



Published in final edited form as:

Semin Immunol. 2019 April ; 42: 101305. doi:10.1016/j.smim.2019.101305.

Lymphocyte-Activation Gene 3 (LAG3): the Next Immune Checkpoint Receptor

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Abstract

Immune checkpoint therapy has revolutionized cancer treatment by blocking inhibitory pathways in T cells that limits the an effective anti-tumor immune response. Therapeutics targeting CTLA-4 and PD1/PDL1 have progressed to first line therapy in multiple tumor types with some patients exhibiting tumor regression or remission. However, the majority of patients do not benefit from checkpoint therapy emphasizing the need for alternative therapeutic options. Lymphocyte Activation Gene 3 (LAG3) or CD223 is expressed on multiple cell types including CD4⁺ and CD8⁺ T cells, and T_{regs}, and is required for optimal T cell regulation and homeostasis. Persistent antigen-stimulation in cancer or chronic infection leads to chronic LAG3 expression, promoting T cell exhaustion. Targeting LAG3 along with PD1 facilitates T cell reinvigoration. A substantial amount of pre-clinical data and mechanistic analysis has led to LAG3 being the third checkpoint to be targeted in the clinic with nearly a dozen therapeutics under investigation. In this review, we will discuss the structure, function and role of LAG3 in murine and human models of disease, including autoimmune and inflammatory diseases, chronic viral and parasitic infections, and cancer, emphasizing new advances in the development of LAG3-targeting immunotherapies for cancer that are currently in clinical trials.

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DISCLOSURE OF CONFLICT OF INTEREST

The authors declare competing financial interests. D.A.A.V and C.J.W. have submitted patents covering LAG3 that are licensed or pending and are entitled to a share in net income generated from licensing of these patent rights for commercial development.

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Keywords

LAG3; immune checkpoint; inhibitory receptor; cancer immunotherapy; autoimmunity; chronic viral infection

1: Introduction

Inhibitory receptors (IRs) play a pivotal role in modulating the immune response and are mediators of T cell dysfunction in autoimmunity and chronic disease [1, 2]. In fact, targeting IRs such as Programmed Cell Death Protein 1 (PD1) and Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) in cancer patients is able to re-invigorate the anti-tumor immune response, which has been reported in several landmark clinical trials [3–5]. Despite tumor regression and remission in some patients, response to these treatments is still limited with >80% of patients not responding [6, 7]. In addition, some immune quiescent tumors, such as pancreatic and prostate cancer, are still resistant to these approaches [8], ultimately offering further incentive for new immunotherapeutic interventions. One of the most promising new IR targets currently in the clinic is Lymphocyte Activation Gene 3 (LAG3) or CD223, an immune checkpoint receptor expressed by both activated and exhausted CD4⁺ and CD8⁺ T cells as well as regulatory T cells (T_{regs}) [2, 9, 10]. LAG3 functions by delivering inhibitory signals that regulate immune cell homeostasis, T cell activation, proliferation, cytokine production, cytolytic activity and other functions [2]. When LAG3 regulated homeostasis is perturbed, such as in the setting of chronic autoimmunity, there is rapid, immune-mediated tissue damage [9, 11]. Further, persistent antigen-stimulation, such as in cancer and chronic viral infection, results in elevated levels of chronic LAG3 expression, which leads to T cell exhaustion and subsequent impairment of T cell function [12]. Numerous immunotherapies targeting LAG3 are in clinical trials in combination with antibodies against other IRs, such as PD1/PDL1, to treat cancer [13].

2: LAG3 structure, function and ligands

LAG3 was discovered in 1990 [14] as a transmembrane molecule that is expressed on CD4⁺ and CD8⁺ T cells, natural killer T (NKT) cells, natural killer (NK) cells, plasmacytoid dendritic cells (pDCs), and regulatory T cells (T_{regs}) [15, 16]. In most cell types, LAG3 expression is regulated via activation with the exception of pDCs and T_{regs} in which expression appears to be constitutive [17, 18]. The majority of studies on LAG3 outlined below have been described in murine models. However, there has been some analysis of LAG3 expression and function in humans with many more studies likely underway given the number of LAG3-targeting immunotherapies that are currently in clinic.

2.1: Structure and Ligands

LAG3 resides on chromosome 12 (12p13.32) in humans and chromosome 6 in mice, encoding a 498-amino acid protein [19]. The LAG3 locus is adjacent to the gene encoding the CD4 co-receptor and has a similar intron/exon organization. Like CD4, LAG3 binds to MHC class II but with a much higher affinity (Fig 1). Combined, these observations suggesting that LAG3 may have evolved from a gene duplication event of the CD4 locus.

Both CD4 and LAG3 consist of four extracellular immunoglobulin superfamily-like domains (D1-D4), although structure of LAG3 has yet to be solved. However, LAG3 does appear to possess two unique structural features. First, unlike CD4 the interaction between LAG3 and MHC class II is mediated through a unique, proline-rich, thirty amino acid loop within D1 [20]. Second, LAG3 has a longer connecting peptide between the fourth Ig domain and the transmembrane region rendering it more susceptible to cell surface shedding by a disintegrin and metalloproteinase domain-containing protein (ADAM) [20, 21].

The cytoplasmic tail of LAG3 consists of three conserved motifs (Fig.1). However, the function and downstream signaling events remain unknown. The first motif contains a putative serine phosphorylation site, which contains two serine residues in humans and one in mice [Ser454]). Thus far, no function has been ascribed to this motif. The second motif is a highly unique and conserved six amino acid sequence (KIEELE) that has been shown to be required for LAG3 to downregulate T cell function [9, 22]. The third motif is a glutamic acid and proline dipeptide repeat (EP) that is phylogenetically conserved. It has been suggested that this motif binds LAG3-associated protein (LAP) which permits LAG3 co-localization with CD3, CD4, and/or CD8 molecules within lipid rafts [23]. However, mutations in the EP motifs maintain LAG3 activity and function, suggesting that it may not be essential [9] and no follow-up studies have been reported to date.

The primary, canonical ligand for LAG3 is MHC class II, which as noted above binds to a conserved, extended loop in the LAG3 D1 domain [24]. Once LAG3 is bound to MHC class II, it transmits inhibitory signals via its cytoplasmic domain, although the mechanism of signal transduction remains obscure [9]. Human melanoma cancer cells exhibit elevated expression of MHC class II which has been linked to poor patient prognosis [25]. LAG3:MHC class II binding contributes to tumor escape from apoptosis [26], and recruitment of tumor-specific CD4⁺ T cells, which subsequently leads to a reduction of the CD8⁺ T cell response [27].

However, over the past five years, other potential ligands have emerged. Galectin-3 (Gal-3), a 31 kDa galactose-binding lectin that regulates T cell activation, has been shown to bind to LAG3, which appears to be required for optimal inhibition of CD8⁺T cell cytotoxic function [28] (Fig 1). Gal-3 can be expressed on different cell types thus exerting its regulatory function on CD8⁺ T cells via multiple mechanisms [29]. LSECTin has also been proposed as LAG3 ligand. It binds to the four glycosylated sites on LAG3 and is a member of the DC-SIGN family of molecules. LSECTin is expressed in the liver and melanoma tumor cells, suggesting a mechanism by which LAG3 can regulate CD8⁺ T cells and NK cell function in these environments [30].

Fibrinogen-like protein 1 (FGL1) was recently described as a new ligand for LAG3 [31] (Fig 1). FGL1 is a member of the fibrinogen family, sharing a similar structure with fibrinogen beta and gamma, with no known role on platelets or in clot formation [32]. FGL1 is normally secreted by hepatocytes in the liver, however tumor cells can also express high levels of FGL1, which correlates with poor patient prognosis and resistance to immunotherapy [31]. Strikingly, blocking the interaction between LAG3 and FGL1 with a monoclonal antibody increases intratumoral T cell responses, which leads to decreased

tumor size in murine models of melanoma, presenting a new mechanism for targeted immunotherapy. In summary, LAG3 appears to have multiple ligands but it remains to be determined if all are valid ligands, and when and where each are critical for LAG3 function.

2.2: Regulation of LAG3 expression

Cell surface expression of LAG3 is regulated by two mechanisms. First, LAG3 is stored in lysosomal compartments to facilitate rapid translocation to the cell surface following TCR stimulation to control T cell responses [33, 34]. In resting T cells, LAG3 is degraded in the lysosomal compartments. This degradation is the limiting step for the surface expression of LAG3, and inhibition of lysosomal activity increases its surface expression. Moreover, its translocation to the cell surface is mediated by protein kinase C signaling through the cytoplasmic domain [34].

Second, LAG3 cell surface expression is regulated by proteolytic cleavage, resulting in the shedding of a soluble form of LAG3 (sLAG3) [20]. LAG3 cleavage is mediated by a disintegrin and metalloproteinase domain-containing protein 10 and 17 (ADAM10 and ADAM17), whose activity can also induce the cleavage of other immune receptors such as TNF α , CD62L, TIM3 and VEGFR2 [35]. Cell surface expression of ADAM10 results in constitutive LAG3 cleavage while the activity of ADAM17 is controlled by serine phosphorylation in a TCR- and PKC θ -dependent manner [21]. Shedding occurs at the connecting peptide between the D4 domain and the transmembrane region. There does not appear to be a function for sLAG3 activity as it does not compete with surface LAG3 for binding to bind MHC II and it is rapidly degraded *in vivo* [20]. However, its cleavage is necessary for optimal T cell function as prevention of LAG3 shedding by non-cleavable LAG3 mutants reduced T cell function, as exhibited by decreased proliferation and attenuated IL-2 and IFN- γ production [21]. Overall, expression of LAG3 is tightly regulated through two separate mechanisms highlighting the importance of tightly controlling LAG3 expression and function to ensure optimal immune homeostasis.

3: Role of LAG3 in murine models and human disease

LAG3 is an IR expressed on activated and dysfunctional T cells. When engaged, it negatively regulates T cell function. Thus, LAG3 is an emerging target for modulating T cell responses in disease. Below, we will discuss studies that support the role of LAG3 as an important IR that impacts autoimmunity, chronic infection (viral and parasitic) and cancer. While LAG3 has been studied in multiple disease models, given the emergence and prominence of immunotherapy for cancer, the majority of studies to date in mice and humans have examined the role of LAG3 in cancer (Fig 2).

3.1: Autoimmune and Inflammatory Diseases

IRs play a pivotal role in regulating autoimmune and inflammatory diseases [36]. In fact, symptoms of autoimmunity are often a side effect of checkpoint blockade for solid tumors due to loss of self-tolerance [37, 38]. Loss of LAG3 does not induce autoimmunity unless the mice are on a permissive genetic background [39, 40]. However, deletion of both LAG3 and PD1 leads to substantive, lethal autoimmunity [41]. On a non-obese diabetic (NOD)

background, LAG3 is required for controlling T cell expansion in the islet. Antigen-specific Lag3^{-/-} CD4⁺ and CD8⁺ T cells infiltrate the pancreas faster in younger mice, showing accelerated development of autoimmune diabetes compared to age-matched wild-type mice. Moreover, in wild-type NOD mice, administration of an anti-LAG3 blocking antibody also accelerates type 1 diabetes [39]. In contrast, T_{reg}-specific deletion of LAG3 in NOD mice leads to a significant delay in the development of autoimmune diabetes [18]. This is due to an increase in T_{reg} proliferation and survival via enhanced IL-2/STAT5 signaling and Eos (*Irf4*)-driven transcription, which ultimately improves T_{reg} suppressive function. This suggests that the immunologic effect of LAG3 deletion or blockade on all T cells is more pronounced on enhancing the function of diabetogenic CD4⁺ effector T cells over T_{regs}. However, in autoimmune environments where chronic inflammation dominates, LAG3 may be constitutively expressed on T_{regs}, ultimately limiting their capacity to suppress auto-reactive effector T cells.

Targeting LAG3 has also generated interest in clinical applications for autoimmune disease in humans. An anti-LAG3 mAb (GSK2831781; GlaxoSmithKline [GSK]) has been developed, which is a humanized Antibody Dependent Cell Cytotoxicity (ADCC) enhanced monoclonal afucosylated antibody, that depletes LAG3-expressing immune cells in patients with autoimmune disease. This agent has completed a phase 1, first-in-human study in patients with plaque psoriasis () and will soon proceed to a phase 2 study. Another agonistic monoclonal antibody (mAb) targeting LAG3 (IMP761; Immutep) is currently in pre-clinical development for the treatment of autoimmune disease. These novel agents highlight that modulation of the immune response via enhanced LAG3 activity may have therapeutic potential in treating autoimmune and inflammatory diseases.

3.2: Chronic Viral and Parasitic Infections

IRs are highly expressed on dysfunctional and exhausted T cells in chronic viral and parasitic infections. The role of LAG3 in acute and chronic infections *in vivo* has been highlighted by multiple groups [16, 42, 43]. We initially demonstrated that LAG3 deficient CD4⁺ and CD8⁺ T cells displayed increased proliferation and IFN- γ production in acute (Sendai) and chronic (γ -herpesvirus) viral infections, further underlining the role of LAG3 as a negative regulator of the immune system [10]. T cell exhaustion and chronic viral infection has also been robustly studied in the LCMV clone 13 model. In this model, the severity of the infection was directly proportional to LAG3 expression. Although LAG3 blockade alone only reduced viral load slightly [16, 42], co-blockade of LAG3 and PDL1 synergistically enhanced CD8⁺ T cell responses and significantly reduced viral load [16].

In a nonhuman primate model (rhesus macaques) of mycobacterium tuberculosis (MTB) infection, LAG3 is highly induced on CD4⁺ T cells and NK cells in the lungs and particularly in the granulomatous lesions of macaques. This coincided with high bacterial burden and changes in the host Th1 response [44]. Similarly, in malaria (*Plasmodium falciparum*) infection in mice, LAG3 is expressed by exhausted, parasite-specific CD4⁺ T cells [45]. *In vivo* LAG3 blockade in murine models has demonstrated synergy with PDL1 blockade to restore CD4⁺ T cell function, amplify the number of follicular helper T cells, germinal-center B cells, and plasmablasts, and enhance protective antibodies that led to

rapid clearance of blood-stage malaria. Correlative studies done in humans have shown that infection with *Plasmodium falciparum* resulted in higher expression of LAG3 and PD1 associated with T cell dysfunction [45].

In summary, although there is limited efficacy in targeting LAG3 alone in treating chronic infections, targeting LAG3 for a synergistic effect with anti-PD1/PDL1 holds significant promise as an immuno-therapeutic approach for the treatment of chronic viral, bacterial or parasitic infections in humans. Further clinical studies in patients will be needed to define the feasibility, safety and efficacy of this approach.

3.3: Cancer

In cancer, T cells are constantly exposed to antigen, leading to a progressive loss of cytokine production and the capacity of CD8⁺ T cells to specifically kill tumor cells [46]. LAG3 expression is elevated on tumor-infiltrating lymphocytes (TILs) in many types of cancer [13, 43, 47]. In the context of vaccination, LAG3 blockade is specifically required for antigen-specific CD8⁺ T cell responses. Mice vaccinated with vaccinia virus–HA (VV-HA) in a tolerance model in combination with anti-LAG3 blocking antibody demonstrate accumulation of CD8⁺ T cells in areas of high antigen expression [48]. In several pre-clinical murine models of melanoma, colon adenocarcinoma and ovarian cancer, co-blockade of LAG3 and PD1, expressed on both CD4⁺ and CD8⁺ TILs, induced an increased anti-tumor response [41, 49]. LAG3 is also expressed on intratumoral T_{regs}, which can contribute to tumor immune escape. LAG3 expression on T_{regs} induces production of IL-10 and TGF-β1 [50]. Thus, blockade of LAG3 on T_{regs} may reduce their suppressive function, leading to reinvigoration of CD8⁺ TIL activity.

LAG3 reduces T cell proliferation and cytokine secretion, as demonstrated by enhanced T cell activation following addition of a blocking LAG3 antibody [22]. Chronic tumor antigen stimulation leads to persistent up-regulation of LAG3 and PD1 on CD8⁺ tumor antigen-specific T cells, which leads to their functional exhaustion [13, 46]. Expression of LAG3 on CD8⁺ T cells defines a more exhausted state of CD8⁺ T cells than PD1 expression alone. For example, tumor-specific (Melan-A/MART-1) CD8⁺ TIL isolated from melanoma metastases expressed higher levels of LAG3 and CTLA-4 when compared to the tumor-specific CD8⁺ T cells in the patient peripheral blood lymphocytes (PBL) [51]. They also failed to produce IFN-γ after peptide stimulation, compared to the tumor-specific CD8⁺ T cells in the PBL, which retained competent effector function. In ovarian cancer patients, the tumor antigen (NY-ESO-1)-specific CD8⁺TIL expressed higher levels of PD1, with some populations co-expressing PD1 and LAG3, as compared to the same NY-ESO-1-specific CD8⁺ T cells in the matched patient PBL [49]. Functionally, CD8⁺PD1⁺LAG3⁺ ovarian cancer TIL have significantly decreased production of IFN-γ, TNF-α and IL-2, compared to PD1⁺LAG3⁻ or LAG3⁻PD1⁻ subsets. Interestingly, the co-expression of LAG3 and PD1 on the CD8⁺ ovarian cancer TIL was driven by IL-6/IL-10 and tumor-derived APCs, known immunosuppressive components of the TME found in malignant ascites. Blockade of LAG3 in addition to PD1 was found to synergistically enhance proliferation and cytokine production of NY-ESO-1-specific CD8⁺ TIL when compared to PD1 blockade alone [49].

LAG3 expression in the TME is also correlated with increased tumor mutational burden. For instance, cancers with high microsatellite instability (MSI^{hi}), such as a subset of colorectal cancer patients, exhibit higher somatic mutations and higher levels of immunogenic neoantigens. The tumor microenvironment of these MSI^{hi} tumors are characterized by increased expression of multiple IRs including LAG3, PD1, PDL1, CTLA4 and IDO, as compared with microsatellite stable (MSS) tumors [52]. These data may suggest a correlation between tumor mutational burden, anti-tumor immune response, and the co-expression of LAG3 and other IRs. In fact, the presence of LAG3 and other co-expressed IRs in the TME may explain why MSI^{hi} tumors are not naturally eliminated despite a hostile immune microenvironment [52].

Studies to evaluate the predictive potential of LAG3 in the context of immunotherapy-treated patients is an area of active research. Currently, short-term expression of LAG3 after immunotherapy treatment can serve as a marker of T cell infiltration and general immune activation, and thus, better responsiveness to immune checkpoint blockade. In a study of longitudinal tissue biopsies from 46 metastatic melanoma patients initially treated with CTLA4 blockade followed by PD1 blockade at progression, the early on-treatment biopsies of responders displayed significantly increased expression of LAG3, as well as other immune biomarkers (PD1, PDL1, CD3, CD8, CD4), as compared to the same patients' pre-treatment biopsies [53]. In contrast, significant expression of these immune biomarkers on pre-treatment or early post-treatment biopsies was not observed in non-responders. In a more recent study comparing global gene expression profiles of melanoma patient tumors pre- and post-anti-PD1 (nivolumab) therapy, it was found that many immune checkpoint genes, with LAG3 in particular, exhibited increased expression (LAG3, PD1, PDL1, CTLA-4, CD80, ICOS, 4-1BB) regardless of response to therapy [54]. Further analysis of responders vs. non-responder tumors identified a broader spectrum of differentially-expressed immune-related genes, including additional immune checkpoint molecules, as well as genes involved in lymphocyte activation, chemotaxis, cytokine signaling and immune cytolytic activities [54]. More in depth analyses must be performed to evaluate the role of LAG3 in the context of immunotherapy at the protein level, as all current studies focus on gene expression. Ultimately, the expression of LAG3, in concert with other IRs such as PD1, could serve as a prognostic immune biomarker for multiple solid tumors.

4: Clinical development of therapeutic agents to target LAG3 in the tumor microenvironment

There are currently ten experimental therapeutic agents targeting LAG3 that are being tested in clinical trials for various human cancers (Table 1). The initial first-in-class agent was IMP321 (Immutep), which is a 200 kDa soluble chimeric recombinant fusion protein of the extracellular portion of LAG3 and antibody Fc domain, which is proposed to activate antigen-presenting cells (APCs) via interaction with its canonical ligand, MHC class II. In pre-clinical studies, IMP321 has been found to activate APCs to promote the proliferation of dendritic cells (DCs), ameliorate T_{reg} immunosuppression, and improve antigen cross-presentation to CD8⁺ T cells [55]. IMP321 has completed three clinical trials in renal cell carcinoma, metastatic breast carcinoma and melanoma, but has only exhibited limited

success with small patient cohorts. However, in a completed phase 1 trial for metastatic breast cancer (), preliminary efficacy of an IMP321 plus chemotherapy (paclitaxel) combination exhibited a 50% overall response rate (ORR) compared to 25% with paclitaxel alone [56]. The efficacy of IMP321 was correlated with increased activation of multiple immune cell types in patients. There are ongoing clinical trials in other cancer types to further evaluate its therapeutic potential (Table 1).

LAG3-specific blocking mAbs have dominated the therapeutic modalities currently under investigation. There are now seven mAbs in development: Relatlimab (BMS-986016, Bristol-Myers Squibb; fully human IgG4 mAb), LAG525 (Novartis; humanized IgG4), MK-4280 (Merck; humanized IgG4), REGN3767 (Regeneron; human IgG4), TSR-033 (Tesaro; humanized IgG4), Sym022 (Symphogen; Fc-inert human mAb), and INCAGN02385 (InCyte; Fc-engineered IgG1 κ) (Table 1). There is considerable interest in the development of mAbs targeting LAG3 for human cancer, particularly in combination with anti-PD1. This is based on preclinical findings that systemic PD1 and LAG3 expression is generally limited in mice and humans but is highly upregulated on intratumoral, dysfunctional TILs [41, 49, 57–59]. Therefore, co-blockade of both IRs may have a more restricted effect within the tumor microenvironment, limiting adverse events [2, 5, 13, 41, 60–62].

Two bispecific antibodies have also recently entered the clinic that target LAG3 and PD1 (MGD013; MacroGenics) or PDL1 (FS118; F-star) (Table 1). MGD013 consists of humanized high-affinity anti-PD1 and anti-LAG3 mAbs that are assembled into an Fc-bearing DART (Dual-affinity Re-targeting) protein. It targets PD1/PDL1, PD1/PDL2 and LAG3/MHC class II interactions. Further *in vitro* functional characterization has demonstrated enhanced cytokine secretion (i.e. in response to antigenic re-challenge of prior superantigen-stimulated T cells), as compared to the same T cells with either anti-PD1 or anti-LAG3 blocking alone. This agent also demonstrated a prolonged circulating half-life in cynomolgus macaques. FS118 was generated by incorporating an engineered Fc region with anti-LAG3 antigen binding capability (Fcab) onto a PDL1-specific antibody. Upon administration, FS118 simultaneously targets LAG3 expressed on T cells in the TME and PDL1 on tumor cells or APCs. *In vitro* functional assays demonstrated increased activation of human CD8⁺ T cells in response to MHC class I restricted peptides. *In vivo* studies of this agent in MC38 tumor-bearing mice demonstrated significant anti-tumor activity equivalent to a combination of antibodies targeting LAG3 and PDL1. This correlated with loss of LAG3 surface expression on CD4⁺ and CD8⁺ T cells, and an increase in the CD8:T_{reg} ratio. These novel bispecific agents are currently being tested in phase I clinical trials.

4.1: Clinical trials with anti-LAG3 blocking antibodies in cancer

Relatlimab (BMS-986016) is a fully human LAG3-specific antibody that was isolated following immunization of transgenic mice expressing human immunoglobulin (Ig) genes. It is expressed as an immunoglobulin G4 (IgG4) isotype antibody and includes a stabilizing hinge mutation (S228P). It is the first anti-LAG3 humanized mAb to be developed and, of all the anti-LAG3 mAbs, is the furthest along in clinical development. The initial phase I/IIa trial launched in 2013 was designed to evaluate the safety and efficacy of LAG3 blockade as

a monotherapy or in combination with Nivolumab for patients with advanced solid malignancies (cervical, ovarian, bladder, colorectal, HPV-positive HNSCC, gastric, hepatocellular, RCC), who were not previously exposed to immunotherapy (). It is now being evaluated in 18 phase I and II/III trials in a variety of solid and hematological malignancies.

The combination of Relatlimab and Nivolumab (anti-PD1) demonstrated exciting preliminary efficacy in melanoma patients who were refractory to previous immunotherapy () [63]. In this phase I/IIa dose escalation and cohort expansion study, these pre-treated patients, all of whom had progressed on immunotherapy, and 47% of whom had failed 3 or more prior therapies, were treated with the combination of Nivolumab and Relatlimab. Among 48 evaluable patients, the objective response rate (ORR) was 12.5%. Of note, patients with LAG3 expression >1% on tumor-infiltrating lymphocytes (TIL) had a response rate of 20%, compared to 7.1% among patients with <1% LAG3 expression. Interestingly, the combination of Nivolumab and Relatlimab was well tolerated, with grade 3 or 4 treatment related immune adverse events seen in 9% of patients, which is similar to the frequency seen with Nivolumab alone. This suggest that an Nivolumab/Relatlimab combination may be safer than a Nivolumab/Ipilimumab (anti-CTLA4) combination. Among those patients with evaluable tumor specimens, the rate of LAG3 positivity, defined as the percentage of LAG3⁺ immune cells >1% of tissue, was approximately 60%. It is not known whether LAG3 positivity correlates with poorer response to Nivolumab or whether LAG3 expression increases at disease progression, as these were not tested in this trial. Based on these promising findings, there is now an ongoing phase II/III trial of Nivolumab + Relatlimab versus Nivolumab alone in the front-line setting against previously untreated unresectable/metastatic melanoma ().

Preliminary results were recently reported for the first-in-human phase I dose-finding study for the MK-4280 anti-LAG3 mAb (Merck) as monotherapy or in combination with Pembrolizumab for patients with advanced solid tumors ([64]). MK-4280 monotherapy demonstrated 6% ORR, with 17% Disease Control Rate (DCR) in 18 patients, whereas MK-4280 + Pembrolizumab demonstrated 27% ORR, with 40% DCR in 15 patients. The majority of patients in both groups had received two or more lines of prior therapy. The MK-4280 monotherapy or in combination with Pembrolizumab were well-tolerated, with no dose-limiting toxicities.

Taken together, these early clinical trial findings are congruent with the findings in the murine studies and although anti-LAG3 blockade by itself does not currently demonstrate significant anti-tumor efficacy, there may be a synergistic anti-tumor immune response when combined with anti-PD1 blockade. In murine tumor models, as well as in some cancer patients who are refractory to anti-PD1 therapy, a combination of anti-LAG3 with anti-PD1 may help to overcome resistance to anti-PD1 immunotherapy. These are of significant interest for the ongoing exploration of LAG3 as an alternative immune checkpoint target, as well as a potential predictive biomarker of clinical response [65]. Randomized phase III clinical trials in the near future with rigorous comparison between the anti-LAG3 + anti-PD1 versus anti-PD1-treated groups, will be required to determine if there is clinical efficacy in multiple cancer types.

5: Conclusions and Future Directions

LAG3 is an IR that helps to maintain homeostasis in the immune system. We are just beginning to understand the complex biology of LAG3. Vital questions remain regarding how to apply and optimize the efficacy of LAG3-targeted immunotherapy, alone or in combination with anti-PD1/L1 and other immunotherapies, for autoimmune and inflammatory diseases, chronic infections, and cancer.

First, how LAG3 negatively regulates TCR signaling is essentially unknown. The unique intracellular cytoplasmic domain of LAG3 with the conserved 'EP' and 'KEELE' motifs, and absence of a tyrosine-based motif, distinguishes it from all other immune inhibitory receptors. We need to have a better understanding of how LAG3 works, as this will serve as an important conceptual advance and provide vital information for future immunotherapeutic development.

Second, the discovery of fibrinogen-like protein (FGL1) as a novel ligand for LAG3 [31] also offers an exciting possibility of dual blockade of FGL1 and LAG3 to enhance cancer immunotherapy. It also remains to be determined how important LAG3:FGL1 interaction is in human cancer and if the current LAG3-targeting therapeutics block LAG3:FGL1 interaction.

Third, given that LAG3 is expressed on multiple immune cell types, understanding the difference and similarities in the function of LAG3 in these cell types and how they are impacted by anti-LAG3 blockade in the setting of autoimmunity, chronic infections and cancer is imperative. Of particular importance is understanding how anti-LAG3 would affect the function of CD4⁺ and CD8⁺ T_{eff} versus T_{reg} sub-populations, and the role of LAG3 on pDCs, NK cells and other cells types remains obscure.

Fourth, there are still important basic and translational questions that remain unanswered: (a) Can anti-LAG3 agonistic antibodies be generated and can they suppress autoimmune and inflammatory diseases, and if so, which immune cell population or subset mediates this effect? (b) What signaling pathway(s) underlie the complex synergistic interactions between LAG-3 and other immune checkpoints such as PD1? (c) How does anti-LAG3 alone or in combination with anti-PD1 alter the transcriptional program of exhausted T cells in the setting of chronic infections and cancer? (d) Can LAG3 expression and/or sLAG3 in plasma serve as a prognostic or predictive biomarker to inform combinatorial immunotherapy and would this be more important in certain tumor types or disease settings?

Elucidating these questions is critical for designing efficacious immunotherapeutic strategies that target LAG3. Based on the promising pre-clinical human and murine studies with LAG3-targeted therapies in combination with anti-PD1/PDL1, numerous clinical studies are ongoing to fully evaluate their safety and efficacy. It will also be intriguing to evaluate the results from ongoing clinical trials using LAG3 depleting or agonistic mAb for autoimmune and inflammatory disorders. Lastly, identifying approaches that combine anti-LAG3 with other therapeutic modalities, such as chemotherapy, radiation therapy and targeted therapy, may unleash further potential therapeutic approaches. As the third inhibitory receptor to be targeted in the clinic, behind CTLA4 and PD1, LAG3 remains an exciting target for

immunotherapy in numerous diseases including chronic infection, autoimmunity, inflammatory disease, and of course cancer.

ACKNOWLEDGEMENTS

The authors wish to thank Lawrence Andrews for helpful comments. This work was supported by the National Institutes of Health [P01 AI108545 and R01 CA203689 to D.A.A.V, and R01 AI144422 to D.A.A.V. and C.J.W.]; NCI Comprehensive Cancer Center Support CORE grant [CA047904 to D.A.A.V.], HNSCC SPORE grant [P50 CA097190 to D.A.A.V.]; Associazione Italiana Per La Ricerca Sul Cancro (AIRC) postdoctoral fellowship [Project code: 22321 to E.R.]; and NCI Institutional National Research Service Award in Cancer Therapeutics [T32 CA193205 supporting R.W.; to E. Chu].

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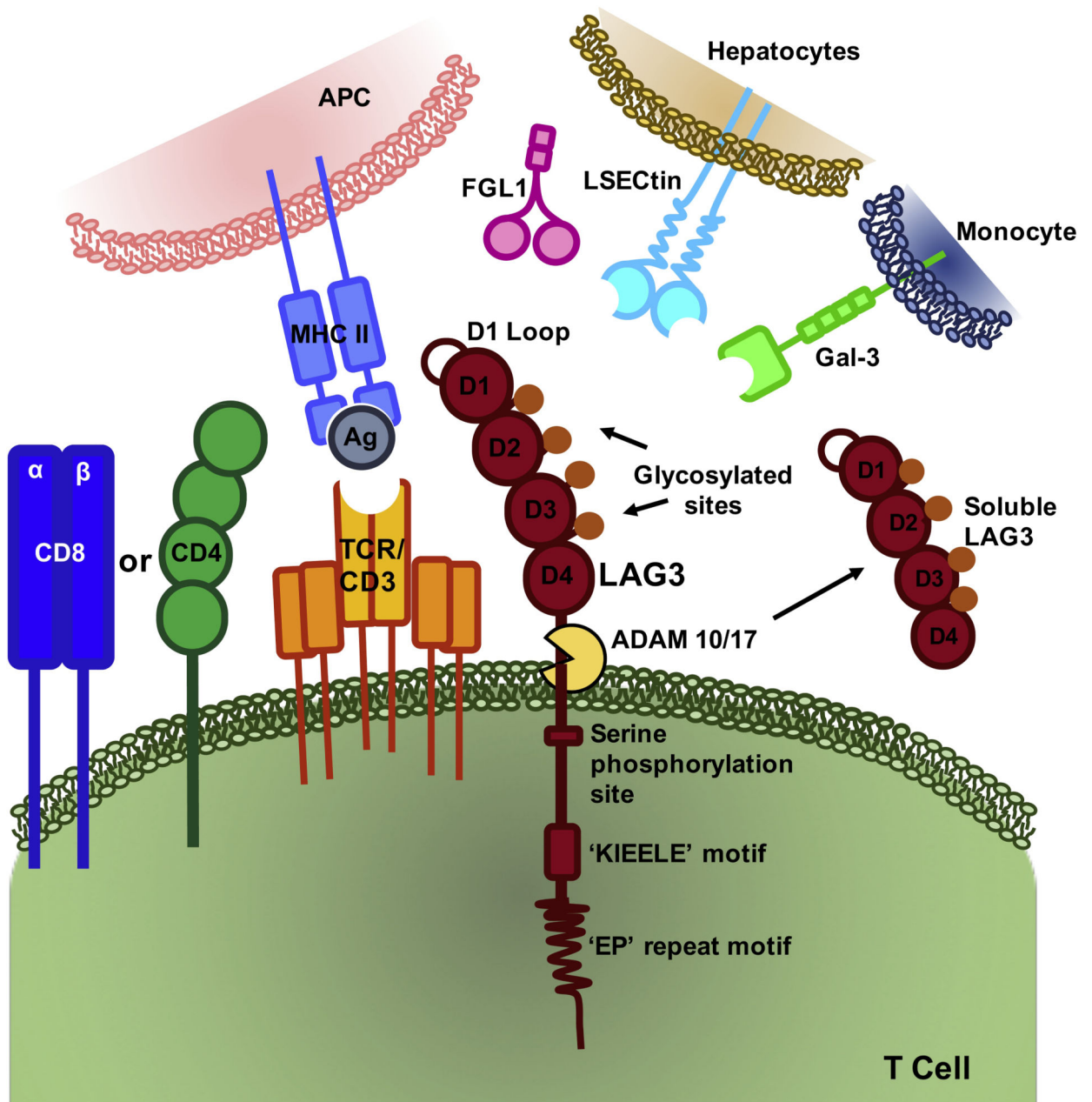


Figure 1: LAG3 Structure and Ligands

LAG3 is composed of four Ig-like domains and contains three highly conserved regions in the cytoplasmic tail. LAG3 binds MHC class II through a thirty amino acid loop in the D1 domain. Galectin-3, LSEctin and FGL1 have also been reported to bind to LAG3.

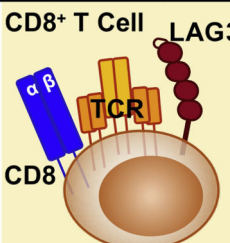
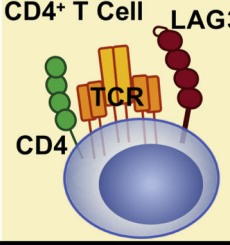
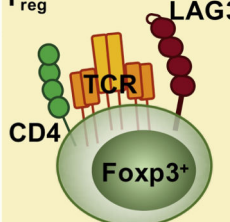
Cell Type	Autoimmunity	Cancer	Infection
 <p>CD8⁺ T Cell</p>	<p>Activation:</p> <p>↓ T cell expansion^{39,40,41}</p> <p>↓ Autoimmunity^{39,40,41}</p>	<p>Activation:</p> <p>↑ T cell exhaustion^{13,43,46-48,49}</p> <p>↓ Tumor killing^{13,43,46-48,49, 51-52}</p>	<p>Activation:</p> <p>↓ T cell response in acute and chronic infections^{10,16,42,43}</p>
	<p>Blockade:</p> <p>↑ CD8⁺ T cell expansion and tissue infiltration^{39,40,41}</p>	<p>Blockade:</p> <p>↑ T cell function^{41,48-49,60-62}</p> <p>↑ Tumor killing^{41,48-49,60-62}</p>	<p>Blockade:</p> <p>↑ T cell response in acute and chronic infections^{10,16,42,43}</p>
 <p>CD4⁺ T Cell</p>	<p>Activation:</p> <p>↓ T cell expansion^{39,40,41}</p> <p>↓ Autoimmunity^{39,40,41}</p>	<p>Activation:</p> <p>↑ T cell exhaustion^{41,49}</p> <p>↓ Anti-tumor response^{41,49}</p>	<p>Activation:</p> <p>↓ T cell response in acute and chronic infections^{10,44-46}</p>
	<p>Blockade:</p> <p>↑ CD4⁺ T cell expansion and tissue infiltration^{39,40,41}</p>	<p>Blockade:</p> <p>↑ T cell function^{41,49}</p> <p>↑ Anti-tumor response^{41,49}</p>	<p>Blockade:</p> <p>↑ T cell response in acute and chronic infections^{10,45}</p>
 <p>T_{reg}</p>	<p>Activation:</p> <p>↓ Suppressive function¹⁸</p> <p>↑ Autoimmunity¹⁸</p>	<p>Activation:</p> <p>↑ Immunosuppressive cytokines⁵⁰</p>	<p>Activation:</p> <p>↑ Activation of Treg</p> <p>↑ Suppressive function</p>
	<p>Blockade:</p> <p>↑ Suppressive function¹⁸</p> <p>↓ Autoimmunity¹⁸</p>	<p>Blockade:</p> <p>↓ Suppressive function</p>	<p>Blockade:</p> <p>↓ Suppressive function</p>

Figure 2:
Role of LAG3 on Different Cell Types in Multiple Diseases

Table 1:

Clinical trials for LAG-3-targeted immunotherapy in human cancer

Trial number/Ref	Study population	Interventions	Phase	Status	Immune Outcome	Patient outcome
IMP321 (a soluble LAG-3 Ig)						
[66]	Stage IV renal Cell Carcinoma	IMP321	I	Completed 10/2008	- Increased CD8 ⁺ percentage/activation at higher (>6 mg) doses	-Better PFS and tumor growth reduction at higher (>6 mg) doses
[67]	Metastatic breast carcinoma patients receiving first-line paclitaxel	IMP321	I	Completed 1/2010	-Increase in the number/activation of APCs (monocytes and DCs), NK, and CD8 ⁺ T cells.	-Objective tumor response rate:50% (vs. 25% with paclitaxel alone) -Clinical benefit in 90% of patients
[68]	Melanoma	Lympho-depletion, vaccine, IMP321 adjuvant	I	Completed 11/2011	-Expansion of MART-1 specific CD8 ⁺ T cells in 83% of patients. -Reduced T _{regs} expansion	- No confirmed responses per RECIST criteria
	Solid tumors Peritoneal carcinomatosis	IMP321	I	Recruiting Estimated completion date: 2/2019	N/A	N/A
	Hormone receptor-positive metastatic breast cancer	Paclitaxel + IMP321/Placebo	II	Recruiting Estimated completion date: 12/2020	N/A	N/A
(TACTI-mel) [69]	Unresectable/metastatic Stage III/IV Melanoma	IMP321 + Pembrolizumab (anti-PD-1)	I	Active, not recruiting Estimated completion date: 12/2018	N/A	- ORR: 33% (from the start of combination therapy). DCR: 66%.
	Untreated or recurrent/refractory unresectable/metastatic NSCLC, HNSCC	IMP321 + Pembrolizumab	II	Not yet recruiting Estimated completion date: 6/2021	N/A	N/A
BMS-986016 (Relatlimab, anti-LAG3 mAb)						
(CA224-020) [70-72]	Advanced solid tumors	Relatlimab ± Nivolumab (BMS-936558, anti-PD-1)	I/IIa	Recruiting Estimated completion date: 10/2019	-Increased proliferation/activation of NK and CD8 ⁺ T cells in the treated patients	-ORR: 11.5% (3-fold higher RR in melanoma patients with 1% LAG3) DCR: 49%
(CA224-034)	Advanced solid tumors	Relatlimab ± Nivolumab	I	Recruiting Estimated completion date: 7/2020	N/A	N/A
(CA224-047)	Previously untreated metastatic or unresectable melanoma	Nivolumab ± Relatlimab	II/III	Active, not recruiting	N/A	N/A

Trial number/Ref	Study population	Interventions	Phase	Status	Immune Outcome	Patient outcome
				Estimated completion date: 3/2022.		
(CA224-048)	Advanced malignant tumors	Relatlimab ± Nivolumab + BMS-986205 (anti-IDO1)/Ipilimumab (anti-CTLA4)	I/II	Recruiting Estimated completion date: 5/2022.	N/A	N/A
	Treatment-naïve metastatic melanoma	1 cycle of lead-in phase (Nivolumab, Relatlimab, Nivolumab + Relatlimab) followed by Nivolumab + Relatlimab combination	II	Recruiting Estimated completion date: 10/2022.	N/A	N/A
	Resectable stage IIIB or IV (oligometastatic) melanoma	Neoadjuvant Nivolumab ± Relatlimab/Ipilimumab	II	Recruiting Estimated completion date: 2/2020	N/A	N/A
	Advanced chordoma	Relatlimab + Nivolumab	II	Not yet recruiting Estimated completion date: 7/2022	N/A	N/A
	Virus associated solid tumors	Relatlimab ± nivolumab	I/II	Recruiting Estimated completion date: 12/2019	N/A	N/A
	Recurrent and metastatic microsatellite high (MSI-H) and microsatellite-stable (MSS) colon cancer	Relatlimab ± Nivolumab	II	Active, not recruiting Estimated completion date: 12/2019	N/A	N/A
	Metastatic/locally advanced microsatellite stable (MSS) colon cancer	Relatlimab + Nivolumab	II	Recruiting Estimated completion date: 10/2022	N/A	N/A
	Advanced mismatch repair deficient solid tumors resistant to prior PD-(L)1 inhibitor	Relatlimab + Nivolumab	II	Recruiting Estimated completion date: 10/2022	N/A	N/A
	Relapsed/refractory Hematologic Malignancies	Relatlimab ± Nivolumab	I/IIa	Recruiting Estimated completion date: 1/2020	N/A	N/A
	Glioblastoma Gliosarcoma Recurrent brain neoplasm	A1: Relatlimab A2: Urelumab (anti-CD137) B1: Relatlimab ± Nivolumab B2: Urelumab + Nivolumab	I	Recruiting Estimated completion date: 12/2019	N/A	N/A
	Stage II/III resectable esophageal/GE junction cancer	Pre-operative nivolumab ± relatlimab combined with chemoradiation prior to surgical resection	Ib	Recruiting Estimated completion date: 2/2025	N/A	N/A
	Advanced gastric cancer	BMS-986016 + Nivolumab	II	Recruiting Estimated completion date: 11/2021	N/A	N/A
	Advanced non-small cell lung cancer	Nivolumab ± Relatlimab	II	Recruiting	N/A	N/A

Trial number/Ref	Study population	Interventions	Phase	Status	Immune Outcome	Patient outcome
	Advanced renal cell carcinoma	Nivolumab + Relatlimab/BMS-986205 (anti-IDO1)/Ipilimumab	II	Recruiting Estimated completion date: 1/2022	N/A	N/A
	Advanced solid tumors	Adaptive matching design: A: Nivolumab + Lirilumab (anti-KIR) B: Nivolumab + Relatlimab C: Nivolumab + Cabiralizumab (anti-CSF1R) D: Nivolumab + Ipilimumab E: Nivolumab + BMS-986156 (anti-GTTR) F: Nivolumab + BMS-986205 (anti-IDO1) G: Nivolumab + Radiation therapy	I	Recruiting Estimated completion date: 4/2022	N/A	N/A
LAG525 (anti-LAG-3 mAb)						
[73]	Advanced solid and hematologic malignancies	LAG525 + PDR001 (spartalizumab, anti-PD-1)	II	Active, not recruiting Estimated completion date: 2/2021	N/A	N/A
	Advanced solid tumors	LAG525 + Spartalizumab (anti-PD-1)	I/II	Active, not recruiting Estimated completion date: 4/2019	N/A	N/A
	Triple-negative breast cancer	A1: LAG525 + Spartalizumab A2: LAG525 + Spartalizumab + Carboplatin A3: LAG525 + Carboplatin	II	Recruiting Estimated completion date: 2/2019	N/A	N/A
	Triple-negative breast cancer	LAG3 + Spartalizumab ± NIR-178 (anti-A2AR)/INC-280 (Capmatinib, anti-c-Met)/MCS110 (anti-M-CSF)/Canakinumab (anti-IL1β)	Ib	Recruiting Estimated completion date: 7/2020	N/A	N/A
	Previously treated unresectable or metastatic melanoma	A1: LAG525 + Spartalizumab A2: Capmatinib + Spartalizumab A3: Canakinumab + Spartalizumab	II	Recruiting Estimated completion date: 3/2022	N/A	N/A
MK-4280 (anti-LAG3 mAb)						
	Hodgkin's and Non-Hodgkin's B-cell lymphomas	MK-4280 ± Pembrolizumab	I/II	Recruiting Estimated completion date: 12/2025	N/A	N/A
[74, 75]	Advanced solid tumors	MK-4280 ± Pembrolizumab	I	Recruiting Estimated completion date: 7/2021 - Preliminary results reported at 2018 SITC	N/A	- MK-4280: ORR: 6% DCR: 17% - Combination with Pembrolizumab: ORR: 27% DCR: 40%

Trial number/Ref	Study population	Interventions	Phase	Status	Immune Outcome	Patient outcome
	Advanced NSCLC	Pembrolizumab + Relatlimab/Lenvatinib based on GEP and TMB	II	Recruiting Estimated completion date: 5/2022	N/A	N/A
REGN3767 (anti-LAG-3 mAb)						
[76]	Advanced solid/hematologic malignancies	REGN3767 ± REGN2810 (anti-PD-1)	I	Recruiting Estimated completion date: 10/2020	N/A	N/A
TSR-033 (anti-LAG-3 mAb)						
[77]	Advanced solid tumors	TSR-033 ± anti-PD-1	I	Recruiting Estimated completion date: 5/2021	-Increased proliferation and cytokine production of CD8 ⁺ and CD4 ⁺ T cells <i>in vitro</i> and <i>in vivo</i> -Reduced M2 TAMs <i>in vivo</i>	N/A
Sym022 (anti-LAG-3 mAb)						
[78]	Advanced solid tumors and lymphomas	Sym022	I	Recruiting Estimated completion date: 8/2020	-Increased cytokine production by T-cells <i>in vitro</i> and tumor growth inhibition <i>in vivo</i> - Decreased total LAG3 surface levels via internalization/Shedding	N/A
	Advanced solid tumors and lymphomas	Sym021 (anti-PD-1) ± Sym022/Sym023 (anti-Tim3)	I	Recruiting Estimated completion date: 11/2020	N/A	N/A
INCAGN02385 (anti-LAG-3 mAb)						
[79]	Selected advanced solid tumors and lymphomas	INCAGN02385	I	Recruiting Estimated completion date: 5/2020	-Enhanced T-cell responsiveness to TCR stimulation <i>in vivo</i>	N/A
MGD013 (a PD-1/LAG-3 bispecific DART®) protein						
[80]	Advanced solid tumors, hematologic malignancies	MGD013	I	Recruiting Estimated completion date: 8/2022	-Enhanced cytokine production upon antigenic re-challenge of prior superantigen-stimulated T cells <i>in vitro</i> .	N/A
	Advanced that have progressed on or after prior PD-1/PD-L1 containing therapy			Recruiting date: 5/2020	CD8 ⁺ T cells <i>in vitro</i> . - Increased CD8 ⁺ T _{regs} ratio <i>in vivo</i> . - Induced loss of LAG3 surface expression on CD4 ⁺ and CD8 ⁺ T cells <i>in vivo</i> .	

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Abbreviations: PD-1=Programmed death 1; PD-L1 = Programmed death ligand 1; CTLA-4=Cytotoxic T lymphocyte antigen 4; Treg: T-regulatory cells; NK: Natural Killer cells; TAM: Tumor-associated macrophages. M-CSF: Macrophage colony-stimulatory factor; IDO-1= Indoleamine 2,3-dioxygenase; mAb=monoclonal antibody; DART= Dual-Affinity Re-Targeting. PFS: Progression-free survival; ORR: Overall response rate; DCR: Disease-control rate.