ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

IOTECH IMMUNO-ONCOLOGY AND TECHNOLOGY

REVIEW

Clinical landscape of LAG-3-targeted therapy

L. Chocarro[1*], E. Blanco[1,2], H. Arasanz[1,3], L. Fernández-Rubio[1], A. Bocanegra[1], M. Echaide[1], M. Garnica[1], P. Ramos[1], G. Fernández-Hinojal[1,4], R. Vera[3], G. Kochan[1*] & D. Escors[1*]

[1]Oncoimmunology Research Unit, Navarrabiomed-Fundación Miguel Servet, Universidad Pública de Navarra (UPNA), Hospital Universitario de Navarra (HUN), Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona; [2]Division of Gene Therapy and Regulation of Gene Expression, Cima Universidad de Navarra, Instituto de Investigación Sanitaria de Navarra (IdISNA), Pamplona; [3]Medical Oncology Unit, Hospital Universitario de Navarra (HUN), Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona; [4]Medical Oncology Department, Hospital Clínico San Carlos, Madrid, Spain

Available online 17 March 2022

Lymphocyte-activated gene 3 (LAG-3) is a cell surface inhibitory receptor and a key regulator of immune homeostasis with multiple biological activities related to T-cell functions. LAG-3 is considered a next-generation immune checkpoint of clinical importance, right next to programmed cell death protein 1 (PD-1) and cytotoxic T-cell lymphocyte antigen-4 (CTLA-4). Indeed, it is the third inhibitory receptor to be exploited in human anticancer immunotherapies. Several LAG-3-antagonistic immunotherapies are being evaluated at various stages of preclinical and clinical development. In addition, combination therapies blocking LAG-3 together with other immune checkpoints are also being evaluated at preclinical and clinical levels. Indeed, the co-blockade of LAG-3 with PD-1 is demonstrating encouraging results. A new generation of bispecific PD-1/LAG-3-blocking agents have also shown strong capacities to specifically target PD-1+ LAG-3+ highly dysfunctional T cells and enhance their proliferation and effector activities. Here we identify and classify preclinical and clinical trials conducted involving LAG-3 as a target through an extensive bibliographic research. The current understanding of LAG-3 clinical applications is summarized, and most of the publically available data up to date regarding LAG-3-targeted therapy preclinical and clinical research and development are reviewed and discussed.

Key words: LAG-3, immune checkpoint, immunotherapy, targeted therapy, cancer treatment

INTRODUCTION

Lymphocyte activation gene 3 (LAG-3, CD223) is a cell surface inhibitory receptor that regulates a wide range of T-cell effector functions.[1-5] LAG-3 plays similar roles to other immune checkpoint molecules such as programmed cell death protein 1 (PD-1) and cytotoxic T-cell lymphocyte antigen-4 (CTLA-4). LAG-3 is expressed by T cells, some activated B cells, plasmacytoid dendritic cells (DCs) and neurons and subjected to epigenetic regulation.[6,7] In addition, LAG-3 in activated T cells delivers co-stimulatory signals to DCs, licensing them to produce interleukin-12p70 (IL-12p70).[8,9]

LAG-3 ligands include major histocompatibility complex (MHC)-II, galectin-3 (Gal-3) and fibrinogen-like protein 1 (FGL1).[10-13] MHC-II is considered the canonical ligand.[3,4] LAG-3 binds to MHC with higher affinity than CD4, disrupting CD4—MHC-II interactions.[11,14] LAG-3 binding induces MHC-II signal transduction in DCs, activating phospholipase C γ2, p72syk, PI3K/AKT, p42/44 and p38 protein kinase.[15] Engagement with Gal-3 and FGL1 also exerts T-cell inhibitory functions possibly by other means. Gal-3 can be highly expressed on tumor cells and activated T cells; it is required for CD8 T-cell and plasmacytoid DC suppression.[12,16-18] LAG-3 binding to FGL1 is non-redundant to MHC-II binding, and contribute to poor responses to anti-PD-1/anti-programmed death-ligand 1 (PD-L1) immunotherapies.[13] In addition, the Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin family member LSECtin acts as an LAG-3 ligand in melanoma cells, inhibiting antitumor T-cell responses by reducing the expression of CDK2, CDK4 and CDK6.[19] LAG-3 inhibits T-cell receptor (TCR) signal transduction by association to the TCR—CD3 complex.[20] However, the exact mechanisms of intracellular negative signal transduction are still uncharacterized.[5,21-23]

Elevated LAG-3 expression is considered a T-cell exhaustion marker, associated to immune homeostasis disruption in a broad spectrum of human diseases.[6] Importantly, LAG-3 and PD-1 co-expression in T cells is a biomarker of strong

*Correspondence to: Luisa Chocarro, Tel.: +34 848 423 322
E-mail: luisa.chocarro.deerauso@navarra.es (L. Chocarro).
*Dr Grazyna Kochan
E-mail: grazyna.kochan@navarra.es (G. Kochan).
*Dr David Escors, Tel : +34 848 425 742
E-mail: david.escors.murugarren@navarra.es (D. Escors).

T-cell dysfunctionality in cancer and it is associated with resistance to anti-PD-1/anti-PD-L1 immunotherapies.[24-30] PD-1 and LAG-3 co-blockade increases many T-cell anti-tumor activities.[26,28,31-33] Several immunotherapies targeting LAG-3 are at various stages of clinical development.[6] Here, we review the publically available data on LAG-3-targeted therapy.

PRECLINICAL AND CLINICAL DEVELOPMENT OF LAG-3-TARGETED THERAPY

Sixteen LAG-3-targeted therapies are tested at 97 clinical trials by Bristol-Myers Squibb (BMS-986016), Regeneron Pharmaceuticals (REGN3767 and 89Zr-DFO-REGN3767), Merck (MK-4280), Novartis (LAG525), Tesaro (GSK) (TSR-033), Symphogen (Sym022), GlaxoSmith (GSK2831781), Incyte Biosciences International Sàrl (INCAGN02385), Prima BioMed/Immutep (IMP321), MacroGenics (MGD013), F-Star (FS118), Hoffmann-La Roche (RO7247669), Shanghai EpimAb Biotherapeutics (EMB-02), Xencor (XmAb841) and Innovent Biologics (IBI323). Figure 1 summarize the publically available data (Supplementary Table 1, available at https://doi.org/10.1016/j.iotech.2022.100079). These therapies are categorized into monoclonal antibodies, soluble LAG-3—immunoglobulin (Ig) fusion proteins and anti-LAG-3 bispecific drugs (Figure 1A). With the exception of the IgG1 antibody etigilimab, most anti-LAG-3 monoclonal antibodies are fully humanized IgG4 monoclonal blocking antibodies. IMP321 is the only soluble recombinant LAG-3 clinically studied. Additionally, bispecific anti-LAG-3-targeted drugs are being studied with very promising results, especially dual PD-1/LAG-3 blockade. Preliminary evidence from clinical trials is providing encouraging results for the treatment of cancers in terms of efficacy, safety, tolerance and pharmacokinetics.

Most trials are phase I (34), I/II (21) and II (35). Few of them are early phase I (2) and phase II/III (3) trials, and only two of them have reached phase III for BMS-986016 (NCT05002569) and MK-4280 drugs (NCT05064059) (Figure 1B). All of them are interventional and investigational trials, still active and recruiting, and considered as applicable clinical trials by the Food and Drug Administration Amendments Act of 2007 (Figure 1C). Most are open-label, randomized and parallel studies (Figure 1C). The treated solid tumors are varied and summarized in Supplementary Table 1. Treated hematologic malignancies include lymphoma, myeloma and leukemia among others. Interestingly, some LAG-3 inhibitors are also being tested for psoriasis (NCT02195349), ulcerative colitis (NCT03893565), hepatitis B (NCT00354861) and influenza vaccines (Phase I Study of IMP321 Given Alone; https://clinicaltrials.gov/ct2/show/NCT00354263). As LAG-3 is involved in the pathogenesis of a wide range of non-neoplastic disease, it could be a therapeutic target for the treatment of selected pathologies. Of note, a recent controversial study with therapeutic anti-LAG-3 antibodies showed that LAG-3 is not expressed in human and murine neurons and does not modulate α-synucleinopathies.[34] LAG-3 expression is included in several clinical trials as a biomarker of disease status (Supplementary Table 2, available at https://doi.org/10.1016/j.iotech.2022.100079).

Anti-LAG-3 monoclonal antibodies

BMS-986016, relatlimab. BMS-986016 fully human monoclonal antibody is an anti-LAG-3 fully human monoclonal IgG4-κ antibody, which was the first LAG-3 blocker to be clinically developed.[35] Relatlimab binds human LAG-3 with high affinity (Kd = 0.12-0.5 nM) and inhibits its binding to MHC-II (WO 2015116539 A1).[36,37] Relatlimab is currently being evaluated in phase I, II, II/III and III trials in solid and hematological malignancies, alone or in combination with anti-PD-1/PD-L1 drugs[6,38] (Supplementary Table 1). Preliminary data are supporting its use either alone or in combination. It is relatively well tolerated and shows clinical efficacy.[14,39] Genomic and immunological differences were found between responder patients and non-responders in a combo treatment with anti-PD-1.[40] The authors monitored interferon (IFN)-γ cytokine concentrations in real time in tumor tissue biopsies, while evaluating the safety of anti-LAG-3/anti-PD-1 combination.[41] Relatlimab and nivolumab (anti-PD-1) combination helps in overcoming treatment resistance. For instance, preliminary results (NCT01968109; NCT03470922) showed promising initial efficacy, safety profile, well tolerability and antitumor activity in patients with melanoma in patients progressing to anti-PD-1/PD-L1 monotherapy.[36,42,43] Relatlimab also restores anti-leukemic T- and natural killer (NK) cell-mediated responses in patients with chronic lymphocytic leukemia. Relatlimab induces leukemic cell depletion, enhances NK and antibody-dependent cytotoxicities and promotes T-cell tumor necrosis factor (TNF)-α, IFN-γ and IL-2 cytokine.[44]

Recent phase III data on relatlimab and nivolumab combo versus nivolumab in untreated advanced melanoma showed greater benefit in progression-free survival: 47.7% at 12 months [95% confidence interval (CI), 41.8% to 53.2%] with the PD-1/LAG-3 combination as compared with 36.0% (95% CI, 30.5% to 41.6%) with the anti-PD-1 monotherapy.[45,46] The combo showed good toxicity profiles.

REGN3767, fianlimab. REGN3767 is an IgG4 fully human, hinge-stabilized, high-affinity, monoclonal antibody developed by Regeneron Pharmaceuticals. It was obtained from VelocImmune mice containing human Ig gene segments.[47,48] Fianlimab blocks LAG-3 binding to MHC class II, activating T cells and enhancing cytotoxic T-cell-mediated tumor cell lysis (https://www.cancer.gov/publications/dictionaries/cancer-drug/def/fianlimab). REGN3767/cemiplimab (anti-PD-1) combo demonstrated good antitumor activities both *in vitro* and in the human PD-1xLAG-3-knockin mice model.[49] Increased activation of tumor-specific T cells was observed, promoting T-cell-mediated immunity. In addition, REGN3767 showed favorable pharmacokinetics and toxicology in cynomolgus monkeys.[49] Two clinical trials are investigating REGN3767 alone and in combination with anti-PD-1 inhibitors (NCT03005782,

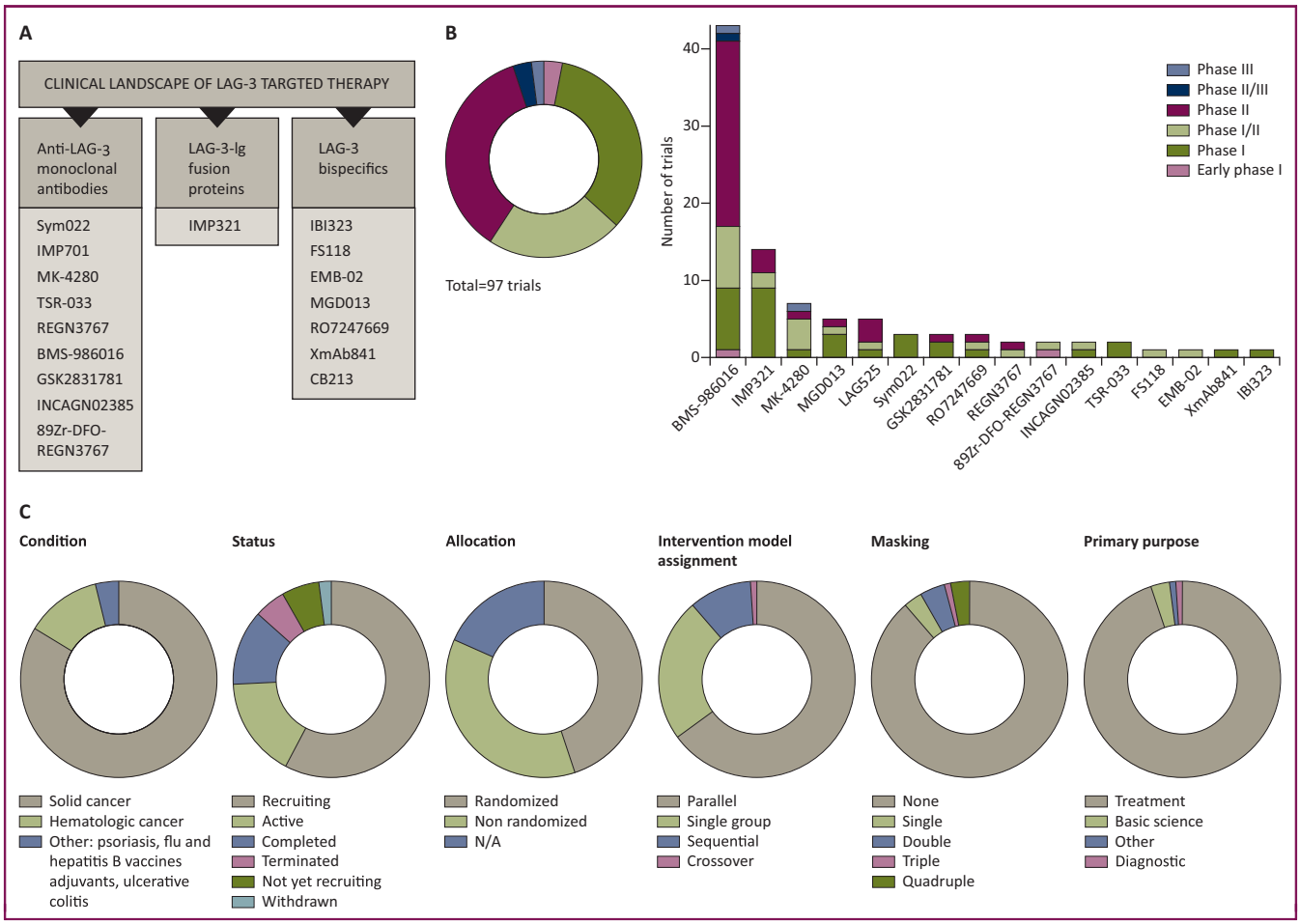

Figure 1. Detailed analysis of the lymphocyte-activated gene 3 (LAG-3)-targeted therapy clinical landscape.
(A) Current LAG-3-targeted therapies can be categorized into three subtypes: anti-LAG-3 monoclonal antibodies, LAG-3—immunoglobulin (Ig) fusion proteins and LAG-3 bispecifics, as indicated in the figure. Some examples of the tested therapeutic drugs are listed. (B) Categorization of LAG-3 clinical trial phases. On the left, a pie chart with the distribution of LAG-3-targeting trials. On the right, a bar chart indicating the clinical phase distributions for the indicated LAG-3-targeted drugs. (C) Pie charts with categorization of LAG-3 clinical trials by the studied pathologies, status and study design as indicated (allocation, intervention model assignment, masking and primary purpose).

NCT01042379). In a phase I, open-label, dose-escalation and cohort expansion first-in-human clinical trial, the combination showed a safety profile similar to other immune checkpoint inhibitors (ICIs) (NCT03005782). Activity and pharmacodynamics were also examined. Preliminary data suggested a dose-dependent expansion of PD-1-expressing memory T-cell subsets by REGN3767/cemiplimab combination. Early efficacy was detected, suggesting that REGN3767 exerts antitumor activity across several tumor types. Thus, a fixed dose was selected for further evaluation.[50] Fianlimab and cemiplimab combo showed a similar safety profile to cemiplimab alone, with one exception, and a clinical activity similar to anti-PD-1/anti-CTLA-4 combination in melanoma patients but with reduced treatment-emergent adverse events (TEAEs).[51] Objective response rate (ORR) was 63.6% (3 complete responses and 18 partial responses) for anti-PD-L1-naïve patients and 13.3% (1 complete response and 1 partial responses) for anti-PD-L1-experienced patients. The REGN3767/cemiplimab combo is being evaluated in a phase II adaptively randomized clinical trial for breast cancer[52] (NCT01042379).

89Zr-DFO-REGN3767, fianlimab tracer. Anti-LAG-3 antibodies are being used for positron emission tomography (PET) scanning as a diagnostic method.[53] 89Zr-DFO-REGN3767 is an anti-LAG-3 PET imaging tracer that integrates the anti-LAG-3 REGN3767 antibody labeled with zirconium,[54] used for monitoring therapy response to anti-LAG-3 treatment. This trial has several aims apart from establishing safety, pharmacokinetics, dosing and timing for PET scanning as a diagnostic method. The objectives include tumor targeting, determination of 89Zr-DFO-REGN3767 biodistribution and dosimetry, optimal time for imaging and tumor uptake after drug administration, evaluation of tumor uptake of the 89Zr-DFO-REGN3767 and correlation with LAG-3 expression (NCT04566978). This study is being carried out in early phase I and phase II imaging clinical trials for solid and hematologic cancer (NCT04706715, NCT04566978).

Sym022. Sym022 is a recombinant, Fc-inert, fully human, monoclonal antibody developed by Symphogen that blocks LAG-3/MHC-II binding. This antibody binds with high

affinity to human and cynomolgus monkey LAG-3 and increases T-cell cytokine production.[55] Three phase I dose-escalation and dose-expansion clinical trials are testing Sym022 for cancer treatment, alone or in combination with Sym021 (anti-PD-1) and Sym023 (anti-T-cell immunoglobulin and mucin domain-3) (NCT03489369, NCT04641871, NCT03311412). Studies in preclinical models have shown that Sym021, Sym022 and Sym023 combinations provide synergistic antitumor activities.[56,57]

GSK2831781, IMP731. GSK2831781 is a humanized anti-LAG-3 monoclonal IgG1 antibody developed by GlaxoSmithKline (GSK), and derived from Immutep's IMP731 antibody. This antibody depletes LAG-3-expressing activated T cells in immuno-inflammatory disorders. Two phase I clinical trials are evaluating safety, tolerability, pharmacokinetics and pharmacodynamics for the treatment of psoriasis (NCT03965533, NCT02195349). A phase II clinical trial has been terminated in ulcerative colitis (NCT03893565). These trials were interrupted based on the assessment of clinical data as part of an interim analysis conducted in consultation with the Data Review Committee of the trial[58] (NCT03893565). Further reporting is being conducted on the efficacy and safety data, although GSK and Immunotep's collaboration remains in place (https://pipelinereview.com/index.php/2021012277234/Antibodies/Ulcerative-Colitis-Phase-II-Study-of-GSK2831781-Discontinued.html). Preliminary results showed that GSK2831781 is pharmacologically active with a tolerable safety profile, and provides early evidence of improvement in psoriasis.[59] GSK2831781 treatment reduced pro-inflammatory gene expression (*IL-17A*, *IL-17F*, *IFNγ*, and *S100A12*), and up-regulated genes associated with skin barrier functions (*CDHR1*).

INCAGN02385. INCAGN02385 is an Fc-engineered IgG1-κ monoclonal antibody developed by Incyte Corporation, which blocks LAG-3 binding to MHC-II, enhancing T-cell responsiveness to TCR stimulation. Studies in cynomolgus monkeys showed acceptable tolerability and pharmacokinetics, with cross-reactivity to cynomolgus monkey LAG-3.[60] INCAGN02385 has been evaluated alone in a completed phase I clinical trial (NCT03538028), and it is being currently studied in a phase I/II clinical trial in combination with INCMGA00012 (anti-PD-1) and INCAGN02390 (anti-TIM-3) in patients with advanced malignancies (NCT04370704).

TSR-033. TSR-033 is an anti-LAG-3 high-affinity human IgG4 monoclonal antibody developed by Tesaro (GSK) (PMID: 30587557), and binds with high affinity to human LAG-3.[61] Complementary determining regions (CDRs) from the original murine antibody were grafted within the germline frameworks of the human ortholog, followed by *in vitro* somatic hypermutation with mammalian cell surface display for further selection of high-affinity variants.[62,63] TSR-033 demonstrated antitumor activities in preclinical models.[61] TSR-033 in combination with anti-PD-1 increased IL-2 production by activated CD4 T cells and improved efficacy. The combo treatment increased total and intra-tumor T-cell proliferation and stimulation, and reduced tumor-associated macrophages. Two dose-escalation phase I clinical trials (NCT03250832, NCT02817633) are investigating TSR-033 alone and in combination with anti-PD-1 antibody. Preliminary data showed good tolerability and safety profiles (NCT03250832).

LAG525, IMP701, ieramilimab. LAG525 is a humanized IgG4 monoclonal antibody developed by Novartis which blocks LAG-3 binding to MHC-II [concentration that causes 50% inhibition of growth (IC_{50}) 5.5 nM].[64] Five trials are studying LAG525 in several phases, administered alone or in combination with anti-PD-1 inhibitors. Preliminary data show that LAG525 exhibits good safety profile and pharmacokinetics, as well as promising antitumor activity.[65,66] The combination was also well tolerated.[67] A phase I/II study in combination with spartalizumab (PDR001) reported well-tolerated dose-escalation results and good antitumor activities.[65] Later, a phase II was conducted in patients with solid or hematologic malignancies that relapsed or were refractory to non-immunotherapies.[66] Promising antitumor activity for neuro endocrine tumors, small-cell lung cancer and diffuse large B-cell lymphoma (DLBCL) were also found. LAG525 combination with spartalizumab (anti-PD-1) exhibited good safety profiles and antitumor activities in melanoma, renal cell cancer and mesothelioma previously treated with PD-1/PD-L1 blockers, suggesting that LAG-3 blockade counteracts prior resistance to these treatments[67] ('NCT02460224').

MK-4280, favezelimab. MK-7684 is a humanized IgG4 monoclonal anti-LAG-3 monoclonal antibody developed by MERCK. Favezelimab increased cytokine (IFN-γ, IL-2, IL-8 and TNF-α) and chemokine (CCL4, CXCL10 and CCL22) production in T cells, and CD69, CD44, CD25, XCL1, GZMB and Nuclear factor of activated T-cells up-regulation.[68] Seven clinical trials are evaluating MK-4280 at various clinical stages. Preliminary findings demonstrated good safety and efficacy profiles, and manageable tolerability when administered alone or in combination with other immune checkpoint blockade agents (NCT05064059).[69] A phase I/II first-in-human trial is evaluating its safety and efficacy in combination with pembrolizumab (anti-PD-1).[70] Preliminary results showed that favezelimab alone or in combination presents good safety profile.[71] The combo showed better antitumor activity than monotherapy with an ORR of 6.3% (one complete response and four partial responses). Treatment-related adverse events were 65% with favezelimab and 65.2% in combination with pembrolizumab, most commonly fatigue, fever and nausea.

Soluble LAG-3—Ig fusion proteins

IMP321, eftilagimod alpha (efti). IMP321, a soluble LAG-3 molecule, is a natural, high-affinity, human and murine MHC-II agonist (hLAG-3Ig) developed by Immutep. It is a dimeric recombinant molecule with LAG-3 extracellular domains fused to a human immunoglobulin Fc region. IMP321 is an atypical ICI as it targets antigen-presenting cells (APCs) such as DCs. Indeed, natural soluble LAG-3 is

associated to protective resistance against tuberculosis and favorable outcome, and to improved disease-free and overall survival rates in some breast cancers expressing estrogen or progesterone receptors.[72,73] IMP321 was firstly developed as an immune modulator for vaccines. It is being evaluated as a treatment for cancer as well. IMP321 increases T-cell responses and vaccine immunogenicity to various diseases, specifically generating type 1 tumor-specific immunity by enhancing the release of Th1 cytokines by APCs.[74] IMP321 demonstrated good safety and tolerability, and increased Th1 responses to influenza vaccine.[75] IMP321 enhanced T-cell responses against an alum-non-absorbed recombinant hepatitis B surface antigen, inducing humoral and T-cell-mediated immunity.[76,77] IMP321 recruits and activates effector innate and adaptive immune cells.[78] Indeed, IMP321 enhanced T-cell proliferation and induced a full Tc1-activated phenotype characterized by IFN-γ, TNF-α, IL-1β, IL-6, CCL4, CCL5 and CCL2 production. Additionally, MP321 treatment programed myeloid cells to produce CCL4 and TNF-α, and CD8 and NK cells to produce IFN-γ and TNF-α.[78] These effects induce monocyte-derived DC maturation and migration *in vitro,* and stimulate naïve and Th1 responses.[77,79,80] IMP321 delayed tumor growth and enhanced tumor rejection, through tumor-specific CD8 effector and memory responses.[81,82] Indeed, IMP321 possesses strong adjuvant activities, expanding tumor and virus antigen-specific T cells and activating APCs, generating long-lasting immunity.[74] Fourteen clinical trials are testing IMP321 at phase I, I/II and II stages in infectious and malignant diseases. It is demonstrating good efficacy, activity and safety, with appropriate pharmacodynamics in combination with other antitumor therapies.[83-87] It has been used as an adjuvant in combination with chemotherapy and autologous adoptive T-cell transfer, achieving long-lasting antitumor immunity. Stronger expansion of antigen-specific effector CD8 T cells and reduced expansion of regulatory T cells were observed.[88] A phase I trial of Melanoma antigen recognized by T cells 1 peptide vaccination plus IMP321 in patients receiving autologous peripheral blood mononuclear cell (PBMC) transfer after lymphodepleting chemotherapy showed that vaccination induced a durable antitumor immune cell response.[88] CD8 T cells did not show exhaustion phenotypes. IMP321 also showed good tolerability and toxicity profiles as front-line therapy in combination with gemcitabine in patients with advanced pancreatic adenocarcinoma.[89] No significant differences were observed in monocytes (CD11b+CD14+), DCs (CD11c+), CD4 T and CD8 T cells or in MHC-II, CD80, and CD86 expression between pre- and post-treatment. Vaccination with IMP321 with immunogenic peptides demonstrated good induction of T-cell responses in metastatic melanoma patients.[90]

A phase IIb study with paclitaxel in metastatic breast cancer is being evaluated.[85,91] Recent results from the stratum D of the INSIGHT platform trial showed that IMP321 combination with avelumab (anti-PD-L1) is feasible, safe and well tolerated in advanced-stage solid tumors. Of the eight patients enrolled by 2020, 50% progressed, 12.5% had partial responses, 12.5% had stable disease and 25% did not undergo tumor assessment by that time.[87,92]

Anti-LAG-3 bispecifics

MGD013, tebotelimab. MGD013 is a humanized, hinge-stabilized, IgG4-κ tetravalent bispecific Fc-bearing dual-affinity re-targeting antibody-like (DART®), binding PD-1 and LAG-3 with high affinity. Tebotelimab targets PD-1 and LAG-3-expressing cells and chronically activated T cells. MGD013 is developed by Macrogenetics, and has demonstrated favorable biophysical and manufacturability properties with a prolonged half-life. PD-1/LAG-3 co-blockade caused increased cytokine secretion and enhanced T-cell responses compared to PD-1 or LAG-3 single blockade.[93] Seven clinical trials are evaluating MGD013 monotherapy and combinations.[94-97] Preliminary data show encouraging responses and acceptable pharmacokinetics.[96] MGD013 dose escalation was well-tolerated with manageable immune-related adverse events, similar to anti-PD-L1. MGD013 monotherapy showed antitumor activity in multiple tumor types, and one complete response after single MGD013 administration in chimeric antigen receptor (CAR)-T-cell therapy. High baseline LAG-3/PD-1 expression and IFN-γ high gene signature (CXCL9, CXCL10, CXC11, STAT1) were associated with objective clinical responses. Furthermore, margetuximab [anti-human epidermal growth factor receptor 2 (HER2)] combination with PD-1xLAG-3 DART® enhanced lytic activity of immune cells. Preliminary results in relapsed or refractory HER2+ solid tumors included an ORR of 42.9%. Further evaluation of MGD013 alone and in combinations is ongoing.[96,98] A phase I study tested MGD013 in patients with relapsed or refractory DLBCL, demonstrating good pharmacodynamics, safety profiles and antitumor activities with and without prior CAR-T-cell treatment.[93]

FS118. FS118 is a first-in-class human tetravalent, full-length human IgG1, anti-LAG-3/PD-L1 bispecific antibody developed by F-star Therapeutics. This bispecific blocks both PD-L1 and LAG-3 with high affinity, and demonstrates comparable activity to single blockades.[99] FS118 overcomes PD-L1- and LAG-3-mediated inhibition of T-cell activation and effector functions *in vitro*. In addition, its surrogate mouse version inhibits tumor growth *in vivo* and contributes to LAG-3 and PD-L1 shedding by the activities of A Disintegrin And Metalloproteinase 10 and A Disintegrin And Metalloproteinase 17.[99,100] Its binding can overcome PD-L1-mediated compensatory up-regulation of LAG-3. FS118 monotherapy is being evaluated in advanced malignancies in a phase I/II clinical trial (NCT03440437). Preliminary data suggest good tolerability and pharmacodynamics, and early signs of clinical efficacy.[101,102] FS118 potently activates primary human T cells *in vitro* and inhibits tumor growth in carcinoma models (https://www.nature.com/articles/d43747-020-00181-6). A phase I study in patients with prior resistance to immune checkpoint therapy exhibited good pharmacodynamic and pharmacokinetic profiles. The treatment was well tolerated, with signs of improved

clinical outcomes, consistent with preclinical data.[101,102] Furthermore, FS119 showed early signs of clinical efficacy with long-term disease control in patients with previous acquired resistances.

RO7247669. RO7247669 is an anti-PD-1-LAG-3 bispecific antibody developed by Hoffmann-La Roche that binds to PD-1 and LAG-3, blocking their inhibitory pathways. RO7247669 is being evaluated in three clinical trials at the recruiting phase for the treatment of solid tumors (NCT04524871, NCT04785820, NCT04140500).

EMB-02. EMB-02 is an anti-PD-1-LAG-3 bispecific antibody developed by EpimAb Biotherapeutics, which blocks their binding to PD-L1, MHC-II and FGL-1. Its binding also induces PD-1 and LAG-3 degradation, and preclinical data show improvement for the treatment of tumor models resistant to PD-1 blockade monotherapies. One phase I/II clinical trial is currently recruiting patients to evaluate EMB-02 in advanced solid tumors (NCT04618393).

XmAb841, pavunalimab. XmAb841 is an anti-CTLA4-LAG-3 bispecific antibody developed by Xencor, which enhances T-cell stimulation and proliferation. Its structure consists of bispecific Fc domains as a scaffold between the two binding domains, conferring stability, ease of purification and manufacture. The Fc domains lack Fcγ receptor binding. This structure promotes heterodimer formation and a long-circulating half-life. XmAb841 enhanced human T-cell activation. This bispecific also enhanced allogeneic antitumor activity and can be combined with anti-PD-1 blockade to promote triple checkpoint blockade (https://investors.xencor.com/static-files/3761fc99-37a0-486e-a5b7-ad94f17eb446; https://investors.xencor.com/static-files/5042016b-70bd-40f5-abf7-862ddd759986; https://investors.xencor.com/static-files/f388d30a-3d0d-4a69-9a43-876a3b38f79f). This biospecific molecule enhanced IL-2 production *in vitro* (https://investors.xencor.com/static-files/f388d30a-3d0d-4a69-9a43-876a3b38f79f). There is only one phase I clinical trial at recruitment stage in selected advanced solid tumors (NCT03849469).

IBI323. IBI323 is an LAG-3/PD-L1 bispecific antibody developed by Innovent Biologic, which preserves the properties of the parental antibodies. IBI323 blocks engagement of PD-1 with PD-L1 and CD80, and of LAG-3 with MHC-II.[103] This bispecific activates T cells, enhancing their activation by cross-linking PD-L1+ APCs with LAG-3+ T cells, and promoting antitumor activities in humanized mouse models. Treatment with IBI323 increased numbers of intra- and extra-tumor T cells, and tumor antigen-specific T cells.[103] A phase I clinical trial is currently studying IBI323 in advanced malignancies.

CB213. Crescendo Biologics Ltd recently announced a clinical development partnership with Cancer Research UK to progress the bispecific PD-1xLAG-3 antagonist Humabody® CB213 into a phase I clinical trial targeting solid tumors (https://www.businesswire.com/news/home/20200505005080/en/Crescendo-Biologics-and-Cancer-Research-UK-sign-Clinical-Development-Partnership-to-develop-CB213-a-novel-bispecific-Humabody%C2%AE-therapeutic). Its structure consists of human nanobodies (VH human bodies) with an asymmetric 2 : 1 binding format using bivalent LAG-3-binding human bodies coupled to a monovalent PD-1 human body. CB213 is a half-life extended version delivering simultaneous PD-1 and LAG-3 checkpoint blockade specifically targeted toward highly dysfunctional LAG-3+ PD-1+ double-positive T cells.[26,104] This approach has been designed to deliver safer, more effective therapeutic interventions in patients with cancers resistant or refractory to PD-1 monotherapies. In preclinical testing, CB213 has demonstrated a potent dual checkpoint blockade activity with the ability to revert the dysfunctionality of patient-derived T cells. Antitumor efficacy was characterized by a potent inhibition of tumor growth and enhancement of tumor antigen-specific CD8+ cytotoxic T cells.[105]

CONCLUSIONS

LAG-3 is one of the most important next-generation immune checkpoint molecule. Currently, 97 clinical trials are evaluating at least 16 LAG-3-targeting molecules (Supplementary Table 1 and Figure 1). Only two trials have reached phase III, one by BMS and the other by Merck, which will be a major part in developing clinical LAG-3 targeting. Phase III studies in melanoma and colorectal cancer are demonstrating encouraging results. Here, we have discussed the positive results of these phase III trials. Other LAG-3-targeting drugs were developed later and have not yet progressed to phase III trials. Most of the trials test LAG-3-antagonistic molecules including combinations with other ICIs. Next-generation bispecifics have been developed, showing stronger capacities for specific dual targeting of LAG-3 with other ICIs. This is significant, because simultaneous co-expression of LAG-3 with other immune checkpoint molecules is a distinguishing feature of highly dysfunctional T cells in cancer patients. LAG-3-targeting cancer immunotherapies have demonstrated good safety profiles, tolerability and adequate pharmacokinetics and pharmacodynamics, with promising antitumor efficacy. In addition, LAG-3 regulates a diversity of biological mechanisms and its use as a cancer target keeps on holding relatively high promises. However, there are never guarantees on clinical efficacies and breakthroughs. Only time will tell whether PD-1 and LAG-3 co-blockade will counteract resistance to current immunotherapies.

FUNDING

The OncoImmunology group is funded by the Spanish Association against Cancer (AECC) [grant number PROYE16001ESCO]; Instituto de Salud Carlos III (ISCIII)-FEDER project grants [grant numbers FIS PI17/02119, FIS PI20/00010, COV20/00000, TRANSPOCART ICI19/00069]; a Biomedicine Project grant from the Department of Health of the Government of Navarre [grant number BMED 050-2019]; strategic projects from the Department of Industry, Government of Navarre (AGATA, Ref. 0011-1411-2020-000013; LINTERNA, Ref. 0011-1411-2020-000033; DESCARTHES, 0011-1411-2019-000058); European Project Horizon 2020 Improved Vaccination for Older Adults

(ISOLDA; ID: 848166); Crescendo Biologics Ltd. supported the OncoImmunology group for the development and testing of PD-1 and LAG-3 bispecifics.

DISCLOSURE

DE declares authorship in the invention 'Therapeutic molecules that bind to LAG3 and PD1' by Crescendo Biologics (GB201802573D0). HA declares to be on the advisory board for AstraZeneca. The remaining authors have declared no conflicts of interest.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.iotech.2022.100079.

REFERENCES

1. Andrews LP, Marciscano AE, Drake CG, Vignali DA. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev*. 2017;276:80-96.
2. Chocarro L, Blanco E, Zuazo M, et al. Understanding LAG-3 signaling. *Int J Mol Sci*. 2021;22:5282.
3. Huard B, Prigent P, Pages F, Bruniquel D, Triebel F. T cell major histocompatibility complex class II molecules down-regulate CD4+ T cell clone responses following LAG-3 binding. *Eur J Immunol*. 1996;26:1180-1186.
4. Huard B, Tournier M, Hercend T, Triebel F, Faure F. Lymphocyte-activation gene 3/major histocompatibility complex class II interaction modulates the antigenic response of CD4+ T lymphocytes. *Eur J Immunol*. 1994;24:3216-3221.
5. Workman CJ, Dugger KJ, Vignali DA. Cutting edge: molecular analysis of the negative regulatory function of lymphocyte activation gene-3. *J Immunol*. 2002;169:5392-5395.
6. Chocarro L, Blanco E, Arasanz H, et al. 55P Clinical landscape of LAG-3-targeted therapy. *Ann Oncol*. 2021;32:S1362.
7. Saleh R, Toor SM, Nair VS, Elkord E. Role of epigenetic modifications in inhibitory immune checkpoints in cancer development and progression. *Front Immunol*. 2020;11:1469.
8. Casati C, Camisaschi C, Novellino L, et al. Human lymphocyte activation gene-3 molecules expressed by activated T cells deliver costimulation signal for dendritic cell activation. *J Immunol*. 2008;180:3782-3788.
9. Sierro S, Romero P, Speiser DE. The CD4-like molecule LAG-3, biology and therapeutic applications. *Expert Opin Ther Targets*. 2011;15:91-101.
10. 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;94:5744-5749.
11. Huard B, Prigent P, Tournier M, Bruniquel D, Triebel F. CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. *Eur J Immunol*. 1995;25:2718-2721.
12. Kouo T, Huang L, Pucsek AB, et al. Galectin-3 shapes anti-tumor immune responses by suppressing CD8+ T cells via LAG-3 and inhibiting expansion of plasmacytoid dendritic cells. *Cancer Immunol Res*. 2015;3:412-423.
13. Wang J, Sanmamed MF, Datar I, et al. Fibrinogen-like protein 1 is a major immune inhibitory ligand of LAG-3. *Cell*. 2019;176:334-347:e312.
14. Long L, Zhang X, Chen F, et al. The promising immune checkpoint LAG-3: from tumor microenvironment to cancer immunotherapy. *Genes Cancer*. 2018;9:176-189.
15. Andreae S, Buisson S, Triebel F. MHC class II signal transduction in human dendritic cells induced by a natural ligand, the LAG-3 protein (CD223). *Blood*. 2013;102:2130-2137.
16. Chung LY, Tang SJ, Wu YC, Sun GH, Liu HY, Sun KH. Galectin-3 augments tumor initiating property and tumorigenicity of lung cancer through interaction with beta-catenin. *Oncotarget*. 2015;6:4936-4952.
17. Li M, Feng YM, Fang SQ. Overexpression of ezrin and galectin-3 as predictors of poor prognosis of cervical cancer. *Braz J Med Biol Res*. 2017;50:e5356.
18. Lu W, Wang J, Yang G, et al. Posttranscriptional regulation of Galectin-3 by miR-128 contributes to colorectal cancer progression. *Oncotarget*. 2017;8:15242-15251.
19. Xu F, Liu J, Liu D, et al. LSECtin expressed on melanoma cells promotes tumor progression by inhibiting anti-tumor T-cell responses. *Cancer Res*. 2014;74:3418-3428.
20. Hannier S, Tournier M, Bismuth G, Triebel F. CD3/TCR complex-associated lymphocyte activation gene-3 molecules inhibit CD3/TCR signaling. *J Immunol*. 1998;161:4058-4065.
21. Iouzalen N, Andreae S, Hannier S, Triebel F. LAP, a lymphocyte activation gene-3 (LAG-3)-associated protein that binds to a repeated EP motif in the intracellular region of LAG-3, may participate in the down-regulation of the CD3/TCR activation pathway. *Eur J Immunol*. 2001;31:2885-2891.
22. Maeda TK, Sugiura D, Okazaki IM, Maruhashi T, Okazaki T. Atypical motifs in the cytoplasmic region of the inhibitory immune co-receptor LAG-3 inhibit T cell activation. *J Biol Chem*. 2019;294(15):6017-6026.
23. Workman CJ, Vignali DA. The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. *Eur J Immunol*. 2003;33:970-979.
24. Datar I, Sanmamed MF, Wang J, et al. 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.
25. Wang Y, Dong T, Xuan Q, Zhao H, Qin L, Zhang Q. Lymphocyte-activation gene-3 expression and prognostic value in neoadjuvant-treated triple-negative breast cancer. *J Breast Cancer*. 2018;21:124-133.
26. Zuazo M, Arasanz H, Fernandez-Hinojal G, et al. Functional systemic CD4 immunity is required for clinical responses to PD-L1/PD-1 blockade therapy. *EMBO Mol Med*. 2019;11:e10293.
27. Chocarro de Erauso L, Zuazo M, Arasanz H, et al. Resistance to PD-L1/PD-1 blockade immunotherapy. A tumor-intrinsic or tumor-extrinsic phenomenon? *Front Pharmacol*. 2020;11:441.
28. 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;107:7875-7880.
29. Zuazo M, Arasanz H, Bocanegra A, et al. Systemic CD4 immunity: a powerful clinical biomarker for PD-L1/PD-1 immunotherapy. *EMBO Mol Med*. 2020;12:e12706.
30. Zuazo M, Arasanz H, Bocanegra A, et al. Systemic CD4 immunity as a key contributor to PD-L1/PD-1 blockade immunotherapy efficacy. *Front Immunol*. 2020;11:586907.
31. Jing W, Gershan JA, Weber J, et al. Combined immune checkpoint protein blockade and low dose whole body irradiation as immunotherapy for myeloma. *J Immunother Cancer*. 2015;3:2.
32. 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.
33. Lino AC, Dang VD, Lampropoulou V, et al. LAG-3 inhibitory receptor expression identifies immunosuppressive natural regulatory plasma cells. *Immunity*. 2018;49:120-133.e129.
34. Emmenegger M, De Cecco E, Hruska-Plochan M, et al. LAG3 is not expressed in human and murine neurons and does not modulate alpha-synucleinopathies. *EMBO Mol Med*. 2021;13:e14745.
35. Albershardt TC, Parsons AJ, Reeves RS, et al. Therapeutic efficacy of PD1/PDL1 blockade in B16 melanoma is greatly enhanced by immunization with dendritic cell-targeting lentiviral vector and protein vaccine. *Vaccine*. 2020;38:3369-3377.
36. Ascierto PA, Bono P, Melero I, et al. LBA18 — Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3

(LAG-3), in combination with nivolumab in pts with melanoma who progressed during prior anti-PD-1/PD-L1 therapy (mel prior IO) in all-comer and biomarker-enriched populations. *Ann Oncol*. 2017;28:v611-v612.
37. Soldevilla MM, Hervas S, Villanueva H, et al. Identification of LAG3 high affinity aptamers by HT-SELEX and Conserved Motif Accumulation (CMA). *PLoS One*. 2017;12:e0185169.
38. Puhr HC, Ilhan-Mutlu A. New emerging targets in cancer immunotherapy: the role of LAG3. *ESMO Open*. 2019;4:e000482.
39. Davar D, Zarour HM. Immunological targets for immunotherapy: inhibitory T cell receptors. *Methods Mol Biol*. 2020;2055:23-60.
40. Jackson C, Choi J, Zhang JJ, et al. Immu-18. Immunogenomic responder phenotype from a phase I trial of anti-LAG3 or anti-CD137 alone and in combination with anti-PD-1 in patients with recurrent GBM. *Neuro-Oncology*. 2019;21:vi122-vi123.
41. Lynes J, Jackson S, Sanchez V, et al. Cytokine microdialysis for real-time immune monitoring in glioblastoma patients undergoing checkpoint blockade. *Neurosurgery*. 2019;84:945-953.
42. 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. *J Clin Oncol*. 2017;35:9520-9520.
43. Lipson EJ, Long GV, Tawbi H, et al. 1302TiP — CA224-047: a randomized, double-blind, phase II/III study of relatlimab (anti-LAG-3) in combination with nivolumab (anti-PD-1) versus nivolumab alone in previously untreated metastatic or unresectable melanoma. *Ann Oncol*. 2018;29:viii464-viii465.
44. Sordo-Bahamonde C, Lorenzo-Herrero S, Gonzalez-Rodriguez AP, et al. LAG-3 blockade with relatlimab (BMS-986016) restores anti-leukemic responses in chronic lymphocytic leukemia. *Cancers*. 2021;13:2112.
45. Lipson EJ, Tawbi HA, Schadendorf D, et al. 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;39:9503-9503.
46. Tawbi HA, Schadendorf D, Lipson EJ, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med*. 2022;386:24-34.
47. Macdonald LE, Karow M, Stevens S, et al. Precise and in situ genetic humanization of 6 Mb of mouse immunoglobulin genes. *Proc Natl Acad Sci U S A*. 2014;111:5147-5152.
48. Murphy AJ, Macdonald LE, Stevens S, et al. Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. *Proc Natl Acad Sci U S A*. 2014;111:5153-5158.
49. Burova E, Hermann A, Dai J, et al. Preclinical development of the anti-LAG-3 antibody REGN3767: characterization and activity in combination with the anti-PD-1 antibody cemiplimab in human PD-1xLAG-3-knockin mice. *Mol Cancer Ther*. 2019;18:2051-2062.
50. Papadopoulos KP, Lakhani NJ, Johnson ML, et al. First-in-human study of REGN3767 (R3767), a human LAG-3 monoclonal antibody (mAb), ± cemiplimab in patients (pts) with advanced malignancies. *J Clin Oncol*. 2019;37:2508.
51. Hamid O, Wang D, Kim TM, et al. Clinical activity of fianlimab (REGN3767), a human anti-LAG-3 monoclonal antibody, combined with cemiplimab (anti-PD-1) in patients (pts) with advanced melanoma. *J Clin Oncol*. 2021;39:9515.
52. Nanda R, Liu MC, Yau C, et al. Effect of pembrolizumab plus neoadjuvant chemotherapy on pathologic complete response in women with early-stage breast cancer: an analysis of the ongoing phase 2 adaptively randomized I-SPY2 trial. *JAMA Oncol*. 2020;6:676-684.
53. Broos K, Keyaerts M, Lecocq Q, et al. Non-invasive assessment of murine PD-L1 levels in syngeneic tumor models by nuclear imaging with nanobody tracers. *Oncotarget*. 2017;8:41932-41946.
54. Zeglis BM, Lewis JS. The bioconjugation and radiosynthesis of 89Zr-DFO-labeled antibodies. *J Vis Exp*. 2015;96:52521.
55. Grandal MM, Melander MC, Bhatia VK, et al. Abstract 5626: Preclinical characterization of Sym022, a novel anti-LAG3 antibody. *Cancer Res*. 2018;78:5626.
56. Spreafico A, Janku F, Rodon JA, et al. 1197P — A phase I study of Sym021, an anti-PD-1 antibody (Ab), alone and in combination with Sym022 (anti-LAG-3) or Sym023 (anti-TIM-3). *Ann Oncol*. 2020;30:v488-v489.
57. Lakhani N, Spreafico A, Tolcher AW, et al. 1019O — Phase I studies of Sym021, an anti-PD-1 antibody, alone and in combination with Sym022 (anti-LAG-3) or Sym023 (anti-TIM-3). *Ann Oncol*. 2020;31:S645-S671.
58. EUCTR2018-003278-28-PL. A multicentre randomized, double-blind (sponsor open), placebo-controlled phase 2 study to evaluate the safety, tolerability, efficacy, dose-response, pharmacokinetics and pharmacodynamics of repeat dosing of an anti-LAG3 cell depleting monoclonal antibody (GSK2831781) in patients with active ulcerative colitis. Cochrane Central Register of Controlled Trials (CENTRAL), 2019. https://www.clinicaltrialsregister.eu/ctr-search/search?query=2018-003278-28
59. Ellis J, Marks DJB, Srinivasan N, et al. Depletion of LAG-3(+) T cells translated to pharmacology and improvement in psoriasis disease activity: a phase I randomized study of mAb GSK2831781. *Clin Pharmacol Ther*. 2021;109:1293-1303.
60. Savitsky D, Ward R, Riordan C, et al. Abstract 3819: INCAGN02385 is an antagonist antibody targeting the co-inhibitory receptor LAG-3 for the treatment of human malignancies. *Cancer Res*. 2018;78:3819.
61. Ghosh S, Sharma G, Travers J, et al. TSR-033, a novel therapeutic antibody targeting LAG-3, enhances T-cell function and the activity of PD-1 blockade in vitro and in vivo. *Mol Cancer Ther*. 2019;18:632-641.
62. Bowers PM, Horlick RA, Neben TY, et al. Coupling mammalian cell surface display with somatic hypermutation for the discovery and maturation of human antibodies. *Proc Natl Acad Sci U S A*. 2011;108:20455-20460.
63. Horlick RA, Macomber JL, Bowers PM, et al. Simultaneous surface display and secretion of proteins from mammalian cells facilitate efficient in vitro selection and maturation of antibodies. *J Biol Chem*. 2013;288:19861-19869.
64. Pauthner M, Yeung J, Ullman C, et al. Antibody Engineering & Therapeutics, the annual meeting of The Antibody Society December 7-10, 2015, San Diego, CA, USA. *mAbs*. 2016;8:617-652.
65. Hong DS, Schoffski P, Calvo A, et al. Phase I/II study of LAG525 ± spartalizumab (PDR001) in patients (pts) with advanced malignancies. *J Clin Oncol*. 2018;36:3012-3012.
66. Uboha NV, Milhem MM, Kovacs C, et al. Phase II study of spartalizumab (PDR001) and LAG525 in advanced solid tumors and hematologic malignancies. *J Clin Oncol*. 2019;37:2553-2553.
67. Lin CC, Garralda E, Schoffski P, et al. 387 A phase II, multicenter study of the safety and efficacy of LAG525 in combination with spartalizumab in patients with advanced malignancies. *J Immunother Cancer*. 2020;8:A412.
68. Bhagwat B, Cherwinski H, Sathe M, et al. Establishment of engineered cell-based assays mediating LAG3 and PD1 immune suppression enables potency measurement of blocking antibodies and assessment of signal transduction. *J Immunol Methods*. 2018;456:7-14.
69. Berry S, Giraldo N, Nguyen P, et al. Correction to: 33rd Annual Meeting & Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2018). *J Immunother Cancer*. 2019;7:46.
70. Gregory GP, Zinzani PL, Palcza J, et al. Abstract CT106: Anti-LAG-3 antibody MK-4280 in combination with pembrolizumab for the treatment of hematologic malignancies: a phase I/II study. *Cancer Res*. 2019;79:CT106.
71. Garralda E, Sukari A, Lakhani NJ, et al. A phase 1 first-in-human study of the anti-LAG-3 antibody MK4280 (favezelimab) plus pembrolizumab in previously treated, advanced microsatellite stable colorectal cancer. *J Clin Oncol*. 2021;39:3584-3584.
72. Lienhardt C, Azzurri A, Amedei A, et al. Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity in vivo. *Eur J Immunol*. 2002;32:1605-1613.
73. Triebel F, Hacene K, Pichon MF. A soluble lymphocyte activation gene-3 (sLAG-3) protein as a prognostic factor in human breast cancer expressing estrogen or progesterone receptors. *Cancer Lett*. 2006;235:147-153.

74. Casati C, Camisaschi C, Rini F, et al. Soluble human LAG-3 molecule amplifies the in vitro generation of type 1 tumor-specific immunity. *Cancer Res*. 2006;66:4450-4460.
75. Brignone C, Grygar C, Marcu M, Perrin G, Triebel F. IMP321 (sLAG-3) safety and T cell response potentiation using an influenza vaccine as a model antigen: a single-blind phase I study. *Vaccine*. 2007;25:4641-4650.
76. Brignone C, Grygar C, Marcu M, Perrin G, Triebel F. IMP321 (sLAG-3), an immunopotentiator for T cell responses against a HBsAg antigen in healthy adults: a single blind randomised controlled phase I study. *J Immune Based Ther Vaccines*. 2007;5:5.
77. El Mir S, Triebel F. A soluble lymphocyte activation gene-3 molecule used as a vaccine adjuvant elicits greater humoral and cellular immune responses to both particulate and soluble antigens. *J Immunol*. 2000;164:5583-5589.
78. Brignone C, Grygar C, Marcu M, Schakel 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.
79. Andreae S, Piras F, Burdin N, Triebel F. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). *J Immunol*. 2002;168:3874-3880.
80. Fougeray S, Brignone C, Triebel F. A soluble LAG-3 protein as an immunopotentiator for therapeutic vaccines: preclinical evaluation of IMP321. *Vaccine*. 2006;24:5426-5433.
81. Cappello P, Triebel F, Iezzi M, et al. LAG-3 enables DNA vaccination to persistently prevent mammary carcinogenesis in HER-2/neu transgenic BALB/c mice. *Cancer Res*. 2003;63:2518-2525.
82. Prigent P, El Mir S, Dreano M, Triebel F. Lymphocyte activation gene-3 induces tumor regression and anti-tumor immune responses. *Eur J Immunol*. 1999;29:3867-3876.
83. 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.
84. Brignone C, Gutierrez M, Mefti F, et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and anti-tumor activity. *J Transl Med*. 2010;8:71.
85. Dirix L, Triebel F. AIPAC: a phase IIb study of eftilagimod alpha (IMP321 or LAG-3Ig) added to weekly paclitaxel in patients with metastatic breast cancer. *Future Oncol*. 2019;15:1963-1973.
86. Goetze TO. 1032P — Safety data from stratum D of the phase I INSIGHT platform trial evaluating feasibility of IMP321 (LAG-3Ig protein, eftilagimod alpha) combined with avelumab in advanced stage solid tumour entities. *Ann Oncol*. 2020;31:S645-S671.
87. Goetze TO, Mueller DW, Rafiyan MR, et al. Open-label, phase I study evaluating feasibility and safety of subcutaneous IMP321 (LAG-3Ig fusion protein, eftilagimod alpha) combined with avelumab in advanced stage solid tumor entities: results from stratum D of the INSIGHT platform trial. *J Clin Oncol*. 2020;38:3099-3099.
88. Romano E, Michielin O, Voelter V, et al. MART-1 peptide vaccination plus IMP321 (LAG-3Ig fusion protein) in patients receiving autologous PBMCs after lymphodepletion: results of a phase I trial. *J Transl Med*. 2014;12:97.
89. Wang-Gillam A, Plambeck-Suess S, Goedegebuure P, et al. A phase I study of IMP321 and gemcitabine as the front-line therapy in patients with advanced pancreatic adenocarcinoma. *Invest New Drugs*. 2013;31:707-713.
90. Legat A, Maby-El Hajjami H, Baumgaertner P, et al. 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.
91. Duhoux FP, Jager A, Dirix L, et al. Combination of paclitaxel and LAG3-Ig (IMP321), a novel MHC class II agonist, as a first-line chemoimmunotherapy in patients with metastatic breast carcinoma (MBC): interim results from the run-in phase of a placebo controlled randomized phase II. *J Clin Oncol*. 2017;35:CN-01397091.
92. Mueller C, Triebel F. 46TiP — AIPAC (Active Immunotherapy PAClitaxel): a phase IIb trial in hormone receptor-positive metastatic breast carcinoma patients receiving IMP321 (LAG-3Ig fusion protein) or placebo as adjunctive to a standard chemotherapy regimen of paclitaxel. *Ann Oncol*. 2016;27:viii14.
93. Wang J, Asch AS, Hamad N, et al. A phase 1, open-label study of MGD013, a bispecific DART® molecule binding PD-1 and LAG-3 in patients with relapsed or refractory diffuse large B-cell lymphoma. *Blood*. 2020;136:21-22.
94. Catenacci D, Rosales MK, Chung H, et al. P-342 Margetuximab combined with anti-PD-1 (MGA012) or anti-PD-1/LAG-3 (MGD013) +/- chemotherapy in first-line therapy of advanced/metastatic HER2+ gastroesophageal junction or gastric cancer. *Ann Oncol*. 2020;31:S201.
95. Catenacci DV, Rosales M, Chung HC, et al. MAHOGANY: margetuximab combination in HER2+ unresectable/metastatic gastric/gastroesophageal junction adenocarcinoma. *Future Oncol*. 2021;17:1155-1164.
96. Luke JJ, Patel MR, Hamilton EP, et al. A phase I, first-in-human, open-label, dose-escalation study of MGD013, a bispecific DART molecule binding PD-1 and LAG-3, in patients with unresectable or metastatic neoplasms. *J Clin Oncol*. 2020;38:3004-3004.
97. Powderly JD, Hurwitz H, Ryan DP, et al. A phase 1, first-in-human, open label, dose escalation study of MGD007, a humanized gpA33 x CD3 DART molecule, in patients with relapsed/refractory metastatic colorectal carcinoma. *J Clin Oncol*. 2016;34.
98. LaMotte-Mohs R, Shah K, Smith D, et al. Abstract 3217: MGD013, a bispecific PD-1 x LAG-3 Dual-Affinity Re-Targeting (DART®) protein with T-cell immunomodulatory activity for cancer treatment. *Cancer Res*. 2016;76:3217.
99. Kraman M, Faroudi M, Allen N, et al. FS118, a bispecific antibody targeting LAG-3 and PD-L1, enhances T-cell activation resulting in potent anti-tumor activity. *Clin Cancer Res*. 2020;26:3333-3344.
100. Everett KL, Kraman M, Wollerton FPG, et al. Generation of Fcabs targeting human and murine LAG-3 as building blocks for novel bispecific antibody therapeutics. *Methods*. 2019;154:60-69.
101. Yap T, Wong D, Hu-Lieskovan S, et al. 395 A first-in-human study of FS118, a tetravalent bispecific antibody targeting LAG-3 and PD-L1, in patients with advanced cancer and resistance to PD-(L)1 therapy. *JITC*. 2020;8.
102. Yap TA, Papadopoulos KP, LoRusso P, et al. A first-in-human phase I study of FS118, an anti-LAG-3/PD-L1 bispecific antibody in patients with solid tumors that have progressed on prior PD-1/PD-L1 therapy. *J Clin Oncol*. 2019;37.
103. Jiang H, Ni H, Zhang P, et al. PD-L1/LAG-3 bispecific antibody enhances tumor-specific immunity. *Oncoimmunology*. 2021;10:1943180.
104. Edwards CJ, Sette A, Cox C, et al. The multi-specific V_H-based Humabody CB213 co-targets PD1 and LAG3 on T cells to promote anti-tumour activity. *Br J Cancer*. 2021;30.
105. Legg JW, McGuinness B, Arasanz H, et al. Abstract 930: CB213: A half-life extended bispecific Humabody VH delivering dual checkpoint blockade to reverse the dysfunction of LAG3+PD-1+ double-positive T cells. *Cancer Res*. 2020;80:930-930.