BINDING MOLECULES BINDING PD-L1 AND LAG-3

Related Applications

The present case is related to US 62/352482 filed on 20 June 2016, the contents of which are hereby incorporated by reference in their entirety.

Field of the Invention

The present invention relates to antibody molecules which bind to programmed death-ligand 1 (PD-L1) and lymphocyte-activation gene 3 (LAG-3). The antibody molecules preferably comprise a CDR-based antigen binding site for PD-L1, and a LAG-3 antigen binding site which may be located in two or more structural loops of a CH3 domain of the antibody molecule. The antibody molecules of the invention find application, for example, in cancer therapy.

Background to the invention

Lymphocyte Activation Gene-3 (LAG-3; CD223) is a member of the Ig superfamily, and is genetically and structurally related to CD4 (though with only 20% sequence identity). Like CD4, LAG-3 binds to MHC class II molecules but with higher affinity than CD4 (K_D = 60 nM). LAG-3 is expressed on activated T cells, NK cells, pDCs, B cells, γδ T cells and participates in immune suppression, particularly through persistent strong expression in a percentage of regulatory T cells (Tregs) (Liang et al, 2008).

The LAG-3 gene is located on human chromosome 12, adjacent to the CD4 gene, and spans 8 exons. There are five alternative transcripts, two of which generate protein products: a full length transmembrane protein and an alternatively spliced soluble monomeric form. The full-length transcript encodes a 525 amino acid protein with a molecular weight of 70 kDa and has functional activity, while the soluble form appears not to bind MHC class II molecules and its function is unknown. Human full-length LAG-3 protein has 93% sequence identity to *Macaca fascicularis* (cynomolgus monkey) LAG-3 and 70% sequence identity to *Mus musculus* (house mouse) LAG-3.

LAG-3 is a transmembrane protein with four extracellular Ig-like domains (D1-D4), and a cytoplasmic portion responsible for LAG-3 signalling. The cytoplasmic domain has an EP

(glutamic acid/proline) motif that associates with LAG-3-associated protein (LAP) as well as a KIEELE motif thought to be required for LAG-3 modulation of T cell function. Reports on the role of the EP motif suggest that it may be responsible for trafficking of LAG-3 to the T cell surface membrane (Bae et al, 2014), or may be directly responsible for modulating downstream signalling of STAT5 during T cell activation (Durham et al, 2014), or possibly both.

The immuno-suppressive mechanism of LAG-3 on T cells is thought to be driven by cross-linking of LAG-3 on activated T cells resulting in decreased calcium flux and IL-2 release during T cell activation (Huard et al, 1997). On Antigen Presenting Cells (APCs), binding to MHC II molecules by LAG-3 positive regulatory T cells causes decreased IL-12 secretion and down regulation of CD86 (Liang et al, 2008), a “secondary signal” of activation, resulting in T cell anergy from improper activation and/or reduced antigen presentation by the APCs. LAG-3 knock out mouse models are viable, with only mild lympho-hyperproliferation (Workman et al, 2003), indicating that LAG-3 acts as a modest immune “brake”.

This suppressive interaction between LAG-3 and MHC class II has also been proposed to occur between Tregs and CD4 positive T cells (Sega et al, 2014). Tregs supress the immune response either by release of suppressive cytokines (such as IL-10 and TGFβ), manipulation of inflammatory metabolism (such as CD73 catabolised adenosine), regulating APC maturation, or direct interaction between regulatory T cells and effector T cells. There is evidence in humans that MHC class II positive Tregs are more suppressive than MHC class II negative Tregs (Baecher-Allen et al, 2006) and actively suppress the immune response through direct interaction with LAG-3 expressed on effector T cells. While LAG-3 negative Tregs can suppress conventional T cell proliferation, LAG-3 negative CD4 and CD8 T cells are resistant to Treg immune suppression. This process was described to occur between human T cells through a process known as trogocytosis (Sega et al, 2014) whereby Tregs not only prevent APC maturation but also acquire MHC class II to suppress primed LAG-3 positive CD4 T cells.

LAG-3 expression is also a marker of repeated antigen stimulation. In cancer, T cells commonly adopt an “exhausted” phenotype, involving expression of immuno-suppressors such as PD-1, CTLA-4, TIM-3, and LAG-3 (Wherry et al, 2011), where the cells have a general inability to properly proliferate and secrete chemokines in response to antigen. Inhibition of these immune-suppressors lowers the immune threshold and (re-)enables a proper anti-cancer response by the T cells. In preclinical models, this has been borne out using antagonist antibodies against LAG-3, CTLA-4 and PD-1 where a decrease in tumour

burden was seen. LAG-3 inhibition by antagonistic antibodies is thought to reactivate the immune response in the tumour microenvironment, where expression of LAG-3 on CD4 positive T cells and CD8 positive T cells is associated with an exhausted phenotype, and LAG-3 expression on Tregs is associated with potent immuno-suppressive capabilities. Antibodies blocking LAG-3 increase T effector cell proliferation, cytokine production, cytotoxicity, and decrease Treg suppressor activity leading to a decrease in tumour growth.

In human tumours, increased expression of LAG-3 was found on tumour-infiltrating lymphocytes (TILs) from human renal cell carcinomas and other tumours, such as melanomas and lymphomas (Demeure et al, 2001; Wolchock et al, 2013). Importantly, LAG-3 is also closely correlated with T cell dysfunction in patients with chronic viral infection (Workman et al, 2005) and cancer (Workman et al, 2003). LAG-3 has also been identified as a surface marker for tumour-infiltrating Tregs in a variety of human cancers (Camisachi et al. 2010; Gandhi et al, 2006).

Monoclonal antibodies to human LAG-3 are in clinical development to abrogate immune suppression and potentially enhance antigen presentation in cancers (solid and haematological malignancies).

LAG-525 and IMP-701 (Novartis AG), are human antibodies against LAG-3 and have advanced to Phase II and I clinical studies, respectively, in kidney cancer (Renal Cell Cancer); Non-Small Cell Lung Cancer (NSCLC); Nasopharyngeal Cancer; Colorectal Cancer; Melanoma; Gastric Cancer and Adenocarcinoma of the Gastroesophageal Junction.

Anti-LAG-3 antibody BMS-986016 (Bristol-Myers Squibb Company), is currently in Phase I clinical testing for Ovarian Cancer; NSCLC; Colorectal Cancer; Cervical Cancer; Melanoma; Gastric Cancer; Bladder Cancer; Head And Neck Cancer Squamous Cell Carcinoma; Renal Cell Carcinoma and in Phase II studies in NSCLC; Relapsed Chronic Lymphocytic Leukemia (CLL); Refractory Chronic Lymphocytic Leukemia (CLL); Melanoma; Non-Hodgkin Lymphoma; Hodgkin Lymphoma; Diffuse Large B-Cell Lymphoma; Indolent Lymphoma; Mantle Cell Lymphoma; Refractory Multiple Myeloma; and Relapsed Multiple Myeloma as either monotherapy or as part of combination therapies.

Further antibodies against LAG-3 are also in preclinical development.

Programmed cell death 1 (PD-1) and its ligands PD-L1 (CD274, B7-H1) and PD-L2 (B7-DC) deliver inhibitory signals that regulate the balance between T cell activation, tolerance, and

immunopathology. PD-L1 is transiently expressed on all immune cells and some tumour cells.

PD-L1 is a type I transmembrane protein with two Ig-like domains within the extracellular region, a transmembrane domain and a short cytoplasmic domain. The cytoplasmic domain has no known signal transduction motif suggesting that there is no signalling by PD-L1 on interaction of the ligand with its receptor. Its molecular weight is 40 kDa (290 amino acids) and it is encoded by the CD274 gene on mouse chromosome 19 and human chromosome 9, respectively. PD-L1 is a member of the B7 protein family and shares approximately 20 % amino acid sequence identity with B7.1 and B7.2. Human PD-L1 shares 70 % and 93 % amino acid identity with the murine and cynomolgus orthologs of PD-L1, respectively.

PD-L1 binds to its receptor PD-1 with an affinity (K_D) of 770 nM. PD-1 is expressed on activated T cells, B cells, and myeloid cells, and modulates activation or inhibition of cellular immune responses. Binding of PD-L1 to PD-1 delivers an inhibitory signal, reducing cytokine production and proliferation of T cells. Consequently, PD-L1 expression by cells can mediate protection against cytotoxic T lymphocyte (CTL) killing and is a regulatory mechanism that dampens chronic immune responses during viral infections. Cancer, as a chronic and pro-inflammatory disease subverts this immune-protective pathway through up-regulation of PD-L1 expression to evade the host immune response. In the context of an active immune response, IFNγ also upregulates the expression of PD-L1.

PD-L1 also mediates immune suppression through interaction with another protein, B7.1 (also known as CD80), blocking its ability to deliver one of the secondary signals of activation on T cells through CD28. In terms of PD-L1 expression on tumour cells and its engagement with B7.1, the relevance of this specific interaction in tumour immune resistance is still unclear.

PD-L1 expression has been shown in a wide variety of solid tumours. Of 654 samples examined in one study, spanning 19 tumours from different sites, 89 (14%) were PD-L1 positive (≥5% frequency). The highest PD-L1 positive frequencies were seen in head and neck (17/54; 31%), cervical (10/34; 29%), cancer of unknown primary origin (CUP; 8/29; 28%), glioblastoma multiforme (GBM; 5/20; 25%), bladder (8/37; 21%), oesophageal (16/80; 20%), triple negative (TN) breast (6/33; 18%), and hepatocarcinoma (6/41; 15%) (Grosso et al, 2013). Tumour-associated expression of PD-L1 has been shown to confer immune resistance and potentially protect tumour cells from T cell mediated apoptosis.

Therapies targeting PD-L1 have shown excellent results in murine *in vivo* studies. In the B16 murine model of melanoma, treatment with anti-PD-L1 combined with either GVAX or FVAX vaccination strategies resulted in a significant effect both on survival (30 days for control vs 52 days for PD-L1-treated) and percentage of tumour-free (5%) animals upon conclusion of the study (Curran et al, 2010). Anti-PD-L1 therapy has also been used to study the mechanism of immune suppression in the P815 murine mastoma model. P815 cells injected into mice normally trigger a strong immune response, which results in their rejection. When PD-L1 is expressed on P815 cells, these cells escape immune attack, which in turn can be negated through administration of anti-PD-L1 antibodies (Iwai et al, 2002). It is evident that targeting the PD-1/PD-L1 axis in immunogenic human cancers (Herbst et at, 2014) results in a survival advantage through stimulation of an anti-cancer immune response (Wolchock et al, 2013; Larkin et al, 2015).

Atezolizumab (MPDL3280A, RG7466, TECENTRIQ) is a humanized IgG1 antibody which binds PD-L1. It is in clinical trials as a single agent and also in combination with other biologic and/or small molecule therapies for treatment of solid cancers, including colorectal cancer, breast cancer, non-small-cell lung carcinoma, bladder cancer, and renal cell carcinoma. Treatment with atezolizumab resulted in objective response rates (ORR) of 23% in NSCLC, 36% melanoma, 33% bladder, 14% in RCC, and 13% in head and neck cancers (Herbst et al, 2014; Powles et al, 2014).

Avelumab (MSB0010718C) is a fully human IgG1 antibody which binds to PD-L1 and is undergoing clinical testing in a number of cancers including bladder cancer, gastric cancer, head and neck cancer, mesothelioma, non-small-cell lung carcinoma, ovarian cancer, renal cancer and Merkel-cell carcinoma.

Durvalumab (MEDI4736) is a human IgG1 antibody which binds to PD-L1 and is being tested in clinical trials alone or in combination with tremelimumab in non-small-cell lung cancer, squamous cell carcinoma of the head and neck, bladder cancer, pancreatic cancer and with other biologic and small molecules in trials for additional solid cancers such as gastric cancers, melanoma and unresectable hepatocellular carcinoma.

Further anti-PD-L1 antibodies have been tested in clinical trials, including BMS-936559 and others are in preclinical testing.

However, few anti-LAG-3 therapies are currently in clinical testing and none have been approved for therapy so there remains a need to develop additional molecules which target LAG-3. Whilst there are some anti-PD-L1 therapeutics in development, current data shows that overall treatment with anti-PD-L1 monotherapy results in a response in less than 50% of cancer patients. Thus, there remains a need in the art for additional molecules which can target LAG-3 and/or PD-L1 and which find application in cancer therapy.

Statements of invention

Anti-PD-1 and anti-PD-L1 antibodies are predominantly involved in breaking immune tolerance and activating an anti-tumour immune response. LAG-3, expressed on T cells following activation, and constitutively expressed on exhausted T cells, further maintains these cells in a suppressive state. Blockade of LAG-3, when employed in combination with other established immune suppressive molecules (i.e.: PD-1, PD-L1) has also been shown to provide a synergistic improved immune response in murine tumour models (Woo et al, 2012). The present inventors postulated that therapies targeting both of these pathways simultaneously will directly address mechanisms which promote and maintain T cell exhaustion. In addition, the inventors expect that targeting LAG-3 may suppress antigen presentation through the action of LAG-3 expressing regulatory T cells on APCs and the published research documenting down-regulation of CD86 (Grosso et al, 2013). Blocking this interaction is expected to maintain antigen presentation, while blocking PD-L1 signalling is expected to break tolerance, resulting in a significant anti-tumour response when both pathways are inhibited at the same time.

Published data on anti-LAG-3 and anti-PD-L1 antibody combinations is limited though there are some results from preclinical syngeneic mouse tumour and viral load models. In a murine model of myeloma, a combination of anti-PD-L1 and anti-LAG-3 blocking antibodies was administered following low dose whole body irradiation and improved survival rates to greater than 80% (Jing et al, 2015). No evidence of systemic or organ-specific autoimmunity was observed. LAG-3 and PD-1 knock-out mice showed markedly increased survival from and clearance of multiple transplantable tumours (Woo et al, 2012).

The present inventors postulated that bispecific antibodies which bind to both LAG-3 and PD-L1 would confer a number of advantages over the combination of monoclonal antibodies against these antigens, including:

1. Directed Therapy

Activated T cells express LAG-3 in the lymph nodes. One part of the anti-LAG-3/PD-L1 bispecific antibody targets primed LAG-3-positive T cells in the lymph node, which then traffic to the site of the tumour, transporting the bispecific antibody. Once within the tumour microenvironment, T cells carrying the bispecific antibody are able to immediately engage and block PD-L1 on tumour cells via the anti-PD-L1 portion. Consequently, all T cells traveling to the tumour site would be resistant to both LAG-3 and PD-L1/PD-1 signalling.

2. Bridging

Primed CD8 positive T cells encounter tumour antigens within the tumour microenvironment, where they respond by killing the tumour cell in the absence of suppressive signals. Bispecific antibodies are expected to be superior over combinations of individual monoclonal therapies by maintaining or prolonging this contact between T cells and tumour cells. Signal strength in the activation of T cells is essential, which in the case of presented antigen in cancers may be key (Engels et al, 2013) and the presence of a bispecific anti-LAG-3/PD-L1 antibody bound to targets on APC or cancer cells is expected to increase the time in which the T cells can successfully recognise antigen and become activated.

3. Localisation

In areas of inflammation and ongoing immune responses, PD-L1 expression is significantly increased because of localised IFN-γ release. This is true whether on target cancer cells, tumour-associated macrophages (TAM), or repeated stimulation of T cell populations. A bispecific antibody that antagonises PD-L1 and LAG-3 is expected to localise and concentrate to the areas of greatest PD-L1 expression in the tumour whilst allowing the anti-LAG-3 portion to bind and prevent LAG-3-mediated suppression of T cells.

Following an extensive screening and affinity maturation programme, the present inventors were able to identify ten specific binding members comprising a binding site specific for LAG-3 in the CH3 domain of the molecule. These molecules were shown to have a high affinity for both human and cynomolgus LAG-3. The high affinity for human LAG-3 is expected to be advantageous in the treatment of e.g. cancers containing tumour-infiltrating lymphocytes (TILs) expressing LAG-3 in human patients, while the high affinity for cynomolgus LAG-3, which is comparable to the affinity for human LAG-3, is expected to be useful in the evaluation of the properties of the specific binding members in cynomolgus

monkey disease models. The reason for this is that the results obtained are more likely to be predictive of the effects of the specific binding member in human patients than when a molecule which has a higher variability in its affinity for human and cynomolgus LAG-3 is tested in cynomolgus monkey models.

The specific binding members were also shown to have high activity in a T cell activation assay, which is expected to be predictive of improved efficacy in human patients through enhanced inhibition of LAG-3.

The present inventors further combined these specific binding members comprising a binding site specific for LAG-3 in the CH3 domain with an antibody Fab domain comprising a CDR-based antigen binding site for PD-L1 to create bispecific antibody molecules comprising binding sites for both LAG-3 and PD-L1, which are expected to have the advantages detailed above. Surrogate murine versions of these antibody molecules which bind to murine LAG-3 and murine PD-L1 were also prepared by the inventors and shown to be capable of significantly inhibiting tumour growth in syngeneic mouse models of cancer. In particular, use of these surrogate murine molecules demonstrated there is a synergistic effect on tumour growth suppression when an antibody molecule comprising binding sites for both LAG-3 and PD-L1 is administered to mice in the mouse models tested. Based on the similar mechanism of action of mouse and human LAG-3 and PD-L1 in the tumour environment, murine studies that show efficacy in diminishing tumour burden are expected to translate into clinical therapeutic benefits in human cancer patients. Based on these results, it is therefore expected that the antibody molecules of the invention will show a superior effect in the treatment of cancer in human patients, in particular in supressing tumour growth, than, for example, administration of two separate molecules which bind LAG-3 and PD-L1, respectively.

Thus, in a first aspect the present invention provides an antibody molecule which binds to both to PD-L1 and LAG-3. Specifically, these antibodies comprise:

(i) a CDR-based antigen binding site for PD-L1; and

(ii) a LAG-3 antigen binding site located in, or engineered into, two or more structural loops of a CH3 domain of the antibody molecule. The LAG-3 binding site preferably comprises the amino acid sequences WDEPWGED (SEQ ID NO: 1) and PYDRWVWPDE. (SEQ ID NO: 3).

The antibody molecule preferably comprises the amino acid sequence set forth in SEQ ID NO: 1 in the AB loop, and the amino acid sequence set forth in SEQ ID NO: 3 in the EF loop of the CH3 domain.

Thus, in a first aspect, the present invention provides an antibody molecule which binds to programmed death-ligand 1 (PD-L1) and lymphocyte-activation gene 3 (LAG-3). The antibody molecule preferably comprises (i) a CDR-based antigen binding site for PD-L1; and (ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule.

The LAG-3 binding site preferably comprises the amino acid sequences WDEPWGED (SEQ ID NO: 1) and PYDRWVWPDE (SEQ ID NO: 3). The amino acid sequence WDEPWGED is preferably located in a first structural loop of the CH3 domain and the amino acid sequence PYDRWVWPDE is preferably located in a second structural loop of the CH3 domain.

For example, the LAG-3 antigen-binding site may be located in a structural loop region of a CH3 domain of the antibody molecule, wherein the structural loop region preferably comprises two or more structural loops, and wherein the LAG-3 binding site preferably comprises the amino acid sequences WDEPWGED (SEQ ID NO: 1) and PYDRWVWPDE (SEQ ID NO: 3).

As a further example, the LAG-3 antigen-binding site may be engineered into two or more structural loops of a CH3 domain of the antibody molecule, wherein the LAG-3 binding site preferably comprises the amino acid sequences WDEPWGED (SEQ ID NO: 1) and PYDRWVWPDE (SEQ ID NO: 3).

As mentioned above, the sequences of the LAG-3 binding site are preferably located in two or more structural loops of the CH3 domain of the antibody molecule. In a preferred embodiment the LAG-3 antigen-binding site comprises the amino acid sequence set forth in SEQ ID NO: 1 in the AB loop, and the amino acid sequence set forth in SEQ ID NO: 3 in the EF loop of the CH3 domain.

The amino acid sequence set forth in SEQ ID NO: 1 is preferably located at residues 11 to 18 of the CH3 domain; and/or the amino acid sequence set forth in SEQ ID NO: 3 is located at residues 92 to 101 of the CH3 domain; wherein the amino acid residue numbering is according to the ImMunoGeneTics IMGT) numbering scheme.

The LAG-3 antigen-binding site of the antibody molecule may further comprise one of the following sequences, preferably in the CD loop of the CH3 domain of the antibody molecule:

(i) SNGQPENNY (SEQ ID NOS 2, 8 and 18);
(ii) SNGQPEDNY (SEQ ID NO: 13);
(iii) SNGYPEIEF (SEQ ID NO: 23);
(iv) SNGIPEWNY (SEQ ID NO: 28);
(v) SNGYAEYNY (SEQ ID NO: 33);
(vi) SNGYKEENY (SEQ ID NO: 38);
(vii) SNGVPELNV (SEQ ID NO: 43); or
(viii) SNGYQEDNY (SEQ ID NO: 48).

Preferably, the LAG-3 antigen-binding site of the antibody molecule further comprise one of the following sequences, preferably in the CD loop of the CH3 domain of the antibody molecule: the amino acid sequence set forth in SEQ ID NO: 2, 28, or 38 in the CD loop of the CH3 domain. More preferably, the LAG-3 antigen-binding site of the antibody molecule further comprises the amino acid sequence set forth in SEQ ID NO: 2 in the CD loop of the CH3 domain

The amino acid sequence set forth in SEQ ID NO: 2, 8, 13, 18, 23, 28, 33, 38, 43, or 48 is preferably located at residues 43 to 78 of the CH3 domain of the antibody molecule, wherein the residues are numbered according to the IMGT numbering scheme.

The sequence of the CH3 domain of the antibody molecule, other the sequences of the LAG-3 antigen-binding site, is not particularly limited. Preferably, CH3 domain is a human immunoglobulin G domain, such as a human IgG1, IgG2, IgG3, or IgG4 CH3 domain, most preferably a human IgG1 CH3 domain. The sequences of human IgG1, IgG2, IgG3, or IgG4 CH3 domains are known in the art.

In a preferred embodiment, the antibody molecule comprises the CH3 domain set forth in SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50, more preferably the CH3 domain set forth in SEQ ID NO: 5, 30, or 40, most preferably the CH3 domain set forth in SEQ ID NO: 5. Alternatively, the antibody molecule may comprise a CH3 domain with an amino acid sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50, preferably SEQ ID NO: 5, 30, or 40, more preferably SEQ ID NO: 5.

The antibody molecule may further comprise a CH2 domain. The CH2 domain is preferably located at the N-terminus of the CH3 domain, as in the case in a human IgG molecule. The CH2 domain of the antibody molecule is preferably the CH2 domain of human IgG1, IgG2, IgG3, or IgG4, more preferably the CH2 domain of human IgG1. The sequences of human IgG domains are known in the art. In a preferred embodiment, the antibody molecule comprises an IgG CH2 domain with the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54, or a CH2 domain with an amino acid sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 53 or SEQ ID NO: 54.

In a preferred embodiment, the antibody molecule comprises the sequence set forth in SEQ ID NO: 6, 7, 11, 12, 16, 17, 21, 22, 26, 27, 31, 32, 36, 37, 41, 42, 46, 47, 51, or 52, or a sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence set forth in SEQ ID NO: 6, 7, 11, 12, 16, 17, 21, 22, 26, 27, 31, 32, 36, 37, 41, 42, 46, 47, 51, or 52. More preferably, the antibody molecule comprises the sequence set forth in SEQ ID NO: 6, 7, 31, 32, 41, or 42, or a sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence set forth in SEQ ID NO: 6, 7, 31, 32, 41, or 42. Yet more preferably, the antibody molecule comprises the sequence set forth in SEQ ID NO: 6 or 7, or a sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence set forth in SEQ ID NO: 6 or 7.

In a preferred embodiment, the antibody molecule is a human immunoglobulin G molecule, such as a human IgG1, IgG2, IgG3 or IgG4 molecule, more preferably a human IgG1 molecule. The sequences of human immunoglobulin G molecules is known in the art and introducing a CH3 domain or CH3 domain sequence as disclosed here into such a molecule would not present any difficulty to the skilled person.

The antibody molecule preferably comprise the CDRs of the VH and/or VL domains set forth in SEQ ID NOs 92 and 93. Methods for determining CDR sequence in a given VH or VL domain are known in the art and include the Kabat and IMGT numbering systems. More preferably, the antibody molecule preferably comprise one or more, two or more, three or more, four or more, five or more, or all six of the complementarity determining regions set forth in SEQ ID NOs 86 to 91. Preferably, the antibody molecule comprises the VH and/or VL domains set forth in SEQ ID NOs 92 and 93, respectively.

In a preferred embodiment, the antibody molecule comprises the heavy chain sequence set forth in any one of SEQ ID NOs: 94 to 113, or a heavy chain with an amino acid sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 94 to 113, provided the VH domain of the heavy chain sequence remains unchanged. More preferably, the antibody molecule comprises the heavy chain sequence set forth in any one of SEQ ID NOs: 94, 95, 104, 105, 108, and 109, or a heavy chain with an amino acid sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 94, 95, 104, 105, 108, and 109, provided the VH domain of the heavy chain sequence remains unchanged.. Yet more preferably, the antibody molecule comprises the heavy chain sequence set forth in SEQ ID NO: 94 or 95, or a heavy chain with an amino acid sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NOs: 94 or 95, provided the VH domain of the heavy chain sequence remains unchanged..

In a further preferred embodiment, the antibody molecule may in addition, or alternatively, comprise the light chain sequence set forth in SEQ ID NO: 116, or a light chain with an amino acid sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 116, provided the VL domain of the light chain sequence remains unchanged..

The antibody molecule is preferably capable of simultaneously binding to PD-L1 and LAG-3. The PD-L1 and LAG-3 may, for example, be present on two different cells. Without wishing to be limited by theory, it is thought that this results in cross-linking between cells and internalization of PD-L1 and/or LAG-3 making them unavailable for stimulation.

The present inventors have shown that an antibody molecule comprising (i) a CDR-based antigen binding site for PD-L1; and (ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule, in accordance with the present invention, FS18-7-9/84G09, surprisingly mediated Complement Dependent Cytotoxicity (CDC) of PD-L1 expressing cells but not of LAG-3 expressing cells, even when a mixture of LAG-3 and PD-L1 expressing cells were present in the sample. This property is expected to be useful where, as is the case with FS18-7-9/84G09, the CDR-based antigen binding of the antibody molecule targets

a tumour cell and the binding site located in a constant domain of the antibody molecule targets an immune cell, as the immune cell would be protected from CDC mediated by binding to the antibody molecule, while tumour cells would be subject to CDC.

Thus, in a further embodiment, the present invention relates to an antibody molecule which binds to tumour antigen and an immune cell antigen, wherein the antibody molecule comprises:

(i) a CDR-based antigen binding site for a tumour antigen; and

(ii) an antigen binding site for an immune cell antigen located in a constant domain, preferably a CH3 or CH2 domain, more preferably a CH3 domain, of the antibody molecule,

wherein the antibody molecule does not mediate, or does not mediate significant, complement dependent cytotoxicity of an immune cell comprising said immune cell antigen when said immune cell is bound by the antibody molecule.

Preferably, the antibody molecule further mediates complement dependent cytotoxicity of a tumour cell comprising said tumour antigen when said tumour cell is bound by the antibody molecule.

Methods for measuring CDC of an antibody molecule are known in the art and described herein.

The present inventors have further shown that an antibody molecule comprising (i) a CDR-based antigen binding site for PD-L1; and (ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule, in accordance with the present invention, FS18-7-9/84G09, surprisingly mediated low Antibody Dependent Cellular Cytotoxicity (ADCC) of LAG-3 expressing cells compared with ADCC of PD-L1 expressing cells. This property is again expected to be useful where, as is the case with FS18-7-9/84G09, the CDR-based antigen binding of the antibody molecule targets a tumour cell and the binding site located in a constant domain of the antibody molecule targets an immune cell, as the immune cell would be subjected to lower ADCC than a tumour cell bound by the antibody.

As explained herein, mutations for reducing or abrogating ADCC activity of antibody molecules are known in the art. One such mutations is the LALA mutation described herein. It was unexpectedly found that FS18-7-9/84G09 has low ADCC activity towards LAG-3-expressing cells. Where it is not necessary to completely abrogate ADCC activity, this may represent an advantage.

Thus, in a further embodiment, the present invention relates to an antibody molecule which binds to tumour antigen and an immune cell antigen, wherein the antibody molecule comprises:

(i) a CDR-based antigen binding site for a tumour antigen; and

(ii) an antigen binding site for an immune cell antigen located in a constant domain, preferably a CH3 or CH2 domain, more preferably a CH3 domain, of the antibody molecule,

wherein the antibody molecule causes less ADCC with respect to immune cells comprising said immune cell antigen when said immune cells are bound by the antibody molecule than of tumour cells comprising said tumour antigen when said tumour cells are bound by the antibody molecule. Preferably, the antibody molecule does not mediate, or not mediate significant, ADCC of an immune cell comprising said immune cell antigen when said immune cell is bound by the antibody molecule. The antibody molecule may further not mediate, or not mediate significant, complement dependent cytotoxicity of an immune cell comprising said immune cell antigen when said immune cell is bound by the antibody molecule and/or may mediate complement dependent cytotoxicity of a tumour cell comprising said tumour antigen when said tumour cell is bound by the antibody molecule.

Methods for measuring ADCC of an antibody molecule are known in the art and described herein.

Various tumour antigens and immune cell antigens are known in the art. The tumour antigen and immune cell antigen are preferably cell-surface antigens. The immune cell antigen is preferably an antigen present on tumour-infiltrating lymphocytes.

The antigen binding site for the immune cell antigen preferably comprises one or more modifications in one or more structural loops of the constant domain of the antibody molecule, such as the AB, CD, and/or EF loop of the constant domain. For example, the binding site may be a LAG-3 binding site as herein described.

The antibody molecule of the invention may be conjugated to an immune system modulator, cytotoxic molecule, radioisotope, or detectable label. The immune system modulator may be cytotoxic molecule is a cytokine.

The present invention also provides a nucleic acid encoding an antibody molecule of the invention, as well as a vector comprising such a nucleic acid.

A recombinant host cell comprising a nucleic acid or the vector of the invention is also provided. Such a recombinant host cell may be used to poduce an antibody molecule. Thus, also provided is a method of producing an antibody molecule of the invention, the method comprising culturing the recombinant host cell under conditions for production of the antibody molecule. The method may further comprise a step of isolating and/or purifying the antibody molecule.

The antibody molecules of the present invention are expected to find application in therapeutic applications, in particular therapeutic applications in humans, such as cancer treatment. Thus, also provided is a pharmaceutical composition comprising an antibody molecule according to the invention and a pharmaceutically acceptable excipient.

The present invention also provides an antibody molecule of the invention, for use in a method of treating cancer in a patient. Also provided is a method of treating cancer in a patient, wherein the method comprises administering to the patient a therapeutically effective amount of an antibody molecule according to the invention. Further provided is the use of an antibody molecule according to the invention for use in the manufacture of a medicament for the treatment of cancer in a patient. The treatment may further comprise administering an anti-tumour vaccine and/or a chemotherapeutic agent to the patient.

Brief Description of the Figures

Figure 1: **A** shows a sequence alignment of the nine Fcabs identified following the second affinity maturation, FS18-7-32; FS18-7-33; FS18-7-36; FS18-7-58; FS18-7-62; FS18-7-65; FS18-7-78; FS18-7-88; and FS18-7-95, against the parental Fcab, FS18-7-9. **B** shows the percentage sequence identity of each of these Fcabs with the sequence of the parental Fcab, FS18-7-9.

Figure 2 shows the results of a T cell activation assay. Specifically, Figure 2 shows representative plots of IL-2 release, which is indicative of T cell activation, in the panel of cell lines treated with mAb^2 FS18-7-9/84G09 (**Figure 2A-C**), or FS18-7-62/84G09 or FS18-7-78/84G09 (**Figure 2D-F**), and control antibodies. **Figure 2A:** The assay with LAG-3 and PD-L1 required inhibition of both targets (FS18-7-9/84G09LALA, FS18-7-9/4420LALA+84G09, 25F7+84G09LALA or 25F7+S1LALA) to have activation. **Figure 2B**: The assay with LAG-3 only required inhibition of LAG-3 for activation (FS18-7-9/84G09LALA, FS18-7-9/4420LALA+84G09, 25F7+84G09LALA, 25F7+S1LALA, 25F7, FS18-7-9/4420 LALA). **Figure 2C:** The assay with PD-L1 only required the inhibition of PD-

L1 for activation (FS18-7-9/84G09LALA, FS18-7-9/4420LALA+84G09, 25F7+84G09LALA, 25F7+S1LALA, 84G09LALA). **Figure 2D:** The assay with LAG-3 and PD-L1 required inhibition of both targets (FS18-7-62/84G09LALA, FS18-7-78/84G09LALA, FS18-7-62/4420LALA+84G09, FS18-7-78/4420LALA+84G09, 25F7+84G09LALA or 25F7+S1LALA) to have activation. **Figure E:** The assay with only LAG-3 required inhibition of LAG-3 for activation (FS18-7-62/84G09LALA, FS18-7-62/4420LALA, FS18-7-78/84G09LALA, FS18-7-78/4420LALA, FS18-7-62/4420LALA+84G09, FS18-7-78/4420LALA+84G09, 25F7+84G09LALA or 25F7+S1LALA). **Figure 2F:** The assay with only PD-L1 required the inhibition of PD-L1 for activation (FS18-7-62/84G09LALA, FS18-7-78/84G09LALA, FS18-7-62/4420LALA+84G09, FS18-7-78/4420LALA+84G09, 25F7+84G09LALA or 25F7+S1LALA).

Figure 3 shows that FS18-7-9/84G09 was able to induce T cell activation, as indicated by IL-2 release, in the presence of both cLAG-3 + cPD-L1 and cLAG-3 or cPD-L1 alone showing functionally cynomolgus cross-reactivity.

Figure 4 shows a representative plot from the SEB assay. LAG-3/PD-L1 mAb^2 and the combination of the LAG-3/4420 mAb^2 + 84G09LALA showed greater activation that the 84G09LALA mAb alone, whereas the LAG-3/4420 mAb^2 or 4420 mAb did not show significant activation.

Figure 5 shows the end tumour weights on day 20 in the non-established MC38 syngeneic tumour model. LAG-3/PD-L1 mAb^2 (FS18-29/S1) treated mice had end tumours with significantly smaller weights than those treated with a combination of benchmark mAbs, C9B7W and S1.

Figure 6 shows growth curves of the non-established MC38 syngeneic tumour model. LAG-3/PD-L1 mAb^2 (FS18-29/S1) treated mice had smaller tumours than those treated with a combination of benchmark mAbs, C9B7W and S1, or those treated with S1 alone. The LAG-3/4420 mAb^2 and benchmark anti-LAG-3 mAb had little impact on tumour growth.

Figure 7 shows the end tumour weights on day 24 in the established MC38 syngeneic tumour model. LAG-3/PD-L1 mAb^2 (FS18-29/S1) was just as effective in suppressing tumour growth as the combination of benchmark antibodies, C9B7W and S1. FS18-29/4420 alone had no noticeable impact on tumour growth and S1 and C9B7W both had a mild effect on resulting tumour growth.

Figure 8 shows tumour growth curves of the established MC38 syngeneic tumour model. LAG-3/PD-L1 mAb^2 (FS18-29/S1) treated mice had tumours volumes similar to those treated with a combination of benchmark mAbs, C9B7W and S1. Mice treated with S1 or C9B7W alone showed intermediate tumour volumes whereas LAG-3/4420 mAb^2 treatment had no impact on tumour growth.

Figure 9 shows the end tumour weights on day 20 in the non-established CT26 syngeneic tumour model. LAG-3/PD-L1 mAb^2 (FS18-29/S1 and FS18-35/S1) supressed tumour growth to a greater extent than combination of benchmark antibodies, C9B7W and S1.

Figure 10 shows tumour growth curves of the non-established CT26 syngeneic tumour model. There was a demonstrated statistically significant difference in FS18-35/S1 versus IgG control in suppressing tumour growth. Such a statistically significant difference was not observed with the combination of benchmark antibodies, versus the IgG control group.

Figure 11 shows the end tumour weights on day 22 in the non-established MC38 syngeneic tumour model used to compare the effect of the LALA mutation in the mAb^2 on tumour growth inhibition. There was no statistically significant difference in end tumour weight between mice treated with mAb^2 with and without the LALA mutation.

Figure 12 shows tumour growth curves of the non-established MC38 syngeneic tumour model used to compare the effect of mAb^2 with and without the LALA mutation on tumour growth inhibition. There was no statistically significant difference in the tumour growth curves between mice treated with mAb^2 with and without the LALA mutation, however there was a trend toward increased tumour growth inhibition by molecules containing the LALA mutation.

Figure 13 shows the effect of mAb^2 treatment on T cell LAG-3 expression. LAG-3 expression on CD8 (**A**), CD4 (**B**) and FoxP3 (**C**) tumour infiltrating lymphocytes (TILs) treated with mAb^2 FS18-29/S1, FS18-29/4420, S1, FS18-29/4420 and S1, or control antibody 4420 is shown at day 19 and 23 after tumour inoculation, corresponding to days 3 and 7 after the last mAb^2/antibody dosing, respectively. LAG-3 expression was decreased after treatment with mAb^2 FS18-29/S1 at day 19 and 23. Animals given a combination of FS18-29/4420 and S1 also showed a decrease in LAG-3 expression but the effect was delayed until day 23, while FS18-29/4420 or S1 administered individually resulted in little to no decrease in LAG-3 expression.

Figure 14 shows the percentage of lysis of PD-L1 and LAG-3 expressing Raji cells following various antibody/mAb^2 treatments using a lactate dehydrogenase (LDH) release CDC assay. **A** and **B** show CDC-mediated lysis of PD-L1 and LAG-3 expressing Raji cells, respectively. Cells were incubated with anti-LAG-3 antibody 25F7, anti-PD-L1 antibody 84G09, anti-PD-L1 antibody 84G09 comprising the LALA mutation, anti-CD20 antibody Rituximab, mAb^2 FS18-7-9/84G09, mAb^2 FS18-7-9/84G09 comprising the LALA mutation, or Rituximab comprising the LALA mutation. Release of LDH was measured 4 hrs after treatment with baby rabbit complement and expressed as percentage compared to total lysis. The concentration of the antibody/mAb^2 treatment is indicated on the X-axis. A shared value was set for the slopes of all curves in order to be able to compare across the curves.

Figure 15 shows the percentage of dead PD-L1 and LAG-3 expressing Raji cells following various treatments using a flow cytometry based CDC assay. A mixture of differentially fluorescently labelled PD-L1 and LAG-3 expressing cells were incubated with control antibody 4420, anti-CD20 antibody Rituximab (RIT), a combination of anti-LAG-3 antibody 25F7 and anti-PD-L1 antibody 84G09, or mAb^2 FS18-7-9/84G09. Cells were then treated with baby rabbit complement and stained using a dye only staining dead cells. The percentage of dead cells in the two cell populations was evaluated as a percentage of total cells. The cell type (PD-L1 expressing or LAG-3 expressing, identified by the differential fluorescent labels) evaluated following each treatment is indicated in **Figure 15** after the name of the relevant treatment, see e.g. 4420 PDL1, which refers to evaluation of viability of PD-L1 expressing Raji cells following treatment with control antibody 4420. The concentration of the antibody/mAb^2 treatment is indicated on the X-axis

Figure 16 shows the percentage lysis (cytotoxicity) of PD-L1 and LAG-3 expressing Raji cells following various treatments using a lactate dehydrogenase (LDH) release ADCC assay. Cells were treated with anti-CD20 antibody Rituximab, anti-PD-L1 antibody 84G09, mAb^2 FS18-7-9/84G09, Rituximab comprising the LALA mutation, mAb^2 FS18-7-9/84G09 comprising the LALA mutation, anti-LAG-3 antibody 25F7, anti-PD-L1 antibody 84G09 comprising the LALA mutation, control antibody 4420, or control antibody 4420 comprising the LALA mutation. Treated cells were then co-incubated with primary NK cells and specific LDH release was measured as a percentage compared to total lysis of target cells. The concentration of the antibody/mAb^2 treatment is indicated on the X-axis.

Detailed Description

The present invention relates to antibody molecules which bind both to PD-L1 and LAG-3. Specifically, the antibody molecules of the present invention comprise a CDR-based antigen binding site for PD-L1 and a LAG-3 antigen binding site located in a constant domain of the antibody molecule. The terms “PD-L1” and “LAG-3” may refer to human PD-L1 and human LAG-3, murine PD-L1 and murine LAG-3, and/or cynomologus monkey PD-L1 and cynomologus monkey LAG-3, unless the context requires otherwise. Preferably the terms “PD-L1” and “LAG-3” refer to human PD-L1 and human LAG-3, unless the context requires otherwise.

The term “antibody molecule” describes an immunoglobulin whether natural or partly or wholly synthetically produced. The antibody molecule may be human or humanised. The antibody molecule is preferably a monoclonal antibody molecule. Examples of antibodies are the immunoglobulin isotypes, such as immunoglobulin G, and their isotypic subclasses, such as IgG1, IgG2, IgG3 and IgG4, as well as fragments thereof.

The term “antibody molecule”, as used herein, thus includes antibody fragments, provided said fragments comprise a CDR-based antigen binding site for PD-L1 and a LAG-3 antigen binding site located in a constant domain, such as a CH1, CH2, or CH3 domain, preferably a CH3 domain, of the antibody molecule. Unless the context requires otherwise, the term “antibody molecule”, as used herein, is thus equivalent to “antibody molecule or fragment thereof”.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing the CDRs, or variable regions, and/or the constant domain sequences providing the LAG-3 antigen binding site, into a different immunoglobulin. Introduction of the CDRs of one immunoglobulin into another immunoglobulin is described for example in EP-A-184187, GB 2188638A or EP-A-239400. Similar techniques could be employed for the relevant constant domain sequences. Alternatively, a hybridoma or other cell producing an antibody molecule may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody molecule" should be construed as covering antibody fragments, derivatives, functional equivalents and

homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A- 0120694 and EP-A-0125023.

An example of an antibody fragment comprising both CDR sequences and CH3 domain is a minibody, which comprises an scFv joined to a CH3 domain (Hu et al. (1996), Cancer Res., 56(13):3055-61).

The antibody molecule of the present invention binds to PD-L1 and LAG-3. Binding in this context may refer to specific binding. The term "specific" may refer to the situation in which the antibody molecule will not show any significant binding to molecules other than its specific binding partner(s), here PD-L1 and LAG-3. The term “specific” is also applicable where the antibody molecule is specific for particular epitopes, such as epitopes on PD-L1 and LAG-3, that are carried by a number of antigens in which case the antibody molecule will be able to bind to the various antigens carrying the epitope.

LAG-3 shares 40% sequence identity with CD4, its most closely related protein. The present inventors tested the FS18-7-9 Fcab, which comprises the amino acid sequences set forth in SEQ ID NOs 1 to 3, for binding to CD4. The FS18-7-9 Fcab showed no binding to CD4, demonstrating that this molecule binds LAG-3 specifically. Thus, in a preferred embodiment, the LAG-3 binding site of an antibody molecule of the present invention does not bind, or does not show any significant binding, to CD4.

An antibody molecule of the invention preferably comprises a LAG-3 antigen binding site. The LAG-3 antigen binding site is located in a constant domain of the antibody molecule, such as a CH1, CH2, CH3 or CH4 domain. Preferably, the LAG-3 antigen binding site is located in the CH3 domain of the antibody molecule. The LAG-3 binding site preferably comprises the amino acid sequences WDEPWGED (SEQ ID NO: 1) and PYDRWVWPDE. (SEQ ID NO: 3). These sequences were present in all of the lead anti-LAG-3 Fcab clones identified by the present inventors following an extensive screening and characterisation program as described in the examples.

The amino acid sequences set forth in SEQ ID NOs 1 and 2 are preferably located in structural loops of the constant domain of the antibody molecule. The introduction of

sequences into the structural loop regions of antibody constant domains to create new antigen-binding sites is described, for example, in WO2006/072620 and WO2009/132876.

The structural loops of antibody constant domains include the AB, CD and EF loops. In the CH3 domain, the AB, CD, and EF loops are located at residues 11-18, 43-78 and 92-101 of the CH3 domain, where the amino acid residue numbering is according to the ImMunoGeneTics (IMGT) numbering scheme. The amino acid sequence set forth in SEQ ID NO: 1 is preferably located in the AB loop of the constant domain. The amino acid sequence set forth in SEQ ID NO: 3 is preferably located in the EF loop of the constant domain. More preferably, the amino acid sequence set forth in SEQ ID NO: 1 is located at residues 11 to 18 of the CH3 domain; and/or the amino acid sequence set forth in SEQ ID NO: 3 is located at residues 92 to 101 of the CH3 domain, wherein the amino acid residue numbering is according to the IMGT numbering scheme.

In addition, the antibody molecule preferably comprises the amino acid sequence set forth in SEQ ID NO: 2, 8, 13, 18, 23, 28, 33, 38, 43, or 48, more preferably SEQ ID NO: 2, 28, or 38, yet more preferably SEQ ID NO: 2, in a structural loop of a constant domain of the antibody molecule. The structural loop is preferably the CD loop and the constant domain is preferably the CH3 domain. The amino acid sequence set forth in SEQ ID NO: 2, 8, 13, 18, 23, 28, 33, 38, 43, or 48 is preferably located at residues 43 to 78 of the CH3 domain, wherein the amino acid residue numbering is according to the IMGT numbering scheme.

An antibody molecule of the invention may further comprise a glutamic acid residue (E) at position 36 and/or a tyrosine residue (Y) at position 85.2 of the CH3 domain (as shown in Figure 1A), wherein the amino acid residue numbering is according to the IMGT numbering scheme. In particular, an antibody molecule which comprises the CD structural loop region set forth in SEQ ID NO: 8 preferably further comprises a glutamic acid residue (E) at position 36 of the CH3 domain. Similarly, an antibody molecule which comprises the CD structural loop region set forth in SEQ ID NO: 18 preferably further comprises a tyrosine residue (Y) at position 85.2 of the CH3 domain.

In a preferred embodiment, the antibody molecule of the invention comprises a CH3 domain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50, preferably a CH3 domain with the sequence set forth in SEQ ID NO: 5, 30, or 40, more preferably, a CH3 domain with the sequence set forth in SEQ ID NO: 5.

The antibody molecule of the invention may comprise a CH3 domain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50, wherein the CH3 domain sequence further comprises a lysine residue (K) at the immediate C-terminus of the sequence shown in SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50. Thus, for example, the antibody molecule of the invention may comprise a CH3 domain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 5 with a lysine residue at the C-terminus of the sequence shown in SEQ ID NO: 5. The sequence of such a CH3 domain would then be as follows:

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 135)

In addition, the antibody molecule of the invention may comprise a CH2 domain of an immunoglobulin G molecule, such as a CH2 domain of an IgG1, IgG2, IgG3, or IgG4 molecule. Preferably the antibody molecule of the invention comprises a CH2 domain of an IgG1 molecule. The CH2 domain may have the sequence set forth in SEQ ID NO: 53.

The CH2 domain of the antibody molecule may comprise a mutation to reduce or abrogate binding of the CH2 domain to one or more Fc γ receptors, such as FcγRI, FcγRIIa, FcγRIIb, FcγRIII and/or to complement. CH2 domains of human IgG domains normally bind to Fc γ receptors and complement and the inventors postulate that reduced binding to Fc γ receptors will reduce the antibody-dependent cell-mediated cytotoxicity (ADCC) and reduced binding to complement will reduce the complement-dependent cytotoxicity (CDC) activity of the antibody molecule. Mutations for reduce or abrogate binding of the CH2 domain to one or more Fc γ receptors and complement are known and include the “LALA mutation” described in Bruhns, *et al*. (2009) and Xu *et al.* (2000). Thus, the antibody molecule may comprise a CH2 domain, wherein the CH2 domain comprises alanine residues at positions 4 and 5 of the CH2 domain, wherein the numbering is according to the IMGT numbering scheme. For example, the antibody molecule comprises an IgG1 CH2 domain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 54.

The antibody molecule of the present invention comprises a CDR-based antigen binding site for PD-L1. The term “CDR-based antigen binding site” refers to the antigen-binding site of an antibody molecule variable region which is composed of six CDRs . The preparation of antibody molecules against PD-L1, and determination of the CDR sequences of such

antibody molecules, is well within the capabilities of the skilled person and many suitable techniques are known in the art.

Preferably, the antibody molecule of the invention comprises the HCDR3 of antibody 84G09. The HCDR3 is known to play a role in determining the specificity of an antibody molecule (Segal et al., (1974), PNAS, 71:4298-4302; Amit et al., (1986), Science, 233:747-753; Chothia et al., (1987), J. Mol. Biol., 196:901-917; Chothia et al., (1989), Nature, 342:877-883; Caton et al., (1990), J. Immunol., 144:1965-1968; Sharon et al., (1990a), PNAS, 87:4814-4817; Sharon et al., (1990b), J. Immunol., 144:4863-4869; Kabat et al., (1991b), J. Immunol., 147:1709-1719).

The antibody molecule may further comprise the HCDR1, HCDR2, LCDR1, LCDR2 and/or LCDR3 of antibody 84G09. The skilled person would have no difficulty in determining the sequences of the CDRs from the VH and VL domain sequences of antibody 84G09 shown in SEQ ID NOs 92 and 93, respectively. The CDR sequences may, for example, be determined according to Kabat (Kabat, E.A. et al., (1991)) or the IMGT numbering scheme.

The sequences of the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 of antibody 84G09, according to the IMGT numbering scheme, are set out in SEQ ID NOs 86, 87, 88, 89, 90, and 91, respectively.

The sequences of the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 of antibody 84G09, according to Kabat, are set out in SEQ ID NOs 136, 137, 138, 139, 140, and 141, respectively.

The antibody may also comprise the VH and/or VL domain of antibody 84G09. The VH and VL domain sequences of antibody 84G09 shown in SEQ ID NOs 92 and 93, respectively.

In a preferred embodiment, the antibody molecule of the invention comprises (i) a CDR-based antigen binding site for PD-L1 comprising the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of antibody 84G09, and (ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule, wherein the LAG-3 binding site comprises the amino acid sequences set forth in SEQ ID NOs 1 and 3, and an amino acid sequence selected from the group consisting of: SEQ ID NOs 2, 8, 13, 18, 23, 28, 33, 38, 43, and 48.

More preferably, the antibody molecule of the invention comprises (i) a CDR-based antigen binding site for PD-L1 comprising the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of antibody 84G09, and (ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule, wherein the LAG-3 binding site comprises the amino acid sequences set forth in SEQ ID NOs 1 and 3, and an amino acid sequence selected from the group consisting of: SEQ ID NOs 2, 28, and 38.

Yet more preferably, the antibody molecule of the invention comprises (i) a CDR-based antigen binding site for PD-L1 comprising the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of antibody 84G09, and (ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule, wherein the LAG-3 binding site comprises the amino acid sequences set forth in SEQ ID NOs 1, 2 and 3.

In a preferred embodiment, the antibody molecule of the invention comprises a VH domain and a VL domain which comprises, has, or consists of the sequence set forth in SEQ ID NOs 92 and 93, respectively, and a CH3 domain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50, preferably a CH3 which comprises, has, or consists of the sequence set forth in SEQ ID NO: 5, 30, or 40, more preferably, a CH3 domain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 5.

In a further preferred embodiment, the antibody molecule comprises a heavy chain which comprises, has, or consists of the sequence set forth in SEQ ID NOs: 94 to 113 and a light chain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 116. More preferably, the antibody molecule comprises a heavy chain which comprises, has, or consists of the sequence set forth in SEQ ID NOs: 94, 95, 104, 105, 108, and 109 and a light chain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 116. Most preferably, the antibody molecule comprises a heavy chain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 94 or 95 and a light chain which comprises, has, or consists of the sequence set forth in SEQ ID NO: 116.

The antibody molecules of the present invention may also comprise variants of the structural loop, CH3 domain, CH2 domain, CH2 and CH3 domain, light chain or heavy chain sequences disclosed herein, provided the VL and VH domains of the light and heavy chain sequences, respectively, remain unchanged. Suitable variants can be obtained by means of methods of sequence alteration, or mutation, and screening. In a preferred embodiment, an antibody molecule comprising one or more variant sequences retains one or more of the

functional characteristics of the parent antibody molecule, such as binding specificity and/or binding affinity for LAG-3 and PD-L1. For example, an antibody molecule comprising one or more variant sequences preferably binds to LAG-3 and/or PD-L1 with the same affinity, or a higher affinity, than the (parent) antibody molecule. The parent antibody molecule is an antibody molecule which does not comprise the amino acid substitution(s), deletion(s), and/or insertion(s) which have been incorporated into the variant antibody molecule.

For example, an antibody molecule of the invention may comprise a structural loop, CH3 domain, CH2 domain, CH2 and CH3 domain, light chain or heavy chain sequence which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% sequence identity to a structural loop, CH3 domain, CH2 domain, CH2 and CH3 domain, , light chain or heavy chain sequence disclosed herein, provided the VL and VH domains of the light and heavy chain sequences, respectively, remain unchanged.

In a preferred embodiment, the antibody molecule of the invention comprises a CH3 domain sequence which has at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% sequence identity to the CH3 domain sequence set forth in SEQ ID NO: 4, 5, or 135.

In a further preferred embodiment, the antibody molecule of the invention comprises a CH3 and CH2 domain sequence, which has at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% sequence identity to the CH2 and CH3 domain sequence set forth in SEQ ID NO: 6 or 7.

Sequence identity is commonly defined with reference to the algorithm GAP (Wisconsin GCG package, Accelerys Inc, San Diego USA). GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul et al. (1990) J. Mol. Biol. 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) PNAS USA 85: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) J. Mol Biol. 147: 195-197), or the TBLASTN program, of Altschul et al. (1990) supra, generally

employing default parameters. In particular, the psi-Blast algorithm (Nucl. Acids Res. (1997) 25 3389-3402) may be used.

An antibody molecule of the invention may also comprise a structural loop, CH3 domain, CH2 domain, CH2 and CH3 domain, light chain or heavy chain sequence which has one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), preferably 20 alterations or fewer, 15 alterations or fewer, 10 alterations or fewer, 5 alterations or fewer, 4 alterations or fewer, 3 alterations or fewer, 2 alterations or fewer, or 1 alteration compared with a structural loop, CH3 domain, CH2 domain, CH2 and CH3 domain, light chain or heavy chain sequence disclosed herein, provided the VL and VH domains of the light and heavy chain sequences, respectively, remain unchanged. In particular, alterations may be made in one or more framework regions of the antibody molecule outside the VH and VL domain sequences.

In a preferred embodiment, the antibody molecule of the invention may comprise a CH3 domain sequence with one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), preferably 20 alterations or fewer, 15 alterations or fewer, 10 alterations or fewer, 5 alterations or fewer, 4 alterations or fewer, 3 alterations or fewer, 2 alterations or fewer, or 1 alteration compared with the CH3 domain sequence set forth in SEQ ID NO: 4, 5, or 135.

In a further preferred embodiment, the antibody molecule of the invention comprises a CH3 and CH2 domain sequence, with one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), preferably 20 alterations or fewer, 15 alterations or fewer, 10 alterations or fewer, 5 alterations or fewer, 4 alterations or fewer, 3 alterations or fewer, 2 alterations or fewer, or 1 alteration compared with the CH2 and CH3 domain sequence set forth in SEQ ID NO: 6 or 7.

Also contemplated is an antibody molecule which competes with an antibody molecule of the invention for binding to LAG-3 and/or PD-L1, or which binds to the same epitope on LAG-3 and/or PD-L1 as an antibody molecule of the invention, wherein the antibody molecule comprises both a CDR-based antigen binding site for PD-L1 and a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule. Methods for determining competition for an antigen by two antibodies are known in the art. For example, competition of binding to an antigen by two antibodies can be determined using BIAcore. Methods for mapping the epitope bound by an antibody are similarly known in the art.

The antibody molecule of the invention preferably binds to LAG-3 with an affinity (K_D) of 1 x 10^{-9} M or an affinity which is greater. For example, the antibody molecule of the invention may bind to LAG-3 with an affinity (K_D) of 8 x 10^{-10} M, or an affinity which is greater.

Fcabs have a smaller binding interface than monoclonal antibodies as the binding sites of Fcabs form a relatively compact antibody fragment with two binding sites situated in close proximity. In contrast, the Fab arms of a typical mAb are separated by a flexible hinge region. The two antigen binding sites of an Fcab are also spatially close to each other, as compared with those of a typical mAb. Based on this smaller binding interface and reduced flexibility of the two binding sites it was surprising that the anti-LAG-3 Fcabs were able to bind to and inhibit LAG-3 with similar affinity and potency as a monoclonal antibody benchmark.

The antibody molecule of the invention preferably binds to PD-L1 with an affinity (K_D) of 1 x 10^{-9} M or an affinity which is greater.

The binding affinity of an antibody molecule to a cognate antigen, such as LAG-3 or PD-L1 can be determined by surface plasmon resonance (SPR), for example. The binding affinity of an antibody molecule to a cognate antigen, such as LAG-3 or PD-L1, expressed on a cell surface can be determined by flow cytometry.

The antibody molecule of the present invention is preferably capable of binding to LAG-3 and PD-L1 expressed on the surface of a cell. The cell is preferably a cancer cell.

The antibody molecule of the present invention is preferably capable of simultaneously binding to LAG-3 and PD-L1. In a preferred embodiment, the antibody molecule of the present invention is capable of simultaneously binding to LAG-3 and PD-L1, wherein the LAG-3 and PD-L1 are expressed on the surface of a single cell, or on the surface of two separate cells.

The antibody molecule of the invention may bind to human LAG-3, murine LAG-3, and/or cynomolgus monkey LAG-3. Preferably, the antibody molecule of the invention binds to human LAG-3. Most preferably, the antibody molecule of the invention binds to human LAG-3 and human PD-L1.

The antibody molecule of the invention comprises (i) a CDR-based antigen binding site for PD-L1; and (ii) a LAG-3 antigen binding site located in a constant domain of the antibody

molecule. Antibody molecules which do not comprise a LAG-3 antigen binding site located in a constant domain, such as a CH3 domain, of the antibody molecule thus do not form part of the present invention. Similarly, a molecule which does not comprise a CDR-based antigen binding site for PD-L1 does not form part of the present invention.

The antibody molecule of the present invention may be conjugated to a therapeutic agent or detectable label. In this case, the antibody molecule may be referred to as a conjugate. For example, the antibody molecule may be conjugated to an immune system modulator, cytotoxic molecule, radioisotope, or detectable label. The immune system modulator or cytotoxic molecule may be a cytokine. The detectable label may be a radioisotope, e.g. a non-therapeutic radioisotope.

The antibody molecule may be conjugated to the therapeutic agent or detectable label, by means of a peptide bond or linker, i.e. within a fusion polypeptide comprising said therapeutic agent or detectable label and the antibody molecule or a polypeptide chain component thereof. Other means for conjugation include chemical conjugation, especially cross-linking using a bifunctional reagent (e.g. employing DOUBLE-REAGENTS™ Cross-linking Reagents Selection Guide, Pierce).

The antibody molecule and the therapeutic agent or detectable label may thus be connected to each other directly, for example through any suitable chemical bond or through a linker, for example a peptide linker.

The peptide linker may be a short (2-20, preferably 2-15, residue stretch of amino acids). Suitable examples of peptide linker sequences are known in the art. One or more different linkers may be used. The linker may be about 5 amino acids in length.

The chemical bond may be, for example, a covalent or ionic bond. Examples of covalent bonds include peptide bonds (amide bonds) and disulphide bonds. For example the antibody molecule and therapeutic or diagnostic agent may be covalently linked. For example by peptide bonds (amide bonds). Thus, the antibody molecule and therapeutic or diagnostic agent may be produced (secreted) as a single chain polypeptide.

The invention also provides isolated nucleic acids encoding the antibodies molecules of the invention. The skilled person would have no difficulty in preparing such nucleic acids using methods well-known in the art. An isolated nucleic acid may be used to express the antibody molecule of the invention, for example, by expression in a bacterial, yeast, insect or

mammalian host cell. A preferred host cell is a mammalian cell such as a CHO, HEK or NS0 cell. The nucleic acid will generally be provided in the form of a recombinant vector for expression.

The isolated nucleic acid may, for example, comprise the sequence set forth in SEQ ID NO: 142, 4, 9, 14, 19, 24, 29, 34, 39, 44, or 49, which encode the CH3 domains of FS18-7-9 (CHO codon optimised nucleotide sequence), FS18-7-9 (HEK293-expressed nucleotide sequence), FS18-7-32, FS18-7-33, FS18-7-36, FS18-7-58, FS18-7-62, FS18-7-65, FS18-7-78, FS18-7-88, and FS18-7-95, respectively.

In vitro host cells comprising such nucleic acids and vectors are part of the invention, as is their use for expressing the antibody molecules of the invention, which may subsequently be purified from cell culture and optionally formulated into a pharmaceutical composition. The present invention thus further provides a method of producing the antibody molecule of the invention, comprising culturing the recombinant host cell of the invention under conditions for production of the antibody molecule. Methods for culturing suitable host cells as mentioned above are well-known in the art. The method may further comprise isolating and/or purifying the antibody molecule. The method may also comprise formulating the antibody molecule into a pharmaceutical composition, optionally with a pharmaceutically acceptable excipient or other substance as described below.

PD-L1 is known to be expressed on many cancer cells, while expression of LAG-3 on cancer cells is more limited. Both are expressed on cells of the immune system. In particular, LAG-3 is known to be expressed on exhausted T cells within the tumour environment. In addition, the present inventors have shown that the use of an antibody molecule which binds to both LAG-3 and PD-L1 is effective in suppressing tumour growth in syngeneic mouse models of cancer, and that such antibody molecules are more effective than the administration of two binding molecules which bind LAG-3 and PD-L1, respectively.

Thus, an antibody molecule of the invention may be used in a method of treating cancer in a patient. The patient is preferably a human patient.

Cells of the cancer to be treated using the antibody molecule of the invention may express LAG-3, e.g. on their cell surface. In one embodiment, cells of the cancer to be treated may have been determined to express LAG-3, e.g. on their cell surface. For example, B cell lymphomas have been shown to express LAG-3 on their cell surface. Methods for

determining the expression of an antigen on a cell surface are known in the art and include, for example, flow cytometry.

Example 4 below shows that the antibody molecules of the present invention can be used to treat tumours with high levels of LAG-3-expressing immune cells, such as LAG-3-expressing TILs, in mice. Thus, in addition, or alternatively, tumours of the cancer to be treated using the antibody molecule of the invention may comprise LAG-3 expressing immune cells. LAG-3 expressing immune cells, such as LAG-3 expressing TILs, are present between tumour cells in many cancers. In one embodiment, tumours of the cancer to be treated using the antibody molecule of the invention have been determined to contain LAG-3 expressing immune cells. Methods for determining the presence of LAG-3 expressing immune cells in a tumour or in the periphery of the tumour are known in the art.

Example 4 below also shows that the antibody molecules of the present invention can be used to treat tumours which express PD-L1 on their cell surface. Thus, in addition, or alternatively, cells of the cancer to be treated using the antibody molecule of the invention may express PD-L1, e.g. on their cell surface. In addition, or alternatively, tumours of the cancer to be treated may comprise immune cells, such as TILs, that express PD-L1. Cells of the cancer to be treated may have been determined to express PD-L1, e.g. on their cell surface. In addition, or alternatively, tumours of the cancer to be treated may have been determined to contain immune cells, such as TILs, that express PD-L1.

Cell surface expression of LAG-3 and PD-L1 is expected to allow the antibody molecule to bind to LAG-3 and PD-L1 expressed on the surface of the immune cell and/or the cancer cell. This is thought to result in directed therapy, bridging, and localisation of cancer cells and immune cells.

A cancer to be treated using an antibody molecule of the invention may be selected from the group consisting of Hodgkin's lymphoma, non-Hodgkin's lymphoma (such as diffuse large B-cell lymphoma, indolent non-Hodgkin's lymphoma, mantle cell lymphoma, ovarian cancer, prostate cancer, colorectal cancer, fibrosarcoma, renal cell carcinoma, melanoma, pancreatic cancer, breast cancer, glioblastoma multiforme, lung cancer (such as non-small cell lung cancer), head and neck cancer (such as head and neck squamous cell carcinoma), stomach cancer (gastric cancer), bladder cancer, cervical cancer, uterine cancer, vulvar cancer, testicular cancer, penile cancer, leukemia (such as chronic lymphocytic leukemia, myeloid leukemia, acute lymphoblastoid leukaemia, or chronic lymphoblastoid leukaemia), multiple myeloma, squamous cell cancer, testicular cancer, esophageal cancer (such as

adenocarcinoma of the gastroesophageal junction), Kaposi's sarcoma, and central nervous system (CNS) lymphoma, hepatocellular carcinoma, nasopharyngeal cancer, Merkel cell carcinoma, and mesothelioma. Tumours of these cancers are known, or expected, to express PD-L1 on their cell surface and/or contain immune cells, such as TILs, expressing PD-L1 and/or LAG-3.

Treatment of renal cell carcinoma, lung cancer (such as non-small cell lung cancer), nasopharyngeal cancer, colorectal cancer, melanoma, stomach cancer (gastric cancer), esophageal cancer (such as adenocarcinoma of the gastroesophageal junction), ovarian cancer, cervical cancer, bladder cancer, head and neck cancer (such as head and neck squamous cell carcinoma), leukemia (such as chronic lymphocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma (such as diffuse large B-cell lymphoma, indolent non-Hodgkin's lymphoma, mantle cell lymphoma), and multiple myeloma using anti-LAG-3 antibodies has been investigated in clinical trials and shown promising results. Thus, the cancer to be treated using the antibody molecules of the present invention may be a renal cell carcinoma, lung cancer (such as non-small cell lung cancer), nasopharyngeal cancer, colorectal cancer, melanoma, stomach cancer (gastric cancer), esophageal cancer (such as adenocarcinoma of the gastroesophageal junction), ovarian cancer, cervical cancer, bladder cancer, head and neck cancer (such as head and neck squamous cell carcinoma), leukemia (such as chronic lymphocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma (such as diffuse large B-cell lymphoma, indolent non-Hodgkin's lymphoma, mantle cell lymphoma), or multiple myeloma.

Treatment of melanoma, colorectal cancer, breast cancer, bladder cancer, renal cell carcinoma, bladder cancer, gastric cancer, head and neck cancer (such as squamous cell carcinoma of the head and neck), mesothelioma, lung cancer (such as non-small-cell lung cancer), ovarian cancer, Merkel-cell carcinoma, pancreatic cancer, melanoma and hepatocellular carcinoma using anti-PD-L1 antibodies has also been investigated in clinical trials and shown promising results. Thus, the cancer to be treated using the antibody molecules of the present invention may be a melanoma, colorectal cancer, breast cancer, bladder cancer, renal cell carcinoma, bladder cancer, gastric cancer, head and neck cancer (such as squamous cell carcinoma of the head and neck), mesothelioma, lung cancer (such as non-small-cell lung cancer), ovarian cancer, Merkel-cell carcinoma, pancreatic cancer, melanoma, or hepatocellular carcinoma.

Preferred cancers for treatment using the antibody molecules of the present invention are lung cancer (such as non-small-cell lung cancer), bladder cancer, head and neck cancer

(squamous cell carcinoma of the head and neck), diffuse large B cell lymphoma, gastric cancer, pancreatic cancer and hepatocellular carcinoma. Tumours of these cancers are known to comprise LAG-3 expressing immune cells and to express PD-L1 either on their cell surface or to comprise immune cells expressing PD-L1.

Where the application refers to a particular type of cancer, such as breast cancer, this refers to a malignant transformation of the relevant tissue, in this case a breast tissue. A cancer which originates from malignant transformation of a different tissue, e.g. ovarian tissue, may result in metastatic lesions in another location in the body, such as the breast, but is not thereby a breast cancer as referred to herein but an ovarian cancer.

The cancer may be a primary or secondary cancer. Thus, the antibody molecule of the present invention may be for use in a method of treating cancer in a patient, wherein the cancer is a primary tumour and/or a tumour metastasis.

The antibody molecules of the invention are designed to be used in methods of treatment of patients, preferably human patients. Antibody molecules will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule, such as a pharmaceutically acceptable excipient. For example, a pharmaceutical composition of the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be by injection, e.g. intravenous or subcutaneous. The antibody molecule may be administered intravenously, or subcutaneously.

Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous injection, or injection at the site of affliction, the antibody molecule, or pharmaceutical composition comprising the antibody molecule, is preferably in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's

Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed, as required. Many methods for the preparation of pharmaceutical formulations are known to those skilled in the art. See e.g. Robinson ed., Sustained and Controlled Release Drug Delivery Systems, Marcel Dekker, Inc., New York, 1978.

A composition comprising an antibody molecules according to the present invention may be administered alone or in combination with other treatments, concurrently or sequentially or as a combined preparation with another therapeutic agent or agents, dependent upon the condition to be treated. For example, an antibody molecule of the invention may be administered in combination with an existing therapeutic agent for the disease to be treated, e.g. a cancer as mentioned above. For example, an antibody molecule of the present invention may be administered to the patient in combination with a second anti-cancer therapy, such as chemotherapy, anti-tumour vaccination (also referred to as a cancer vaccination), radiotherapy, immunotherapy, an oncolytic virus, chimeric antigen receptor (CAR) T-cell therapy, or hormone therapy.

It is expected that the antibody molecule of the invention may act as an adjuvant in anti-cancer therapy, such as chemotherapy, anti-tumour vaccination, or radiotherapy. Without wishing to be bound by theory, it is thought that administration of the antibody molecule to the patient as part of chemotherapy, anti-tumour vaccination, or radiotherapy will trigger a greater immune response against the cancer associated antigens LAG-3 and PD-L1, than is achieved with chemotherapy, anti-tumour vaccination, or radiotherapy alone. For example, anti-LAG-3 therapies have shown good efficacy in treating viral based pathologies in mice (Blackburn SD, et al., 2009).

A method of treating cancer in a patient may thus comprise administering to the patient a therapeutically effective amount of an antibody molecule according to the present invention in combination with a chemotherapeutic agent, anti-tumour vaccine, radionuclide, immunotherapeutic agent, oncolytic viruses, CAR-T cells, or agent for hormone therapy. The chemotherapeutic agent, anti-tumour vaccine, radionuclide, immunotherapeutic agent, oncolytic viruses, CAR-T cells, or agent for hormone therapy is preferably a chemotherapeutic agent, anti-tumour vaccine, radionuclide, immunotherapeutic agent, oncolytic viruses, CAR-T cells, or agent for hormone therapy for the cancer in question, i.e. a chemotherapeutic agent, anti-tumour vaccine, radionuclide, immunotherapeutic agent, oncolytic viruses, CAR-T cells, or agent for hormone therapy which has been shown to be effective in the treatment of the cancer in question. The selection of a suitable

chemotherapeutic agent, anti-tumour vaccine, radionuclide, immunotherapeutic agent, oncolytic viruses, CAR-T cells, or agent for hormone therapy which have been shown to be effective for the cancer in question is well within the capabilities of the skilled practitioner.

For example, where the method comprises administering to the patient a therapeutically effective amount of an antibody molecule according to the present invention in combination with a chemotherapeutic agent, the chemotherapeutic agent from the group consisting of: taxanes, cyctotoxic antibiotics, tyrosine kinase inhibitors, PARP inhibitors, B_RAF enzyme inhibitors, alkylating agents, platinum analogs, nucleoside analogs, thalidomide derivatives, antineoplastic chemotherapeutic agents and others. Taxanes include docetaxel, paclitaxel and nab-paclitaxel; cytotoxic antibiotics include actinomycin, bleomycin, anthracyclines, doxorubicin and valrubicin; tyrosine kinase inhibitors include erlotinib, gefitinib, axitinib, PLX3397, imatinib, cobemitinib and trametinib; PARP inhibitors include piraparib; B-Raf enzyme inhibitors include vemurafenib and dabrafenib; alkylating agents include dacarbazine, cyclophosphamide, temozolomide; platinum analogs include carboplatin, cisplatin and oxaliplatin; nucleoside analogs include gemcitabine and azacitidine; antineoplastics include fludarabine. Other chemotherapeutic agents suitable for use in the present invention include methotrexate, defactinib, entinostat, pemetrexed, capecitabine, eribulin, irinotecan, fluorouracil, and vinblastine.

Vaccination strategies for the treatment of cancers has been both implemented in the clinic and discussed in detail within scientific literature (such as Rosenberg, S. 2000 Development of Cancer Vaccines). This mainly involves strategies to prompt the immune system to respond to various cellular markers expressed by autologous or allogenic cancer cells by using those cells as a vaccination method, both with or without granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF provokes a strong response in antigen presentation and works particularly well when employed with said strategies.

Administration may be in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. Thus “treatment” of a specified disease refers to amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular patient being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the type of antibody molecule, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other

medical doctors, and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody molecules are well known in the art (Ledermann et al. (1991) Int. J. Cancer 47: 659-664; and Bagshawe et al. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922). Specific dosages indicated herein, or in the Physician's Desk Reference (2003) as appropriate for an antibody molecule being administered, may be used. A therapeutically effective amount or suitable dose of an antibody molecule can be determined by comparing it's *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the size and location of the area to be treated, and the precise nature of the antibody molecule. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. Treatment may be given before, and/or after surgery, and may be administered or applied directly at the anatomical site of surgical treatment.

Further aspects and embodiments of the invention will be apparent to those skilled in the art given the present disclosure including the following experimental exemplification.

All documents mentioned in this specification are incorporated herein by reference in their entirety.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures described above.

Examples

Example 1 – Selection and characterisation of Fcab molecules

1.1 Naïve selection and affinity maturation of anti-human LAG-3 Fcabs

1.1.1 Naïve selection

Naïve phage libraries displaying the CH3 domain of human IgG1 (IMGT numbering 1.4-130) with randomisation within the AB (residues 14-18) and EF (residues 92-101) loops were used for selection with recombinant Fc-tagged human LAG-3 (LAG-3 Fc) antigen (R&D systems, 2319-L3-050). The libraries were selected in three rounds using antigen captured on Protein A (Life Technologies, 10002D) or Protein G (Life Technologies, 10004D) beads. The outputs were screened by ELISA and positive binders sub-cloned and expressed as soluble Fcabs (containing a truncated hinge) in *Pichia pastoris* using EasySelect *Pichia* Expression Kit (Life Technologies, K1740-01). The Fcabs were then screened for binding to recombinant human LAG-3 Fc on the Biacore 3000 (GE Healthcare). Briefly, LAG-3 Fc (R&D systems, 2319-L3-050) was coupled at a density of 7200 RU to a CM5 chip (GE Healthcare, BR-100012) using amine coupling (GE Healthcare, BR-1000-50). Fcabs were diluted in HBS-P (GE Healthcare, BR100368) buffer and injected at 250 nM, 500 nM and 1000 nM for 3 min and then allowed to dissociate in buffer for 5 min. Reference subtracted data (LAG-3 Fc flow cell 2 –blank flow cell) was analyzed using BIAevaluation 3.2 software to identify binding. Fcabs were then tested for binding to HEK cell-expressed human LAG-3 (LAG-3 cloned into pcDNA5FRT vector [Life Technologies, V6010-20] [See section 1.4.5 for methodology]). Briefly, HEK 293 cells overexpressing human LAG-3 grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) and 1 µg/ml Doxycyclin (Sigma, D9891) were detached from tissue culture flasks using cell dissociation buffer (Life Technologies, 13151-014) and seeded in V-bottom 96-well plates at $2x10^5$ cells/well. Fcabs were incubated with the cells at 5 µM in a 100 µl volume for 1 h at 4 °C. The plates were washed the secondary antibody (Anti-human Fc-488, Jackson ImmunoResearch, 109-546-098) was diluted 1:1000 in PBS and 100 µl was added to the cells and incubated for 30 min at 4 °C. The plates were washed and the cells were resuspended in 100 µl PBS containing 1 µg/ml DAPI (Biotium, 40043). The plate was read on a BD FACSCanto II cytometer (BD Biosciences) and the data analysed using FlowJoX. The Fcabs were then expressed in mammalian cells by transformation using lipofectamine (Life Technologies, 11668-019) into Flp-In T-Rex 293 cells (Life Technologies,

R780-07). The LAG-3 binding Fcabs were tested for inhibition of binding of human MHC class II on A375 cells (ATCC, CRL-1619) to recombinant LAG-3 Fc (using the methodology in example 1.6). 54 unique Fcab sequences were identified from three rounds of phage selection, and 12 of these Fcabs were determined to bind to LAG-3 Fc by BIAcore analysis and/or bind to LAG-3 expressing HEK cells. Three of the selected Fcabs were also able to inhibit the interaction of LAG-3 with MHC class II and were selected for affinity maturation. The three Fcabs were termed FS18-3, FS18-7 and FS18-21.

1.1.2 Affinity Maturation

First Affinity maturation

Six phage display affinity maturation libraries were constructed by randomising five residues in the AB loop (residues 14-18) and either five (residues 92-94 and 97-98) or eight (residues 92-94 and 97-101) residues in the EF loop of each of the three Fcabs identified using the naïve selection process described above.

The affinity maturation libraries were selected using recombinant human LAG-3 Fc (R&D systems, 2319-L3-050) and HEK cells expressing human LAG-3 (as described above). The outputs were screened by phage ELISA, the positive binders were subcloned and expressed as soluble Fcabs (containing a truncated hinge) in HEK Expi293 cells (Fcabs cloned into pTT5 vector [National Research Council of Canada] transfected using ExpiFectamine 293 Transfection kit [Life Technologies, A14524] into Expi293F cells [Life technologies, A14527]). The HEK expressed soluble Fcabs were then screened for binding to cell expressed human LAG-3, binding to cell expressed cynomolgus LAG-3 (methodology as example 1.4.3), and the ability to block MHC class II binding to recombinant LAG-3 Fc (methodology as in example 1.6). The blocking Fcabs were further tested to determine whether they were able to reverse LAG-3 induced inhibition of IL-2 secretion in a T cell activation assay (methodology as in example 2.1). 61 unique anti-LAG-3 Fcabs were identified from the six affinity maturation libraries using these screening methods. Affinity matured Fcabs from the FS18-7 lineage were shown to have the highest level of cross–reactivity with cynomolgus monkey LAG-3. The three Fcabs from this lineage with the strongest binding to cynomolgus monkey LAG-3 Fc and the highest activity in the T cell activation assay (termed FS18-7-7, FS18-7-9, and FS18-7-11) were selected for further affinity maturation. These three Fcabs were also shown to block the interaction of LAG-3 Fc with cell expressed MHC class II.

Second Affinity maturation

A pool of the three Fcabs (FS18-7-7, FS18-7-9, and FS18-7-11) from the first affinity maturation was used to create further affinity maturation libraries. The CD loop was hard randomized using randomized primers from ELLA Biotech. A portion of amino acid positions in the CD loop (residues 45.1-78) was randomized using an equimolar distribution of amino acids excluding cysteine. Error prone PCR was also carried out across the entire CH3 domain sequence to introduce additional mutations that might enhance binding.

The affinity maturation libraries were generated in phage and selections performed against biotinylated recombinant LAG-3 avi-Fc (BPS Bioscience, 71147) and HEK hLAG-3 cells and screened for binding to recombinant LAG-3 Fc (R&D systems, 2319-L3-050) by phage ELISA. 86 unique Fcabs (containing a truncated hinge) were expressed in HEK293F cells. Selected Fcabs were also screened for activity in a T cell activation assay as described above. The nine Fcabs identified during the second affinity maturation with the highest activity in the T cell activation assay (FS18-7-32; FS18-7-33; FS18-7-36; FS18-7-58; FS18-7-62; FS18-7-65; FS18-7-78; FS18-7-88; and FS18-7-95), as well as the parental Fcab clone, FS18-7-9, were then further characterised as described below. A sequence alignment of these nine Fcabs against the parental Fcab clone, FS18-7-9, is shown in **Figure 1A**. **Figure 1B** details the percentage sequence identity of each of the nine Fcab clones to the parental Fcab clone, FS18-7-9. Fcabs originating from affinity maturation of the two other parental Fcab clones, FS18-7-7 and FS18-7-11, were not as promising candidates as those originating from affinity maturation of FS18-7-9 and were therefore not pursued further.

1.2 Selection of surrogate Fcab specific for mouse LAG-3

Fcab FS18-7, which was selected using the naïve selection protocol described above, was used to generate phage libraries to select against mouse LAG-3. Two rounds of affinity maturation were performed, and Fcab clones FS18-7-108-29 and FS18-7-108-35, which showed high–affinity, specific binding to mouse LAG-3 were selected following affinity maturation. The ability of FS18-7-108-29 and FS18-7-108-35 to inhibit mouse LAG-3 in a T cell activation assay was confirmed. Epitope mapping using the Octet (Forteo Bio) showed that the anti-mouse LAG-3 Fcabs compete with the anti-human LAG-3 Fcabs (selected following the second affinity maturation as described above) for binding to human LAG-3. There are between 4 and 8 residue differences between the anti-human LAG-3 and anti-mouse LAG-3 Fcabs. It is therefore expected that the anti-mouse LAG-3 Fcabs represent suitable surrogates for the binding and function of the anti-human LAG-3 Fcabs in mice.

1.3 Construction and expression of mock mAb^2

"mock" mAb^2 comprising the lead anti-human LAG-3 and anti-mouse LAG-3 Fcabs identified in 1.1 and 1.2 above were prepared in order to allow the characterisation of these Fcabs in mAb^2 format. These mock mAb^2 were prepared from the anti-LAG-3 Fcabs and the variable regions of anti-FITC antibody 4420 (see SEQ ID NO: 83, SEQ ID NO: 84, and SEQ ID NO: 85 for details) (Bedzyk, W. D., et al. 1989 and Bedzyk, W. D., et al. 1989). The mock mAb^2 were prepared both with (SEQ ID NO: 63, 65, 67, 69, 71, 73, 75, 77, 79, and 81) and without (SEQ ID NO: 64, 66, 68, 70, 72, 74, 76, 78, 80, and 82) the LALA mutation in the CH2 domain of the heavy chain (see section 1.5 below for details) and further comprised the light chain of the anti-FITC mAb 4420 (SEQ ID NO: 85). The mock mAb^2 were produced by transient expression in HEK293-6E cells and purified using mAb Select SuRe protein A columns.

1.4 Binding affinity of Fcabs to LAG-3

1.4.1 Binding affinity of Fcabs to human LAG-3 as determined by surface plasmon resonance (SPR)

A BIAcore T200 (GE Healthcare) was used to measure the affinity of the anti-human LAG-3 Fcabs in the mock mAb^2 format for human LAG-3. Flow cell 4 of a CM5 sensor chip (GE Healthcare, BR1005-30) was immobilised with human LAG-3-Fc (R&D Systems, 2319-L3-050), and flow cell 3 was immobilised with buffer for reference using the amine coupling kit (GE Healthcare, BR-1000-50). LAG-3-Fc was diluted to 5µg/ml in sodium acetate pH5 (ForteoBio, 18-1069) and injected at a flow rate of 10µl/min for 12 seconds followed by deactivation of the surface by injection of ethanolamine for 420 sec. The Immobilisation level was 158 RU. The mock mAb^2 (or control anti-human LAG-3 mAb, 25F7) were diluted in HBS-P buffer (GE Healthcare, BR-1003-68) in a 2-fold dilution series from 4 µg/ml. The control mAb/mock mAb^2 were injected with an association time of 240 seconds at 30µl/min, and a dissociation time 300 seconds at 30µl/min. The surface was regenerated using 25mM NaOH for 30 seconds at 100 µl/min. The data was double reference subtracted and analysed using the BIAevaluation 3.2 software to calculate kinetic constants. The Fcabs in mock mAb^2 format had affinities for human LAG-3 in the range of 0.8 – 1.1 nM (**Table 1**), which is similar to the affinity of the benchmark anti-human LAG-3 mAb 25F7. This was surprising because Fcabs have a smaller binding interface than monoclonal antibodies as the binding sites of Fcabs form a relatively compact antibody fragment with two binding sites situated in close

proximity. In contrast, the Fab arms of a typical mAb are separated by a flexible hinge region. Based on this smaller binding interface and the associated reduced flexibility of the two binding sites in the Fc region, it was unexpected that the anti-LAG-3 Fcabs were able to bind to and inhibit LAG-3 with similar affinity and potency as the benchmark antibody 25F7.

Table 1: Binding affinity of LAG-3 specific Fcabs in mock mAb^2 format to human LAG-3

Anti-human LAG-3 Fcab in mock mAb^2 format and benchmark anti-human LAG-3 mAb, 25F7	K_D (M)
FS18-7-9	8.3×10^{-10}
FS18-7-62	9.5×10^{-10}
FS18-7-78	8.4×10^{-10}
FS18-7-32	8.6×10^{-10}
FS18-7-36	8.9×10^{-10}
FS18-7-65	1.1×10^{-9}
25F7	3.2×10^{-10}

1.4.2 Binding affinity of surrogate Fcab specific for mouse LAG-3 to mouse LAG-3 as determined by SPR

A Biacore 3000 (GE Healthcare) was used to measure the affinity of the surrogate Fcabs specific for mouse LAG-3 to mouse LAG-3. Amine coupling (amine coupling kit, GE Healthcare, BR-1000-50) was used to coat mLAG-3 Fc (R&D Systems, 3328-L3-050) diluted in 10 mM sodium acetate pH 5.0 (ForteBio, 18-1069) directly to a CM5 chip (GE Healthcare, BR-1000-12). Flow cell 1 was coated with Mouse Fc (SinoBiological, 51094-MNAH), and flow cell 2 was coated with mLAG-3 Fc at 950 RU. Fcabs were diluted in HBS-P buffer (GE Healthcare, BR-1003-68) and injected at various concentrations (fourfold dilutions from 100 nM) for 3 min at 20 µl/min and then allowed to dissociate in buffer for 12 min. The chip was regenerated by injection of 10 mM glycine pH 2.5 for 30 s at 30 µl/min. Data was double reference subtracted and analyzed using BIAevaluation 3.2 software to calculate kinetic constants. The tested surrogate Fcabs bound to mouse LAG-3 with single digit nanomolar affinity as set out in **Table 2**.

Table 2: Binding affinity (K_D) of surrogate LAG-3 specific Fcabs to mouse LAG-3

Surrogate Fcabs specific for mouse LAG-3	Affinity K_D (nM)
FS18-7-108-29	1.5
FS18-7-108-35	2.1

1.4.3 Binding affinity of Fcabs to human LAG-3 expressed on cells as determined by flow cytometry

Production of cell lines over-expressing LAG-3

Lentiviral transduction methodology was used to generate DO11.10 cells (National Jewish Health) over-expressing human, cynomolgus or mouse LAG-3 using the Lenti-X HTX Packaging System (Clontech, Cat. No 631249). Lenti-X expression vector (pLVX) (Clontech, Cat. No 631253), containing the mouse LAG-3 cDNA (SEQ ID NO: 96), human LAG-3 cDNA (SEQ ID NO: 95) or cynomolgus LAG-3 cDNA (SEQ ID NO: 97), was co-transfected with a Lenti-X HTX Packaging Mix into the Lenti-X 293T Cell Line (Clontech, Cat. No 632180) to generate virus. The DO11.10 cell line was transduced using the lentiviral vectors produced with the Lenti-X HTX Packaging System.

The affinity of the anti-human LAG-3 Fcabs in mock mAb^2 format to cells expressing human LAG-3 (DO11.10 cell line transfected with human LAG-3) was measured using flow cytometry. mAb^2 and control mAb dilutions (2 x final concentration) were prepared in triplicate in 1 x DPBS (Gibco, 14190-094). DO11.10:LAG-3 cell suspensions were prepared in PBS+2%BSA (Sigma, A7906) and seeded at 4 x 10^{-6} cell/ml with 50 µl/well in V-bottomed 96-well plates (Costar, 3897). 50µl of the mAb^2 or control mAb (anti human LAG-3 mAb, 25F7) dilutions were added to the wells containing cells (final volume 100 µl) and incubated at 4°C for 1 hour. The plates were washed and 100µl/well of secondary antibody (anti-human Fc-488 antibody, Jackson ImmunoResearch, 109-546-098) diluted 1:1000 in PBS+2% BSA was then added and incubated for 30 mins at 4°C in the dark. The plates were washed and resuspended in 100 µl of PBS containing DAPI (Biotium, 40043) at 1 mg/ml. The plates were read using Canto II flow cytometer (BD Bioscience). Dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was measured. The data was fitted using log (agonist) vs response in GraphPad Prism Software. All tested Fcabs in mock mAb^2 format and the benchmark anti-human LAG-3 mAb, 25F7, bound human LAG-3 with similar affinity (EC_{50}), in the range of 1.2 – 2.1 nM as set out in **Table 3**.

Table 3: Binding affinity of anti-human LAG-3 Fcabs in mock mAb^2 format to DO11.10 cells expressing human LAG-3 as determined by flow cytometry

Anti-human LAG-3 Fcab in mock mAb^2 format and benchmark anti-human LAG-3 mAb, 25F7	EC_{50} (nM)
FS18-7-9	1.2
FS18-7-32	1.6
FS18-7-33	1.5
FS18-7-36	1.5
FS18-7-62	2.1
FS18-7-65	1.6
FS18-7-78	1.7
25F7	2.1

1.4.4 Binding affinity of Fcabs to cynomolgus LAG-3 expressed on cells as determined by flow cytometry

The affinity of the anti-human LAG-3 Fcabs in mock mAb^2 format to cells expressing cynomolgus LAG-3 (DO11.10 cell line transfected with cynomolgus LAG-3) was measured using flow cytometry. mAb^2 and control mAb dilutions (2 x final concentration) were prepared in triplicate in 1 x DPBS (Gibco, 14190-094). DO11.10:LAG-3 cell suspensions were prepared in PBS+2%BSA (Sigma, A7906) and seeded at 4 x 10^{-6} cell/ml with 50 µl/well in V-bottomed 96-well plates (Costar, 3897). 50µl of the mAb^2 or control mAb (anti human LAG-3 mAb, 25F7) dilutions were added to the wells containing cells (final volume 100 µl) and incubated at 4°C for 1 hour. The plates were washed and 100µl/well of secondary antibody (anti-human Fc-488 antibody, Jackson ImmunoResearch, 109-546-098) diluted 1:1000 in PBS+2% BSA was then added and incubated for 30 mins at 4°C in the dark. The plates were washed and resuspended in 100 µl of PBS containing DAPI (Biotium, 40043) at 1 mg/ml. The plates were read using Canto II flow cytometer (BD Bioscience). The dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was measured. The data was fit using log (agonist) vs response in GraphPad Prism Software. The tested Fcabs in mock mAb^2 format bound to cynomolgus LAG-3 with 0.5-0.6 nM affinity indicating that toxicology studies in cynomolgus monkeys would be expected to be predictive of effects seen in humans (see **Table 4**). The benchmark anti-human LAG-3 mAb, 25F7, binds cynomolgus LAG-3 with a 15-fold poorer affinity (EC_{50}) (**Table 4**).

Table 4: Binding affinity of anti-LAG-3 Fcabs to DO11.10 cells expressing cynomolgus LAG-3 by flow cytometry

Anti-human LAG-3 Fcab in mock mAb^2 format and benchmark anti-human LAG-3 mAb, 25F7	EC_{50} (nM)
FS18-7-9	0.6
FS18-7-62	0.5
FS18-7-78	0.5
25F7	9.0

1.4.5 Binding affinity of surrogate anti-mouse LAG-3 Fcabs and anti-human LAG-3 Fcab to mouse LAG-3 expressed on cells as determined by flow cytometry

Production of HEK cells over-expressing mLAG-3

The mouse LAG-3 sequence (SEQ ID NO: 96) was subcloned into pcDNA5FRT vector (Life Technologies, V6010-20) using KpnI (NEB, R0142) and NotI (NEB, R0146) restriction digestion. The vector was then transformed into Flp-In T-REx 293 HEK cell line (Life Technologies, R780-07) using Lipofectamine 2000 (Life Technologies, 11668-019). Transformed Flp-In T-REx 293 cells were grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) for 3-4 weeks until colonies of stably transformed cells were apparent. These colonies were amplified in the presence of 1 µg/ml Doxycyclin (Sigma, D9891) and tested for mouse LAG-3 expression using PE conjugated anti-mouse LAG-3 (clone C9B7W, BD Biosciences, 552380).

The affinity of the surrogate anti-mouse LAG-3 Fcabs (containing the truncated hinge; SEQ ID NO: 58) to cell-expressed mouse LAG-3 was determined using flow cytometry. HEK cells expressing mLAG-3 grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) and 1 µg/ml Doxycyclin (Sigma, D9891) were detached from tissue culture flasks using cell dissociation buffer (Life Technologies, 13151-014) and seeded in V-bottom 96-well plates (Costar, 3897) at $2x10^5$ cells/well. The plates were centrifuged at 1500 rpm for 3 min at 4°C to pellet the cells. A dilution series of the Fcabs (or control mAb) were incubated with the cells in a 100 µl volume

for 1 h at 4 °C. The plates were washed and secondary antibody (Anti-human Fc-488, Jackson ImmunoResearch, 109-546-098 for Fcabs or Anti-Rat IgG (H+L), Alexa Fluor 488 Conjugate, ThermoFisher, A-11006 for C9B7W) was diluted 1:1000 in PBS and 100 µl was added to the cells for 30 min at 4 °C (plates were kept in the dark). The plates were then washed and the cells resuspended in 100 µl PBS containing 1 µg/ml DAPI (Biotium, 40043). The plates were read using Canto II flow cytometer (BD Bioscience). Dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was measured. The data was fit using log (agonist) vs response in GraphPad Prism Software. The tested Fcabs bound to mouse LAG-3 with similar affinity (see **Table 5**). The benchmark LAG-3 mAb, C9B7W (2B Scientific, BE0174-50MG), binds mouse LAG-3 with 17-fold poorer affinity (EC_{50}) than the Fcabs (**Table 5**).

Table 5: Binding affinity of surrogate anti-mouse LAG-3 Fcabs to HEK cells expressing mouse LAG-3 by flow cytometry

Anti-mouse LAG-3 Fcabs and benchmark anti-mouse LAG-3 mAb, C9B7W	EC_{50} (nM)
FS18-7-108-29	4.5
FS18-7-108-35	4.5
C9B7W	79

The affinity of the anti-human LAG-3 Fcab FS18-7-9 in mock mAb^2 format to cell-expressed mouse LAG-3 was determined using flow cytometry. HEK cells expressing mLAG-3 grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) and 1 µg/ml Doxycyclin (Sigma, D9891) were detached from tissue culture flasks using cell dissociation buffer (Life Technologies, 13151-014). Cells were collected by centrifuged at 1500 rpm for 3 min at 4°C to pellet the cells and then resuspended in 1 x DPBS then seeded in V-bottom 96-well plates (Costar, 3897) at $1.2x10^5$ cells/well in 30 µl. A 1:1 volume of a dilution series of the mAb^2 (or control mAb) was added and incubated with the cells for 1 h at 4 °C. The plates were washed and secondary antibody (Anti-human Fc-488, Jackson ImmunoResearch, 109-546-098) was diluted 1:1000 in PBS and 60 µl was added to the cells for 30 min at 4 °C (plates were kept in the dark). The plates were then washed and the cells resuspended in 60 µl PBS containing 1 µg/ml DAPI (Biotium, 40043). The plates were read using Canto II flow cytometer (BD Bioscience). Dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was

measured. The data was fitted using log (agonist) vs response in GraphPad Prism Software. The anti-human LAG-3 Fcab FS18-7-9 in mock mAb^2 format bound to mouse LAG-3 with an EC_{50} of 19 nM compared to an EC_{50} of 2.6 nM for the surrogate anti-mouse LAG-3 Fcab FS18-7-9-108 (**Table 6**). The human mAb, 25F7 does not show any detectable binding to mouse LAG-3, indicating that the human LAG-3 Fcab, FS18-7-9, has a different binding epitope on LAG-3 than that of 25F7.

Table 6: Binding affinity of human anti-LAG-3 Fcab FS18-7-9 to HEK cells expressing mouse LAG-3 by flow cytometry

Anti-human LAG-3 Fcab, anti-mouse LAG-3 Fcab and benchmark anti-human LAG-3 mAb, 25F7	EC_{50} (nM)
FS18-7-108-29	2.6
FS18-7-9	19
25F7	No binding

1.5 Binding affinity of Fcabs to Fc receptors

The introduction of the LALA mutation in the CH2 domain of human IgG1 is known to reduce Fc γ receptor binding (Bruhns, P., *et al*. (2009); and Xu, D. *et al.* (2000)). BIAcore was used to confirm that the LALA mutation had reduced the binding affinity of the Fcabs (in mock mAb^2 format) to Fcγ receptors. The human FcγR binding assay was performed on a Biacore T200 instrument (GE Healthcare) using the Fcabs in the mock mAb^2 format. Human FcγRs (R&D Systems, 1257-FC, 1330-CD, 1875-CD, 4325-FC) were immobilized using amine coupling (amine coupling kit, GE Healthcare, BR-1000-50) onto a Series S CM5 chip (GE Healthcare, BR-1005-30) to a surface density of 370 RU for FcγRI, 264 RU for FcγRIII (high affinity human FcγRs) and 500 RU for FcγRIIa and FcγRIIb (low affinity human FcγRs). For each immobilized chip a flow cell was left blank for background subtraction. FcγR were immobilized using a concentration of 5 µg/ml in sodium acetate pH5 (ForteBio, 18-1069) and injected at a flow rate of 10 µl/min in 15 second cycles until the required immobilization level was reached.

For the high affinity FcγRI and FcγRIII, 200 µg/ml of mAbs or mock mAb^2 were flowed across the chip for 3 min at a flow rate of 30 µl/min and the dissociation was followed for 5 min. Running buffer was HBS-P (0.01 M HEPES pH 7.4, 0.15 M NaCl, 0.005% v/v Surfactant P20, GE Healthcare, BR-1003-68). For the low affinity FcγRIIa and FcγRIIb the concentration of mock mAb^2 was increased to 500 µg/ml.

The positive control was a wild type IgG1 isotype mAb, which was compared to controls LALA IgG1 mAb and monoclonal IgG2 and IgG4 isotype mAbs to irrelevant targets. The flow cells were regenerated by injecting 10 mM sodium hydroxide (VWR, 28244.262) at a flow rate of 100 µl/min for 30 seconds. The data analysis was performed with BiaEvaluation software version 3.2 RC1 by double referencing against the blank flow cell (without immobilized FcγR) and subtracting a buffer cycle from test mAb^2. The results are shown in **Table 7**.

Table 7: Binding response of anti-human LAG-3 Fcabs in mock mAb^2 format (comprising LALA mutation as detailed above) to human Fcγ receptors by SPR

mAb/mock mAb^2	Binding response at end of association (RU)			
	FcγRI	FcγRIII	FcγRIIa	FcγRIIb
FS18-7-9	1.4	6.6	-9.8	-8.5
FS18-7-62	-0.9	0.7	-10	-8.5
FS18-7-78	-0.3	4.0	-10.7	-9.2
mock mAb LALA	2	8.0	-12.7	-9.6
IgG2	0	1.9	9.7	7.4
IgG4	9	3.1	4.3	15.1
mock mAb IgG1	26	44	13	17.7

All mock mAb^2 tested (all comprising the LALA mutation as set out above) showed significantly reduced binding to the tested Fcγ receptors compared to the control antibody (mock mAb IgG1) without the LALA mutation, indicating that the LALA mutation has reduced Fcγ receptor binding by these mock mAb^2 and therefore is expected to reduce ADCC activity of the mAb^2.

1.6 Blocking of MHC class II binding to LAG-3

The ability of the Fcabs (containing the truncated hinge; SEQ ID NO: 58) to block the interaction between recombinant human or mouse LAG-3 Fc and human MHC Class II was studied by measuring binding of LAG-3 Fc to A375 cells, a melanoma cell line that expresses human MHC Class II. A375 (ATCC, CRL-1619) cells grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-106) were detached from cell culture flasks using cell dissociation buffer (Life Technologies, 13151-014) and seeded in V-bottom 96-well plates (Costar, 3897) at 2x10^5 cells/well. The plates

were centrifuged at 1500 rpm for 3 min at 4 °C to pellet the cells. The relevant concentrations of Fcab or control mAb were incubated with 1 µg/ml LAG-3 Fc (human LAG-3-Fc R&D Systems, 2319-L3-050 or mouse LAG-3 Fc R&D Systems, 3328-L3-050) in 100 µl DMEM containing 10% FBS for 1 h at 4 °C. LAG-3/Fcab mix was added to the A375 cells and incubated for 1 h at 4 °C. Cells were washed. Secondary antibody (Alexa Fluor 488 conjugated goat anti-human Fc $F(ab')_2$, Jackson Immunoresearch, 109-546-098 or Goat anti-mouse IgG (H+L) 488 conjugate, Life Technologies, A-1101) was diluted 1:1000 in PBS and 100 µl was added to the cells for 30 min at 4 °C (plates were kept in the dark). Cells were washed once in PBS and resuspended in 100 µl PBS + 1 µg/ml DAPI (Biotium, 40043). The plates were read on a BD FACSCanto II cytometer (BD Biosciences) and the data analysed using FlowJo software.

Both anti-mouse LAG-3 Fcabs were able to inhibit the interaction of human MHC class II with mouse LAG-3, whereas the control anti-mouse LAG-3 mAb (C9B7W, 2B Scientific, BE0174-50MG) was not (see **Table 8**).

Table 8: Surrogate anti-mouse LAG-3 Fcabs inhibit binding of mouse LAG-3 to MHC class II

Surrogate anti-mouse LAG-3 Fcabs and control anti-mouse LAG-3 mAb C9B7W	IC_{50} (nM)
FS18-7-108-29	0.6
FS18-7-108-35	0.7
C9B7W	No blocking

The anti-human LAG-3 Fcabs tested were also able to inhibit the interaction of human MHC class II with human LAG-3 with a similar potency as the control anti-human LAG-3 mAb (25F7).

Table 9: Anti-human LAG-3 Fcabs inhibit binding of human LAG-3 to MHC class II

Anti-human LAG-3 Fcabs and control anti-human LAG-3 mAb 25F7	**IC_{50} (nM)**
FS18-7-108-33	2.6
FS18-7-108-78	2.4
25F7	3.6

Example 2: Preparation and characterisation of mAb and mAb^2 molecules

2.1 Preparation of mAb 84G09

2.1.1 DNA construct generation

DNA inserts encoding variable heavy and light chain regions of 84G09 were codon optimised for mammalian expression and synthesised by DNA2.0 (Menlo Park, CA, USA). The inserts supplied in pJ-Amp-high host vector were sub-cloned into expression vectors pFS-hHC2.1-G1m17(z) LALA (IgG heavy chain containing LALA mutation) or pFS-hHC2.1-G1m17(z) (IgG heavy chain without LALA mutation) and pFShK1.0 (IgG kappa light chain) via *EcoRI* and *NheI* restriction digest.

Fidelity of cloning was verified by colony PCR and subsequent nucleotide sequencing analysis by a third party (GATC Biotech).

2.1.2 Cell maintenance

HEK293-6E cells (NRCC), were sub-cultured in pre-warmed F17 medium (Invitrogen, A13835-01) supplemented with 4mM of GlutaMAX-1 (Invitrogen, 35050-038), 0.1% of Pluronic F-68 (Invitrogen A13835-01) and 25 µg/ml of geneticin (Invitrogen, 10131-027). Cells were incubated at 37 °C, 140 rpm, 5% CO_2 and subcultured at $0.3x10^6$ cells/ml on a three then four day regime.

2.1.3 Transient transfection

HEK293-6E cells were transfected transiently using PEIpro at 1 mg/ml (Polyplus, PPLU115). 24 hours prior transfection cells were seeded at $0.8x10^6$ cells/ml in culture medium. For each 200 ml of cell culture, a DNA mixture was prepared by mixing 10 ml of warmed Opti-MEMI (Invitrogen, 11058-021), 100 µg endotoxin-free DNA encoding the heavy chain and 100 µg endotoxin-free DNA encoding the light chain. PEI mixture was prepared by mixing 10 ml of warmed Opti-MEMI and 200 µl of PEIpro and vortexing. The DNA mixture was quickly added to the vortexed PEI mixture, mixed by vortex pulsing 3 times for 1 s, incubated 3 min at room temperature and added drop by drop to the cells. 48 hours after transfection 20 ml of F17 plus supplements with 0.5% Tryptone N1 (TekniScience Inc., 19553) was added to each flask.

6 days after transfection cells were harvested by centrifugation at 4500 rpm for 40 min. Supernatant was then filtered with 0.22 µm polyethersulfone filter unit (Millipore, SCGPU01RE, SCGPU02RE, SCGPU05RE, SCGPU11RE) and stored at +4 °C until purification.

2.1.4 Protein A chromatography

Clarified supernatants were purified using pre-packed 5ml HiTrap MabSelect SuRe columns (GE Healthcare, 11-0034-95) on an ÄKTAexplorer or ÄKTAxpress. Briefly, the columns were equilibrated with 50mM Tris-HCl, 250mM NaCl at pH 7.0, unbound material washed with the same buffer at 5 ml/min. The products were eluted with 10mM sodium formate pH 3.0 at 5 ml/min. Eluted samples were immediately buffer exchange into PBS pH 7.4 using PD-10 columns (GE Healthcare, 17-0851-01) pre-equilibrated with PBS pH 7.4 according to the manufacturer recommendations.

2.1.5 Product concentration measurement by spectrometry

Absorbance at 280 nm of each purified product was measured using LabChip DS (PerkinElmer, 133089) with DropPlate 96 D+ (PerkinElmer, CLS135136). The product concentration was calculated using extinction coefficient (A280 of 1mg/ml) calculated using VectorNTI Advance v11.5.4 software (Thermofisher Scientific, A13784)

2.1.6 Product concentration

When necessary, purified fractions were concentrated using Amicon Ultra-4 Centrifugal Filter Unit 30K (Millipore, UFC803024). After equilibration of the Ultracel regenerated cellulose membrane with PBS pH 7.4 by centrifugation 10 min at 3000 rpm, samples were loaded to the 4 ml unit and centrifuged at 3000 rpm until desired protein concentration was reached.

2.1.6 Filter sterilisation

Final samples were filtered using pre-wet Millex-GV PVDF syringe filters (Millipore, SLGV013SL).

2.2 Preparation of human LAG-3/PD-L1 mAb^2

The heavy chains of the mAb^2 molecules FS18-7-9/84G09 (SEQ ID NOs 94 and 95), FS18-7-32/84G09 (SEQ ID NOs 96 and 97), FS18-7-33/84G09 (SEQ ID NOs 98 and 99), FS18-7-36/84G09 (SEQ ID NOs 100 and 101), FS18-7-58/84G09 (SEQ ID NOs 102 and 103), FS18-7-62/84G09 (SEQ ID NOs 104 and 105), FS18-7-65/84G09 (SEQ ID NOs 106 and 107), FS18-7-78/84G09 (SEQ ID NOs 108 and 109), FS18-7-88/84G09 (SEQ ID NOs 110 and 111) and FS18-7-95/84G09 (SEQ ID NOs 112 and 113) were prepared by replacing the CH3 domains of the monoclonal antibodies 84G09 (with and without the LALA mutation) with the CH3 domains of the human LAG-3 specific Fcabs FS18-7-9, FS18-7-32, FS18-7-33, FS18-7-36, FS18-7-58, FS18-7-62, FS18-7-65, FS18-7-78, FS18-7-88 and FS18-7-95 within *XhoI* and *BamHI* sites present in the sequence of the unmodified CH3 domain of human IgG1. The heavy chain of the mAb^2 were co-transfected with the light chain of 84G09 (SEQ ID NO: 116) as described for mAb 84G09 in section 2.1 above. The mAb^2 were then expressed and purified as described for mAb 84G09 in section 2.1 above.

2.3 Preparation of human LAG-3/mock mAb^2

The anti-FITC mAb (with and without LALA mutation) was prepared as described for mAb 84G09 in section 2.1 above using the heavy chains (SEQ ID NOs 83 and 84; with and without LALA mutation) and light chain (SEQ ID NO: 85) of mAb 4420.

The heavy chains of the mAb^2 molecules FS18-7-9/4420 (SEQ ID NOs 63 and 64), FS18-7-32/4420 (SEQ ID NOs 65 and 66), FS18-7-33/4420 (SEQ ID NOs 67 and 68, FS18-7-36/4420 (SEQ ID NOs 69 and 70), FS18-7-58/4420 (SEQ ID NOs 71 and 72), FS18-7-62/4420 (SEQ ID NOs 73 and 74), FS18-7-65/4420 (SEQ ID NOs 75 and 76), FS18-7-78/4420 (SEQ ID NOs 77 and 78), FS18-7-88/4420 (SEQ ID NOs 79 and 80) and FS18-7-95/4420 (SEQ ID NOs 81 and 82) were prepared by replacing the CH3 domains of the monoclonal antibodies 4420 (with and without the LALA mutation) with the CH3 domains of the human LAG-3 specific Fcabs FS18-7-9, FS18-7-32, FS18-7-33, FS18-7-36, FS18-7-58, FS18-7-62, FS18-7-65, FS18-7-78, FS18-7-88 and FS18-7-95 within *XhoI* and *BamHI* sites present in the sequence of the unmodified CH3 domain of human IgG1. The heavy chains of the mAb^2 were co-transfected with the light chain of mAb 4420 as described for mAb 84G09 in section 2.1 above. The proteins were then expressed and purified as described for mAb 84G09 in section 2.1 above.

2.4 Preparation of mouse LAG-3/PD-L1 mAb^2

The mouse anti-PD-L1 mAb (with and without LALA mutation) was prepared as described for mAb 84G09 in section 2.1 above using the heavy chain (SEQ ID NOs 122 and 123) and light chain (SEQ ID NO: 119) of mAb S1.

The heavy chain of the mAb^2 molecules FS18-7-108-29/S1 (SEQ ID NOs 117 and 118) and FS18-7-108-35/S1 (SEQ ID NOs 120 and 121) were prepared by replacing the CH3 domains of the monoclonal antibodies S1 (with and without the LALA mutation) with the CH3 domains of the mouse LAG-3 specific Fcabs FS18-7-108-29 and FS18-7-108-35 within *XhoI* and *BamHI* sites present in the sequence of the unmodified CH3 domain of human IgG1. The heavy chain of the mAb^2 were co-transfected with the light chain of S1 as described for mAb 84G09 in section 2.1 above. The proteins were then expressed and purified as described for mAb 84G09 in section 2.1 above.

2.5 Binding affinity and kinetics of mAb^2 for human LAG-3 and human PD-L1

Protein L (Thermo, 21189) was immobilized on flow cells 1 and 2 of a Series S CM5 chip (GE Healthcare, BR-1005-30) by amine coupling (GE Healthcare, BR-1000-50) to a surface density of 2000 RU by following the manufacturer's instructions for the BIAcore T200 instrument. For LAG-3 binding, the mAb^2 samples (all containing the LALA mutation) were captured on flow cell 2 only and human LAG-3Fc (R&D Systems, 2319-L3) at 4 concentrations in a two-fold dilution series starting at 0.5 nM were flowed across both flow cell 1 and 2 at a flow rate of 30 µl/min. The association time was 3 min and the dissociation time was 6 min. Running buffer was HBS-P (GE Healthcare, BR-1003-68). Both flow cells were regenerated by injecting 10 mM sodium hydroxide (NaOH) at a flow rate of 100 µl/min for 20 seconds. The data were analysed by double referencing against the blank flow cell.

For PD-L1 binding, four concentrations in a two-fold dilution series of PD-L1 Fc (R&D Systems, 156-B7), starting at 40 nM, were flowed across mAb^2 captured on the same Protein L chip. All others conditions were the same as for LAG-3 binding (see above).

The binding kinetics were fit with a 1:1 Langmuir model to generate binding association (k_a) and dissociation (k_d) rates. Equilibrium binding constants (K_D) were calculated by dividing the dissociation rate by the association rate for each sample. Data analysis was performed with BiaEvaluation software version 3.2. The results are shown in **Tables 10 and 11.**

Table 10: Binding affinity and kinetics of mAb^2 to human LAG-3 as determined by SPR

mAb^2	K_D (pM)	k_a (1/Ms) x 10^6	k_d (1/s) x 10^{-4}
84G09LALA	No binding	-	-
FS18-7-09/84G09LALA	56	6.3	3.5
FS18-7-32/84G09LALA	49	5.6	2.8
FS18-7-33/84G09LALA	43	4.2	1.8
FS18-7-36/84G09LALA	38	4.4	1.7
FS18-7-62/84G09LALA	36	5.4	2.0
FS18-7-65/84G09 LALA	39	5.9	2.3
FS18-7-78/84G09 LALA	29	4.8	1.4

Table 11: Binding affinity and kinetics of mAb^2 to human PD-L1 as determined by SPR

mAb^2	K_D (nM)	k_a (1/Ms) x10^5	k_d (1/s) x 10^{-4}
84G09LALA	1.2	3.8	4.6
FS18-7-09/84G09LALA	1.0	3.8	3.9
FS18-7-32/84G09LALA	1.0	3.4	3.2
FS18-7-33/84G09LALA	1.1	3.7	4.2
FS18-7-36/84G09LALA	3.5	1.6	5.6
FS18-7-62/84G09LALA	1.3	1.7	2.2
FS18-7-65/84G09 LALA	1.1	1.3	1.4
FS18-7-78/84G09 LALA	1.0	1.0	1.0

The binding affinities for human PD-L1 and human LAG-3 were comparable for all the mAb^2 tested. The mAb^2 binding affinities for human PD-L1 were comparable to 84G09, indicating that introduction of the LAG-3 binding site into the CH3 domain did not affect PD-L1 binding.

2.6 Simultaneous binding of mAb^2 to human LAG-3 and human PD-L1

The ability of mAb^2 (FS18-7-09/84G09, FS18-7-32/84G09, FS18-7-33/84G09, FS18-7-36/84G09, FS18-7-62/84G09, FS18-7-65/84G09, and FS18-7-78/84G09 all with the LALA mutation) to bind simultaneously to LAG-3 and PD-L1 was tested by SPR. Human PD-L1Fc (R&D Systems, 156-B7) was immobilized on flow cell 2 of a Series S CM5 chip (GE Healthcare, BR-1005-30) to a surface density of 150 RU by following the manufacturer's instructions. Flow cell 1 was activated and deactivated without any protein immobilised for background subtraction. For each sample, 10 µg/ml of mAb^2 was flowed across flow cells 1 and 2, at a flow rate of 10 µl/min for 3 min. Subsequently, 40 nM of LAG-3Fc (R&D Systems,

2319-L3) was flowed across both flow cell 1 and 2 at a flow rate of 10 µl/min for 3 min. For each binding step dissociation was followed for 3 min. Sensor chip was regenerated after each cycle with a 15 s injection of 25 mM NaOH at a flow rate of 100 µl/min.

All mAb^2 tested were capable of simultaneously binding to LAG-3 and PD-L1. The parental anti-PD-L1 mAb, 84G09, only binds to PD-L1.

2.7. Simultaneous binding of surrogate mAb^2 to murine LAG-3 and murine PD-L1

The ability of the two surrogate mouse mAb^2 (FS18-7-108-29/S1 and FS18-7-108-35/S1, both containing the LALA mutation) to bind simultaneously to murine LAG-3 and murine PD-L1 was tested by SPR on a BIAcore 3000 (GE Healthcare). Murine PD-L1Fc (R&D Systems, 1019-B7-100) was immobilized on flow cell 4 of a CM5 chip to a surface density of 830 RU according to the manufacturer's instructions. Flow cell 3 was immobilised with 820 RU of human-Fc (R&D system, 110-HG) for background subtraction. For each sample, 50 nM of mAb^2 was flowed across flow cells 1 and 2, at a flow rate of 20 µl/min for 150 sec. Subsequently, 50 nM of murine LAG-3Fc (R&D Systems, 3328-L3-050) was flowed across both flow cell 3 and 4 at a flow rate of 20 µl/min for 150 sec. For each binding step dissociation was followed for 3 min. Sensor chip was regenerated after each cycle with 2 x 10 µl of 50 mM NaOH. Both mAb^2 tested were capable of simultaneously binding to murine LAG-3 and murine PD-L1 and are therefore suitable surrogates for the human LAG-3/PD-L1 mAb^2.

2.8 Binding to human Fcγ receptors by mAb^2 comprising LALA mutation

Human Fcγ receptors were immobilized on a CM5 chip to a surface density of approximately 200 RU for Fcγ RI (R&D Systems, 1257-FC) and Fcγ RIIIa (R&D Systems, 4325-FC) and approximately 500 RU for Fcγ RIIa (R&D Systems, 1330-CD) and Fcγ RIIb/c (R&D Systems, 1875-CD), according to the manufacturer's instruction for the BIAcore3000 instrument. For Fcγ RI and Fcγ RIIIa, 100µg/ml of mAbs or mAb^2 were flowed across the chip for 3 min at a flow rate of 10 µl/min and the dissociation was followed for 5 min. Running buffer was PBS (Lonza, BE17-516F) + 0.05% (v/v) P20 surfactant (GE Healthcare, BR-1000-54). The positive control was wild-type IgG1 4420-mAb. Monoclonal IgG2 and IgG4 mAbs to irrelevant targets (20H4 and MOR7490) and mouse IgG1 (Sigma, P5305) were included as reference points. No regeneration was required due to the fast dissociation rates of the binding complexes. For Fcγ RIIa and Fcγ RIIb/c testing the concentration of mAb^2 was

increased to 500 µg/ml to compensate for the weaker binding to these two receptors. The results are shown in **Table 12.**

Table 12: Binding response of anti-LAG-3/PD-L1 mAb^2 to human Fcγ receptors as determined by SPR

mAb/mAb^2	Binding response at end of association (RU)			
	FcγRI	FcγRIII	FcγRIIa	FcγRIIb/c
FS18-7-9/84G09 LALA	<1	<5	<1	<2
FS18-7-33/84G09 LALA	<1	<5	<1	<2
FS18-7-62/84G09 LALA	<1	<5	<1	<2
FS18-7-78/84G09 LALA	<1	<5	<1	<2
84G09 mAb LALA	<1	<5	<1	<2
IgG2	<1	<5	9	<2
IgG4	8	<5	8	9
4420 mAb IgG1	30	28	29	18
Mouse IgG1	<1	<5	7	<2

As expected, the LALA variant introduced into the mAb or mAb^2 reduces the ability of these molecules to bind human Fcγ receptors.

2.9 Binding of mAb^2 to cells expressing human and cynomolgus LAG-3

The human LAG-3 sequence (SEQ ID NO: 126) or cynomolgus LAG-3 sequence (SEQ ID NO: 128) were subcloned into pcDNA5FRT vector (Life Technologies, V6010-20) using KpnI (NEB, R0142) and NotI (NEB, R0146) restriction digestion. The vector was then transformed into Flp-In T-REx 293 HEK cell line (Life Technologies, R780-07) using Lipofectamine 2000 (Life Technologies, 11668-019). Transformed Flp-In T-REx 293 cells were grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) for 3-4 weeks until colonies of stably transformed cells were apparent. These colonies were amplified in the presence of 1 µg/ml Doxycyclin (Sigma, D9891) and tested for LAG-3 expression was confirmed by flow cytometry.

The affinity of the mAb^2 (all containing the LALA mutation) for cell expressed human or cynomolgus LAG-3 was determined using flow cytometry. HEK cells expressing human or cynomolgus LAG-3 grown in DMEM (Life Technologies, 61965-026) containing 10% FBS

(Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) and 1 µg/ml Doxycyclin (Sigma, D9891) were detached from tissue culture flasks using cell dissociation buffer (Life Technologies, 13151-014) and seeded in V-bottom 96-well plates (Costar, 3897) at $2x10^5$ cells/well. The plates were centrifuged at 1500 rpm for 3 min at 4 °C to pellet the cells. A dilution series of the mAb^2 (or control mAb) were incubated with the cells in a 100 µl volume for 1 h at 4 °C. The plates were washed and secondary antibody (Anti-human Fc-488, Jackson ImmunoResearch, 109-546-098) was diluted 1:1000 in PBS and 100 µl was added to the cells for 30 min at 4 °C (plates were kept in the dark). The plates were washed and then cells were resuspended in 100 µl PBS containing 1 µg/ml DAPI (Biotium, 40043). The plates were read using Canto II flow cytometer (BD Bioscience). Dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was measured. The data was fit using log (agonist) vs response in GraphPad Prism Software. The plates were read on a BD FACSCanto II cytometer (BD Biosciences) and the data analysed using FlowJo. The results are shown in **Table 13.**

Table 13: Binding affinity of anti-LAG-3/PD-L1 mAb^2 to HEK cells expressing human or cynomolgus LAG-3 as determined by flow cytometry

	human LAG-3		cyno LAG-3	
	EC_{50} (nM)	95% Conf. Int.	EC_{50} (nM)	95% Conf. Int.
FS18-7-09/84G09LALA	3.179	2.311 to 4.372	29.9	22.16 to 40.35
FS18-7-62/84G09LALA	4.079	2.736 to 6.083	26.37	15.80 to 44.02
FS18-7-78/84G09LALA	2.526	1.474 to 4.329	24.75	19.31 to 31.73
25F7	4.192	2.791 to 6.297	156.5	107.3 to 228.2

The results confirm mAb^2 binding to human and cynomologus LAG-3 expressed on HEK cells. Regarding the calculated EC_{50} values, tested mAb^2 show better or equal binding to the human LAG-3 and at least two times better binding to the cynomologus LAG-3 when compared with control anti-LAG-3 antibody 25F7. No cross-reactivity with other proteins expressed on the surface of the HEK cell line was observed.

2.10 Binding of mAb^2 to cells expressing human and cynomolgus PD-L1

The human PD-L1 sequence (SEQ ID NO: 129) or cynomolgus PD-L1 sequence (SEQ ID NO: 131) were subcloned into pcDNA5FRT vector (Life Technologies, V6010-20) using KpnI

(NEB, R0142) and NotI (NEB, R0146) restriction digestion. The vector was then transformed into Flp-In T-REx 293 HEK cell line (Life Technologies, R780-07) using Lipofectamine 2000 (Life Technologies, 11668-019). Transformed Flp-In T-REx 293 cells were grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) for 3-4 weeks until colonies of stably transformed cells were apparent. These colonies were amplified in the presence of 1 µg/ml Doxycyclin (Sigma, D9891) and LAG-3 expression was confirmed by flow cytometry.

The affinity of the mAb^2 (all containing the LALA mutation) binding to cell expressed human or cynomolgus PD-L1 or to parental (untransformed cells) was determined using flow cytometry. HEK cells expressing human or cynomolgus PD-L1 grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) and 1 µg/ml Doxycyclin (Sigma, D9891) were detached from tissue culture flasks using cell dissociation buffer (Life Technologies, 13151-014) and seeded in V-bottom 96-well plates (Costar, 3897) at $2x10^5$ cells/well. The plates were centrifuged at 1500 rpm for 3 min at 4 °C to pellet the cells. A dilution series of the mAb^2 (or control mAb) was incubated with the cells in a 100 µl volume for 1 h at 4 °C. The plates were washed and secondary antibody (Anti-human Fc-488, Jackson ImmunoResearch, 109-546-098) was diluted 1:1000 in PBS and 100 µl was added to the cells for 30 min at 4 °C (plates were kept in the dark). The plates were washed then cells were resuspended in 100 µl PBS containing 1 µg/ml DAPI (Biotium, 40043). The plates were read using Canto II flow cytometer (BD Bioscience). Dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was measured. The data was fit using log (agonist) vs response in GraphPad Prism Software. The plates were read on a BD FACSCanto II cytometer (BD Biosciences) and the data analysed using FlowJo. The results are shown in **Table 14.**

Table 14: Binding affinity of anti-LAG-3/PD-L1 mAb^2 to HEK cells expressing human or cynomolgus PD-L1 as measured by flow cytometry

	human PD-L1 HEK		cynomolgus PD-L1	
	EC_{50} (nM)	95% Conf. Int.	EC_{50} (nM)	95% Conf. Int.
84G09	3.107	2.230 to 4.331	1.641	1.244 to 2.164
FS18-7-09/84G09	3.19	2.208 to 4.610	1.674	1.393 to 2.012

FS18-7-09/84G09	3.51	2.570 to 4.794	1.814	1.503 to 2.189
FS18-7-33/84G09	3.448	2.436 to 4.881	1.895	1.566 to 2.292
FS18-7-32/84G09	3.816	2.749 to 5.298	1.984	1.644 to 2.393
FS18-7-58/84G09	3.775	2.957 to 4.820	1.861	1.541 to 2.247
FS18-7-33/84G09	3.225	2.214 to 4.700	1.733	1.397 to 2.150
FS18-7-65/84G09	3.999	2.782 to 5.750	1.845	1.455 to 2.339
FS18-7-36/84G09	3.907	2.844 to 5.367	1.999	1.580 to 2.528
FS18-7-88/84G09	3.492	2.541 to 4.800	1.814	1.464 to 2.247
FS18-7-58/84G09	3.907	2.998 to 5.093	2.033	1.622 to 2.550
84G09 (parental)	3.051	2.181 to 4.267	1.959	1.515 to 2.533

All of the tested LAG-3/PD-L1 mAb^2 bound to human PD-L1 and cyno PD-L1 with an EC_{50} close to that of the 84G09 mAb, demonstrating that the PD-L1 binding affinity was not affected by the introduction of the LAG-3 binding site into the CH3 domain of the mAb^2.

2.11 Binding of surrogate mouse mAb^2 to cells expressing mouse LAG-3 or mouse PD-L1

The murine LAG-3 sequence (SEQ ID NO: 127) or murine PD-L1 sequence (SEQ ID NO: 130) were subcloned into pcDNA5FRT vector (Life Technologies, V6010-20) using KpnI (NEB, R0142) and NotI (NEB, R0146) restriction digestion. The vector was then transformed into Flp-In T-REx 293 HEK cell line (Life Technologies, R780-07) using Lipofectamine 2000 (Life Technologies, 11668-019). Transformed Flp-In T-REx 293 cells were grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) for 3-4 weeks until colonies of stably transformed cells were apparent. These colonies were amplified in the presence of 1 µg/ml Doxycyclin (Sigma, D9891) and LAG-3 or PD-L1 expression was confirmed by flow cytometry.

The affinity of the mAb^2 (all containing the LALA mutations) binding to cell expressed murine LAG-3 or murine PD-L1 was determined using flow cytometry. HEK cells expressing murine LAG-3 or murine PD-L1 grown in DMEM (Life Technologies, 61965-026) containing 10% FBS (Life Technologies, 10270-1-6), 100 µg/ml Hygromycin B (Melford Laboratories Ltd, Z2475), 15 µg/ml Blasticidin (Melford Laboratories Ltd, B1105) and 1 µg/ml Doxycyclin (Sigma, D9891) were detached from tissue culture flasks using cell dissociation buffer (Life Technologies, 13151-014) and seeded in V-bottom 96-well plates (Costar, 3897) at $2x10^5$ cells/well. The plates were centrifuged at 1500 rpm for 3 min at 4 °C to pellet the cells. A dilution series of the mAb^2 (or control mAb) were incubated with the cells in a 60 µl volume

for 1 h at 4 °C. The plates were washed and secondary antibody (Anti-human Fc-488, Jackson ImmunoResearch, 109-546-098 for mAb^2 or Anti-Rat IgG (H+L), Alexa Fluor 488, ThermoFisher, A-11006 for anti-LAG-3 control, C9B7W) was diluted 1:1000 in PBS and 50 µl was added to the cells for 30 min at 4 °C (plates were kept in the dark). The plates were washed then cells were resuspended in 50 µl FACS Cell Fix (BD Bioscience, 340181) for 15 minutes, then washed and resuspended in 100 µl PBS containing 1 µg/ml DAPI (Biotium, 40043). The plates were read using Canto II flow cytometer (BD Bioscience). Dead cells were excluded and the fluorescence in the FITC channel (488nm/530/30) was measured. The data was fit using log (agonist) vs response in GraphPad Prism Software. The plates were read on a BD FACSCanto II cytometer (BD Biosciences) and the data analysed using FlowJo. The results are shown in **Table 15.**

Table 15: Binding affinity of surrogate anti-mouse LAG-3/PD-L1 mAb^2 to HEK cells expressing murine LAG-3 or murine PD-L1 by flow cytometry

	murine LAG-3 HEK		murine PD-L1 HEK	
	EC_{50} (nM)	95% Conf. Int.	EC_{50} (nM)	95% Conf. Int.
C9B7W	27	12.27 to 58.04	N/A	-
FS18-7-108-29/S1	2.27	1.064 to 4.857	11.9	7.627 to 18.68
FS18-7-108-29/4420	2.29	1.256 to 4.164	N/A	-
FS18-7-108-35/S1	3.84	2.126 to 6.926	12.3	8.873 to 17.16
FS18-7-108-35/4420	2.38	0.7819 to 7.239	N/A	-

The surrogate mAb^2 were able to bind to cell-expressed murine LAG-3 and to cell expressed murine PD-L1. The binding affinity of the surrogate mAb^2 to cell expressed murine LAG-3 is approximately the same as the affinity of the anti-human LAG-3/PD-L1 mAb^2 to human LAG-3 (2.3-3.8 nM compared to 2.5-4.2 nM) and the binding affinity of the surrogate mAb^2 to cell-expressed murine PD-L1 is within 3-fold the affinity of the anti-human LAG-3/PD-L1 mAb^2 to human PD-L1 (11.9-12.3 nM compared to 3.1-4.0 nM), demonstrating that these mAb^2 are suitable surrogates for the anti-human LAG-3/PD-L1 mAb^2 for use *in vivo* studies in mice.

Example 3: Activity of mAb^2 molecules in T cell activation assays and an SEB assay

3.1 T cell activation assay

An IL-2 release assay based on the DO11.10 OVA T-lymphocyte and LK35.2 B-lymphocyte hybridoma cell lines was used for functional screening of the mAb^2. IL-2 release is a marker of T cell activation. T cells, expressing endogenous murine PD-1, were transfected with either empty vector (pLVX) or human LAG-3 construct. B-cells were transfected with empty vector (pLVX) or human PD-L1 construct.

Three combinations of these four cell lines were used side by side for testing T cell activation by the mAb^2:

- DO11.10 pLVX + LK35.2 hPD-L1 for anti-PD-L1 activity;
- DO11.10 hLAG-3 + LK35.2 pLVX for anti-LAG-3 activity;
- DO11.10 hLAG-3 + LK35.2 hPD-L1 for simultaneous anti-LAG-3/ anti-PD-L1 activity.

All mAb^2 (all containing the LALA mutation) were tested twice in this T cell activation assay. Cross-reactivity with cynomolgus LAG-3 and PD-L1 was tested in a functional T cell activation assay using cells which overexpress cynomolgus targets (cPD-L1 and cLAG-3).

Production of T cell lines over-expressing LAG-3

Lentiviral transduction methodology was used to generate DO11.10 cells (National Jewish Health) overexpressing human, cynomolgus or mouse LAG-3 using the Lenti-X HTX Packaging System (Cat. No 631249). Lenti-X expression vector (pLVX) (Cat. No 631253), containing the mouse LAG-3 cDNA (SEQ ID NO: 127), human LAG-3 cDNA (SEQ ID NO: 126) or cynomolgus LAG-3 cDNA (SEQ ID NO: 128), was co-transfected with a Lenti-X HTX Packaging Mix into the Lenti-X 293T Cell Line (Cat. No 632180) to generate virus. The DO11.10 cell line was transduced using the lentiviral vectors produced with the Lenti-X HTX Packaging System.

Production of antigen presenting cells over-expressing PD-L1

Lentiviral transduction methodology was used to generate LK35.2 B cell lymphoma (ATCC, HB-98) overexpressing human, cynomolgus or mouse PD-L1 using the Lenti-X HTX Packaging System (Cat. No 631249). Lenti-X expression vector (pLVX) (Cat. No 631253), containing the mouse PD-L1 cDNA (SEQ ID NO: 130), human PD-L1 cDNA (SEQ ID NO: 129 or cynomolgus PD-L1 cDNA (SEQ ID NO: 131), was co-transfected with a Lenti-X HTX Packaging Mix into the Lenti-X 293T Cell Line (Cat. No 632180) to generate virus. The LK35.2 cell line was transduced using the lentiviral vectors produced with the Lenti-X HTX Packaging System.

Media and Peptide

Cell culture medium: DMEM (Gibco, 61965-026) 10% FBS (Gibco, 10270-106), 1 mM Sodium Pyruvate (Gibco, 11360-070), 1 µg/ml puromycin (Gibco, A11138-03)
Experimental medium: complete DO11.10 culture medium without puromycin.
OVA peptide (MW = 1773.9 Da): H-ISQAVHAAHAEINEAGR-OH (Pepscan)

Cells:

- DO11.10 hLAG-3: DO11.10 T cell hybridoma transduced with a lentiviral vector to overexpress human LAG-3;
- DO11.10 pLVX: DO11.10 T cell hybridoma transduced with an empty lentiviral vector;
- DO11.10 cLAG-3: DO11.10 T cell hybridoma transduced with a lentiviral vector to overexpress cynomolgus LAG-3.
- LK 35.2 hPD-L1: B cell hybridoma transduced with a lentiviral vector containing hPD-L1 to overexpress human PD-L1;
- LK 35.2 PLVX: B cell hybridoma transduced with an empty lentiviral (pLVX) vector;
- LK 35.2 cPD-L1: B cell hybridoma transduced with a lentiviral vector to overexpress cynomolgus PD-L1.

DO11.10 cells (either DO11.10 pLVX cells or DO11.10 hLAG-3 cells) at 0.3 x 10^6 cells/ml were mixed at a 1:1 ratio with antibodies at 3 x final concentration. Antibodies and DO11.10 cells were incubated at 37° C, 5% CO_2 for 1 hour. LK 35.2 cells (both pLVX and PD-L1-pLVX) were incubated at 3x10^5 cells/ml experimental media with the OVA peptide at 1.5 µM for 30 min. LK 35.2 cells + OVA were added to DO11.10 cells/treatment mix at a 1:2 ratio in the following combinations:

Human functional screen
DO11.10 pLVX + LK35.2 hPD-L1,
DO11.10 hLAG-3 + LK35.2 pLVX,
DO11.10 hLAG-3 + LK35.2 hPD-L1;

Cynomolgus cross reactivity screen
DO11.10 pLVX + LK35.2 cPD-L1,
DO11.10 cLAG-3 + LK35.2 pLVX,
DO11.10 cLAG-3 + LK35.2 cPD-L1;

Cells were incubated at 37ºC, 5%CO_2 for 24 hours. Supernatants were collected and assayed with mouse IL-2 ELISA kit (eBioscience, 88-7024-88 or R&D systems, SM2000) following the manufacturer's instructions. Plates were read at 450 nm using the plate reader with the Gen5 Software, BioTek. Absorbance values of 570 nm were subtracted from those of 450 nm (Correction). The standard curve for calculation of cytokine concentration was based on four parameter logistic curve fit (Gen5 Software, BioTek). The concentration of mIL-2 was plotted vs the log concentration of Fcab or mAb and the resulting curves were fitted using the log (agonist) vs response equation in GraphPad Prism. **Table 16** shows the EC_{50} values and the maximal IL-2 release of the mAb^2 and control mAbs, calculated as a percentage of the control (84G09 + 25F7). **Figure 2A-F** show representative plots of IL-2 release by the panel of cell lines treated with FS18-7-9/84G09, FS18-7-62/84G09, or FS18-7-78/84G09, and controls. In the T cell activation assay with both targets present (DO11.10 hLAG-3 + LK35.2 hPD-L1), IL-2 release was only induced when both LAG-3 and PD-L1 were inhibited, for example when the mAb^2 or a combination of LAG-3 and PD-L1 antibodies was used. Therefore the mAb^2 has benefit over a single agent alone. In the assays with just LAG-3 (DO11.10 hLAG-3 + LK35.2 pvlx) or PD-L1 (DO11.10 pvlx + LK35.2 PD-L1) the mAb^2 showed similar activity as the single agents in inhibiting these targets, demonstrating that the mAb^2 is the only single molecule which was able to lead to T cell activation in the presence of LAG-3, PD-L1 or LAG-3+PD-L1.

Table 16: EC_{50} and maximal IL2 release (as calculated by percentage of IL2 release compared to control – 84G09+25F7) for nine mAb^2

mAb^2/control mAbs	AVERAGE	SD	AVERAGE	SD
	EC_{50}		Maximal IL2 release calculated as % ctrl (84G09+25F7)	
FS18-7-09/84G09LALA	0.75	0.20	65.89	24.04
FS18-7-33/84G09LALA	0.82	0.35	63.95	36.56
FS18-7-78/84G09LALA	0.82	0.38	64.39	39.28
FS18-7-62/84G09LALA	0.65	0.02	58.97	18.13
FS18-7-65/84G09LALA	0.81	0.20	70.25	2.22
FS18-7-95/84G09LALA	1.10	-	79.77	-
FS18-7-32/84G09LALA	1.19	0.00	82.94	14.67
FS18-7-36/84G09LALA	1.05	0.32	71.56	8.83
FS18-7-58/84G09LALA	1.16	0.21	79.40	0.10
84G09LALA + 25F7	1.32	0.25	100.00	

One of the mAb^2 (FS18-7-9/84G09 containing the LALA mutation) was tested for cynomolgus functional cross reactivity in the DO11.1-/LK35.2 T cell activation assay. **Figure 3 A-C** show the results in the panel of three cell based assays. FS18-7-9/84G09 was able to induce T cell activation in the presence of both cLAG-3 + cPD-L1 and cLAG-3 or cPD-L1 alone, indicating that the mAb^2 are functionally cynomolgus cross-reactive and therefore suitable for use in primate based safety studies.

3.2 Staphylococcal Enterotoxin B assay

Three mAb^2 (all containing the LALA mutation) were tested in the human-PBMC based Staphylococcal Enterotoxin B assay (SEB assay). Staphylococcal Enterotoxin B is a superantigen, and binds to MHC class II molecules on antigen presenting cells (APCs) and the vβ chain of the T cell receptor (TCR), causing non-specific activation of T cells and cytokine release. There is no requirement for antigen to be present to see T cell activation. The SEB assay uses stimulated human cells (PBMCs) with physiological levels of checkpoint inhibitors, and can be used to confirm that T cell activation is enhanced by the mAb^2 in a human system. Three mAb^2 were tested in the SEB system with cells coming from four different donors.

Generation of expanded T cells

PMBCs were isolated from leukocyte cones by Ficoll gradient seperation. CD4+ T cells were isolated using human CD4+ T Cell Isolation Kit (Miltenyi Biotec Ltd, 130-096-533) according to the manufacturer's instruction. Human T-Activator CD3/CD28 Dynabeads (Life technologies, 11131D) were resuspended by vortexing. Beads were transferred to a sterile 15ml tube and 10ml RPMI (Life Technologies, 61870044) with 10% FBS (Life Technologies, 10270106) and 1x Penicillin Streptomycin (Life Technologies, 15140122) was added to wash Dynabeads. The supernatant was discarded. The required amount of CD4+ T cells at 1.0 $x10^6$ cells/ml in RPMI with 10% FBS and 1x Penicillin Streptomycin Solution and 50 IU/ml recombinant human IL2 (Peprotech, 200-02-50ug) with 3:1 bead to cell ratio were transferred to T75 flask (Greiner Bio-one, 690195) and incubated at 37ºC + 5% CO_2. After 3 days the cells were gently resuspended and counted. The cell density was maintained between 0.8-1 x 10^6 cells/ml by adding fresh media (RPMI-10% FBS + Penicillin Streptomycin Solution 1X + 50IU/ml rhuIL2) as needed. On day 7 or 8, the CD3/28 beads were removed and CD4+ T cells were rested overnight at 1 x 10^6 cells/ml fresh media RPMI-10% FBS + Penicillin Streptomycin Solution 1X with reduced 10IU/ml rhuIL2. The cells were stored frozen until required.

Generation of MoiDCs

Untouched monocytes were isolated from human PBMCs using human Pan Monocyte Isolation Kit, (Miltenyi Biotec Ltd, 130-096-537) following the manufacturer's instructions. Monocytes were differentiated to iDCs using human Mo-DC Differentiation Medium (Miltenyi Biotec Ltd, 130-094-812) following the manufacturer's instructions.

SEB assay

Expanded T cells were thawed one day before the experiment, washed with AIM medium (Gibco, 12055-091) and incubated at 37ºC, 5% CO_2 overnight at $1x10^6$ cells/ml in AIM medium. 2 µM concentration of each antibody/mixture was prepared in DPBS (Gibco, 14190-169) and diluted 1:10 in medium (30 µl + 270 µl) to obtain 200 nM. In a 96 well plate, serial dilutions were carried out at 1:10 (30 µl + 270 µl experimental medium; 2x final conc.). MoiDCs were thawed, washed with AIM medium and mixed with T cells from the same donor at a 1:10 ratio (5 ml of iDCs at $2x10^5$ cells/ml were combined with 5ml of T cells at $2x10^6$ cells/ml). 20 µl of SEB (Sigma, S4881) at 0.1 µg/ml was added to 10 ml of the cells. In

a round bottom 96 well plate, 100 µl of the cell/SEB mixture was added to 100 µl of the antibody dilution, giving a ratio of 10^4 iDC cells to 10^5 T cells with 0.1 ng/ml SEB in 200 µl of AIM media per well with final antibody concentrations of 100, 10, 1, 0.1, 0.01, 0.001 nM. Cells were incubated at 37°C, 5% CO2 for 3 days. Supernatants were collected and assayed immediately with human IFN$_\gamma$ ELISA kit (R&D Systems, PDIF50) or frozen down at -20°C for further analysis. The assay was performed according to the kit manufacturer's instructions using supernatants diluted 1:30 with PBA (DPBS, 2% BSA (Sigma, A7906-100G)). The concentration of human IFN$_\gamma$ was plotted vs the log concentration of mAb^2 or mAb and the resulting curves were fitted using the log (agonist) vs response equation in GraphPad Prism software. **Table 17** shows the EC_{50} values and the span of the IFNγ release in the SEB assay with cells from four different cell donors (Donors A to D). **Figure 4** shows a representative plot of the SEB assay with a single donor. In all six assays the LAG-3/PD-L1 mAb^2 and the combination of the LAG-3/FITC mAb^2 + 84G09LALA showed greater activation than the 84G09LALA mAb alone, whereas when the LAG-3/FITC mAb^2 or 4420 mAb were assayed they did not show significant activation. The results of the SEB assay therefore confirm the results of the DO11.10/LK35.2 assay in a more physiological system.

Table 17. EC_{50} values calculated for six mAb^2 based on six SEB assays with 4 different cell donors.

Assay 1 Donor A				
	EC_{50} (nM)	95% Conf. Int.	SPAN	95% Conf. Int.
FS18-7-09/84G09LALA	0.1551	0.08521 to 0.2824	12280	10802 to 13758
FS18-7-62/84G09LALA	0.1424	0.08665 to 0.2340	12120	10897 to 13342
FS18-7-78/84G09LALA	0.2774	0.1220 to 0.6310	14156	11970 to 16341
84G09 LALA	0.1884	0.08960 to 0.3962	10077	8612 to 11542
FS18-7-09/4420LALA	-	-	1010	384.8 to 1635
FS18-7-62/4420LALA	-	-	416.1	-443.1 to 1275
FS18-7-78/4420LALA	-	-	411	-1103 to 1925
84G09 LALA + FS18-7-09/4420LAL	0.2455	0.1030 to 0.5850	11836	9888 to 13785
84G09 LALA + FS18-7-62/4420LAL	0.1601	0.05399 to 0.4750	11448	8958 to 13937
84G09 LALA + FS18-7-78/4420LAL	0.1774	0.06863 to 0.4587	12250	9955 to 14544
4420 LALA	-	-	218.6	-35.91 to 473.1

Assay 1 Donor B				
	EC_{50} (nM)	95% Conf. Int.	SPAN	95% Conf. Int.
FS18-7-09/84G09LALA	0.07316	0.02255 to 0.2374	11825	8919 to 14730
FS18-7-62/84G09LALA	0.03236	0.007146 to 0.1465	12170	8419 to 15921
FS18-7-78/84G09LALA	0.09718	0.03128 to 0.3019	15198	11594 to 18802
84G09 LALA	0.08562	0.03403 to 0.2154	12343	9957 to 14730
FS18-7-09/4420LALA	-	-	1337	-195.8 to 2870
FS18-7-62/4420LALA	-	-	2508	-2118 to 7133
FS18-7-78/4420LALA	-	-	1425	-58.52 to 2908
84G09 LALA + FS18-7-09/4420LAL	0.2861	0.1005 to 0.8143	16037	12890 to 19184
84G09 LALA + FS18-7-62/4420LAL	0.1431	0.02857 to 0.7172	13907	9358 to 18455
84G09 LALA + FS18-7-78/4420LAL	0.3775	0.08929 to 1.596	14418	10502 to 18334
4420 LALA	-	-	2293	1028 to 3558
Assay 2 Donor A				
	EC_{50} (nM)	95% Conf. Int.	SPAN	95% Conf. Int.
FS18-7-09/84G09LALA	0.2761	0.1654 to 0.4611	20727	18729 to 22724
FS18-7-33/84G09LALA	0.1934	0.07990 to 0.4681	171 03	14156 to 20049
FS18-7-78/84G09LALA	0.2384	0.1596 to 0.3560	19822	18311 to 21333
84G09 LALA	0.3788	0.1166 to 1.230	11745	9138 to 14352
FS18-7-09/4420LALA	-	-	1991	-1927 to 5909
FS18-7-33/4420LALA	-	-	~ 1.053e+006	(Very wide)
FS18-7-78/4420LALA	-	-	~ 72657	(Very wide)
84G09 LALA + FS18-7-09/4420LALA	0.5202	0.2624 to 1.031	23346	20230 to 26462
84G09 LALA + FS18-7-33/4420LALA	0.2881	0.1529 to 0.5428	22529	19853 to 25205
84G09 LALA + FS18-7-78/4420LALA	0.4335	0.2089 to 0.8996	20955	18038 to 23873
Assay 2 Donor B				
	EC_{50} (nM)	95% Conf. Int.	SPAN	95% Conf. Int.
FS18-7-09/84G09LALA	0.139	0.04157 to 0.4646	21572	16269 to 26874

FS18-7-33/84G09LALA	0.07278	0.01615 to 0.3280	19554	13408 to 25699
FS18-7-78/84G09LALA	0.1356	0.03552 to 0.5178	22319	16212 to 28426
84G09 LALA	0.1959	0.03365 to 1.140	12077	7938 to 16216
FS18-7-09/4420LALA	-	-	1272	-963.4 to 3507
FS18-7-33/4420LALA	-	-	2272	-190.8 to 4735
FS18-7-78/4420LALA	-	-	1960	-1287 to 5207
84G09 LALA + FS18-7-09/4420LALA	0.2889	0.07772 to 1.074	23661	17835 to 29488
84G09 LALA + FS18-7-33/4420LALA	0.3	0.06936 to 1.298	26145	18971 to 33320
84G09 LALA + FS18-7-78/4420LALA	0.271	0.03980 to 1.845	25489	16292 to 34686
Assay 1 Donor C				
	EC_{50} (nM)	95% Conf. Int.	SPAN	95% Conf. Int.
FS18-7-09/84G09LALA	0.06739	0.01933 to 0.2349	2987	15107 to 27710
84G09 LALA	0.08347	0.0331 to 0.2105	1509	11046 to 17414
84G09 LALA + FS18-7-09/4420LALA	0.1618	0.06635 to 0.3944	1744	13346 to 20704
Assay 1 Donor D				
	EC_{50} (nM)	95% Conf. Int.	SPAN	95% Conf. Int.
FS18-7-09/84G09LALA	0.04373	0.01797 to 0.1064	1850	15995 to 23799
84G09 LALA	0.07043	0.03483 to 0.1424	1045	10996 to 15405
84G09 LALA + FS18-7-09/4420LALA	0.1351	0.08359 to 0.2182	968	15318 to 19403

Example 4: *In vivo activity of mAb^2 molecules in murine tumour models*

4.1 Activity of mAb^2 molecules in a MC38 non-established tumour model

The MC38 syngeneic tumour model was used in this experiment as MC38 tumours are known to express PD-L1 on their cell surface and to be highly immunogenic resulting in increased LAG-3 expression on immune cells in the tumour and tumour periphery.

The surrogate mouse mAb^2 FS18-7-108-29/S1 containing the LALA mutation (SEQ ID NO: 117 and 119) referred to as FS18-29/S1 was tested for *in vivo* activity using a MC38

syngeneic mouse tumour growth model. The ability of the mAb^2 to inhibit tumour growth was compared to that of the LAG-3/mock mAb^2, FS18-7-108-29/4420 containing the LALA mutation (SEQ ID NO: 132 and 85) referred to as FS18-29/4420, the benchmark anti-LAG-3 mAb C9B7W (2B scientific; Catalogue Number BE0174-50MG), the benchmark anti-PD-L1 mAb S1 containing the LALA mutation (SEQ ID NO: 122 and 119) and to a combination of mAbs C9B7W and S1.

C57BL/6 female mice (Charles River) aged 8-10 weeks and weighing 20-25 g each were rested for one week prior to the study start. All animals were micro-chipped and given a unique identifier. Each cohort had 10 mice. The MC38 colon carcinoma cell line (S. Rosenberg, NIH) was initially expanded, stored, and then pre-screened by IDEXX Bioresearch for pathogens using the IMPACT I protocol and shown to be pathogen free. MC38 cells were thawed from -150 ^{o}C storage and added to 20 ml DMEM (Gibco, 61965-026) with 10% FCS (Gibco, 10270-106) in a T175 tissue culture flask. Mice were anaesthetised using isoflurane (Abbott Laboratories) and each animal received $2x10^6$ cells injected subcutaneously in the left flank. 7-8 days following tumour cell inoculation, mice which did not have tumours at this point were removed from the study.

All of the mAb^2 molecules and the control antibodies were analysed within 24 hours prior to injection by SEC-HPLC profiling and checked for impurities. Antibodies were prepared at a final concentration of 10 mg/kg in PBS and combined with a second antibody in the combination studies. The mAb^2 molecules and the control antibodies were administered to the mice by intraperitoneal (IP) injection on days 8, 11, and 14 following tumour inoculation. Accurate measurements of tumours were taken, any drug dosing due on the day in question was performed, and the mice subjected to close observation for the remainder of the trial. Tumour volume measurements were taken with callipers to determine the longest axis and the shortest axis of the tumour. The following formula was used to calculate the tumour volume:

$L \times (S^2) / 2$
Where L = longest axis; S= shortest axis

The trial was halted at day 20 when the tumour burden was considered close to restrictions. All mice were humanely sacrificed and the tumours were excised and weighed.

The results are shown in **Figures 5 and 6.** LAG-3/PD-L1 mAb^2 (FS18-29/S1) treated mice had end tumours with significantly smaller weights than those treated with a combination of benchmark mAbs, C9B7W and S1. Specifically, statistical analysis of end tumour weights was performed using a two tailed Student's t-test within the GraphPad Prism software package demonstrating a statistical significant difference between administration of FS18-29/S1 and administration of the combination of benchmark antibodies C9B7W and S1 ($p = 0.0125$), indicating that LAG-3 and PD-L1 inhibition provided by the same molecule resulted in a synergistic effect on end tumour weights compared with LAG-3 and PD-L1 inhibition provided by separate molecules.

The surrogate mAb^2 FS18-29/S1 also had a marked effect on tumour growth preventing the establishment in 6 of 8 growing MC38 tumours, and slowing the growth of the remaining 2. Administration of the benchmark anti-LAG-3 and PD-L1 antibodies in combination slowed tumour growth in 7 animals with no animals being tumour free.

FS18-29/4420 alone had no marked effect on tumour growth, indicating that for maximal efficacy, the mAb^2 requires the anti-PD-L1 Fab. The benchmark anti-mouse LAG-3 antibody alone had little or no effect on resulting tumour growth while the benchmark anti-mouse PD-L1 prevented establishment of 1 of 7 tumours in this cohort, and had some overall effect of slowing tumour growth.

Syngeneic mouse models are accepted as appropriate murine systems for testing the anti-tumour effect of inhibiting therapeutic targets and have been used extensively to validate development of human therapeutics.

4.2 Activity of mAb2 molecules in a MC38 established tumour model

The surrogate mouse mAb^2 FS18-7-108-29/S1 containing the LALA mutation, (SEQ ID NO: 117 and 119) referred to as FS18-29/S1, was tested for *in vivo* activity in a MC38 syngeneic mouse tumour growth model. The ability of the mAb^2 to inhibit tumour growth was compared to that of the LAG-3/mock mAb^2, FS18-7-108-29/4420 containing the LALA mutation (SEQ ID NO: 132 and 85), referred to as FS18-29/4420, the benchmark LAG-3 mAb C9B7W, the benchmark PD-L1 mAb S1 containing the LALA mutation (SEQ ID NO: 122 and 119) and to a combination of C9B7W and S1.

C57BL/6 female mice (Charles River) aged 8-10 weeks and weighing 20-25 g each were rested for one week prior to the study start. All animals were micro-chipped and given a

unique identifier. Each cohort had 10 mice. The MC38 colon carcinoma cell line (S. Rosenberg, NIH) was initially expanded, stored, and then pre-screened by IDEXX Bioresearch for pathogens using the IMPACT I protocol and shown to be pathogen free. MC38 cells (approximately 3-5x10^6) was thawed from -150 ^{o}C storage and added to 20 ml DMEM (Gibco, 61965-026) with 10% FCS (Gibco, 10270-106) in a T175 tissue culture flask. Mice were anaesthetised using isofluorane (Abbot Laboratories) and 2x10^6 cells in 100 µl were injected subcutaneously into the left flank of each mouse. 7-8 days following tumour cell inoculation, mice were routinely monitored for health and tumour growth appropriate for initiation of the study. When the majority of mice exhibited tumours of 5-10 mm diameter, they were sorted and randomised back into study cohorts. Any mice which did not have appropriately sized tumours at this point were removed from the study.

All of the mAb^2 molecules and the control antibodies were analysed within 24 hours prior to injection by SEC-HPLC profiling and checked for impurities. Antibodies were prepared at a final concentration of 10 mg/kg in PBS and combined with the second antibody for the combination studies. The mAb^2 molecules and the control antibodies were administered to the mice on days 15, 18, and 21 following tumour inoculation by IP injection. Animals were health screened under anaesthesia three times a week in a blinded fashion, during which time accurate measurements of tumours were taken. Tumour volume measurements were taken with callipers to determine the longest axis and the shortest axis of the tumour. The formula used to calculate the tumour volume was as set out in section 4.1 above.

The trial was halted at day 24 when the tumour burden was considered close to restrictions. All mice were humanely sacrificed and the tumours were excised and weighed. The results are shown in **Figures 7 and 8**.

Effective control or suppression of tumour growth in syngeneic tumour models in mice is best accomplished through therapeutic intervention at early time points (starting tumour volumes of less than 40mm^3). The later the intervention is administered, the more difficult it is to observe positive effects with respect to tumour growth, though perhaps this is more akin to the situation in the human clinical setting.

FS18-29/S1 had a positive effect in both suppressing tumour growth and preventing the establishment of MC38 colon carcinoma in immune competent mice when given at early time points in C57BL/6 mice. When administered at later time points (starting tumour volumes of 50-125mm^3), FS18-29/S1 was just as effective in suppressing tumour growth as

the combination of benchmark antibodies. FS18-29/4420 alone had no noticeable impact on tumour growth and S1 and C9B7W both had a mild effect on resulting tumour growth.

4.3 Activity of mAb^2 molecules in a CT26 non-established tumour model

The CT26 syngeneic tumour model was used in this experiment as CT26 tumours are known to express PD-L1 on their cell surface and to be highly immunogenic resulting in increased LAG-3 expression on immune cells in the tumour and periphery.

The surrogate mouse mAb^2 FS18-7-108-29/S1 containing the LALA mutation (SEQ ID NO: 117 and 119), referred to as FS18-29/S1, and FS18-7-108-35/S1 containing the LALA mutation (SEQ ID NO: 120 and 119), referred to as FS18-35/S1, were tested for *in vivo* activity in a CT26 syngeneic mouse tumour growth model. The ability of the mAb^2 to inhibit tumour growth was compared to that of the LAG-3/mock mAb^2, FS18-7-108-29/4420 containing the LALA mutation (SEQ ID NO: 132 and 85), referred to as FS18-29/4420, and FS18-7-108-35/4420 containing the LALA mutation (SEQ ID NO: 133 and 85), referred to as FS18-35/4420, the benchmark LAG-3 mAb C9B7W, the benchmark PD-L1 mAb S1 containing the LALA mutation (SEQ ID NO: 122 and 119) and to a combination of C9B7W and S1.

BALB/c female mice (Charles River) aged 8-10 weeks and weighing 20-25 g each were rested for one week prior to the study start. All animals were micro-chipped and given a unique identifier. Each cohort had 10 mice. The CT26 colon carcinoma cell line (ATCC, CRL-2638) was initially expanded, stored, and then pre-screened by IDEXX Bioresearch for pathogens using the IMPACT I protocol and shown to be pathogen free. CT26 cells (approximately 3-5x10^6) were thawed from -150 ^{o}C storage and added to 20 ml DMEM (Gibco, 61965-026) with 10% FCS (Gibco, 10270-106) in a T175 tissue culture flask. 7-8 days following tumour cell inoculation, mice were routinely monitored for health and tumour growth appropriate for initiation of the study. When the majority of mice exhibited tumours with a diameter of 3-5 mm^3, they were sorted and randomised back into study cohorts. Any mice which did not have tumours at this point were removed from the study.

All of the mAb^2 molecules and the control antibodies were analysed within 24 hours of injection by SEC-HPLC profiling and checked for impurities. Antibodies were prepared at a final concentration of 10 mg/kg in PBS and combined with a second antibody in the combination studies. The mAb^2 molecules and the control antibodies were administered to

the mice on days 8, 11, and 14 following tumour inoculation. Animals were health screened during which time accurate measurements of tumours were taken. Tumour volume measurements were taken with callipers to determine the longest axis and the shortest axis of the tumour. The formula used to calculate the tumour volume was as set out in section 4.1 above:

The trial was halted at day 20 when the tumour burden was considered close to restrictions. All mice were humanely sacrificed and the tumours were excised and weighed. The results are shown in **Figures 9 and 10**.

Statistical analysis of the end tumour weights was performed using a two tailed Student's t-test within the GraphPad Prism software package. Statistical analysis of the tumour growth curves was determined using compare Growth Curves function from the Statistical Modeling package, **statmod** (Elso *et al.*, 2004 and Baldwin *et al*., 2007), available from the R Project for Statistical Computing.

There was a demonstrated statistical significant difference between FS18-35/S1 mAb^2 and IgG control (normal growth) in suppressing tumour growth. Such a statistically significant difference was not observed with either the combination of benchmark antibodies, or FS18-29/S1 mAb^2 versus the IgG control group, or versus any other cohort in this trial.

The CT26 tumour model is an aggressive, fast growing tumour model, one that is inherently prone to mice developing intestinal metastasis, and as a result has a very limited therapeutic window.

Surprisingly the combination of benchmark LAG-3 and PD-L1 antibodies did not significantly suppress tumour growth compared to the IgG control cohort. However, the FS18-35/S1 treated cohort did reveal a significant suppression of growth compared to IgG control. FS18-29/S1, while it did suppress tumour growth as well compared to IgG control, it was not statistically significant. This trial shows a second tumour model in which the mouse reactive LAG-3/PD-L1 mAb^2 has demonstrated a positive effect in slowing tumour growth at least to the same degree as administration of a combination of benchmark monoclonal antibodies.

4.4 Effect of LALA mutation on tumour growth inhibition in the MC38 non-established tumour model

Two mAb^2 (FS18-7-108-29/S1 LALA and FS18-7-108-29/S1) were tested to examine potential differences in anti-tumour activity of these mAb^2 with and without the LALA mutation in the Fc region. The surrogate mouse mAb^2 FS18-7-108-29/S1 referred to as FS18-29/S1 with (SEQ ID NO: 117 and 119) and without LALA mutation (SEQ ID NO: 118 and 119) were tested for *in vivo* activity using a MC38 syngeneic mouse tumour growth model. The ability of the mAb^2 to inhibit tumour growth was compared to that of the LAG-3/mock mAb^2, FS18-7-108-29/4420 with (SEQ ID NO: 132 and 85) and without LALA mutation (SEQ ID NO: 134 and 85) referred to as FS18-29/4420LALA and FS18-29/4420 and a combination of the LAG-3/mock mAb^2 with and without LALA mutation with the benchmark PD-L1 mAb S1 with (SEQ ID NO: 122 and 119) and without the LALA mutation (SEQ ID NO: 123 and 119).

C57BL/6 female mice (Charles River) aged 8-10 weeks and weighing 20-25 g each were rested for one week prior to the study start. All animals were micro-chipped and given a unique identifier. Each cohort had 10 mice. The MC38 colon carcinoma cell line (S. Rosenberg, NIH) was initially expanded, stored, and then pre-screened by IDEXX Bioresearch for pathogens using the IMPACT I protocol and shown to be pathogen free. MC38 cells (approximately $3\text{-}5x10^6$) were thawed from -150 ^{o}C storage and added to 20 ml DMEM (Gibco, 61965-026) with 10% FCS (Gibco, 10270-106) in a T175 tissue culture flask. Mice were anaesthetised using isofluorane (Abbot Laboratories) and $2x10^6$ cells in 100 µl were injected subcutaneously into the left flank of each mouse. Mice were allowed to recover under observation and the inoculation date noted as Day 0. 7-8 days following tumour cell inoculation, mice were routinely monitored for health and tumour growth appropriate for initiation of the study. Any mice which did not have tumours at this point were removed from the study.

All of the mAb^2 molecules and the control antibodies were analysed within 24 hours prior to injection by SEC-HPLC profiling and checked for impurities. Antibodies were prepared at a final concentration of 10 mg/kg in PBS and combined with a second antibody in the combination studies. The mAb^2 molecules and the control antibodies were administered to the mice on days 8, 11, and 14 following tumour inoculation by IP injection. Animals were health screened under anaesthesia three times a week in a blinded fashion, during which time accurate measurements of tumours were taken. Tumour volume measurements were taken with callipers to determine the longest axis and the shortest axis of the tumour. The formula used to calculate the tumour volume was as set out in section 4.1.

All mice were humanely sacrificed and the tumours were excised and weighed. The results are shown in **Figures 11 and 12**.

This trial confirms that the presence or absence of the LALA mutation, which abrogates ADCC activity, has no statistically significant effect on tumour growth in the MC38 colon carcinoma model, however those mAb^2 which included the LALA mutation tended to result in increased tumour growth suppression. Nevertheless, the rationale for including the mutation to potentially inhibit ADCC activity against T cells expressing LAG-3 or PD-L1 is justified as the LALA mutation has no detrimental effect on the anti-tumour activity of LAG-3/PD-L1 mAb^2. There was some evidence to suggest that inclusion of the LALA mutation was only critical for the PD-L1 antibody.

This trial also examined whether the LAG-3/PD-L1 mAb^2 may have increased efficacy over administration of the individual antibodies (LAG-3 LALA + PD-L1 LALA). In this case there was no significant difference between these two cohorts. Both groups suppressed growth in the MC38 colon carcinoma model.

4.5 Conclusion

Overall, it is clear from the above results that there is a synergistic effect on tumour growth suppression when a mAb^2 molecule comprising binding sites for both LAG-3 and PD-L1 is administered to mice in the mouse models tested. Based on these results, it is expected that the antibody molecules of the invention will show a superior effect in the treatment of cancer in human patients, in particular in supressing tumour growth, than administration of two separate molecules which bind LAG-3 and PD-L1, respectively.

Example 5: Effect of mAb2 treatment on T cell LAG-3 expression

The mechanism by which the surrogate mouse mAb^2 FS18-7-108-29/S1 containing the LALA mutation, (SEQ ID NOs: 117 and 119) referred to as FS18-29/S1 led to decreased tumour burden was tested in a MC38 syngeneic mouse tumour growth model expressing ovalbumin (MC38.OVA). The effect of FS18-29/S1 was compared to that of the LAG-3/mock mAb^2, FS18-7-108-29/4420 containing the LALA mutation (SEQ ID NOs: 132 and 85), referred to as FS18-29/4420, the benchmark PD-L1 mAb S1 containing the LALA mutation (SEQ ID NOs: 122 and 119) and to a combination of FS18-29/4420 and S1.

On the day of implant, cultured MC38.OVA cells were harvested during log phase growth (Confluency ~75%) and resuspended in PBS at a concentration of 1 x 10^7 cells/mL. Tumours were initiated by first anesthetizing each animal with isoflurane, then subcutaneously implanting 1 x 10^6 MC38.OVA cells (0.1 mL suspension) into the left flank of each test animal. Eleven days after tumour cell implantation animals were randomised, using a deterministic randomisation method, into five groups with individual tumour volumes of 32 to 62.5 mm^3. Animals were dosed at 10 mg/kg antibody or mAb^2 on day 12, 14 and 16 after tumour inoculation, and tumours collected from three animals/group at days 19 and 23 after tumour inoculation. GentleMACS™ Dissociator was used to dissociate the tumour with cells subsequently sieved through a 70 μm cell strainer to obtain a single cell suspension. 1x10^6 cells/well on a 96-well plate were resuspended in FACS buffer with 1:3000 viability stain and Fc block (anti-CD16/32 antibody). Cells for FACS analysis were stained using a Master Mix that included labelled antibodies against CD43, CD8a, CD4, FoxP3, and LAG-3. For the FoxP3 intracellular staining cells were fixed and permeabilized prior to staining with the FoxP3 antibody. Samples were run on the Canto II flow cytometer with a compensation matrix and a minimum of 500,000 events counted.

In this experiment, TILs were examined for LAG-3 expression after the third dose of antibody/mAb^2 had been administered, when a separation in the growth of the tumour between control and mAb^2 treatments is seen but before there is a large difference between tumour sizes which might skew results. At this time point, LAG-3 expression on TILs was found to be markedly decreased in animals treated with the mAb^2 FS18-29/S1 or with the combination of FS18-29/4420 and S1. Specifically, as shown in **Figure 13**, LAG-3 expression on CD8, CD4 and FoxP3 tumour infiltrating lymphocytes (TILs) was decreased after treatment with FS18-29/S1 at day 19 and 23 after tumour inoculation, which corresponds to 3 and 7 days after the last antibody/mAb^2 dosing, respectively. The decrease in LAG-3 expression was more pronounced at day 23 but was still evident at day 19. Animals given a combination of FS18-29/4420 and S1 also show a decrease in LAG-3 expression on TILs but the effect was delayed until day 23, while treatment with FS18-29/4420 or S1 administered individually had little to no effect on LAG-3 expression on TILs.

These results show that dual inhibition of LAG-3 and PD-L1 is required for a decrease in LAG-3 expression by TILs, as this phenomenon was not seen in animals treated with single agents against LAG-3 or PD-L1. Without wishing to be bound by theory, it is thought that dual anti-LAG-3 and anti-PD-L1 treatment leads to a decrease in LAG-3 expression on TILS, thereby reducing the inhibitory effect of LAG-3 and allowing the TILs to overcome

exhaustion. Once the TILs become activated, they are able to recognise neo-antigens expressed by the tumour and mount a response against it. This is therefore thought to be the mechanism by which treatment with anti-LAG3/PD-L1 mAb^2 results in a reduction in the tumour burden.

Example 6: *Antibody Dependent Cellular Cytotoxicity and Complement Dependent Cytotoxicity activity of mAb^2*

IgG1 antibodies usually exhibit effector functions via conserved interaction sites within the constant region of the molecule. These include Antibody Dependent Cellular Cytotoxicity (ADCC), mediated by binding to FcγR expressed on Monocytes/Macrophages, dendritic cells, NK cells, neutrophils and other granulocytes, and Complement Dependent Cytotoxicity (CDC), mediated by induction of the complements cascade initiated by binding to C1q complement component. Since LAG-3 is predominantly expressed on activated T cells and PD-L1 is expressed on these but also on tumour cells at high levels, the ability of mAb^2 FS18-7-9/84G09 (SEQ ID NOs 94 and 116) to induce ADCC and CDC was investigated.

Specifically, it was tested whether FS18-7-9/84G09 treatment of LAG-3 or PD-L1 expressing cells, followed by incubation with either NK cells or complement, induces lysis of the respective target cells. In addition, since FS18-7-9/84G09 is a bispecific antibody, it was also tested whether target engagement on one of the specificities affects effector function towards cells expressing the target for the other specificity.

Understanding the effector functions of mAb^2 FS18-7-9/84G09 is useful for a number of reasons, including determining whether a mutation which reduces effector functions, such as the LALA mutation, should be included in the molecule to protect LAG-3 expressing effector T cells engaged in tumour killing from FS18-7-9/84G09-mediated ADCC and/or CDC.

6.1 Study design

Raji cells recombinantly expressing LAG-3 or PD-L1 were used for all assays, using their endogenous expression of CD20 as a control for targeting with a generic version of Rituximab to demonstrate functional activity of the added complement and NK cell preparations independent of the recombinant expression of the target proteins. Target expression was confirmed prior to these experiments.

To determine the basic CDC activity towards LAG-3 or PD-L1 expressing cells, this activity was measured using LDH release, measured by conversion of a substrate into a fluorescent dye (CytoTox-ONE™ by Promega). To measure which target cells in a cell mixture comprising both LAG-3 expressing cells and PD-L1 expressing cells were being lysed, differential CDC was measured by flow cytometry of differentially fluorescently labelled target cells after incubation with the mAb^2/antibody, detecting dead cells using a fluorescent dye excluded from live cells.

In order to determine ADCC activity towards LAG-3 or PD-L1 expressing cells, this activity was measured using NK cells isolated from frozen PBMC and LDH release measured colourimetrically (CytoTox 96 by Promega). For all these studies, Rituximab in various isotypes and Fc configurations was used as a control. No reliable method for measuring ADCC differentially is known, so differential ADCC activity was not measured.

In all experiments, the PD-L1 specific mAb (84G09) and the LAG-3 specific mAb (25F7) were used as controls. An IgG isotype control (4420) which was either used as negative control or to generate the CDC background activity was also used. The LALA versions of the respective antibodies and mAb^2 (excluding 25F7) were also compared to the IgG1 wild-type versions in the CDC and ADCC assays to determine the effect of this mutation on these effector functions.

6.2 Materials and Methods

6.2.1 CDC assays

All antibodies/mAb^2, including Rituximab, were diluted in 10 point 1 in 2 serial dilutions. Control wells containing the IgG (4420 LALA) at the highest concentration used were also prepared. Cell suspensions of Raji cells recombinantly expressing LAG-3 or PD-L1 respectively, were prepared in serum free medium for the LDH release assay and added to an equal volume of the prepared antibodies/mAb^2.

For the Flow Cytometry based CDC assay, cell suspensions of 5×10^7 cells were prepared and resuspended in either 0.5 µM CellTracker deep red (CellTracker™ Deep Red, Thermo Fisher, #C34565) or 5 µM for CellTracker Green (CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate, Thermo Fisher, # C7025) in serum free medium. After a 30 min incubation at 37°C, cells were washed in serum free medium and either added to the

prepared antibody/mAb^2-containing wells directly, or combined with the other differentially stained cell line at equal volumes and then added to the antibody/mAb^2-containing wells as described above. For both assays, after a 30 min incubation under cell culture conditions, wells were topped up with an equal volume of baby rabbit complement, 10% in serum free medium (Baby Rabbit Complement,TEBU-bio, #CL3441). Plates were incubated for 4 hrs in cell culture conditions. For the LDH release CDC assay, 100% lysis controls were generated by adding Triton X 100 to half of the 4420 LALA treated wells and the Cytotox assay was performed according to manufacturer's instructions (CytotoxOne,Promega, G7891). After obtaining the reads, the signal from the 100% lysis controls was set to 100% and the signals from the sample wells were calculated as a percentage of that level.

For the Flow Cytometry based CDC assay, at the end of the incubation period, the dead cell dye (SYTOX® Blue Dead Cell Stain, Thermo Fisher, #S34857) was diluted 1 in 500 in PBS and wells were topped up with an equal volume. Flow Cytometry was performed on a Cytoflex flow cytometer gating on the intact cell populations based on FSC and SSC and detecting the percentage of Sytox positive cells (channel PB450) of both the CellTracker™ Deep Red positive and the CellTracker™ Green CMFDA positive cell populations.

6.3.2 ADCC assay

ADCC was measured as described previously (Broussas, Matthieu; Broyer, Lucile; and Goetsch, Liliane. (2013) Evaluation of Antibody-Dependent Cell Cytotoxicity Using Lactate Dehydrogenase (LDH) Measurement in *Glycosylation Engineering of Biopharmaceuticals: Methods and Protocols,* Methods in Molecular Biology. New York: Springer Science + Business Media. Volume 988, pp 305-317) . Briefly, target cells were pre-incubated with the antibodies before adding primary NK cells isolated from human PBMCs (NK cell isolation kit, Miltenyi Biotec, 130-092-657) at a ratio of 20 to 1 for 4 hrs. The cytotoxicity assay was performed following manufacturer's instructions (CytoTox 96 Non-Radioactive Cytotoxicity Assay, Promega, G1780). % lysis was calculated based on 100% target cell lysis, taking into account spontaneous lysis of effector and target cells.

6.3 Results and conclusions

6.3.1 CDC assay

PD-L1 expressing Raji cells were targeted by the anti-CD20 antibody Rituximab, which resulted in a maximum lysis of <60% when measuring CDC by generic LDH release. Anti-PD-L1 antibody 84G09 (which comprises the $F(ab)_2$ portion of mAb^2 FS18-7-9/84G09), and FS18-7-9/84G09 in IgG1 format, showed higher maximum lysis and also higher lysis potency, with estimated half maximal doses of about half of that required by Rituximab in IgG1 format. This shows that the introduction of the LAG-3 binding site into the 84G09 antibody did not change its PD-L1 targeting activity with respect to potency or max response, as both were very similar when 84G09 and FS18-7-9/84G09 were compared. The introduction of the LALA mutation resulted in a reduced max response for Rituximab, 84G09 and FS18-7-9/84G09, however, the potency was only reduced for 84G09 and FS18-7-9/84G09. As expected, the anti-LAG-3 antibody 25F7 had no effect on cell viability of the PD-L1 expressing Raji cells, as these cells did not express human LAG-3. These results are shown in **Figure 14A**.

LAG-3 expressing Raji cells were targeted for CDC by the anti-CD20 antibody Rituximab, however, the LAG-3 antibody 25F7 showed even better potency with an estimated half maximal dose of about half of that required for Rituximab. None of the other antibodies showed any CDC activity against LAG-3 expressing Raji cells, including FS18-7-9/84G09. The introduction of the LALA mutation had very limited effect on the CDC activity of Rituximab (**Figure 14B**).

6.3.2 Differential CDC assay

A differential CDC assay, employing flow cytometry, was developed by the inventors to distinguish which target expressing cells were lysed when treated with FS18-7-9/84G09 or control antibodies. This assay was used to confirm the results from the basic LDH release CDC assay described above. Compared to the IgG isotype control antibody (4420), which had no effect on the percentage of live cells, Rituximab mediated a reduction of live cells and an increase of dead cells, both of PD-L1 and LAG-3 expressing cells. However, FS18-7-9/84G09 had no effect on LAG-3 expressing cells, but very efficiently lysed PD-L1 expressing cells. Similarly, the mixture of the LAG-3 specific antibody 25F7 and the PD-L1 antibody 84G09 also showed a dose-dependent decrease in live cells and a reciprocal increase in dead cells, however, the maximal lysis of LAG-3 expressing cells was only just

over 50% of cells, but was reached already at a concentration around 1 nM, the lowest dose to achieve maximal lysis of all antibodies tested. This confirms the previous finding that the LAG-3 binding site in the CH3 domain of FS18-7-9/84G09 does not induce CDC mediated lysis of LAG-3 expressing target cells. In addition, this experiment shows that the presence of LAG-3 expressing cells has no effect on the CDC activity of FS18-7-9/84G09 towards PD-L1 expressing cells. The results are shown in **Figure 15**.

6.3.3 ADCC assay

PD-L1 expressing Raji cells were targeted for ADCC by the anti-CD20 antibody Rituximab, FS18-7-9/84G09, and 84G09 with very similar efficacy and potency, resulting in a maximum lysis of around 40% of cells. Rituximab and 84G09 containing the LALA mutation showed no ADCC-mediated lysis and FS18-7-9/84G09 containing the LALA mutation showed no or very low ADCC-meditated lysis of PD-L1 expressing target cells. The LAG-3 specific antibody 25F7 and the isotype control 4420 with and without the LALA mutation showed no activity in this assay.

These results show that the introduction of the LAG-3 binding site into antibody 84G09 did not change its PD-L1 targeted ADCC activity in potency or max response, since both were very similar to the PD-L1 specific antibody 84G09. The introduction of the LALA mutation resulted in abrogation of ADCC activity (**Figure 16A**).

LAG-3 expressing Raji cells were targeted by Rituximab and 25F7 for ADCC-mediated lysis, resulting in maximum lysis of about 40%. FS18-7-9/84G09 also mediated lysis of LAG-3 expressing cells by ADCC, be it with a much lower potency and efficacy, only reaching just under 20% lysis at 2.5 nM concentration. The introduction of the LALA mutation abrogated all ADCC activity of Rituximab and FS18-7-9/84G09. 84G09 and the isotype control 4420 with and without the LALA mutation showed no ADCC activity in this assay as expected, as these antibodies do not bind LAG-3 (**Figure 16B**).

Sequence listing

Amino acid sequences of Fcab FS18-7-9 loop regions

FS18-7-9 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-9 CD loop – SNGQPENNY (SEQ ID NO: 2)

FS18-7-9 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-9 CH3 domain (SEQ ID NO: 4)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC
TTCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

CHO codon optimised nucleotide sequence of Fcab FS18-7-9 CH3 domain (SEQ ID NO: 142)

GGCCAGCCCCGGGAACCCCAGGTGTACACACTGCCTCCATCCTGGGATGAGCCCTGGGGCGA
GGATGTGTCTCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAG
AGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACAGCAAGCTGACAGTGCCCTACGACAGATGGGTGTGGCCCGACGAGTTCTCCT
GCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCCG
GC

Amino acid sequence of Fcab FS18-7-9 CH3 domain (SEQ ID NO: 5)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-9 CH3 domain comprising C-terminal lysine (SEQ ID NO: 135)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPGK

Amino acid sequence of Fcab FS18-7-9 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 6)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-9 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 7)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-32 loop regions

FS18-7-32 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-32 CD loop – SNGQPENNY (SEQ ID NO: 8)

FS18-7-32 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-32 CH3 domain (SEQ ID NO: 9)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAAATCGCCGTGGAGTGGGAG
AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC
TTCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-32 CH3 domain (SEQ ID NO: 10)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSEIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-32 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 11)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSEIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-32 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 12)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSEIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-33 loop regions

FS18-7-33 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-33 CD loop – SNGQPEDNY (SEQ ID NO: 13)

FS18-7-33 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-33 CH3 domain (SEQ ID NO: 14)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGCAGCCGGAGGACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC
TTCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-33 CH3 domain (SEQ ID NO: 15)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGQPEDNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-33 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 16)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGQPEDNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-33 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 17)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGQPEDNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-36 loop regions

FS18-7-36 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-36 CD loop – SNGQPENNY (SEQ ID NO: 18)

FS18-7-36 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-36 CH3 domain (SEQ ID NO: 19)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC
TACTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCAT
GCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGG
T

Amino acid sequence of Fcab FS18-7-36 CH3 domain (SEQ ID NO: 20)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSYFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of CH2+CH3 of Fcab FS18-7-36 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 21)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSYFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-36 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 22)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSYFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-58 loop regions

FS18-7-58 AB loop – WDEPWGED (SEQ ID NO: 1)
FS18-7-58 CD loop – SNGYPEIEF (SEQ ID NO: 23)
FS18-7-58 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-58 CH3 domain (SEQ ID NO: 24)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGTATCCAGAAATCGAATTCAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGCCTTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-58 CH3 domain (SEQ ID NO: 25)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGYPEIEFKTTPPVLDSDGSFFLY
SKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-58 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO:26)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGYPEIEFKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMH
EALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-58 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 27)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGYPEIEFKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHEA
LHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-62 loop regions

FS18-7-62 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-62 CD loop – SNGIPEWNY (SEQ ID NO: 28)

FS18-7-62 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-62 CH3 domain (SEQ ID NO: 29)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGATCCCAGAATGGAACTATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-62 CH3 domain (SEQ ID NO: 30)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGIPEWNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-62 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 31)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGIPEWNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMH
EALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-62 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 32)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGIPEWNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHEA
LHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-65 loop regions

FS18-7-65 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-65 CD loop – SNGYAEYNY (SEQ ID NO: 33)

FS18-7-65 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-65 CH3 domain (SEQ ID NO: 34)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGTATGCAGAATATAACTATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-65 CH3 domain (SEQ ID NO: 35)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGYAEYNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-65 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 36)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGYAEYNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-65 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 37)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGYAEYNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-78 loop regions

FS18-7-78 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-78 CD loop – SNGYKEENY (SEQ ID NO: 38)

FS18-7-78 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-78 CH3 domain (SEQ ID NO: 39)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGTATAAAGAAGAAAACTATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-78 CH3 domain (SEQ ID NO: 40)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGYKEENYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-78 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 41)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGYKEENYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-78 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 42)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGYKEENYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-88 loop regions

FS18-7-88 AB loop – WDEPWGED (SEQ ID NO: 1)
FS18-7-88 CD loop – SNGVPELNV (SEQ ID NO: 43)
FS18-7-88 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-88 CH3 domain (SEQ ID NO: 44)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGGTTCCAGAACTGAACGTTAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-88 CH3 domain (SEQ ID NO: 45)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGVPELNVKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-88 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 46)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGVPELNVKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMH
EALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-88 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 47)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGVPELNVKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHEA
LHNHYTQKSLSLSPG

Amino acid sequences of Fcab FS18-7-95 loop regions

FS18-7-95 AB loop – WDEPWGED (SEQ ID NO: 1)

FS18-7-95 CD loop – SNGYQEDNY (SEQ ID NO: 48)

FS18-7-95 EF loop – PYDRWVWPDE (SEQ ID NO: 3)

Nucleotide sequence of Fcab FS18-7-95 CH3 domain (SEQ ID NO: 49)

GGCCAGCCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCTGGGATGAGCCGTGGGGTGAA
GACGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGTATCAGGAAGATAACTATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGCCGTATGATAGGTGGGTTTGGCCGGATGAGTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGT

Amino acid sequence of Fcab FS18-7-95 CH3 domain (SEQ ID NO: 50)

GQPREPQVYTLPPSWDEPWGEDVSLTCLVKGFYPSDIAVEWESNGYQEDNYKTTPPVLDSDGSFFL
YSKLTVPYDRWVWPDEFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-95 CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 51)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGED
VSLTCLVKGFYPSDIAVEWESNGYQEDNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVM
HEALHNHYTQKSLSLSPG

Amino acid sequence of Fcab FS18-7-95 CH2 and CH3 domains without LALA mutation (SEQ ID NO: 52)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSWDEPWGEDVS
LTCLVKGFYPSDIAVEWESNGYQEDNYKTTPPVLDSDGSFFLYSKLTVPYDRWVWPDEFSCSVMHE
ALHNHYTQKSLSLSPG

Amino acid sequence of the wild-type human IgG1 CH2 domain (SEQ ID NO: 53)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

Amino acid sequence of the human IgG1 CH2 domain comprising the “LALA mutation” (underlined) (SEQ ID NO: 54)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

Amino acid sequence of “wild-type” Fcab CH2 and CH3 domains without LALA mutation (SEQ ID NO: 55).

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPG

Amino acid sequence of “wild-type” Fcab CH2 and CH3 domains, comprising LALA mutation (underlined) (SEQ ID NO: 56)

APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG

Amino acid sequence of the human IgG1 hinge region (SEQ ID NO: 57)

EPKSCDKTHTCPPCP

Amino acid sequence of the human IgG1 truncated hinge region (SEQ ID NO: 58)

TCPPCP

Amino acid sequence anti-mouse LAG-3 Fcab FS18-7-108-29, comprising LALA mutation (underlined) (SEQ ID NO: 59)

The CH3 domain is shown in italics. The AB, CD and EF loops of the CH3 domain are shown in bold and underlined.

TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK*GQPREPQVYTLPPS**WDEP**
WGEDVSLTCLVKGFYPSDIVVEWE**SNGQPENNY**KTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FS
CSVMHEALHNHYTQKSLSLSPG*

Amino acid sequence of the anti-mouse LAG-3 Fcab FS18-7-108-29 without LALA mutation (SEQ ID NO: 60)

The CH3 domain is shown in italics. The AB, CD and EF loops of the CH3 domain are shown in bold and underlined.

TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK*GQPREPQVYTLPPS**WDEP**
WGED*VSLTCLVKGFYPSDIVVEWE**SNGQPENNY**KTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FS*
CSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-mouse LAG-3 Fcab FS18-7-108-35, comprising LALA mutation (underlined) (SEQ ID NO: 61)

The CH3 domain is shown in italics. The AB, CD and EF loop regions are shown in bold and underlined.

TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK*GQPREPQVYTLPPS**WDEP**
WGED*VSLTCLVKGFYPSDISVEWE**SNGQPENNY**KTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FS*
CSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-mouse LAG-3 Fcab FS18-7-108-35 without LALA mutation (SEQ ID NO: 62)

The CH3 domain is shown in italics. The AB, CD and EF loop regions are shown in bold and underlined.

TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK*GQPREPQVYTLPPS**WDEP**
WGED*VSLTCLVKGFYPSDISVEWE**SNGQPENNY**KTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FS*
CSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-9/4420 comprising LALA mutation (SEQ ID NO: 63)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-9/4420 without LALA mutation (SEQ ID NO: 64)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-32/4420 comprising LALA mutation (SEQ ID NO: 65)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSEIAVEWE**SNGQPENNY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-32/without LALA mutation (SEQ ID NO: 66)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSEIAVEWE**SNGQPENNY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-33/4420 comprising LALA mutation (SEQ ID NO: 67)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV

DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPEDNY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-33/4420 without LALA mutation (SEQ ID NO: 68)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPEDNY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-36/4420 comprising LALA mutation (SEQ ID NO: 69)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTTPP
VLDSDGSYFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-36/4420 without LALA mutation (SEQ ID NO: 70)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTTPPVL
DSDGSYFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-58/4420 comprising LALA mutation (SEQ ID NO: 71)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYPEIEF**KTTPPV
LDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-58/4420 without LALA mutation (SEQ ID NO: 72)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYPEIEF**KTTPPVLD
SDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-62/4420 comprising LALA mutation (SEQ ID NO: 73)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGIPEWNY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-62/4420 without LALA mutation (SEQ ID NO: 74)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGIPEWNY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-65/4420 comprising LALA mutation (SEQ ID NO: 75)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYAEYNY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-65/4420 without LALA mutation (SEQ ID NO: 76)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYAEYNY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-78/4420 comprising LALA mutation (SEQ ID NO: 77)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYKEENY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-78/4420 without LALA mutation (SEQ ID NO: 78)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYKEENY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-88/4420 comprising LALA mutation (SEQ ID NO: 79)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGVPELNV**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-88/4420 without LALA mutation (SEQ ID NO: 80)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGVPELNV**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-95/4420 comprising LALA mutation (SEQ ID NO: 81)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYQEDNY**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-human LAG-3/FITC mAb^2 FS18-7-95/4420 without LALA mutation (SEQ ID NO: 82)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYQEDNY**KTTPPVL
DSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-FITC mAb 4420 comprising LALA mutation (SEQ ID NO: 83)

Position of the CDRs are underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-FITC mAb 4420 without LALA mutation (SEQ ID NO: 84)

Position of the CDRs are underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSDS
VKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-FITC mAb 4420 light chain (SEQ ID NO: 85)

Position of the CDRs are underlined.

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLRWYLQKPGQSPKVLIYKVSNRFSGVPDRF
SGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Amino acid sequences of the CDRs of anti-PD-L1 antibody 84G09 (according to IMGT)

HCDR1	GFTFDDYA	(SEQ ID NO: 86)
HCDR2	ISWKSNII	(SEQ ID NO: 87)
HCDR3	ARDITGSGSYGWFDP	(SEQ ID NO: 88)
LCDR1	QSISSY	(SEQ ID NO: 89)
LCDR2	VAS	(SEQ ID NO: 90)
LCDR3	QQSYSNPIT	(SEQ ID NO: 91)

Amino acid sequences of the CDRs of anti-PD-L1 antibody 84G09 (according to Kabat)

HCDR1	DYAMH	(SEQ ID NO: 136)
HCDR2	GISWKSNIIGYADSVKG	(SEQ ID NO: 137)
HCDR3	DITGSGSYGWFDP	(SEQ ID NO: 138)
LCDR1	RASQSISSYLN	(SEQ ID NO: 139)
LCDR2	VASSLQS	(SEQ ID NO: 140)

LCDR3 QQSYSNPIT (SEQ ID NO: 141)

Amino acid sequence of the anti-PD-L1 antibody 84G09 VH domain (SEQ ID NO: 92)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSS

Amino acid sequence of the anti-PD-L1 antibody 84G09 VL domain (SEQ ID NO: 93)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKPLIYVASSLQSGVPSSFSGSGS
GTDFTLTISSLQPEDFATYYCQQSYSNPITFGQGTRLEIK

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-9/84G09 with LALA mutation heavy chain (SEQ ID NO: 94)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-9/84G09 heavy chain (SEQ ID NO: 95)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-32/84G09 with LALA mutation heavy chain (SEQ ID NO: 96)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL

APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSEIAVEWE**SNGQPENNY**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-32/84G09 heavy chain (SEQ ID NO: 97)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSEIAVEWE**SNGQPENNY**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-33/84G09 with LALA mutation heavy chain (SEQ ID NO: 98)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPEDNY**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-33/84G09 heavy chain (SEQ ID NO: 99)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL

PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPEDNY**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-36/84G09 with LALA mutation heavy chain (SEQ ID NO: 100)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTT
PPVLDSDGSYFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-36/84G09 heavy chain (SEQ ID NO: 101)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGQPENNY**KTTP
PVLDSDGSYFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-58/84G09 with LALA mutation heavy chain (SEQ ID NO: 102)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYPEIEF**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-58/84G09 heavy chain (SEQ ID NO: 103)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYPEIEF**KTTPP
VLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-62/84G09 with LALA mutation heavy chain (SEQ ID NO: 104)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGIPEWNY**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-62/84G09 heavy chain (SEQ ID NO: 105)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGIPEWNY**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-65/84G09 with LALA mutation heavy chain (SEQ ID NO: 106)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYAEYNY**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-65/84G09 heavy chain (SEQ ID NO: 107)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYAEYNY**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-78/84G09 with LALA mutation heavy chain (SEQ ID NO: 108)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYKEENY**KTT
PPVLDSDGSFFLYSKLTV***PYDRWVWPDE***FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-78/84G09 heavy chain (SEQ ID NO: 109)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV

VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYKEENY**KTTP
PVLDSDGSFFLYSKLTV***PYDRWVWPDE***FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-88/84G09 with LALA mutation heavy chain (SEQ ID NO: 110)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGVPELNV**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-88/84G09 heavy chain (SEQ ID NO: 111)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGVPELNV**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb^2 FS18-7-95/84G09 with LALA mutation heavy chain (SEQ ID NO: 112)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYQEDNY**KTT
PPVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human LAG-3/PD-L1 mAb[2] FS18-7-95/84G09 heavy chain (SEQ ID NO: 113)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIAVEWE**SNGYQEDNY**KTTP
PVLDSDGSFFLYSKLTV**PYDRWVWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human PD-L1 mAb 84G09 with LALA heavy chain (SEQ ID NO: 114)

Position of the CDRs are underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human PD-L1 mAb 84G09 heavy chain (SEQ ID NO: 115)

Position of the CDRs are underlined.

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQTPGKGLEWVSGISWKSNIIGYADSVKG
RFTISRDNAKNSLYLQMNSLRAEDTALYYCARDITGSGSYGWFDPWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-human PD-L1 mAb 84G09 light chain (SEQ ID NO:116)

Position of the CDRs are underlined.

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKPLIYVASSLQSGVPSSFSGSGS
GTDFTLTISSLQPEDFATYYCQQSYSNPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC

Amino acid sequence of the anti-mouse LAG-3/PD-L1 mAb^2 FS18-7-108-29/S1 with LALA mutation heavy chain (SEQ ID NO:117)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIVVEWE**SNGQPENNY**KTTPP
VLDSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-mouse LAG-3/PD-L1 mAb^2 FS18-7-108-29/S1 heavy chain (SEQ ID NO:118)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIVVEWE**SNGQPENNY**KTTPPVL
DSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-mouse PD-L1 mAb S1 light chain (SEQ ID NO:119)

Position of the CDRs are underlined.

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQYLFTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC

Amino acid sequence of the anti-mouse LAG-3/PD-L1 mAb^2 FS18-7-108-35/S1 with LALA mutation heavy chain (SEQ ID NO:120)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP

APIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDISVEWE**SNGQPENNY**KTTPP
VLDSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the anti-mouse LAG-3/PD-L1 mAb[2] FS18-7-108-35/S1 heavy chain (SEQ ID NO:121)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDISVEWE**SNGQPENNY**KTTPPVL
DSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the control PD-L1 mAb S1 with LALA mutation heavy chain (SEQ ID NO:122)

Position of the CDRs are underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the control PD-L1 mAb S1 heavy chain (SEQ ID NO:123)

Position of the CDRs are underlined. Position of LALA mutation is in bold.

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the control anti-human LAG-3 mAb 25F7 heavy chain (SEQ ID NO:124)

Position of the CDRs are underlined.

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEINHRGSTNSNPSLKSR
VTLSLDTSKNQFSLKLRSVTAADTAVYYCAFGYSDYEYNWFDPWGQGTLVTVSSASTKGPSVFPLAP

SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the control anti-human LAG-3 mAb 25F7 light chain (SEQ ID NO:125)
Position of the CDRs are underlined.
EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSG
TDFTLTISSLEPEDFAVYYCQQRSNWPLTFGQGTNLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGEC

Amino acid sequence of human LAG-3 (SEQ ID NO: 126)
MWEAQFLGLLFLQPLWVAPVKPLQPGAEVPVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVTWQHQ
PDSGPPAAAPGHPLAPGPHPAAPSSWGPRPRRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRGD
FSLWLRPARRADAGEYRAAVHLRDRALSCRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRP
ASVHWFRNRGQGRVPVRESPHHHLAESFLFLPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLGLE
PPTPLTVYAGAGSRVGLPCRLPAGVGTRSFLTAKWTPPGGGPDLLVTGDNGDFTLRLEDVSQAQAG
TYTCHIHLQEQQLNATVTLAIITVTPKSFGSPGSLGKLLCEVTPVSGQERFVWSSLDTPSQRSFSGPW
LEAQEAQLLSQPWQCQLYQGERLLGAAVYFTELSSPGAQRSGRAPGALPAGHLLLFLILGVLSLLLLV
TGAFGFHLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEPEPEPEPEPEPEQL

Amino acid sequence of mouse LAG-3 (SEQ ID NO: 127)
MREDLLLGFLLLGLLWEAPVVSSGPGKELPVVWAQEGAPVHLPCSLKSPNLDPNFLRRGGVIWQHQ
PDSGQPTPIPALDLHQGMPSPRQPAPGRYTVLSVAPGGLRSGRQPLHPHVQLEERGLQRGDFSLWL
RPALRTDAGEYHATVRLPNRALSCSLRLRVGQASMIASPSGVLKLSDWVLLNCSFSRPDRPVSVHW
FQGQNRVPVYNSPRHFLAETFLLLPQVSPLDSGTWGCVLTYRDGFNVSITYNLKVLGLEPVAPLTVYA
AEGSRVELPCHLPPGVGTPSLLIAKWTPPGGGPELPVAGKSGNFTLHLEAVGLAQAGTYTCSIHLQG
QQLNATVTLAVITVTPKSFGLPGSRGKLLCEVTPASGKERFVWRPLNNLSRSCPGPVLEIQEARLLAE
RWQCQLYEGQRLLGATVYAAESSSGAHSARRISGDLKGGHLVLVLILGALSLFLLVAGAFGFHWWRK
QLLLRRFSALEHGIQPFPAQRKIEELERELETEMGQEPEPEPEPQLEPEPRQL

Amino acid sequence of cynomolgus LAG-3 (SEQ ID NO: 128)
MWEAQFLGLLFLQPLWVAPVKPPQPGAEISVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVTWQHQ
PDSGPPAAAPGHPPVPGHRPAAPYSWGPRPRRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRG
DFSLWLRPARRADAGEYRATVHLRDRALSCRLRLRVGQASMTASPPGSLRTSDWVILNCSFSRPDR
PASVHWFRSRGQGRVPVQGSPHHHLAESFLFLPHVGPMDSGLWGCILTYRDGFNVSIMYNLTVLGL
EPATPLTVYAGAGSRVELPCRLPPAVGTQSFLTAKWAPPGGGPDLLVAGDNGDFTLRLEDVSQAQA
GTYICHIRLQGQQLNATVTLAIITVTPKSFGSPGSLGKLLCEVTPASGQEHFVWSPLNTPSQRSFSGP

WLEAQEAQLLSQPWQCQLHQGERLLGAAVYFTELSSPGAQRSGRAPGALRAGHLPLFLILGVLFLLL
LVTGAFGFHLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPELEPEPELERELGPEPEPGPEPEP
EQL

Amino acid sequence of human PD-L1 (SEQ ID NO: 129)
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFV
HGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPY
NKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTT
TNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKK
CGIQDTNSKKQSDTHLEET

Amino acid sequence of murine PD-L1 (SEQ ID NO: 130)
MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFV
AGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYR
KINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSLRVNATA
NDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVE
KCGVEDTSSKNRNDTQFEET

Amino acid sequence of cynomolgus PD-L1 (SEQ ID NO: 131)
MRIFAVFIFTIYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLTSLIVYWEMEDKNIIQFVH
GEEDLKVQHSNYRQRAQLLKDQLSLGNAALRITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYN
KINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLLNVTSTLRINTTA
NEIFYCIFRRLDPEENHTAELVIPELPLALPPNERTHLVILGAIFLLLGVALTFIFYLRKGRMMDMKKCG
IRVTNSKKQRDTQLEET

Amino acid sequence of the heavy chain of anti-mouse LAG-3/FITC mAb[2] FS18-7-108-29/4420 comprising LALA mutation (SEQ ID NO: 132)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSD
SVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIVVEWE**SNGQPENN**
YKTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-mouse LAG-3/FITC mAb^2 FS18-7-108-35/4420 comprising LALA mutation (SEQ ID NO: 133)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined. Position of LALA mutation is in bold.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSD
SVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDISVEWE**SNGQPENN**
YKTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequence of the heavy chain of anti-mouse LAG-3/FITC mAb^2 FS18-7-108-29/4420 (SEQ ID NO: 134)

Position of the CDRs are underlined, and the AB, CD, and EF loop sequences are in bold and underlined.

EVKLDETGGGLVQPGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYETYYSD
SVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPS**WDEPWGED**VSLTCLVKGFYPSDIVVEWE**SNGQPENNY**
KTTPPVLDSDGSFFLYSKLTV**PFERWMWPDE**FSCSVMHEALHNHYTQKSLSLSPG

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Claims

1. An antibody molecule which binds to programmed death-ligand 1 (PD-L1) and lymphocyte-activation gene 3 (LAG-3), wherein the antibody molecule comprises:

(i) a complementarity determining region (CDR)-based antigen binding site for PD-L1 comprising the CDRs set forth in SEQ ID NOs 86 to 91; and
(ii) a LAG-3 antigen binding site located in a CH3 domain of the antibody molecule, wherein the LAG-3 binding site comprises the amino acid sequences WDEPWGED (SEQ ID NO: 1) and PYDRWVWPDE (SEQ ID NO: 3), and wherein the amino acid sequence WDEPWGED is located in the AB loop of the CH3 domain and the amino acid sequence PYDRWVWPDE is located in the EF loop of the CH3 domain.

2. An antibody molecule according to claim 1, wherein the LAG-3 antigen binding site further comprises one of the following sequences in the CD loop of the CH3 domain:

(i) SNGQPENNY (SEQ ID NOS 2, 8 and 18);
(ii) SNGQPEDNY (SEQ ID NO: 13);
(iii) SNGYPEIEF (SEQ ID NO: 23);
(iv) SNGIPEWNY (SEQ ID NO: 28);
(v) SNGYAEYNY (SEQ ID NO: 33);
(vi) SNGYKEENY (SEQ ID NO: 38);
(vii) SNGVPELNV (SEQ ID NO: 43); or
(viii) SNGYQEDNY (SEQ ID NO: 48);

preferably wherein the LAG-3 antigen binding site comprises the amino acid sequence set forth in SEQ ID NO: 2, 28, or 38,
preferably wherein the LAG-3 antigen binding site comprises the amino acid sequence set forth in SEQ ID NO: 2.

3. An antibody molecule according to claim 1 or 2, wherein the antibody molecule is an immunoglobulin G (IgG) molecule, preferably an IgG1, IgG2, IgG3, or IgG4 molecule, preferably an IgG1 molecule.

4. An antibody molecule according to any one of claims 1 to 3, wherein the antibody molecule comprises the CH3 domain set forth in SEQ ID NO: 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50, preferably SEQ ID NO: 5, 30, or 40, preferably SEQ ID NO: 5.

5. An antibody molecule according to any one of claims 1 to 4, wherein the antibody molecule comprises a CH2 domain and the CH2 domain has the sequence set forth in SEQ ID NO: 53 or SEQ ID NO: 54.

6. An antibody molecule according to any one of claims 1 to 5, wherein the antibody molecule comprises the sequence set forth in SEQ ID NO: 6, 7, 11, 12, 16, 17, 21, 22, 26, 27, 31, 32, 36, 37, 41, 42, 46, 47, 51, or 52, preferably SEQ ID NO: 6, 7, 31, 32, 41, or 42, preferably SEQ ID NO: 6 or 7.

7. An antibody molecule according to any one of claims 1 to 6, wherein the antibody molecule comprises the VH and/or VL domains set forth in SEQ ID NOs 92 and 93.

8. An antibody molecule according to any one of claims 1-7, wherein the antibody molecule comprises the heavy chain sequence set forth in any one of SEQ ID NOs: 94 to 113, preferably SEQ ID NO: 94, 95, 104, 105, 108, or 109, preferably SEQ ID NO: 94 or 95.

9. An antibody molecule according to claim 7 or 8, wherein the antibody molecule comprises the light chain sequence set forth in SEQ ID NO: 116.

10. An antibody molecule according to claim 9, wherein the antibody molecule comprises the heavy chain sequence set forth in SEQ ID NO: 94 and the light chain sequence set forth in SEQ ID NO: 116.

11. An antibody molecule according to any one of claims 1 to 10, wherein the antibody molecule is conjugated to an immune system modulator, cytotoxic molecule, radioisotope, or detectable label, optionally wherein the immune system modulator or cytotoxic molecule is a cytokine.

12. A nucleic acid encoding an antibody molecule according to any one of claims 1 to 11, or a vector comprising a nucleic acid encoding a antibody molecule according to any one of claims 1 to 11.

13. A recombinant host cell comprising the nucleic acid or vector of claim 12.

14. A method of producing an antibody molecule according to any one of claims 1 to 11, comprising culturing the recombinant host cell of claim 13 under conditions for production of

the antibody molecule, wherein the method optionally further comprises isolating and/or purifying the antibody molecule.

15. A pharmaceutical composition comprising an antibody molecule according to any one of claims 1 to 11 and a pharmaceutically acceptable excipient.

16. An antibody molecule according to any one of claims 1 to 11, for use in a method of treating cancer in a patient, optionally wherein the method further comprises administering to the patient:

(a) an anti-tumour vaccine; or
(b) a chemotherapeutic agent.