti-Tumor Efficacy. Int J Mol Sci 2022; 23.

43) Freeman GJ, Sharpe AH. A new therapeutic strategy for malaria: targeting T cell exhaustion. Nat Immunol 2012; 13: 113-115.

44) Pilon-Thomas S, Kuhn L, Ellwanger S, Janssen W, Royster E, Marzban S, Kudchadkar R, Zager J, Gibney G, Sondak VK, Weber J, Mulé JJ, Sarnaik AA. Efficacy of adoptive cell transfer of tumor-infiltrating lymphocytes after lymphopenia induction for metastatic melanoma. J Immunother 2012; 35: 615-620.

45) Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 2015; 15: 486-499.

46) Anzengruber F, Ignatova D, Schlaepfer T, Chang YT, French LE, Pascolo S, Contassot E, Bobrowicz M, Hoetzenecker W, Guenova E. Divergent LAG-3 versus BTLA, TIGIT, and FCRL3 expression in Sézary syndrome. Leuk Lymphoma 2019; 60: 1899-1907.

47) Datar I, Sanmamed MF, Wang J, Henick BS, Choi J, Badri T, Dong W, Mani N, Toki M, Mejías LD, Lozano MD, Perez-Gracia JL, Velcheti V, Hellmann MD, Gainor JF, McEachern K, Jenkins D, Syrigos K, Politi K, Gettinger S, Rimm DL, Herbst RS, Melero I, Chen L, Schalper KA. Expression Analysis and Significance of PD-1, LAG-3, and TIM-3 in Human Non-Small Cell Lung Cancer Using Spatially Resolved and Multiparametric Single-Cell Analysis. Clin Cancer Res 2019; 25: 4663-4673.

48) Chen BJ, Dashnamoorthy R, Galera P, Makarenko V, Chang H, Ghosh S, Evens AM. The im-

mune checkpoint molecules PD-1, PD-L1, TIM-3 and LAG-3 in diffuse large B-cell lymphoma. Oncotarget 2019; 10: 2030-2040.

49) Park Y, Seo AN, Koh J, Nam SK, Kwak Y, Ahn SH, Park DJ, Kim HH, Lee HS. Expression of the immune checkpoint receptors PD-1, LAG3, and TIM3 in the immune context of stage II and III gastric cancer by using single and chromogenic multiplex immunohistochemistry. Oncoimmunology 2021; 10: 1954761.

50) Zhang Y, Qiao HX, Zhou YT, Hong L, Chen JH. Fibrinogen-like-protein 1 promotes the invasion and metastasis of gastric cancer and is associated with poor prognosis. Mol Med Rep 2018; 18: 1465-1472.

51) Li N, Jilisihan B, Wang W, Tang Y, Keyoumu S. Soluble LAG3 acts as a potential prognostic marker of gastric cancer and its positive correlation with CD8+T cell frequency and secretion of IL-12 and INF-γ in peripheral blood. Cancer Biomark 2018; 23: 341-351.

52) Ohmura H, Yamaguchi K, Hanamura F, Ito M, Makiyama A, Uchino K, Shimokawa H, Tamura S, Esaki T, Mitsugi K, Shibata Y, Oda H, Tsuchihashi K, Ariyama H, Kusaba H, Oda Y, Akashi K, Baba E. OX40 and LAG3 are associated with better prognosis in advanced gastric cancer patients treated with anti-programmed death-1 antibody. Br J Cancer 2020; 122: 1507-1517.

53) Mimura K, Kua LF, Xiao JF, Asuncion BR, Nakayama Y, Syn N, Fazreen Z, Soong R, Kono K, Yong WP. Combined inhibition of PD-1/PD-L1, Lag-3, and Tim-3 axes augments antitumor immunity in gastric cancer-T cell coculture models. Gastric Cancer 2021; 24: 611-623.

54) Cen S, Xu H, Liu Z, Zhao R, Pan H, Han W. Immune microenvironment characteristics and their implications for immune checkpoint inhibitor efficacy in HER2-overexpressing gastric cancer. Clin Exp Immunol 2022; 207: 318-328.

55) Lv K, Li R, Cao Y, Gu Y, Liu X, He X, Jin K, Fang H, Fei Y, Shi M, Liu H, Li H, He H, Lin C, Zhang H, Xu J. Lymphocyte-activation gene 3 expression associates with poor prognosis and immunoevasive contexture in Epstein-Barr virus-positive and MLH1-defective gastric cancer patients. Int J Cancer 2021; 148: 759-768.

56) Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014; 513: 202-209.

57) Lin DC, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, Garg M, Liu LZ, Yang H, Yin D, Shi ZZ, Jiang YY, Gu WY, Gong T, Zhang Y, Xu X, Kalid O, Shacham S, Ogawa S, Wang MR, Koeffler HP. Genomic and molecular characterization of esophageal squamous cell carcinoma. Nat Genet 2014; 46: 467-473.

58) Perez-Santos M, Anaya-Ruiz M, Cebada J, Bandala C, Landeta G, Martínez-Morales P, Villa-Ruano N. LAG-3 antagonists by cancer treatment: a patent review. Expert Opin Ther Pat 2019; 29: 643-651.

59) Legat A, Maby-El Hajjami H, Baumgaertner P, Cagnon L, Abed Maillard S, Geldhof C, Iancu EM, Lebon L, Guillaume P, Dojcinovic D, Michielin O, Romano E, Berthod G, Rimoldi D, Triebel F, Luescher I, Rufer N, Speiser DE. Vaccination with LAG-3Ig (IMP321) and Peptides Induces Specific CD4 and CD8 T-Cell Responses in Metastatic Melanoma Patients--Report of a Phase I/IIa Clinical Trial. Clin Cancer Res 2016; 22: 1330-1340.

60) Hahn AW, Gill DM, Pal SK, Agarwal N. The future of immune checkpoint cancer therapy after PD-1 and CTLA-4. Immunotherapy 2017; 9: 681-692.

61) Long L, Zhang X, Chen F, Pan Q, Phiphatwatchara P, Zeng Y, Chen H. The promising immune checkpoint LAG-3: from tumor microenvironment to cancer immunotherapy. Genes Cancer 2018; 9: 176-189.

62) Puhr HC, Ilhan-Mutlu A. New emerging targets in cancer immunotherapy: the role of LAG3. ESMO Open 2019; 4: e000482.