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*Review Article*

# Lymphocyte-activation gene-3, an important immune checkpoint in cancer

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**Immunotherapy has recently become widely used in lung cancer. Many oncologists are focused on cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death ligand-1 (PD-L1) and programmed cell death-1 (PD-1). Immunotherapy targeting the PD-1/PD-L1 checkpoints has shown promising efficacy in non-small cell lung cancer (NSCLC), but questions remain to be answered. Among them is whether the simultaneous inhibition of other checkpoints could improve outcomes. Lymphocyte-activation gene-3 (LAG-3) is another vital checkpoint that may have a synergistic interaction with PD-1/PD-L1. Here we review the LAG-3 function in cancer, clinical trials with agents targeting LAG-3 and the correlation of LAG-3 with other checkpoints.**

In 2013, *American Science* ranked cancer immunotherapy as one of the most important scientific breakthroughs.[1] Cancer immunotherapy can reverse tumor immune escape by suppressing immune checkpoint pathways. It is possible that the inhibition of pathway checkpoints, including cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1), could activate T cells to attack and eliminate cancer.[2–7]

In recent years, several trials of agents that block CTLA-4, PD-L1 and PD-1 have demonstrated durable efficacy against melanoma, renal, lung and other cancers.[1,4–17] In March 2015, immunotherapy reached another milestone when the FDA approved nivolumab as a second line treatment for metastatic lung squamous carcinoma. Previously, nivolumab had been approved for use in patients with melanoma that is either not resectable or had not responded to other therapies.[6–8,10] For reasons that remain unclear, not all PD-1 or PD-L1 positive patients have good outcomes with the treatment of anti-PD-1 or PD-L1 monoclonal antibody. It remains to be analyzed whether PD-1 or PD-L1 have synergistic effects with other checkpoints in a clinical setting (Fig. 1).

Preliminary data indicates that another important checkpoint, lymphocyte-activation gene-3 (LAG-3) (CD 223) may have a synergistic effect with PD-1/PD-L1.[18–20]

## Lymphocyte-activation gene-3 and soluble lymphocyte-activation gene-3

LAG-3 (CD223) is encoded by the LAG-3 gene. LAG-3 is a member of the immunoglobulin superfamily (IgSF) and exerts a wide variety of biologic impacts on T cell function.[21] LAG-3 is expressed on cell membranes of natural killer cells (NK),[21] B cells,[22] tumor-infiltrating lymphocytes (TIL), a subset of T cells,[23] and dendritic cells (DC)[24,25]. The LAG-3 gene encompasses 8 exons, and the cDNA encodes a 498 amino acid membrane protein. Human LAG-3 is highly

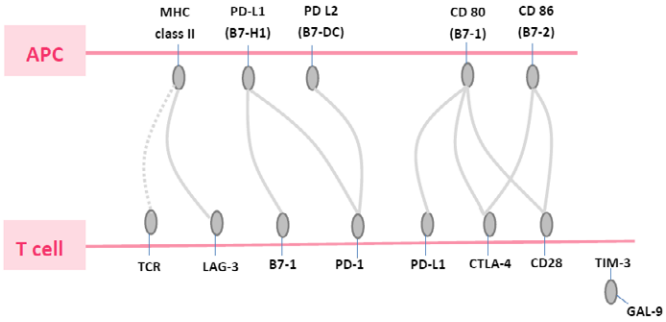


**Fig. 1.** Some checkpoint pathways in cancer. The lymphocyte-activation gene-3 (LAG-3) protein binds a nonholomorphic region of MHC class II.

homologous with both murine (70%) and pig (78%) LAG-3.[21,26]

LAG-3 is closely related to CD 4.[27] LAG-3 is located on the human chromosome 12 (12p13.32) adjacent to the CD 4 gene, and its sequence is approximately 20% identical to CD 4.[21] The LAG-3 protein binds a nonholomorphic region of major histocompatibility complex 2 (MHC class II) with greater affinity than CD 4.[28–35] LAG-3 is one of the various immune-checkpoint receptors that are coordinately upregulated on both regulatory T cells (Tregs) and anergic T cells, and the simultaneous blockade of these receptors can result in an enhanced reversal of this anergic state relative to the blockade of one receptor alone.[18] The LAG-3/MHC class II molecule interaction leads to the downregulation of CD4+ Ag-specific T cell clone proliferation and cytokine secretion. T cell MHC class II molecules downregulate T cell proliferation following LAG-3 binding and suggest a role for LAG-3 in control of the CD4+ T cell response.[31] LAG-3 can negatively regulated T cell proliferation, activation and homeostasis.

LAG-3 plays a complicated role in the immune pathway. Soluble lymphocyte-activation gene-3 (sLAG-3) likely performs different functions from LAG-3. sLAG-3 is a Th1 activity marker in serum that can be detected by ELISA.[36,37] sLAG-3 causes DCs to mature [38–44] and attack tumor cells.[43,44] Studies of the mechanisms that underlie monocyte and DC activation[38,40] by sLAG-3 indicate that sLAG-3 induces protein phosphorylation in immature DC that triggers the functional maturation.[38,39] This process requires sLAG-3 binding with MHC class II.[28]

## Lymphocyte-activation gene-3 in disease

Beyond the role it plays in a variety of autoimmune diseases, LAG-3 can also reduce the body's ability to resist infection and promote chronic infection. LAG-3 prevents autoimmune disorders in the eye by inducing anterior chamber-associated immune deviation.[45] LAG-3 may regulate the functions of CD4+ and CD8+ T cells during autoimmune diabetes, and limit autoimmunity in disease-prone environments.[46] In bone marrow transplant (BMT) patients, LAG-3 can regulate CD8+ cells involved in alloreactivity, T cell proliferation and activation after BMT.[47,48] In patients with chronic viral infection, the blockade of both PD-1 and LAG-3 could synergistically activate T cell responses and control the virus.[49] LAG-3

**Table 1. LAG-3 and cancer**

| Year | Disease | Finding | References |
|---|---|---|---|
| 1999 | Cancer | sLAG-3 could be a vaccine since it could active antigen presenting cells (APC). | (41) |
| 2001 | Cancer | sLAG-3 could improve interactions between *in situ* T cells and DC, and potentiate Th1-type response to target tumors, and, thus, could be a cancer vaccine. | (40) |
| 2003 | Breast cancer | Therapy involving LAG-3 relative could block the progression of mammary carcinogenesis in an animal model. | (42) |
| 2005 | Cancer | LAG-3 related anti-cancer therapy was effective and shared a similar mechanism with IL-12 | (27) |
| 2006 | Cancer (melanoma or colorectal cancer) | Human LAG-3 Ig induced specific CD8+ T-cell activity. The activation of this protein is a potential adjuvant treatment for cancer vaccines | (56) |
| 2006 | Cancer | sLAG-3, used as a cancer vaccine, bound MHC class $II^+$ APC, induced DC maturation and was well tolerated | (57) |
| 2006 | Hodgkin's lymphoma (HL) | LAG-3 played important roles in the suppression of EBV immunity in HL | (58) |
| 2010 | Ovarian cancer | Inhibiting both LAG-3 and PD-1 pathways could efficiently improve T-cell function | (59) |
| 2010 | Chronic lymphocytic leukemia (CLL) | High LAG-3 expression indicated poor treatment outcomes in CLL | (60) |
| 2010 | Cancer | LAG-3 defined Tregs were more numerous in tumor sites | (61) |
| 2010 | Multiple myeloma | LAG-3 gene single nucleotide polymorphism (SNP) increased susceptibility to multiple myeloma | (62) |
| 2011 | Melanoma | LAG-3/MHC class II interaction in MHC class II-positive melanoma tumors might serve as a bidirectional immune escape pathway shared by tumor cells and immune cells and renews the interest in MHC class II phenotyping for more efficient therapeutic strategies | (63) |
| 2012 | Cancer | LAG-3/Pdcd1 mice lived markedly longer than wild type and could eliminate multiple transplantable tumors | (20) |
| 2012 | Hepatocellular carcinoma (HCC) | Increased LAG-3 expression was observed in TIL in HCC | (64) |
| 2014 | Melanoma | LAG-3 activated pDC were found in tumor areas, which could suppress the immune environment | (65) |
| 2015 | Gastric cancer | In gastric cancer, expression of PD-1 and LAG-3 on CD4+ and CD8+ T cells was elevated and might impair cell-mediated immunity after surgery | (66) |

DC, dendritic cells; LAG-3, lymphocyte-activation gene-3; sLAG-3, soluble LAG-3; TIL, tumor-infiltrating lymphocytes.

negatively regulates CD8+ T cells in chronic hepatitis C patients.[50] In tuberculosis, sLAG-3 is elevated both in healthy people who have been exposed to the bacteria and in tuberculosis patients with good prognoses,[51] indicating that sLAG-3 could modulate an anti-bacterial immune response in mycobacterium tuberculosis.[52] In acquired immune deficiency, high expression of LAG-3 was correlated with impaired invariant natural killer T cell cytokine production for the duration of chronic human immunodeficiency virus (HIV)-1 infection and treatment.[53,54] Targeting the LAG-3 pathway has an immune regulatory effect and can enhance immune reconstitution in HIV-infected patients.[55]

### Lymphocyte-activation gene-3 in cancer

LAG-3 expression was also observed in various cancer types. Vital preclinical studies have demonstrated that LAG-3 antibodies have potential for cancer immunotherapy (Table 1).

### Lymphocyte-activation gene-3 and treatment

LAG-3 may be an even more promising target in cancer immunotherapy, because anti-LAG-3 antibodies can activate T effector cells and affect Tregs function.[67] Many companies are now focusing on the LAG-3 immune checkpoint in their search for novel approaches to treat malignant tumors and autoimmune disorders, many of which are now in clinical development (Table 2).

### Correlation of lymphocyte-activation gene-3 and other checkpoints

LAG-3 and CTLA-4 function similarly.[19,68,69] CTLA-4 inhibits T cell activation, suppresses T cell receptor signaling, and promotes cell cycle arrest.[70] Activated LAG-3−/− T cells extend cell cycle progression and increase T cell death. The similarity of function between LAG-3 and CTLA-4 may be related to some intersection in their signal transduction pathways. Tetravalent CTLA-4-Ig and LAG-3-Ig could have a synergistic effect in preventing acute graft-versus-host disease (GVHD). The combination therapy could more effectively inhibit T cell proliferation and reduce GVHD lethality.[71]

LAG-3 has synergistic action with PD-1/PD-L1.[20,48,72] LAG-3 and PD-1 are critical for the prevention of autoimmunity. Their synergistic function reverses autoimmune disease.[19] A deficiency of LAG-3 and PD-1 caused lethal myocarditis in a mouse model. The respective ligand receptor interactions between PD-L1 and LAG-3, together with the molecules themselves, synergistically inhibit T cell responses during persistent plasmodium. Blockade of PD-L1 and LAG-3 activated CD 4+ T cells, increased helper T cells and B cells, enhanced protective antibodies and rapidly cleared blood-stage malaria in mice.[73] In chronic viral infection, LAG-3 and PD-

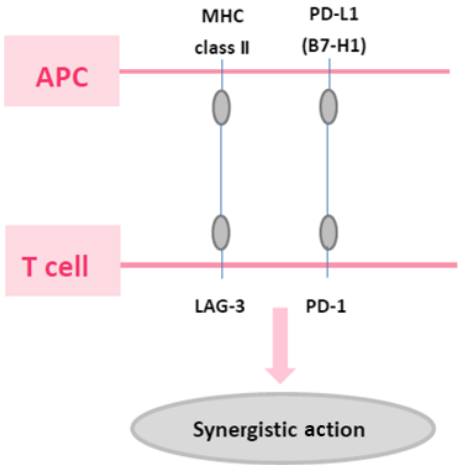


**Fig. 2.** Lymphocyte-activation gene-3 (LAG-3)/MHC class II and PD-1/PD-L1 pathways. LAG-3 has synergistic action with PD-1/PD-L1.

**Table 2. Clinical trials with LAG-3**

| Year | Drug | Phase | Company | Type | Objective | Clinical trial.gov identifier |
|---|---|---|---|---|---|---|
| 2006 | IMP321 | I | Immutep S.A. | sLAG-3 | IMP321 given alone or with a reference flu antigen | NCT00354263 |
| 2006 | IMP321 | I | Immutep S.A. | sLAG-3 | IMP321 combined with a hepatitis B antigen | NCT00354861 |
| 2006 | IMP321 | I | Immutep S.A. | sLAG-3 | IMP321 metastatic breast carcinoma receiving first line paclitaxel | NCT00349934 |
| 2006 | IMP321 | I | Immutep S.A. | sLAG-3 | IMP321 in metastatic renal cell carcinoma | NCT00351949 |
| 2008 | IMP321 | I | Immutep S.A. | sLAG-3 | IMP321 and gemcitabine in advanced pancreatic cancer | NCT00732082 |
| 2015 | IMP321 | II | Immutep S.A. | sLAG-3 | Adjunctive IMP321 to paclitaxel in metastatic breast carcinoma | NCT02614833 |
| 2013 | BMS-986016 | I | BMS | Anti-LAG-3 | The safety of anti-LAG-3 alone or with anti-PD-1 in solid tumors | NCT01968109 |
| 2014 | BMS-986016 | I | BMS | Anti-LAG-3 | The safety of anti-LAG-3 in hematological malignant tumors | NCT02061761 |
| 2016 | BMS-986016 | I | BMS | Anti-LAG-3 | Anti-LAG-3 or urelumab alone or with nivolumab in recurrent glioblastoma | NCT02658981 |
| 2014 | GSK2831781 | I | GSK | Anti-LAG-3 | GSK2831781 in healthy people and patients with plaque psoriasis | NCT02195349 |

1 maintain CD8+ T cell exhaustion.[18,49] *In vivo* research has shown that the blockade of PD-1 and LAG-3 pathways can activate T cells to achieve better viral control compared to either blockade alone.[49] Co-expression of LAG-3 and PD-1 can induce greater T cell exhaustion and more severe infection.[23] PD-1 and LAG-3 signaling pathways can inhibit CD 8 by antigen and cytokine signaling.[18] In ovarian cancer, CD8+ TIL could be negatively regulated by LAG-3 and PD-1. CD8+LAG-3+PD-1+ T cells significantly reduced IFN-γ/TNF-α. Blockade of both LAG-3 and PD-1 could increase specific CD8+ T cells producing cytokine.[59] It was also reported that LAG-3 and PD-1 synergistically regulate T-cell function, blunting the anti-tumor immune response. Lag-$3^{-/-}$Pdcd-$1^{-/-}$ mice developed an early onset, lethal autoimmune condition, but not a single knockout or wild-type mice. Cytokine analysis revealed high levels of IFN-γ, TNF-α and MCP-1 in the serum of Lag-$3^{-/-}$Pdcd-$1^{-/-}$ recipients but not a single knockout or wild-type control recipient. Although CTLA-4, PD-1 and LAG-3 are all negative regulators expressed during T-cell activation, high level, dual LAG-3/PD-1 expression is largely restricted to infiltrating TIL. Thus, LAG-3/PD-1 combinatorial immunotherapy may promote the tumor-specific responses relative to nonspecific or self-antigen-specific immune responses and, thus, may be less toxic than the CTLA-4 blockade.[20] Dual anti-LAG-3 and anti-PD-1 antibody therapy has a better prognosis than single antibody therapy. Dual knockout mice survive longer than single knockout mice. The strong synergy between the PD-1 and LAG-3 inhibitory pathways could be the foundation for novel cancer treatments (Fig. 2).[20]

## Summary

Immune checkpoints play vital roles in tumor immune escape. However, the mechanisms of the synergy between various immune checkpoints remain unknown. Cancer treatments related to CTLA-4 and PD-1/PD-L1 have achieved remarkable results. Another important immune checkpoint, LAG-3, which is closely related to CD4, can regulate T cell proliferation, activation and homeostasis. LAG-3 plays an important role in a variety of autoimmune diseases and promotes chronic infection and cancer. LAG-3's synergistic function with PD-1 and PD-L1 warrants further exploration.

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## Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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