Gene of the month: lymphocyte-activation gene 3 (LAG-3)

Mark P Lythgoe,[1] Daniel Si Kit Liu ,[1] Nicola E Annels,[2] Jonathan Krell,[1] Adam Enver Frampton [1,2,3]

[1]Department of Surgery and Cancer, Imperial College London, London, UK
[2]Department of Clinical and Experimental Medicine, University of Surrey, Faculty of Health and Medical Sciences, Guildford, UK
[3]HPB Surgical Unit, Royal Surrey NHS Foundation Trust, Guildford, UK

Correspondence to
Adam Enver Frampton, Department of Surgery and Cancer, Imperial College London, London, UK; a.frampton@imperial.ac.uk

MPL and DSKL contributed equally.

Accepted 11 June 2021
Published Online First 28 June 2021

To cite: Lythgoe MP, Liu DSK, Annels NE, *et al*. *J Clin Pathol* 2021;**74**:543–547.

ABSTRACT

Lymphocyte-activation gene 3 (LAG-3) is a coreceptor found on activated T-lymphocytes activated B-lymphocytes and natural killer (NK) cells. It is closely related to CD4 where it shares multiple common and divergent features. It contains specific binding sites with high affinity to major histocompatibility complex (MHC) Class II and functions as an inhibitor of T-cell signalling. Tumour-infiltrating lymphocytes with high LAG-3 expression have been found in many solid tumours including ovarian cancer, melanoma, colorectal cancer and haematological malignancies including Hodgkin and diffuse large B-cell lymphoma. LAG-3 antagonism has been demonstrated to restore the anti-tumourigenic function of T-cells in vivo, however, mechanistic knowledge remains relatively poorly defined. As other immune checkpoint inhibitors have transformed the management of difficult to treat cancers, such as melanoma, it is hoped that LAG-3 might have the same potential. This review will explore LAG-3 modulation as an anticancer therapy, highlighting recent clinical developments.

INTRODUCTION

Lymphocyte-activation gene 3 (LAG-3; also known as CD223) was first identified over 30 years ago.[1] Although broadly expressed and selectively transcribed in activated natural killer (NK) and T-lymphocytes, it was recognised as encoding a protein that had key features linking it to CD4, and therefore was thought to play a role in regulating the immune response. LAG-3 joins a class of genes, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which are also receptors regulating immune checkpoints and involved in the recognition of novel antigens expressed in disease, as self or otherwise.

Following the recent successes in monoclonal antibodies (mAbs) targeting these immune checkpoints to initiate or abrogate the immune response (eg, ipilimumab, as well as the older abatacept), there is a large amount of interest in developing novel immunotherapies that act on the body's own response to antigens expressed in diseases, including cancer.

STRUCTURE

The LAG-3 gene spans ~6.6 kb and includes eight exons. Interestingly, its chromosomal location is adjacent to the gene for CD4 on the distal part of the short arm of chromosome 12. The LAG-3 gene encodes a type I transmembrane protein, which is made up of 498 amino acids, and shares significant structural homology with CD4, although it shares less than 20% similarity at the primary amino acid sequence. In addition, the LAG-3 protein can be grouped within the immunoglobulin superfamily containing four extracellular Ig regions, with one variable (V type) and three constant (C type) Ig-like domains (see figure 1).

It has been noted that there are similarities in the exon/intron organisation, as well as sharing internal sequence homologies between LAG-3 and the CD4 coreceptor between domains 1 and 2, and between domains 2 and 4, indicating that LAG-3 may have arisen from a common ancestor. However, it is the differences between the two genes which are important and may give rise to important functional variances between their mechanisms.

Of particular interest is the membrane distal D1 domain which houses an extra loop comprising 30 amino acids (see figure 1) and encoded by exon 3 in a region that in CD4 seems to be important for interactions with class II major histocompatibility complex (MHC) molecules and HIV gp120.[2] This proline-rich loop is constitutively expressed on LAG-3 positive lymphocytes and allows it to bind with higher affinity to MHC Class II than CD4 alone.[3]

Comparison of the intracellular domain with CD4 also shows an absence of a binding site for the tyrosine kinase p56Lck, which facilitates signal transduction downstream of the T-cell receptor. Instead, definable motifs within LAG-3 have been found conserved in many other mammalian species, which indicates some importance in the cytoplasmic domain for its function.[4] Three definable motifs have been specifically identified including a potential serine phosphorylation site (S454), a conserved 'KIEELE' motif with no homology to any other known protein and an unusual glutamic acid-proline repetitive sequence. Mutant cell work using a murine T-cell hybridoma line shows that a single lysine residue (K468) within the 'KIEELE' motif appears to be essential for LAG-3 activity.[5]

FUNCTION

LAG-3 receptors have been found expressed on activated CD4 +and CD8+T cells (including T regulatory cells), and are also present on the surface of NK cells and invariant NK T-cells, activated B lymphocytes[6] and plasmacytoid dendritic cells.[7] LAG-3, CTLA-4 and PD-1 function as negative regulators of T-cell expansion and homoeostasis,

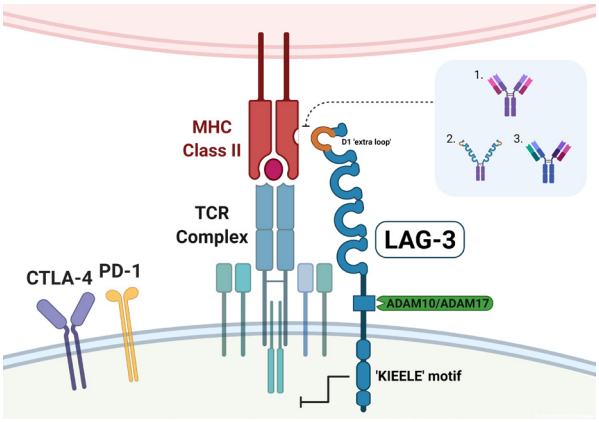

Figure 1 Lymphocyte-activation gene 3 (LAG-3) structure contains four extracellular Ig domains with a proline-rich extra loop present on domain one which acts as a high affinity binding site for major histocompatibility complex (MHC) class II. Along with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Programmed cell death protein 1 (PD-1), LAG-3 is seen as an immune checkpoint contributing to immune escape in carcinogenesis and inhibits downstream signalling of the T-cell receptor (TCR) complex by an as yet unknown mechanism. therapies to block the activity of LAG-3 involve (1) monoclonal antibodies, such as relatimab; (2) fusion proteins, such as eftilagimod alpha and (3) bispecific monoclonal antibodies, such as FS-118.

as shown using in vivo inhibition of these proteins by antibody blockade. Similarly, these proteins show a mixed cellular distribution, predominantly retained in intracellular compartments, with nearly half the cellular content of LAG-3 in close proximity to microtubules and recycling endosomes which facilitates rapid translocation after cell activation.[8]

LAG-3 functions in T-cells specifically via association with the CD3/T-cell receptor (TCR) complex, resulting in decreased T-cell proliferation and cytokine synthesis.[9] This downregulation, rather than termination through apoptosis, may allow the immune system to generate memory responses. This is induced by MHC class II binding in both CD4 +and CD8+T cell subsets.[10]

Interestingly, antibodies that do not block LAG-3 to MHC-II binding can nonetheless promote T-cell functions, and this supports the evidence that there remain other ligands for LAG-3 that also contribute to immune regulation. This includes fibrinogen-like protein 1,[11] which is upregulated in human solid tumours (normally expressed in the liver and pancreas) and galectin-3,[11] which has been identified as modulating CD4 +and CD8+T cell response.[12] LAG-3 expression on conventional T effector cells is controlled by cleavage by two transmembrane metalloproteases, ADAM10 and ADAM17. The resultant cleavage product, soluble LAG-3 (sLAG-3) has a short half-life and does not seem to have any global function.[13] It is possible that selective agonists of LAG-3 cleavage could be another potential candidate for therapeutic intervention.[14]

LAG-3 EXPRESSION IN MALIGNANCY

The immune system plays an important role in removing abnormal and malignant cells.[15] Upregulated expression of inhibitory receptors are pivotal to balance costimulatory receptor activity and limit T-cell activation, preventing autoimmunity and tissue damage.[16] Malignant cells may hijack immune checkpoint mechanisms to protect against antitumouricidal responses elicited by CD4 +and CD8+T cells[16]. Expression of immune checkpoints, such as PD-1 and LAG-3 correlate with intratumoural T-cell dysfunction in patients.[4 17] Furthermore, tumours may recruit regulatory T-cells (Tregs) to further reduce immune activation.[18 19]

Expression of LAG-3 has been identified predominantly on infiltrating immune cells by immunohistochemistry or next generation sequencing in a range of different tumour types.[20] High expression of LAG-3 can be found in tumour infiltrating T-cells in ovarian cancer, Hodgkin Lymphoma, melanoma, non-small cell lung cancer (NSCLC), diffuse large B-cell lymphoma, follicular lymphoma, head and neck squamous cell carcinoma (HNSCC), correlating alongside other immune checkpoint receptors with an aggressive tumour phenotype and overall poor prognosis.[21–33] It also plays a role in chronic viral infection such as hepatitis B virus and therefore indirectly in viral-induced cancers, such as hepatocellular carcinoma.[31 34] However, paradoxically, studies in breast cancer, gastric cancer and oesophageal squamous cell carcinoma have shown opposite results, suggesting further studies investigating the use of LAG-3 as a putative cancer biomarker are required.[35–37]

In these circumstances, LAG-3 expression can be described as a marker of T-cell exhaustion, a process characterised by progressive loss of function and sustained inhibitory receptor expression which commonly develops under conditions of antigen persistence. These also include expression of T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and T-cell immunoreceptor with Ig and ITIM domains, which are also being considered as targets for immune checkpoint inhibitors (ICPIs) in their own right. T-cell exhaustion was first identified in chronic viral infection and more recently in tumourigenesis characterised by dysfunctional CD8 +T cells, alterations in CD4 +T cell cytokine secretion and inhibition of Treg expression.[38 39] However, there are important differences in antigen exposure and metabolic constraints between chronic infection and the processes involved in the tumour microenvironment, meaning that the processes underlying each disease require specific investigation.[40] What remains clear is that this hyporesponsiveness can be transiently restored by PD-1 blockade, and there may be potential synergistic effects in combination with LAG-3 immunotherapy to allow immune cells to exert antitumour effects.

LAG-3 contributes to 'immune escape' in carcinogenesis, mirroring observations seen with other ICPIs, such as anti-PD-L1 and anti-CTLA-4.[4 16] Furthermore, upregulation is likely to contribute to the acquired resistance seen in patients treated with ICPIs who initially respond.[17 18] Preclinical studies have demonstrated that blockade of LAG-3 strongly supports anticancer immune responses, significantly reducing tumour growth in several murine models compared with controls.[26 41] These findings suggest that antagonism of LAG-3 may be a promising therapeutic target for novel cancer immunotherapy development.

Notably, LAG-3 blockade appears to more effective when combined with other anti-cancer therapies, and particular synergy has been noted in combination with other ICPIs. Concomitant blockade or genetic deletion of both PD-1 and LAG-3 has demonstrated increased anticancer efficacy preclinically, providing further validation for clinical investigation.[42]

CLINICAL MODULATION OF LAG-3

The success of other ICPIs and the rationale of targeting LAG-3 has fuelled the development of therapies to modulate this pathway, despite lack of functional understanding.[43] LAG-3 modulation is primarily focused on exploring anticancer efficacy, either as a monotherapy or in combination with other ICPIs, cytotoxic chemotherapy or other novel agents.[20 33] Most

of the combination studies combine LAG-3 antagonism with an anti-PD-L1 or anti-PD-1 drug. In addition, LAG-3 modulation is being explored in autoimmune conditions, with efficacy demonstrated in diseases such as ulcerative colitis and psoriasis.[44]

Currently, over 100 clinical trials are ongoing (at date of censoring) with at least 15 novel molecular and biological agents in clinical development (see table 1). The majority (57%) are antagonistic mAbs against LAG-3, but other agents include LAG-3 depleting antibodies, sLAG-3 fusion protein and bispecific mAbs (BMAs). Despite the wealth of favourable pre-clinical data demonstrating anticancer efficacy, as monotherapy or in combination, only a limited amount of this work has been recapitulated in the clinical setting thus far.

LAG-3 mAbs

Antagonistic LAG-3 mAbs block the interaction between MHC class II molecules and LAG-3, attenuating downstream signalling.[33] The first LAG-3 mAb to enter clinical evaluation was relatimab (BMS-986016). A phase 1/2 study (NCT01968109) to assess the tolerability of relatimab with nivolumab (anti-PD-1) in patients with advanced melanoma with progression on prior anti-PD-1/PD-L1 therapy showed an overall response rate (ORR) of 11.5%.[45] Subanalysis has demonstrated the ORR in patients with LAG-3 expression of >1% on tumour infiltrating lymphocytes (TILs) was over three times greater (18% ORR) than patients with LAG-3 negative TILs (5% ORR), irrespective of PD-L1 status. A further phase 2 study (NCT03470922) of relatimab with nivolumab in treatment-naïve melanoma patients showed a significantly longer progression-free survival (10.1 months) compared with nivolumab monotherapy (4.6 months).[46] However, an increase in treatment-related adverse events according to the Common Terminology Criteria for Adverse Events grades 3 and 4 was seen in the combination (18.4%) versus nivolumab monotherapy (9.7%).

An alternative LAG-3 mAb, Ieramilimab (LAG-525) has been investigated in a phase 1/2 dose-escalation study (NCT02460224) in combination with the investigational anti-PD-1 inhibitor spartalizumab (PDR001). Durable responses were reported in 9.9% (10/121) of patients with a variety of tumours, including mesothelioma and triple negative breast cancer.[47] Biopsies from the patients with breast cancer demonstrated an overall trend in conversion of immune-cold towards an immune-activated profile, suggesting LAG-3 blockade may modulate the tumour microenvironment favourably.

Table 1 Principal LAG-3 checkpoint Inhibitors in clinical trials

Drug	Description	Tumour type	Combination(s) with
Relatlimab (BMS-986016)	Human IgG4	Melanoma, hepatocellular, sarcoma, chordoma, head and neck, mismatch repair deficiency and basal cell carcinoma	Nivolumab (anti-PDL1) and ipilimumab (anti-CTLA4)
Sym022	Fc-inert monoclonal antibody	Solid tumours and lymphoma	Sym021* (anti-PD1)
Eftilagimod alpha (IMP-321)	LAG-3 fused to Fc region of IgG1	Non-small cell lung cancer, head and neck, melanoma, renal, pancreas, breast and solid tumours	Pembrolizumab (anti-PDL1), avelumab (anti-PD1) and gemcitabine (cytotoxic chemotherapy)
Leramilimab (LAG 525/IMP-701)	Humanised IgG4	Melanoma, breast cancer, haematological cancers and solid tumours	Spartalizumab* (anti-PD1), carboplatin (cytotoxic chemotherapy), NIR178* (AA2a inhibitor), capmatinib (MET inhibitor), lacnotuzumab* (CSF-1) and canakinumab (anti-IL1β)
Favezelimab (MK-4280)	Humanised IgG4	Renal cell carcinoma, non-small cell lung cancer, lymphoma and solid tumours	Pembrolizumab (anti-PDL1), lenvatinib (anti-VEGF), irinotecan (cytotoxic chemotherapy), 5-flurouracil (cytotoxic chemotherapy) and oxaliplatin (cytotoxic chemotherapy
REGN-3767	Humanised IgG4	Breast cancer and solid tumours	Cemplimab (anti-PD1)
BI-754111	Humanised IgG4	Head and Neck cancer, non-small cell lung cancer and solid cancers	BI 754091* (anti-PD-1)
FS-118	Tetravalent bispecific antibody (PD-L1 and LAG-3) IgG1	Head and Neck cancer and solid cancers	None
Tebotelimab (MGD-013)	Bispecific antibody (PD-L1 and LAG-3) IgG4k	Hepatocellular carcinoma, melanoma, gastric cancer, gastro-oesophageal cancer, breast cancer, biliary tract cancer, head and neck cancer, endometrial and solid cancers	Brivanib alaninate* (anti-VEGF), niraparib (PARP inhibitor), margetuximab (anti-HER2), enobiltuzumab* (anti-B7H3)
TSR-033	Humanised IgG4	Colorectal cancer and solid cancers	Dostarlimab (anti-PDL1), bevacizumab (anti-VEGF), irinotecan (cytotoxic chemotherapy, 5-flurouracil (cytotoxic chemotherapy) and oxaliplatin (cytotoxic chemotherapy
INCAGN2385	Fc-engineered IgG1κ	Solid cancers	None
XmAb22841	Bispecific antibody (CTLA-4 and LAG-3)	Solid cancers	Pembrolizumab (anti-PDL1)
EMB-02	Bispecific antibody (PD-1 and LAG-3)	Solid cancers	None
GSK2831781	Humanised IgG1	Ulcerative colitis and psoriasis	None

*Investigational drug not approved by the United States Food and Drug Administration (FDA) or European Medicines Agency (EMA) at date of censoring.
AA2a, adenosine A2a receptor antagonist; anti-B7H3, humanised antibody targeting B7-H3; anti-IL1β, anti-interleukin 1β antibod; Anti-PDL1, anti-programmed cell death-ligand one antibody; CSF-1, colony-stimulating factor 1 antibody; CTLA4, cytotoxic T-lymphocyte-associated protein 4; HER2, human epidermal growth factor 2 antibody; LAG-3, lymphocyte-activation gene 3; MET inhibitor, c-MET tyrosine kinase inhibitor; PARP, Poly adenosine diphosphate ribose polymerase inhibitor; VEGF, vascular endothelial growth factor tyrosine kinase inhibitor.

LAG-3 fusion proteins

Eftilagimod alpha (IMP321) a soluble recombinant LAG-3 fusion protein, comprising the extracellular region of LAG-3 and the Fc portion of human IgG.[20] It binds to a subset of MHC class II molecules, mediates antigen-presenting cell activation, followed by CD8 +T cell activation. Several clinical trials have investigated efficacy with variable results.

In melanoma, a phase 1 study (NCT02676869) of eftilagimod alpha in combination with pembrolizumab (anti-PD-1) demonstrated an ORR of 33% and 50% in pembrolizumab refractory and anti-PD-1 naïve subgroups, respectively.[48] Positive results have also been reported in NSCLC and HNSCC, both in combination with pembrolizumab.[49 50] However, in hormone positive breast cancer a phase 2 trial (NCT02614833) did not prolong overall progression-free survival when used as an adjunct to the cytotoxic chemotherapy drug paclitaxel.[51]

LAG-3 BMAs

Several first-in-class BMAs targeting LAG-3 and other immune targets are being investigated clinically. BMAs target two different antigens simultaneously and are effective alternatives to combining two or more different therapies. Due to the marked therapeutic synergy demonstrated when combining LAG-3 and other ICPIs, the development of BMAs is an attractive therapeutic strategy.

Preclinical studies have demonstrated similar efficacy to combination therapy.[52] Current agents in development include FS-118 (targeting LAG-3/PD-L1), R07247669 (targeting LAG-3/PD-L1), tebitelimab/MGD013 (targeting LAG-3/PD-1) and XmAb22841 (targeting LAG-3/CTLA-4). Several other agents are in preclinical development.[33]

CONCLUSIONS

LAG-3 is a protein which shares particular similarities with the CD4 coreceptor in structure but is present only on activated T-cells and other lymphocytes. As a marker of immune exhaustion, downregulation of LAG-3 as an anticancer therapy is progressing rapidly through clinical development and holds potential to become a third class of ICPIs. However, important unanswered questions remain relating to identification of important downstream signalling mechanisms. Deeper mechanistic understanding of the LAG-3 modulation pathway may lead to more efficacious therapies and the identification of biomarkers that would facilitate the stratification of patients responsive to LAG-3 blockade.

Take home messages

- Lymphocyte-activation gene 3 (LAG-3) is a protein member of the Ig superfamily, closely related to CD4 and is a co-receptor for MHC class II.
- It is found expressed on activated CD4 +and CD8+T cells, NK cells and invariant NK T-cells, activated B lymphocytes and plasmacytoid dendritic cells.
- Although its mechanism is yet to be fully elucidated, a proline-rich loop present on domain 1 is necessary for binding with high affinity to MHC class II.
- Blockade of LAG-3 strongly supports anticancer immune responses with over 100 clinical trials in clinical development.
- While the majority are classical monoclonal antibodies against LAG-3, other novel agents include LAG-3 depleting antibodies, soluble LAG-3 fusion proteins and bispecific monoclonal antibodies.

Handling editor Runjan Chetty.

Twitter Mark P Lythgoe @mlythoe

Acknowledgements Figures produced with BioRender.

Contributors Conception or design: MPL and DSKL. Acquisition, analysis or interpretation of data: MPL and DSKL. Drafting the work or revising it critically for important intellectual content: NEA, AEF and JK. Final approval of the version to be published: MPL, DSKL, NEA, JK and AEF.

Funding This study was funded by Royal College of Surgeons of England.

Competing interests JK has received honoraria from Clovis Oncology, Tesaro and AstraZeneca, outside the submitted work. None of the aforementioned companies had any input into the study design, data collection, reporting or preparation of the manuscript.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

ORCID iDs
Daniel Si Kit Liu http://orcid.org/0000-0002-4153-504X
Adam Enver Frampton http://orcid.org/0000-0002-1392-2755

REFERENCES

1 Triebel F, Jitsukawa S, Baixeras E, *et al*. LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med* 1990;171:1393–405.
2 Huard B, Mastrangeli R, Prigent P, *et al*. Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. *Proc Natl Acad Sci U S A* 1997;94:5744–9.
3 Huard B, Prigent P, Tournier M, *et al*. CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. *Eur J Immunol* 1995;25:2718–21.
4 Andrews LP, Marciscano AE, Drake CG, *et al*. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev* 2017;276:80–96.
5 Workman CJ, Dugger KJ, Vignali DAA. Cutting edge: molecular analysis of the negative regulatory function of lymphocyte activation gene-3. *J Immunol* 2002;169:5392–5.
6 Kisielow M, Kisielow J, Capoferri-Sollami G, *et al*. Expression of lymphocyte activation gene 3 (LAG-3) on B cells is induced by T cells. *Eur J Immunol* 2005;35:2081–8.
7 Workman CJ, Wang Y, El Kasmi KC, *et al*. LAG-3 regulates plasmacytoid dendritic cell homeostasis. *J Immunol* 2009;182:1885–91.
8 Woo S-R, Li N, Bruno TC, *et al*. Differential subcellular localization of the regulatory T-cell protein LAG-3 and the coreceptor CD4. *Eur J Immunol* 2010;40:1768–77.
9 Hannier S, Tournier M, Bismuth G, *et al*. CD3/TCR complex-associated lymphocyte activation gene-3 molecules inhibit CD3/TCR signaling. *J Immunol* 1998;161:4058–65.
10 Maçon-Lemaître L, Triebel F. The negative regulatory function of the lymphocyte-activation gene-3 co-receptor (CD223) on human T cells. *Immunology* 2005;115:170–8.
11 Wang J, Sanmamed MF, Datar I, *et al*. Fibrinogen-Like protein 1 is a major immune inhibitory ligand of LAG-3. *Cell* 2019;176:e12:334–47.
12 Demotte N, Wieërs G, Van Der Smissen P, *et al*. A galectin-3 ligand corrects the impaired function of human CD4 and CD8 tumor-infiltrating lymphocytes and favors tumor rejection in mice. *Cancer Res* 2010;70:7476–88.
13 Li N, Wang Y, Forbes K, *et al*. Metalloproteases regulate T-cell proliferation and effector function via LAG-3. *Embo J* 2007;26:494–504.
14 Lambrecht BN, Vanderkerken M, Hammad H. The emerging role of ADAM metalloproteinases in immunity. *Nat Rev Immunol* 2018;18:745–58.
15 Villadolid J, Amin A. Immune checkpoint inhibitors in clinical practice: update on management of immune-related toxicities. *Transl Lung Cancer Res* 2015;4:560–75.
16 Sharma P, Allison JP. The future of immune checkpoint therapy. *Science* 2015;348:56–61.
17 Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. *Nat Immunol* 2019;20:1425–34.
18 Huang C-T, Workman CJ, Flies D, *et al*. Role of LAG-3 in regulatory T cells. *Immunity* 2004;21:503–13.
19 Ohue Y, Nishikawa H, Regulatory T. Regulatory T (Treg) cells in cancer: can Treg cells be a new therapeutic target? *Cancer Sci* 2019;110:2080–9.
20 Lecocq Q, Keyaerts M, Devoogdt N, *et al*. The Next-Generation Immune Checkpoint LAG-3 and Its Therapeutic Potential in Oncology: Third Time's a Charm. *Int J Mol Sci* 2020;22:75–17.
21 Matsuzaki J, Gnjatic S, Mhawech-Fauceglia P, *et al*. Tumor-Infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc Natl Acad Sci U S A* 2010;107:7875–80.
22 Gandhi MK, Lambley E, Duraiswamy J, *et al*. Expression of LAG-3 by tumor-infiltrating lymphocytes is coincident with the suppression of latent membrane antigen-specific CD8+ T-cell function in Hodgkin lymphoma patients. *Blood* 2006;108:2280–9.

23 Baitsch L, Baumgaertner P, Devêvre E, *et al*. Exhaustion of tumor-specific CD8⁺ T cells in metastases from melanoma patients. *J Clin Invest* 2011;121:2350–60.
24 Scurr M, Ladell K, Besneux M, *et al*. Highly prevalent colorectal cancer-infiltrating LAP⁺ Foxp3⁻ T cells exhibit more potent immunosuppressive activity than Foxp3⁺ regulatory T cells. *Mucosal Immunol* 2014;7:428–39.
25 Chen J, Chen Z. The effect of immune microenvironment on the progression and prognosis of colorectal cancer. *Med Oncol* 2014;31:82.
26 Deng W-W, Mao L, Yu G-T, *et al*. LAG-3 confers poor prognosis and its blockade reshapes antitumor response in head and neck squamous cell carcinoma. *Oncoimmunology* 2016;5:e1239005.
27 Guo M, Yuan F, Qi F, *et al*. Expression and clinical significance of LAG-3, FGL1, PD-L1 and CD8⁺T cells in hepatocellular carcinoma using multiplex quantitative analysis. *J Transl Med* 2020;18:306.
28 He Y, Yu H, Rozeboom L, *et al*. LAG-3 protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes. *J Thorac Oncol* 2017;12:814–23.
29 Hemon P, Jean-Louis F, Ramgolam K, *et al*. Mhc class II engagement by its ligand LAG-3 (CD223) contributes to melanoma resistance to apoptosis. *J Immunol* 2011;186:5173–83.
30 Keane C, Law SC, Gould C, *et al*. LAG3: a novel immune checkpoint expressed by multiple lymphocyte subsets in diffuse large B-cell lymphoma. *Blood Adv* 2020;4:1367–77.
31 Li F-J, Zhang Y, Jin G-X, *et al*. Expression of LAG-3 is coincident with the impaired effector function of HBV-specific CD8(+) T cell in HCC patients. *Immunol Lett* 2013;150:116–22.
32 Long L, Zhang X, Chen F, *et al*. The promising immune checkpoint LAG-3: from tumor microenvironment to cancer immunotherapy. *Genes Cancer* 2018;9:176–89.
33 Maruhashi T, Sugiura D, Okazaki I-M, *et al*. LAG-3: from molecular functions to clinical applications. *J Immunother Cancer* 2020;8:e001014.
34 Dong Y, Li X, Zhang L, *et al*. CD4⁺ T cell exhaustion revealed by high PD-1 and LAG-3 expression and the loss of helper T cell function in chronic hepatitis B. *BMC Immunol* 2019;20:27.
35 Gebauer F, Krämer M, Bruns C, *et al*. Lymphocyte activation gene-3 (LAG3) mRNA and protein expression on tumour infiltrating lymphocytes (TILs) in oesophageal adenocarcinoma. *J Cancer Res Clin Oncol* 2020;146:2319–27.
36 Ohmura H, Yamaguchi K, Hanamura F, *et al*. Ox40 and LAG3 are associated with better prognosis in advanced gastric cancer patients treated with anti-programmed death-1 antibody. *Br J Cancer* 2020;122:1507–17.
37 Sidaway P. Breast cancer: LAG3 expression indicates favourable outcomes. *Nat Rev Clin Oncol* 2017;14:712.
38 Simoni Y, Becht E, Fehlings M, *et al*. Bystander CD8⁺ T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature* 2018;557:575–9.
39 Yi JS, Cox MA, Zajac AJ. T-cell exhaustion: characteristics, causes and conversion. *Immunology* 2010;129:474–81.
40 Blank CU, Haining WN, Held W, *et al*. Defining 'T cell exhaustion'. *Nat Rev Immunol* 2019;19:665–74.
41 Que Y, Fang Z, Guan Y, *et al*. LAG-3 expression on tumor-infiltrating T cells in soft tissue sarcoma correlates with poor survival. *Cancer Biol Med* 2019;16:331–40.
42 Lichtenegger FS, Rothe M, Schnorfeil FM, *et al*. Targeting LAG-3 and PD-1 to enhance T cell activation by antigen-presenting cells. *Front Immunol* 2018;9:385.
43 Lythgoe MP, Krell J, Mahmoud S, *et al*. Development and economic trends in anticancer drugs licensed in the UK from 2015 to 2019. *Drug Discov Today* 2021;26:301–7.
44 Angin M, Brignone C, Triebel F. A LAG-3-Specific agonist antibody for the treatment of T cell-induced autoimmune diseases. *J Immunol* 2020;204:810–8.
45 Ascierto PA, Melero I, Bhatia S, *et al*. Initial efficacy of anti-lymphocyte activation gene-3 (anti–LAG-3; BMS-986016) in combination with nivolumab (nivo) in PTS with melanoma (MEL) previously treated with anti–PD-1/PD-L1 therapy. *JCO* 2017;35:9520.
46 Evan J, Lipson HA-HT, Schadendorf D. Relatlimab (RelA) plus nivolumab (NIVO) versus NIVO in first-line advanced melanoma: primary phase III results from RELATIVITY-047 (CA224-047). *J Clin Oncol* 2021.
47 Hong DS, Schoffski P, Calvo A, *et al*. Phase I/II study of LAG525 ± spartalizumab (PDR001) in patients (PTS) with advanced malignancies. *JCO* 2018;36:3012.
48 Atkinson V, Khattak A, Haydon A, *et al*. Eftilagimod alpha, a soluble lymphocyte activation gene-3 (LAG-3) protein plus pembrolizumab in patients with metastatic melanoma. *J Immunother Cancer* 2020;8:e001681.
49 Clay TD, Majem M, Felip E, *et al*. Results from a phase II study of eftilagimod alpha (soluble LAG-3 protein) and pembrolizumab in patients with PD-L1 unselected metastatic non-small cell lung carcinoma. *JCO* 2021;39:9046.
50 Brana I, Forster M, Lopez-Pousa A, *et al*. Results from a phase II study of eftilagimod alpha (soluble LAG-3 protein) and pembrolizumab in patients with PD-L1 unselected metastatic second-line squamous head and neck carcinoma. *JCO* 2021;39:6028.
51 Wildiers H, Armstrong A, Cuypere E. *Abstract PD14-08: primary efficacy results from AIPAC: a double-blinded, placebo controlled, randomized multinational phase IIb trial comparing Weekly paclitaxel plus eftilagimod alpha (soluble LAG-3 protein) vs Weekly paclitaxel plus placebo in HR-positive metastatic breast cancer patients*. American Association for Cancer Research (AACR), 2021.
52 Kraman M, Faroudi M, Allen NL, *et al*. FS118, a bispecific antibody targeting LAG-3 and PD-L1, enhances T-cell activation resulting in potent antitumor activity. *Clin Cancer Res* 2020;26:3333–44.