(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 October 2020 (08.10.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/205875 A1

(51) International Patent Classification:
C07K 16/24 (2006.01) *C07K 16/28* (2006.01)

(21) International Application Number:
PCT/US2020/025979

(22) International Filing Date:
31 March 2020 (31.03.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/827,381 01 April 2019 (01.04.2019) US

(71) Applicant: **IMMETAS THERAPEUTICS, INC.** [US/US]; 120 Eagle Rock Avenue, Suite 141, East Hanover, NJ 07936 (US).

(72) Inventors: **WANG, Jin**; C/o Immetas Therapeutics, Inc., 120 Eagle Rock Avenue, Suite 141, East Hanover, NJ 07936 (US). **RAINEY, Godfrey, Jonah**; C/o Immetas Therapeutics, Inc., 120 Eagle Rock Avenue, Suite 141, East Hanover, NJ 07936 (US).

(74) Agent: **BOOTH, Paul, M.** et al.; Edell, Shapiro & Finnan, LLC, 9801 Washingtonian Blvd, Suite 750, Gaithersburg, MD 20878 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: BISPECIFIC BINDING MOLECULES THAT TARGET THE TUMOR MICROENVIRONMENT AND AN IMMUNE CHECKPOINT PROTEIN

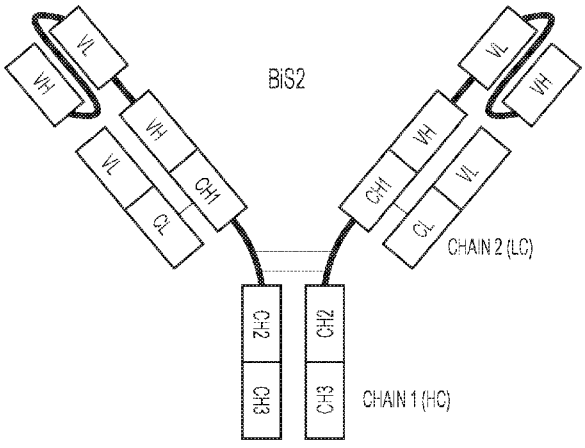

FIG.1

(57) Abstract: Bispecific binding proteins that bind IP-1β or IL-1R and a checkpoint protein are provided, together with methods of making the proteins. The checkpoint protein may be PD-1 or PD-L1. The binding proteins may have immunoglobulin-like structures that contain human Fab or scFv domains. A novel bispecific antibody format is provided. Methods of making the binding proteins are provided, together with pharmaceutical compositions containing the proteins. The binding proteins and pharmaceutical compositions may be used for treating or preventing diseases such as cancer.

[Continued on next page]

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UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

Bispecific Binding Molecules That Target the Tumor Microenvironment and an Immune Checkpoint Protein

Field of the invention

Bispecific binding molecules are provided that are useful for treating cancer and other diseases.

Background

Immune checkpoints proteins act as immune system regulators and are a key part of the mechanism of self-tolerance. Inhibitory immune checkpoint proteins include: Programmed cell death protein 1 (PD-1); programmed cell death ligand 1 (PD-L1); Adenosine A2A receptor (A2AR); B7-H3 (CD276); B7-H4 (VTCN1), B and T Lymphocyte Attenuator (BTLA); Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4, CD152); Indoleamine 2,3-dioxygenase (IDO); Killer-cell Immunoglobulin-like Receptor (KIR); Lymphocyte Activation Gene-3 (LAG3); Nicotinamide adenine dinucleotide phosphate NADPH oxidase isoform 2 (NOX2); T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3); V-domain Ig suppressor of T cell activation (VISTA); Sialic acid-binding immunoglobulin-type lectin 7 (SIGLEC7, CD328); and Sialic acid-binding immunoglobulin-type lectin 9 (SIGLEC9, CD329).

Programmed cell death protein 1 (PD-1) is a receptor protein expressed on T-cells that acts as an immune checkpoint. PD-1 expression is upregulated on activated T cells as part of the mechanism of immune tolerance. The ligand for PD-1 is programmed cell death ligand 1 (PD-L1), and binding of PD-L1 to PD-1 transmits an inhibitory signal that reduces the activation and proliferation of antigen-specific T-cells in lymph nodes, and reduces apoptosis in regulatory T cells. Tumor cells often overexpress PD-L1 as a mechanism for avoiding immune surveillance. Monoclonal antibodies that inhibit binding between PD-1 and PD-L1 - by binding either the ligand or receptor - have been shown to be effective either as monotherapy or in combination with other agents, in subpopulations of patients with a number of cancers, while being ineffective or refractory in many other cancers. Pembrolizumab is a humanized antibody that was first approved by the FDA in 2014 and that is used for treatment of a variety of cancers where the tumor cells express elevated PD-L1. Robert *et al., N Engl. J Med.* 372:2521-2532 (2015) Nivolumab is a fully human antibody that was first approved by the FDA in 2014 and also is used for treating a variety of cancers.

Tumor-associated macrophages (TAMs) are a class of immune cells present in high numbers in the tumor microenvironment (TME), and are associated with cancer-related inflammation. Expression of PD-1 on TAM cells has been shown to decrease macrophage phagocytosis of tumor cells and confers "immunity" on the tumor cells. Gordon *et al, Nature* 545:495 (2017).

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that is associated with chronic and acute inflammation and plays an important role in multiple inflammation-associated diseases. Elevated levels of IL-1β have also been shown to recruit TAM cells and myeloid-derived suppressor cells (MDSC) to the TME and to promote tumor growth and metastasis in breast cancer. Guo *et al., Sci. Rep.* 6, 36107; doi: 10.1038/srep36107 (2016). In other studies, lung lesions have been shown to be populated with TAM whose pro-tumor activity is up-regulated by activation of the NLRP3 inflammasome and the release of IL-1β. (Terlizzi *et al., Oncotarget* 7:58181 (2016)). Finally, IL-1β has been shown to promote a pro-tumor phenotype of TAM cells and the level of the cytokine has been correlated to tumor size & stage in renal cell carcinoma (Chittezhath *et al., Immunity* 41:815 (2014)).

In mice deficient in IL-1β, fewer animals developed tumors and tumor development was slower. Apte, *et al., European Journal of Cancer*, 42:751 (2006). Additionally, it was shown that the lung cancer risk genotype IL-1β-31TT results in increased expression of IL-1β, providing a microenvironment with elevated inflammatory stimuli and increase lung cancer risk. Bhat *et al., Meta Gene* 2:123 (2014). An IL-1 receptor antagonist was shown to suppress metastasis and tumor proliferation by inhibiting angiogenic factors such as VEGF and IL-8. Konishi *et al., Oncology* 68:138 (2005); Lewis *et al, J. Transl. Med.* 4:48 (2006). Canakinumab, a monoclonal antibody that inhibits IL-1β activity, has been shown to reduce incident lung cancer and lung cancer mortality. Ridker *et al., Lancet,* 390:*P1833-1842,* (2017).

Engineered bispecific monoclonal antibodies are non-naturally occurring proteins containing immunoglobulin domains that can simultaneously bind to two different types of antigen. Bispecific antibodies can be made in a variety of format and have been used, for example, for cancer immunotherapy and drug delivery. See, for example, Fan *et al., J. Hemat. Oncol.* 8:130 (2015); Brinkmann and Kontermann, *mAbs* 9:182 (2017); and Spiess *et al., Molecular Immunology*, 67: 95-106 (2015).

Summary of the Invention

What is provided is a binding protein that may contain a first binding domain and a second binding domain, where the first binding domain specifically binds and inhibits activation of an immune checkpoint protein, and where the second binding domain specifically binds and inhibits the activity of IL-β or IL-1R. The immune checkpoint protein may be, for example, PD-1 or PD-L1. The first and second binding domains may contain immunoglobulin binding domains, such as human immunoglobulin binding domains. These binding protein may further contain a third binding domain that specifically binds and inhibits activation of an immune checkpoint protein, such as PD-1 or PD-L1, and a fourth binding domain that specifically binds and inhibits the activity of IL-β or IL-1R. The first and the third binding domains may contain the same CDR regions and the second and the fourth binding domains may contain the same CDR regions.

In one embodiment these protein the first binding domain may contain a) a heavy chain CDR1 of SEQ ID NO.:1; (b) a heavy chain CDR2 of SEQ ID NO.:2; (c) a heavy chain CDR3 of SEQ ID NO.:3; (d) a light chain CDR1 of SEQ ID NO.:4; (e) a light chain CDR2 of SEQ ID NO.:5; and (f) a light chain CDR3 of SEQ ID NO.:6. In this binding protein the first binding domain may contain the heavy chain variable region of SEQ ID NO: 37 and the light chain variable region of SEQ ID NO:38.

In another embodiment the first binding domain may contain a) a heavy chain CDR1 of SEQ ID NO.:7; (b) a heavy chain CDR2 of SEQ ID NO.8; (c) a heavy chain CDR3 of SEQ ID NO.:9; (d) a light chain CDR1 of SEQ ID NO.:10; (e) a light chain CDR2 of SEQ ID NO.:11; and (f) a light chain CDR3 of SEQ ID NO.:12. In this binding protein the first binding domain may contain the heavy chain variable region of SEQ ID NO: 39 and the light chain variable region of SEQ ID NO:40.

In a further embodiment the first binding domain may contain a) a heavy chain CDR1 of SEQ ID NO.:13; (b) a heavy chain CDR2 of SEQ ID NO.15; (c) a heavy chain CDR3 of SEQ ID NO.:15; (d) a light chain CDR1 of SEQ ID NO.:16; (e) a light chain CDR2 of SEQ ID NO.:17; and (f) a light chain CDR3 of SEQ ID NO.:18. In this binding protein the first binding domain may contain the heavy chain variable region of SEQ ID NO: 41 and the light chain variable region of SEQ ID NO:42.

In a still further embodiment the first binding domain may contain (a) a heavy chain CDR1 of SEQ ID NO.:19; (b) a heavy chain CDR2 of SEQ ID NO.:20; (c) a heavy chain CDR3 of SEQ ID NO.:21; (d) a light chain CDR1 of SEQ ID NO.:22; (e) a light chain CDR2 of SEQ ID NO.:23; and (f) a light chain CDR3 of SEQ ID NO.:24. In this binding protein the

first binding domain may contain the heavy chain variable region of SEQ ID NO: 43 and the light chain variable region of SEQ ID NO:44.

In yet a further embodiment the first binding domain may contain (a) a heavy chain CDR1 of SEQ ID NO.:25; (b) a heavy chain CDR2 of SEQ ID NO.:26; (c) a heavy chain CDR3 of SEQ ID NO.:27; (d) a light chain CDR1 of SEQ ID NO.:28; (e) a light chain CDR2 of SEQ ID NO.:29; and (f) a light chain CDR3 of SEQ ID NO.:30. In this binding protein the first binding domain may contain the heavy chain variable region of SEQ ID NO: 45 and the light chain variable region of SEQ ID NO:46.

In a further embodiment the first binding domain may contain (a) a heavy chain CDR1 of SEQ ID NO.:31; (b) a heavy chain CDR2 of SEQ ID NO.:32; (c) a heavy chain CDR3 of SEQ ID NO.:33; (d) a light chain CDR1 of SEQ ID NO.:34; (e) a light chain CDR2 of SEQ ID NO.:35; and (f) a light chain CDR3 of SEQ ID NO.:36. In this binding protein the first binding domain may contain the heavy chain variable region of SEQ ID NO: 47 and the light chain variable region of SEQ ID NO:48.

In any of the these binding proteins the second binding domain may contain (a) a heavy chain CDR1 of SEQ ID NO.:49; (b) a heavy chain CDR2 of SEQ ID NO.:50; (c) a heavy chain CDR3 of SEQ ID NO.:51; (d) a light chain CDR1 of SEQ ID NO.:52; (e) a light chain CDR2 of SEQ ID NO.:53; and (f) a light chain CDR3 of SEQ ID NO.:54. In this binding protein the first binding domain may contain the heavy chain variable region of SEQ ID NO: 55 and the light chain variable region of SEQ ID NO:56.

Further provided are binding proteins where the first binding domain is an antibody binding domain that specifically binds and inhibits activation of PD-1 or PD-L1, and where the second binding domain contains an interleukin-1β-binding domain from an interleukin-1 receptor type 1 (IL-1R1) or interleukin-1 receptor type 2 (IL-1R2), optionally coupled to a ligand binding domain from IL-1R accessory protein . For example, such a binding protein may contain a first protein chain containing (a) an interleukin-1β-binding domain of IL-1R1 linked to (b) a VH domain of an immunoglobulin that binds and inhibits PD-1 or PD-L1 linked to (c) an immunoglobulin Fc domain; and a second protein chain containing a VL domain of the immunoglobulin that binds PD-1 or PD-L1. In another example, the binding protein may contain a first protein chain containing (a) a VH domain of an immunoglobulin that binds that binds and inhibits PD-1 or PD-L1 linked to (b) an interleukin-1β-binding domain of IL-1R1 linked to (c) an immunoglobulin Fc domain; and a second protein chain containing a VL domain of the immunoglobulin that binds and inhibits PD-1 or PD-L1. In yet another example, the binding protein may contain two identical protein chains where each

protein chain contains (a) an interleukin-1β-binding domain of IL-1R1 linked to (b) an immunoglobulin Fc domain linked to (c) an scFV domain that binds and inhibits activation of PD-1 or PD-L1. In a further example, the binding protein may contain: a first protein chain containing (a) an interleukin-1β-binding domain of IL-1R1 linked to (b) VL and CL domains of an immunoglobulin that binds and inhibits PD-1 or PD-L1 linked to (c) an immunoglobulin Fc domain; and a second protein chain containing VH and CH1 domains of the immunoglobulin that binds and inhibits PD-1 or PD-L1. In still another example, the binding protein may contain: a first protein chain containing (a) VL and CL domains of an immunoglobulin that binds that binds and inhibits PD-1 or PD-L1 linked to (b) an interleukin-1β-binding domain of IL-1R1 linked to (c) an immunoglobulin Fc domain; and a second protein chain containing VH and CH1 domains of the immunoglobulin that binds and inhibits PD-1 or PD-L1.

Nucleic acid molecules that encode the protein chains described above are provided, together with vectors, including expression vectors that contain these nucleic acid molecules.

Methods also are provided for the preparation of a binding protein as described above, where the method includes the steps of a) transforming a host cell with vectors that containing nucleic acid molecules encoding the first binding domains and the second binding domain; b) culturing the host cell under conditions that allow synthesis of the binding protein; and c) recovering the binding protein from the culture. The host cell may contain vectors containing nucleic acid molecules encoding the first binding domain and second binding domain.

Pharmaceutical compositions also are provided that contain a binding protein as described above, together with a pharmaceutically acceptable excipient.

Also provided are bispecific binding proteins ("FAT" binding proteins) that contain a first protein chain, a second protein chain and a third protein chain, where the first protein chain contains a heavy chain having VH, CH1, CH2, and CH3 domains, and a first Fab domain (Fab1) at a solvent exposed loop in the CH2 domain, the CH3 domain, or at the interface of the CH2 and CH3 domains; where the second chain contains a second Fab domain and where the third chain contains a third Fab domain. In these binding proteins the second chain Fab domain associates with the VH and CH1 domains of the first protein chain to form a first binding domain, and the third chain Fab domain associates with the first Fab domain at the solvent exposed loop in the first protein to form a second binding domain. In these binding proteins the solvent exposed loop may contain an amino acid sequence from the CH2 domain, such as ISRTP (SEQ ID NO:57). The solvent exposed loop may contain an

amino acid sequence from the CH3 domain, such as SNG. The solvent exposed loop may contain an amino acid sequence from the interface of the CH2 domain and the CH3 domain, such as AKGQP (SEQ ID NO:58). The CH1 domain may be connected to the CH2 domain via an antibody hinge region. The CH2 and CH3 domains may contain an Fc region, such as an Fc region from an IgGl, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD. The first protein chain further may contain a first peptide linker between a first terminus of the first Fab domain and the CH2 domain, CH3 domain, or interface of the CH2 and CH3 domains, and/or a second peptide linker between the second terminus of the first Fab domain and the CH2 domain, CH3 domain, or interface of the CH2 and CH3 domains. The first and the second peptide linker may be, for example, $(G4S)_2$ (SEQ ID NO:59), $(G4S)_3$, (SEQ ID NO:60), and $(G4S)_4$ (SEQ ID NO:61). In a first embodiment, the first protein chain may contain the following polypeptide domains, from N-terminus to C-terminus: VH1-CH1-hinge-CH2(N-term)-Fab1-CH2(C-term)-CH3, or in a second embodiment may contain the following polypeptide domains, from N-terminus to C-terminus: VH1-CH1-hinge-CH2-CH3(N-term)-Fab1-CH3(C-term). In a third embodiment the first protein chain may contain the following polypeptide domains, from N-terminus to C-terminus: VH1-CH1-CH2-Fab1-CH3. The first binding domain may bind specifically to PD-1 or PD-L1 and the second binding domain may bind specifically to IL-1β or IL-1R, or the first binding domain may bind specifically to IL-1β or IL-1R and the second binding domain may bind specifically to PD-1 or PD-L1.

In some embodiments of a FAT binding protein the CDR regions of the first binding domain may be selected from the group consisting of the CDR domains of SEQ ID NO:1-6: the CDR domains of SEQ ID NO:7-12; the CDR domains of SEQ ID NO:13-18; the CDR domains of SEQ ID NO:19-24, the CDR domains of SEQ ID NO:25-30; and the CDR domains of SEQ ID NO:31-36 and the CDR regions of the second binding domain may be the CDR domains of SEQ ID NO:49-54.

In further embodiments of the FAT binding proteins the CDR regions of the first binding domain may be the CDR domains of SEQ ID NO:49-54 and the CDR regions of the second binding domain may be selected from the group consisting of: CDR domains of SEQ ID NO:1-6: the CDR domains of SEQ ID NO:7-12; the CDR domains of SEQ ID NO:13-18; the CDR domains of SEQ ID NO:19-24, the CDR domains of SEQ ID NO:25-30; and the CDR domains of SEQ ID NO:31-36.

Nucleic acid molecules that encode the FAT binding protein chains described above are provided, together with vectors, including expression vectors that contain these nucleic acid molecules.

Also provided are pharmaceutical compositions containing one or more FAT binding proteins and a pharmaceutically acceptable carrier.

Methods for the preparation of a FAT binding protein are provided that include the steps of a) transforming a host cell with vectors that may contain nucleic acid molecules encoding the first, second and third protein chains; b) culturing the host cell under conditions that allow synthesis of the binding protein; and c) recovering the FAT binding protein from the culture. The vectors may contain nucleic acid molecules encoding the first, second and protein chains of the FAT protein.

Methods of treating cancer in a subject are provided that include administering a binding protein or pharmaceutical composition as described above to a subject in need thereof. These methods optionally include administering an antitumor agent to the subject in addition to the binding protein.

In these methods of treating cancer, the subject may previously have been treated with cancer immune therapy or have been found to be resistant to the therapy. The subject may have previously been treated with cancer immune therapy or been found to be refractory to cancer immune therapy. The cancer immune therapy may be, for example, treatment with at least one immune checkpoint inhibitor.

The methods of treating cancer may also include administering an additional anti-tumor therapy to the subject, such as chemotherapy, immune therapy, treatment with biologics or small molecules, vaccination, and/or a cell therapy.

Also provided are methods of preventing or reducing the risk of cancer in a subject at risk thereof, that include administering to the subject an effective amount of a binding protein or pharmaceutical composition as described above. The subject may previously have been diagnosed with cancer and be in remission or may previously have been treated for cancer. The subject may be considered to be at risk of cancer due to environmental exposure, tobacco use or exposure, genetic mutation, or a family history of cancer.

In these methods of cancer treatment, the cancer may be lung cancer, such as small cell lung cancer, combined small-cell lung carcinoma, and/or non-small cell lung cancer. The non-small cell lung cancer may be, for example, squamous cell lung carcinoma, large cell lung carcinoma, lung adenocarcinoma, pulmonary pleomorphic carcinoma, lung carcinoid tumor, salivary gland carcinoma, or carcinoma NOS (not otherwise specified). The cancer may be combined small-cell lung carcinoma, extrapulmonary small-cell carcinoma, extrapulmonary small-cell carcinoma localized in the lymph nodes or small-cell carcinoma of the prostate, or may be a cancer with microsatellite instability.

Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

Brief description of the Drawings

Figure 1 shows the chain structure of a BiS2 bispecific antibody.

Figure 2A shows the chain structure of a BiS3 bispecific antibody.

Figure 2B shows the chain structure of a binding molecule containing (a) an IL-1β binding domain derived from IL-1R1 and IL-1R accessory protein and (b) an antibody binding domain.

Figure 3A shows the chain structure of a FIT-Ig bispecific antibody.

Figure 3B-3E show the chain structures of four binding molecule containing(a) an IL-1β binding domain derived from IL-1R1 and IL-1R accessory protein and (b) an antibody binding domain

Figure 4 shows the chain structure of a FAT-Ig bispecific antibody.

Figure 5 shows the amino acid sequences of bispecific antibodies that bind IL-1β and PD-1.

Figure 6A and 6B show a table of known antibodies and binding molecules that bind IL-1β, IL-1R, PD-1 or PD-L1.

Figure 7 shows binding of bispecific antibodies to cell membrane-bound PD-1 and soluble IL-1β simultaneously in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of ITA series of bispecific antibodies are demonstrated, and all the binding affinities are substantially higher than control human IgG.

Figure 8 shows binding of bispecific antibodies to cell membrane-bound PD-1 and soluble IL-1β simultaneously in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of ITC, ITD and ITE series of bispecific antibodies are demonstrated, and all the binding affinities are substantially higher than control human IgG.

Figures 9A-C show binding of bispecific antibodies to cell membrane-bound PD-1 in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of ITB and ITF series of bispecific antibodies are demonstrated,and all the binding affinities are substantially higher than control human IgG.

Figure 10 shows binding of bispecific antibodies to cell membrane-bound PD-1 and soluble IL-1β simultaneously in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of ITB and ITF series of bispecific antibodies are demonstrated. All the binding affinities are substantially higher than control human IgG.

Figure 11 shows that bispecific antibodies block PD-1 activity in a PD-1/PD-L1 reporter assay. Variable blockade activities are demonstrated for the ITA series of bispecific antibodies. All the blockade activies are substantially higher than control human IgG.

Figures 12A and 12B show that bispecific antibodies block IL-1β activity in a IL-1β functional assay. Variable blockade activities are demonstrated of the ITA series of bispecific antibodies. All the blockade activies are substantially higher than control human IgG.

Detailed Description

Bispecific binding proteins are provided that contain at least one first binding domain that specifically binds an immune checkpoint protein and at least one second binding protein that binds IL-1β. The immune checkpoint protein may be, for example, PD-1, PD-L1, A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA, CTLA-4 (CD152), IDO. KIR, LAG3, NOX2, TIM-3, VISTA, SIGLEC7, (CD328), or SIGLEC9 (CD329). Advantageously, the checkpoint protein is PD-1 or PD-L1.

A binding protein simultaneously binds the checkpoint protein PD-1 or PD-L1 and IL-1β or IL-1R and thereby inhibits binding between PD-1 on CD8 T-cells and PD-L1 on a target cell, such as a tumor cell, and IL-1β activity. Simultaneous inhibition of IL-1β activity and PD-1/PD-L1 binding in this fashion provides for improved methods for cancer treatment. The first and second binding domains advantageously are human antibody variable domains. Novel bispecific binding protein formats also are provided that allow for specific binding of two antigens including, but not limited to, a checkpoint protein and a cytokine such as IL-1β. Methods of using the bispecific binding proteins for treating disease, such as cancer, also are provided.

Binding domains

The binding domains that may be used in the binding proteins as described herein may be in any format that specifically binds the target protein. For example, for binding IL-1β the ligand binding domain of the interleukin type I or type II receptor may be used, optionally fused to a sequence from the IL-1 receptor accessory protein, as in Rilonacept.

Advantageously, however, the binding domains are derived from the variable domains of human immunoglobulin molecules. Specifically, the binding domains may be derived from an antibody that binds a checkpoint protein and an antibody that binds IL-1β or IL-1R. Methods of making fully human antibodies that bind a preselected antigen are well known in the art. For example, human antibodies can be selected from large libraries of antibodies displayed on filamentous phage, and the heavy and light chain variable regions of the selected antibodies identified by methods that are well known. See, for example, Winter *et al., Annual Review of Immunology* 12:433-455 (1994). The nucleic acids encoding these variable regions are then used in constructing the genes encoding the bispecific binding proteins described herein. Alternatively, antibody variable domains from known antibodies may be used. In particular, human antibodies again IL-1β, PD-1 and PD-L1 have been approved for use in treating a variety of disease states in humans, and the variable regions from these antibodies can be used to construct the bispecific binding proteins described herein. Examples of suitable antibodies are shown below in Tables 1 and 6.

Alternatively, the CDR regions from an antibody with known specificity against IL-1β, IL-1R, PD-1 or PD-L1 can be inserted into known human framework regions using methods of CDR grafting that are well known in the art. See, for example, Williams and Matthews, "Humanising Antibodies by CDR Grafting" in *Antibody Engineering* (Kontermann and Dübel, Eds.) pp 319-339 (Springer, 2010). These CDR regions may be derived from the CDR regions shown or Figure 1 or from the CDR regions of the antibodies described in Table 6. The skilled artisan will recognize that other antibodies that specifically bind IL-1β, IL-1R, PD-1 or PD-L1 exist in addition to those described herein, and that the CDR regions of those antibodies may be used in constructing the binding proteins as described herein.

With respect to IL-1β, canakinumab is an FDA-approved human antibody that binds IL-1β, and the amino acid sequences of the heavy and light chain variable regions are well known. See Rondeau *et al., MAbs* 7:1151 (2015). The entire heavy and light chain variable regions of canakinumab can be used to construct the bispecific protein proteins as described herein; alternatively, the CDR regions of canakinumab can be inserted into alternative human variable framework sequences using methods of CDR grafting that are well known in the art. See, for example, Winter and Harris, *Trends in Pharmacological Sciences* 14:139-143 (1993). An alternative IL-1β binding antibody is the humanized SK48-E26 antibody described in WO1995/01997.

With respect to IL-1R, anakinra is an FDA-approved, recombinant, nonglycosylated form of human interleukin -1 receptor antagonist (IL-1Ra). Compared to native human IL-1Ra, anakinra contains an extra N-terminal methionine residue. Anakinra competitively inhibits binding of IL-1α and IL-1β to the IL-1 receptor type 1. This IL-1R1 binding domain can be used in construction of bispecific proteins to block binding of IL-1β to the IL-1 receptor. The sequence of the IL-1R1 binding portion of anakinra, which may be used as a suitable binding domain, is

MRPSGRKSSKMQAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVVPIEPHALFLGIH GGKMCLSCVKSGDETRLQLEAVNITDLSENRKQDKRFAFIRSDSGPTTSFESAACPGWFLCTAMEADQP VSLTNMPDEGVMVTKFYFQEDE (SEQ ID NO:72)

With respect to PD-1, pembrolizumab is a humanized antibody that was first approved by the FDA in 2014 and that is used for treatment of a variety of cancers where the tumor cells express elevated PD-1. Nivolumab is a fully human antibody that was first approved by the FDA in 2014 and also is used for treating a variety of cancers. Cemiplimab is a human antibody that was first approved in 2018 for treatment of metastatic cutaneous squamous cell carcinoma. The amino acid sequences for the heavy and light chain variable domains of pembrolizumab, nivolumab, and cemiplimab, are known, as are the sequences of the CDR regions.

With respect to PD-L1, durvalumab is a human antibody that was first approved in 2017 for treatment of metastatic urothelial cancer. Atezolizumab was first approved in 2016 and is used for treating lung cancer. The amino acid sequences for the heavy and light chain variable domains of durvalumab and atezolizumab are known, as are the sequences of the CDR regions.

The sequences of the variable domains and CDR regions of pembrolizumab, nivolumab, cemeplimab, atezolizumab, avelumab, durvalumab and canakinumab are shown in Table 1 below. A list of other known antibodies again IL-1β, PD-1 and PD-L1 is shown in Figure 6.

Table 1:

Antibody	Domain	Sequence	SEQ ID NO
Pembrolizumab	VH CDR1	NYYMY	1
	VH CDR2	GINPSNGGTNFNEKFKN	2
	VH CDR3	RDYRFDMGFDY	3
	VL CDR1	RASKGVSTSGYSYLH	4
	VL CDR2	LASYL	5

Antibody	Domain	Sequence	SEQ ID NO
	VL CDR3	QHSRDLPLT	6
	VH	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYM YWVRQAPGQGLEWMGGINPSNGGTNFNEKFKN RVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDY RFDMGFDYWGQGTTVTVSS	37
	VL	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYL HWYQQKPGQAPRLLIYLASYLESGVPARFSGSGSG TDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVE IK	38
Nivolumab	VH CDR1	NSGMH	7
	VH CDR2	VIWYDGSKRYYADSVKG	8
	VH CDR3	NDDY	9
	VL CDR1	RASQSVSSYLA	10
	VL CDR2	DASNRAT	11
	VL CDR3	QQSSNWPRT	12
	VH	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGM HWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGR FTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSS	39
	VL	s	40
Cemiplimab	VH CDR1	GFTFSNFG	13
	VH CDR2	ISGGGRDT	14
	VH CDR3	VKWGNIYFDY	15
	VL CDR1	LSINTF	16
	VL CDR2	AAS	17
	VL CDR3	QQSSNTPFT	18
	VH	EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMT WVRQAPGKGLEWVSGISGGGRDTYFADSVKGRF TISRDNSKNTLYLQMNSLKGEDTAVYYCVKWGNIY FDYWGQGTLVTVSS	41
	VL	DIQMTQSPSSLSASVGDSITITCRASLSINTFLNWY QQKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDF TLTIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDFR	42
SK48-E26	VH CDR1	SYDMS	62
	VH CDR2	YISSGGGGTYYPDTVKG	63
	VH CDR3	GGVRRGYFDV	64
	VL CDR1	RASGNIHNYLT	65
	VL CDR2	NAKTLAD	66
	VL CDR3	QHFWSIPYT	67
	VH	EVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYDMS WVRQAPGKGLEWVAYISSGGGGTYYPDTVKGRFT ISRDNSKNTLFLQMDSLRPEDTGVYFCARGGVRRG YFDVWGQGTPVTVSS	68

Antibody	Domain	Sequence	SEQ ID NO
	VL	DIQMTQSPSSLSASVGDRVTITCRASGNIHNYLTW YQQTPGKAPKLLIYNAKTLADGVPSRFSGSGSGTD YTFTISSLQPEDIATYYCQHFWSIPYTFGQGTKLQIT	69
Atezolizumab	VH CDR1	GFTFSDSWIH	19
	VH CDR2	AWISPYGGST	20
	VH CDR3	RHWPGGFDY	21
	VL CDR1	RASQDVSTAVA	22
	VL CDR2	SASFLYS	23
	VL CDR3	QQYLYHPAT	24
	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIH WVRQAPGKGLEWVAWISPYGGSTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARRHWP GGFDYWGQGTLVTVSS	43
	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVE IK	44
Avelumab	VH CDR1	SYIM	25
	VH CDR2	SIYPSGGITFYADTVKG	26
	VH CDR3	IKLGTVTTVDY	27
	VL CDR1	TGTSSDVGGYNYVS	28
	VL CDR2	DVSNRPS	29
	VL CDR3	SSYTSSSTRV	30
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMM WVRQAPGKGLEWVSSIYPSGGITFYADTVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARIKLGTVT TVDYWGQGTLVTVSS	45
	VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVS WYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGTK VTVL	46
Durvalumab	VH CDR1	RYWMS	31
	VH CDR2	NIKQDGSEKYYVDSVKG	32
	VH CDR3	EGGWFGELAFDY	33
	VL CDR1	RASQRVSSSYLA	34
	VL CDR2	DASSRAT	35
	VL CDR3	QQYGSLPWT	36
	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWM SWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGR FTISRDNAKNSLYLQMNSLRAEDTAVYYCAREGG WFGELAFDYWGQGTLVTVSS	47
	VL	EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAW YQQKPGQAPRLLIYDASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK	48

Antibody	Domain	Sequence	SEQ ID NO
Canakinumab	VH CDR1	VYGMN	49
	VH CDR2	IIWYDGDNQYYADSVKG	50
	VH CDR3	DLRTGP	51
	VL CDR1	RASQSIGSSLH	52
	VL CDR2	ASQSFS	53
	VL CDR3	HQSSSLP	54
	VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGM NWVRQAPGKGLEWVAIIWYDGDNQYYADSVKG RFTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLR TGPFDYWGQGTLVTVSS	55
	VL	EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWY QQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFT LTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDIK	56

The IL-1 Receptor type has a binding domain with the sequence:

KCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVSTEQASRIHQHKEKLWFVPAK VEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNAQAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKL QWYKDCKPLLLDNIHFSGVKDRLIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVS PANETMEVDLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITVLNISEI ESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTN (SEQID NO:70)

Rilonacept is an immunoglobulin fusion protein, where the binding domain contains the IL-1 receptor accessory protein (IL-1RAP) fused to the type I IL-1 receptor. The sequence of the IL-1-binding portion of Rilonacept, which may be used as a suitable binding domain, is:

SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLEEPIN FRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGI QRITCPNVDGYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLT RTLTVKVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDVT INESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQKVPAPRYTVEKCKEREEKIILV SSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVSTEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSY CLRIKISAKFVENEPNLCYNAQAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHF SGVKDRLIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEVDLGSQIQL ICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITVLNISEIESRFYKHPFTCFAKNTH GIDAAYIQLIYPVTN (SEQ ID NO:71)

Bispecific binding protein structure

Once the appropriate binding domains are selected, they are incorporated into a format that contains at least one binding domain that specifically binds IL-1β or IL-1R and at least one domain that specifically binds a checkpoint protein. Advantageously, the format of the binding protein is that of a bivalent or multivalent bispecific antibody, containing immunoglobulin variable and constant chain domains arranged to contain two different binding domains, as opposed to the naturally occurring homodimeric structure of a bivalent but monospecific human antibody.

Methods of making bispecific antibodies are well known in the art and are described in, for example, Brinkmann and Kontermann, *mAbs* 9:182 (2017) and Spiess *et al., Molecular Immunology*, 67: 95-106 (2015). The bispecific binding proteins described herein can be in any format known in the art that is stable, suitable for administering to a subject, and contains at least one binding domain the binds IL-1β or IL-1R and at least one binding domain that binds a checkpoint protein.

Examples of suitable bispecific binding protein formats known in the art include:

Fc-less bispecific antibody formats, including: two scFv molecules joined by a linker (Kontermann, *Acta Pharmacol Sin* 26:1-9 (2005)); bispecific single-domain antibody fusion proteins (Weidle *et al., Cancer Genomics Proteomics* 10:155-68 (2013;)) and diabodies (Atwell *et al., Mol Immunol* 33:1301-12 (1996)); Fab fusion proteins (*Schoonjans et al., J Immunol*; 165:7050-7 (2000); and miniantibodies (Pluckthun and Pack, *Immunotechnology* 3:83-105 (1997) and Muller *et al., FEBS Lett* 432:45 49(1998));

Asymmetric IgGs with heavy and light chains from two different antibodies (Suresh *et al, Methods Enzymol*; 121:210-28 (1986)); and

Bispecific IgGs with an asymmetric Fc region, *e.g.* asymmetric Fc regions using the "knobs into holes" method (Ridgway *et al., Protein Eng*; 9:617-21 (1996); Shatz *et al., MAbs*; 5:872-81 (*2013*)); Sampei *et al., PLoS One*; 8:e57479 (2013); Spiess *et al., Biotechnol*; 31:753-8 (20130; Juntilla *et al., Cancer Res.* 74:5561-71 (2014); and Sun *et al., J Clin Invest.*125):4077-4090 (2015)).

The skilled artisan will recognize that a large number of bispecific antibody formats in addition to the specific formats described above can be used to construct a bispecific antibody that binds IL-1β or IL-1R and a checkpoint protein. Advantageously, the bispecific antibody is either a 2+2 scFv-based structure or a 2+2 Fab-based structure as described in more detail below.

2+2 scFv-based structures

A first 2+2 scFv-based structure is the structure shown in Figure 1, referred to herein as BiS2. As shown in Figure 1, the BiS2 format contains two protein chains:

(1) a heavy chain that contains (from N- to C-terminus): a single chain Fv containing a first VH domain and a first VL domain (arranged VH-VL or VL-VH, *i.e.* the domains can be in either order), where the scFv binds the first target (IL-1β, IL-1R or the checkpoint protein, respectively); a second VH domain; and CH1, CH2, and CH3 domains, and

(2) a light chain that contains (from N- to C-terminus): a second VL domain and a CL domain.

The BiS2 protein assembles via non-covalent homodimeric binding of the CH3 and CH2 domains and heterodimeric binding between the CH1 and CL domains on the heavy chain and the second VH and VL domains on the light chain. The binding between the CH1 and CL domains and the VH and VL domains forms a Fab domain that binds the second target (the checkpoint protein or IL-1β/IL-1R, respectively). Advantageously, disulfide bonds also form between the hinge regions, and between the CH1 and CL domains in the same manner as are found in naturally-occurring IgG molecules.

A second 2+2 sc Fv-based structure is the structure shown in Figure 2A, referred to herein as BiS3. As shown in Figure 2A, the BiS3 format also contains two protein chains:

(1) a heavy chain that contains (from N- to C-terminus): a first VH domain; CH1, CH2, and CH3 domains; and a single chain Fv containing a second VH domain and a second VL domain (where the VH and VL domains can be in either order), where the scFv binds the first target (IL-1β or the checkpoint protein, respectively);

(2) a light chain that contains (from N- to C-terminus): a second VL domain and a CL domain.

The BiS3 protein also assembles via homodimeric binding of the CH3 and CH2 domains and heterodimeric binding between the CH1 and CL domains on the heavy chain and the second VH and VL domains on the light chain. The binding between the CH1 and CL domains and the VH and VL domains forms a Fab domain that binds the second target (the checkpoint protein or IL-1β/IL-1R, respectively). Advantageously, disulfide bonds also form between the CH2 domains, and between the CH1 and CL domains in the same manner as are found in naturally-occurring IgG molecules.

An alternative binding protein structure is the homodimeric structure shown in Figure 2B, in which the heavy chain VH and CH1 domains are replaced by a binding domain derived from the extracellular ligand binding domain of an interleukin-1 receptor. This

binding domain contains all or part of the extracellular binding domain from type 1 or type 2 IL-1R, optionally conjugated to the extracellular protein binding domain from interleukin-1 accessory protein (IL-1RAcP). IL-1RAcP is a receptor subunit of the functional IL-1 receptor and forms a receptor heterodimer with IL-1RI. In the structure shown in Figure 2B the binding protein assembles via non-covalent homodimeric binding of the CH3 and CH2 domains. Advantageously, disulfide bonds also form between the CH2 domains, and between the hinge domains in the same manner as are found in naturally-occurring IgG molecules.

2+2 Fab-based structures

A first 2+2 Fab-based structure is the structure shown in Figure 3A, referred to herein as FIT-Ig (see Gong *et al., MABS,* 2017, 9:1118–1128 (2017)). As shown in Figure 3A, the FIT-Ig format contains three protein chains:

(1) a heavy chain that contains (from N- to C-terminus): a first VL domain; a first CL domain, a first VH domain, a first CH1 domain, and CH2 and CH3 domains;

(2) a light chain that contains (from N- to C-terminus): a second VL domain and a second CL domain; and

(3) an Fd chain that contains (from N- to C-terminus): a second VH domain and second CH1 domain.

In the heavy chain the first CL domain may be linked to the first VH domain via a peptide linker such as, for example, a flexible hydrophilic linker having the sequence $(GGGGS)_x$, where x is 1-5. Advantageously, however, the linker is absent.

The FIT-Ig protein assembles via: non-covalent homodimeric binding of the CH3 and CH2 domains; heterodimeric binding between the first VH and CH1 domains on the heavy chain and the second VL and CL domains on the light chain; and heterodimeric binding between the first VL and CL domains and the second VH and CH1 domains on the Fd chain Two identical Fab binding domains are formed by binding of the heavy chain to the light chain, and two distinct but identical Fab domains are formed by binding of the heavy chain to the Fd chain as shown in Figure 3. Advantageously, disulfide bonds also form between the CH2 domains, and between the hinge domains in the same manner as are found in naturally-occurring IgG molecules.

An alternative 2+2 binding protein is shown in Figures 3B-3E, in which one of the antibody Fab binding domains is replaced by a binding domain derived from the extracellular ligand binding domain of an interleukin-1 receptor. This binding domain contains all or part of the extracellular binding domain from type 1 or type 2 IL-1R, optionally conjugated to the

extracellular protein binding domain from interleukin-1 accessory protein (IL-1RAcP). IL-1RAcP is a receptor subunit of the functional IL-1 receptor and forms a receptor heterodimer with IL-1RI. In the structures shown in Figures 3B-E the binding proteins assemble via non-covalent homodimeric binding of the CH3 and CH2 domains; heterodimeric binding between the VH and CH1 domains on one chain and the VL and CL domains on the second protein chain. Advantageously, disulfide bonds also form between: the CH1 and CL domains, between the CH2 domains, and between the hinge domains in the same manner as are found in naturally-occurring IgG molecules.

A second 2+2 Fab-based structure is the novel structure shown in Figure 4, referred to herein as FAT-Ig. As shown in Figure 4, the FAT-Ig format contains three protein chains:

(1) a heavy chain that contains (from N- to C-terminus): a first VH domain; a first CH1 domain, and CH2 and CH3 domains; plus first VL and a first CL domain, where the first VL and first CL domains are disposed at a solvent exposed loop in the CH2 domain, the CH3 domain, or at the interface of the CH2 and CH3 domains. Advantageously, the first CL and first CL domains are disposed at a solvent-exposed loop in the CH3 domain.

(2) a light chain that contains (from N- to C-terminus): a second VL domain and a second CL domain; and

(3) an Fd chain that contains (from N- to C-terminus): a second VH domain and second CH1 domain.

In the heavy chain, the first VL and CL disposed at the solvent-exposed loop are connected to the CH2 domain, CH3 domain, or interface of the CH2 and CH3 domains via flexible peptide linkers. The linkers can have 4-25 amino acids and advantageously comprise $(GGGGS)_x$ units, where x=1-5. Other linkers may also be used, as described in more detail below.

The FAT-Ig protein assembles as shown in Figure 4 via: non-covalent homodimeric binding of the CH3 and CH2 domains; heterodimeric binding of the heavy chain CH1 and VH domains with the light chain CL and VL domains; and heterodimeric binding of the heavy chain CL and VL domains with the Fd chain CH1 and VH domains. Two identical Fab binding domains are formed by binding of the heavy chain to the light chain, and two distinct but identical Fab domains are formed by binding of the heavy chain to the Fd chain as shown in Figure 4. Advantageously, disulfide bonds also form between the hinge domains, and between the CH1 and CL domains in the same manner as are found in naturally-occurring IgG molecules. One of ordinary skill in the art will recognize that the novel FAT-Ig antibody

format can be used to bind any two desired antigens, and is not limited to IL-1β/IL-1R and a checkpoint protein.

In each of the four specific binding proteins described above the binding domains that specifically bind IL-1β/IL-1R and the checkpoint protein are disposed asymmetrically within the binding protein *i.e.* the structure of the binding protein is different when the first binding domain binds IL-1β/IL-1R and the second binding domain binds the checkpoint protein compared to when the first binding domain binds the checkpoint protein and the second binding domain binds IL-1β/IL-1R. Accordingly, each of the four specific binding proteins can exist in two alternative forms for any given pair of binding domains.

Polypeptide Linkers

The domains of the bispecific binding proteins described herein may be joined into contiguous protein chains using linkers. The linkers may be used, for example, to connect the variable heavy and light chains of an scFv, or to connect the CL/VL domains into the heavy chain constant domains in the FAT-Ig format.

Suitable linkers are well known in the art and, when present, advantageously contain at least four amino acids, although longer or shorter linkers may also be used. The linkers advantageously are flexible, hydrophilic and have little or no secondary structure of their own. Linkers may be approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or approximately 50 residues in length. When multiple linkers are used to interconnect portions of a bispecific protein as described herein, the linkers may be the same, or have different lengths and/or amino acid sequences.

The linker(s) facilitate formation of the desired bispecific binding protein structure. Linkers may contain $(Gly\text{-}Ser)_x$ units, where x=1-5. Glutamic acid or lysine residues may also be placed in the linker sequences to increase solubility if necessary. The length of the linker may be varied to facilitate protein folding, target binding, and/or expression. For example, different multiples of $(Gly\text{-}Ser)_x$ units may be used to improve or optimize protein folding, target binding, and/or expression using methods that are well-known in the art.

Preparation of bispecific binding proteins

Methods of making bispecific binding proteins such as Fc-less bispecific antibodies, asymmetric IgGs with heavy and light chains from two different antibodies and bispecific IgGs with an asymmetric Fc region, are well known in the art and are described in the references provided above.

The particular 2+2 bispecific antibodies described above may each be produced using suitable expression constructs in recombinant host cells. Nucleic acids encoding the heavy, light and Fd chains can be prepared synthetically using, for example, a commercial gene synthesis vendor such as Thermo Fisher (Carlsbad, CA). Advantageously the host cells are eukaryotic cells and the gene for each chain advantageously is synthesized with a sequence that encodes an N-terminal signal sequence that causes secretion of the translated protein from the host cell. The gene for each chain is inserted into a suitable expression vector, for example, pTT5 vector (Durocher *et al., Nucleic Acids Res.* 30:E9 (2002)), and the resulting expression constructs are then transfected into a culture of suitable host cells for transient expression. Methods of efficiently transfecting expression vectors into host cells are well known in the art using, for example, cationic lipids. *See* Felgner *et al., Proc. Nat'l Acad. Sci USA* 84:7413 (1987). Other methods of delivering expression vectors into cells also are well known in the art. Methods of making host cells that provide stable expression of a desired protein by integrating expression constructs into the genome of suitable host cells also are well known, as are methods of stable expression using episomal vectors containing a mammalian origin of replication that act as extrachromosomal elements in the nucleus of the host cell.

The host cells advantageously are eukaryotic cells, for example, a single-celled eukaryote (*e.g.,* a yeast or other fungus), a plant cell (*e.g.,* a tobacco or tomato plant cell), an animal cell (*e.g.,* a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman *et al.*, 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen *et al.,* 1998, *Cytotechnology* 28:31) or CHO strain DX-B11, which is deficient in DHFR (see Urlaub *et al.,* 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan *et al.,* 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Advantageously, the host cell is a CHO cell, such as CHO-3E7.

Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase

"recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. The term "host cell" as used herein refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. Suitable cell culture media for growing eukaryotic host cells are well known in the art and are commercially available from, for example, Thermo Fisher (Grand Island, NY).

The host cells are cultured under suitable conditions to allow assembly of the protein chains in the endoplasmic reticulum of the host cell, followed by secretion of the bispecific binding proteins into the cell culture supernatant. The assembly of the correct structure of the binding protein (as opposed to, for example, non-specific pairing of the chains leading to formation of inactive proteins) can be improved by altering the relative ratios of the expression vectors used to transfect the host cells. This variation in the vector ratio also can be used to counteract, say, less efficient production of one of the chains compared to a different chain. One skilled in the art will recognize that methods of optimizing the vector ratio(s) are well known in the art.

After the host cells are cultured in an appropriate expression medium for a suitable length of time, the conditioned medium containing the bispecific binding protein is collected, and the bispecific binding protein is purified using methods that are well known in the art. For example, the binding protein can be purified using methods that may include ion-exchange chromatography, size-exclusion chromatography, and affinity chromatography, such as protein A affinity chromatography. Methods of protein purification are described in, for example, Burgess and Deutscher (Eds) "Guide to Protein Purification, Volume 436 (Methods in Enzymology) 2nd Edition (2009). Purity of the protein can be confirmed using methods well-known in the art, such as RT-HPLC, SDS-PAGE and the like.

The correct assembly of the multichain binding protein can be shown by, for example, non-denaturing gel electrophoresis, to show that the binding protein has the expected molecular weight. SDS-PAGE can be used to show that each of the expected protein chains is present and has the expected molecular weight. Western blotting using a suitable anti-Human IgG antibody can further show that the measured proteins are immunoglobulin chains.

Pharmaceutical Compositions and Methods of Administration

Methods of preparing and administering the bispecific binding molecules to a subject in need thereof are well known to or are readily determined by those skilled in the art. The route of administration of the binding molecule can be, for example, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, for example, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. However, in other methods compatible with the teachings herein, the binding molecules may be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.

The binding molecules may be administered in a pharmaceutically effective amount for the treatment of diseases such as certain types of cancers. The pharmaceutical compositions can comprise pharmaceutically acceptable carriers, including, for example, water, ion exchangers, proteins, buffer substances, and salts. Preservatives and other additives can also be present. The carrier can be a solvent or dispersion medium. Suitable formulations for use in the therapeutic methods disclosed herein are described in Remington's Pharmaceutical Sciences (Mack Publishing Co.) 16th ed. (1980).

In any case, sterile injectable solutions can be prepared by incorporating the binding molecule(s) by itself or in combination with other active agents in an effective amount in an appropriate solvent followed by filtered sterilization. The preparations may also be packaged and sold in the form of a kit. Such articles of manufacture can have labels or package inserts indicating that the associated compositions are useful for treating a subject suffering from, or predisposed to a disease or disorder.

Parenteral formulations can be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions can be administered at specific fixed or variable intervals, for example, once a week or monthly, or on an "as needed" basis.

The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (for example, a therapeutic or prophylactic response).

The composition may be used for treatment of cell-mediated diseases such as certain types of cancers including for example, bone cancer, pancreatic cancer, cancer of the head and neck, cutaneous or intraocular melanoma, uterine cancer, cancer of the central nervous system (CNS), ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon

cancer, breast cancer, melanoma, colorectal cancer, testicular cancer, Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors of childhood, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, adrenocortical carcinoma, AIDS-related cancers, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Basal Cell Carcinoma, extrahepatic bile duct cancer, osteosarcoma/malignant fibrous histiocytoma bone cancer, brain tumors, bronchial adenomas/carcinoids, carcinoid tumor, gastrointestinal carcinoid tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, CMML, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Disorders, ependymoma, Ewing's Family of Tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, gallbladder cancer, gastrointestinal carcinoid tumor, germ cell tumors, gestational trophoblastic tumor, glioma, hairy cell leukemia, hepatocellular cancer, hypopharyngeal cancer, hypothalamic and visual pathway glioma, islet cell carcinoma, Kaposi's Sarcoma, laryngeal cancer, leukemia, lip and oral cavity cancer, non-small cell lung cancer (including squamous cell lung carcinoma, large cell lung carcinoma, lung adenocarcinoma, pulmonary pleomorphic carcinoma, lung carcinoid tumor, salivary gland carcinoma), small cell lung cancer (including combined small-cell carcinoma), "not otherwise specified" (NOS) lung cancer, extrapulmonary small-cell carcinoma (including extrapulmonary small-cell carcinoma localized in the lymph nodes and small-cell carcinoma of the prostate), lymphoma, Waldenstrom's Macroglobulinemia, medulloblastoma, mesothelioma, metastatic squamous neck cancer with occult primary, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, islet cell pancreatic cancer, parathyroid cancer, pheochromocytoma, pineoblastoma, pituitary tumor, pleuropulmonary blastoma, ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, Sezary Syndrome, skin cancer, non-melanoma skin cancer, Merkel Cell Skin Carcinoma, squamous cell carcinoma, testicular cancer, thymoma, gestational trophoblastic tumor, Wilms' Tumor, and cancers with microsatellite instability.

With respect to esophageal cancer, the cancer may be, for example, esophageal squamous-cell carcinomas (ESCC) or esophageal adenocarcinomas (EAC). With respect to pancreatic cancer, the cancer may be, for example, exocrine cancer, pancreatic adenocarcinoma, pancreatic ductal carcinoma, acinar cell carcinoma of the pancreas, cystadenocarcinoma, pancreatoblastoma, adenosquamous carcinomas, undifferentiated carcinomas, pancreatic mucinous cystic neoplasms, neuroendocrine, or pancreatic neuroendocrine tumors (PanNETs). With respect to hepatic cancer, the cancer may be, for example, hepatocellular carcinoma (HCC), hepatoblastoma, cholangiocarcinoma, cholangiocellular cystadenocarcinoma, angiosarcoma, or leiomyosarcoma. With respect to colorectal cancer the cancer may be adenocarcinoma, carcinoid tumors, gastrointestinal stromal tumors (GIST), lymphoma, sarcomas, adenosquamous carcinoma (Ad-SCC) or squamous carcinoma (SCC). With respect to breast cancer, the cancer may be In situ, ductal carcinoma in situ (DCIS), invasive, invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), triple-negative breast cancer, inflammatory breast cancer, angiosarcoma, or Paget disease of the breast. With respect to ovarian cancer, the cancer may be epithelial tumors, benign epithelial ovarian tumors, borderline epithelial ovarian tumors, malignant epithelial ovarian tumors, germ cell tumors, teratoma, dysgerminoma, endodermal sinus tumor and choriocarcinoma, primary peritoneal carcinoma, fallopian tube cancer or ovarian stromal tumors. With respect to multiple myeloma & precancerous conditions, the cancer may be light chain myeloma, non-secretory myeloma, solitary plasmacytoma, extramedullary plasmacytoma, monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), Immunoglobulin D (IgD) myeloma or Immunoglobulin E (IgE) myeloma.

Therapeutically effective doses of the compositions for treating these diseases vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

The amount of at least one binding molecule to be administered can be readily determined by one of ordinary skill in the art without undue experimentation. Factors influencing the mode of administration and the respective amount of at least one binding molecule include, but are not limited to, the severity of the disease, the history of the disease,

and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of binding molecule, to be administered will depend upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent.

The binding molecule, may also be used in the manufacture of a medicament for treating a type of cancer, including, for example, the cancers listed above.

The subject treated with the compositions described herein may be treatment naïve or may be pretreated with one or more other therapies (for example, at least one other anti-cancer therapy) prior to receiving the medicament comprising the binding molecule. It is not necessary that the subject was a responder to pretreatment with the prior therapy or therapies. Thus, the subject that receives the medicament comprising the binding molecule, could have responded, responded poorly, responded initially but subsequently failed to respond, or could have failed to respond to pretreatment with the prior therapy, or to one or more of the prior therapies where pretreatment comprised multiple therapies. Accordingly, the present disclosure provides methods to treat patients that are poor responders or non-responders to other therapies comprising administering a binding molecule as described herein. Also provided are methods to overcome or prevent resistance to cancer therapies or to prevent or delay relapse, comprising administering a binding molecule as disclosed herein, or a composition as described herein.

Even if a patient has been previously treated with an anti-cancer medicament, a person skilled in the art can determine whether a person showed no response or was refractory to that medicament. For example, a non-response to an anti-cancer medicament may be reflected in an increased suffering from cancer, such as an increased growth of a cancer/tumor and/or increase in the size of a tumor, in the formation of (or increase in) metastases or an increase in the number or size of metastases. A non-response may also be the development of a tumor or metastases, for example after resection of a tumor, in the shortening of time to disease progression, or in the increase in the size of (a) tumor(s) and/or (a) metastases, for example in neoadjuvant therapy. Based on these parameters or other parameters known in the art, a patient group can be identified that does not respond to treatment with anti-cancer medicaments and this group of patients may then be treated with the binding molecules described herein.

The binding molecules and compositions containing the binding molecules may also be used to treat patients that are, for example, poor-responders or non-responders to another therapy. The term "non-responder" as used herein can refer to an individual/patient/subject

that is less likely to respond to a treatment using an anti-cancer medicament. "Less likely to respond" as used herein refers to a decreased likeliness that a pathological complete response will occur in a patient treated with an anti-cancer medicament. In some aspects, a patient can be initially a good responder, and resistance to treatment can develop during treatment with such an anti-cancer medicament, leading to poor or no-response to the treatment.

The term "good responder" as used herein refers to an individual whose tumor does not demonstrate growth, metastases, increase in number or size of metastases, etc. during or after treatment using an anti-cancer medicament, for example based on serial imaging studies, an individual that does not experience tumor growth, metastases, increase in number or size of metastases, etc. over a period of time (for example, about 1 year following initial diagnosis), and/or an individual that experiences a certain life span (for example, about 2 years or more following initial diagnosis).

The term "poor responder" as used herein refers to an individual whose tumor grows or metastasizes during or shortly thereafter standard therapy, for example using an anti-cancer medicament, or who experiences adverse clinical effects attributable to the tumor. The term “poor responder” also includes indivduals who transitioned from “good responder” to ‘poor responder” during treatment with an anti-cancer medicament.

In cases where it is assessed that the subject is a "non-responder," a "poor-responder" or is "less likely to respond" (based, for example, on the presence of certain biomarkers in the cancer cells), the subject could be treated with the binding molecules disclosed herein.

Methods also are provided for the co-administration of a binding molecule as described herein and at least one other therapy. The binding molecule and the at least one other therapy can be co-administered together in a single composition or can be co-administered together at the same time or overlapping times in separate compositions. In some aspects, a binding molecule can be used as an adjuvant therapy.

The binding molecule may also be used in the manufacture of a medicament for treating a subject suffering from a cancer, where the binding molecule is administered before a subject has been treated with at least one other therapy.

The binding molecules can also be used in methods of preventing or reducing the risk of cancer in a subject by administering to the subject an effective amount of a binding protein or composition containing the binding protein. The cancer may be lung cancer and the patient may be in remission from a previously diagnosed and/or previously treated cancer. The patient may be considered to be at risk of cancer due to environmental exposure, tobacco use or exposure, genetic mutation, or a family history of cancer.

Examples:

In the examples below the binding domains are based on heavy and light chain variable regions from human antibodies that have received regulatory approval for use in humans. "O," "E" and "K" indicate domains that bind IL-1β, while "M" and "B" indicate binding domains that bind PD-1. The amino acid sequences of the chains of the binding proteins are shown in Figure 5

Example 1: Expression of bispecific antibodies

1. Plasmid Preparation

Target DNA sequences encoding the binding proteins were synthesized and subcloned into the pTT5 vector (Durocher *et al., Nucleic Acids Res.* 30:E9 (2002) for expression in CHO-3E7 cells. The amino acid sequences of the coding sequences are shown in Figure 5.

2. Cell Culture and Transient Transfection

CHO-3E7 cells were grown in serum-free FreeStyle™ CHO Expression Medium (Life Technologies, Carlsbad, CA, USA). The cells were maintained in Erlenmeyer Flasks (Corning Inc., Acton, MA) at 37°C with 5% CO_2 on an orbital shaker (VWR Scientific, Chester, PA). One day before transfection, the cells were seeded in Corning Erlenmeyer Flasks. On the day of transfection, DNA and transfection reagent were mixed and then added into the cells culture, during which the recombinant plasmids encoding target antibody were transiently transfected into CHO-3E7 cells. The cell culture supernatant collected on day 6 was used for purification. Table 2 lists the heavy chain, light chain and Fd chain combination of each antibody and each of the plasmid ratios that were screened.

Table 2. Summary of heavy chain (HC), light chain (LC) and Fd chain (Fd) combinations and plasmid ratios for each antibody.

Antibody name	Antibody format	HC name	LC name	Fd	Ratio 1	Ratio 2	Ratio 3	Ratio 4
					HC:LC:Fd	HC:LC:Fd	HC:LC:Fd	HC:LC:Fd
ITA101	BiS2-OB	BscFv-OHC	OLC	/	1:1:0	3:1:0	1:3:0	/
ITA102	BiS2-OM	MscFv-OHC	OLC	/	1:1:0	3:1:0	1:3:0	/
ITA103	BiS2-PC	OscFv-BHC	BLC	/	1:1:0	3:1:0	1:3:0	/
ITA104	BiS2-MO	OscFv-MHC	MLC	/	1:1:0	3:1:0	1:3:0	/
ITA201	BiS3-OB	OHC-BscFv	OLC	/	1:1:0	3:1:0	1:3:0	/
ITA202	BiS3-OM	OHC-MscFv	OLC	/	1:1:0	3:1:0	1:3:0	/
ITA203	BiS3-BO	BHC-OscFv	BLC	/	1:1:0	3:1:0	1:3:0	/
ITA204	BiS3-MO	MHC-OscFv	MLC	/	1:1:0	3:1:0	1:3:0	/
ITA301	FiT-OB	BLC-OHC	OLC	BFd	1:1:1	1:3:3	1:1:3	1:3:1
ITA302	FiT-OM	MLC-OHC	OLC	MFd	1:1:1	1:3:3	1:1:3	1:3:1
ITA303	FiT-BO	OLC-BHC	BLC	OFd	1:1:1	1:3:3	1:1:3	1:3:1
ITA304	FiT-MO	OLC-MHC	MLC	OFd	1:1:1	1:3:3	1:1:3	1:3:1
ITA401	FAT-OB	OHC-BLC	OLC	BFd	1:1:1	1:3:3	1:1:3	1:3:1
ITA402	FAT-OM	OHC-MLC	OLC	MFd	1:1:1	1:3:3	1:1:3	1:3:1

ITA403	FAT-BO	BHC-OLC	BLC	OFd	1:1:1	1:3:3	1:1:3	1:3:1
ITA404	FAT-MO	MHC-OLC	MLC	OFd	1:1:1	1:3:3	1:1:3	1:3:1
ITB101	FP2-BK	K BHC	B LC	/	1:1			
ITB102	FP2-MK	K MHC	M LC	/	1:1			
ITB103	FFP1(FIT)- KM	MFd-K-G4P	M LC	/	1:1			
ITB104	FFP1(FIT)-KB	BFd-K-G4P	B LC	/	1:1			
ITB105	FFP3-KM	K-G4P-MscFv	/	/	N/A			
ITB106	FP1-(crossmab)BK	K-BLC-Fc	/	B Fd	1:1			
ITB107	FP1- (crossmab) MK	K-MLC-Fc	/	M Fd	1:1			
ITB108	FFP2-KM	MLC-KG4P	/	M Fd	1:1			
ITB109	FFP2-KB	BLC-K-G4P	/	B Fd	1:1			
ITC401	FAT-O/B 1+5	O HC-B LC 1+5	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITC402	FAT-O/B 3+3	O HC-B LC 3+3	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITC403	FAT-O/B 4+2	O HC-B LC 4+2	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITC404	FAT-O/B 5+1	O HC-B LC 5+1	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITC405	FAT-O/B 4+4	O HC-B LC 4+4	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1

ITC406	FAT-O/B 2+2	O HC-B LC 2+2	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITD401	FAT-O/B 5+5	FAT-O/B 5+5	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITD402	FAT-O/B 4+5	FAT-O/B 4+5	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITD403	FAT-O/B 5+4	FAT-O/B 5+4	O LC	B Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITE101	BiS2-O/M VH/VL DSB	M scFv-O HC VH/VL DSB	O LC	/	1:1:0	3:1:0	1:3:0	/
ITE102	BiS2-O/M VL/VH DSB	M scFv-O HC VL/VH DSB	O LC	/	1:1:0	3:1:0	1:3:0	/
ITE201	BiS3-M/O VH/VL DSB	M HC-O scFv_ VH/VL DSB	M LC	/	1:1:0	3:1:0	1:3:0	
ITE202	BiS3-M/O VL/VH DSB	M HC-O scFv_ VL/VH DSB	M LC	/	1:1:0	3:1:0	1:3:0	
ITE301	FIT-B/O-L3	O LC-B HC-L3	B LC	O Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITE302	FIT-B/O-L7	O LC-B HC-L7	B LC	O Fd	1:1:1	1:3:3	1:1:3	1:3:1
ITF101	BiS2-E/M VL/VH DSB	BiS2-E /M VL/VH DSB	E13 LC	/	1:1:0,	1:3:0	3:1:0	
ITF102	BiS2-M/E VL/VH DSB	BiS2-M/E VL/VH DSB	M LC	/	1:1:0,	1:3:0	3:1:0	
ITF103	BiS2-M/E VH/VL DSB	BiS2-M/E VH/VL DSB	M LC	/	1:1:0,	1:3:0	3:1:0	

ITF201	BiS3-M/E VL/VH DSB	BiS3-M/E VL/VH DSB	M LC	/	1:1:0,	1:3:0	3:1:0	
ITF202	BiS3-M/E VH/VL DSB	BiS3-M/E VH/VL DSB	M LC	/	1:1:0,	1:3:0	3:1:0	
ITF301	FIT-B/E L3	FIT-B/E-L3	B LC	E Fd	1:1:1	1:1:1,	1:1:1,	1:1:1,
ITF302	FIT-B/E L7	FIT-B/ E-L7	B LC	E Fd	1:1:1	1:1:1,	1:1:1,	1:1:1,
ITF401	FAT-E/B	FAT-E/B	E13LC	B Fd	1:1:1	1:1:1,	1:1:1,	1:1:1,
ITF402	FAT-B/E	FAT-B/E	B LC	E Fd	1:1:1	1:1:1,	1:1:1,	1:1:1,
ITF403	FAT-M/E	FAT-M/E	M LC	E Fd	1:1:1	1:1:1,	1:1:1,	1:1:1,

3. Purification and Results

Cell culture supernatant was centrifuged followed by filtration. The filtered supernatant was loaded onto Monofinity A Resin Prepacked Column 1 ml (GenScript, Cat.No.L00433-11) at 1.0 ml/min. After a washing step, the antibodies were eluted and then buffer exchanged to PBS, pH 7.2. The antibodies were further purified by standard techniques. For example, some samples were further purified by size exclusion chromatography (SEC) using a Superdex 200 Increase 10/300 GL column or by ceramic hydroxyapatite chromatography using a CHT™ ceramic hydroxyapatite column (Bio-Rad). For other proteins, Protein A affinity chromatography was used for initial purification, followed by SEC if necessary.

Each of the expressed proteins was analyzed by SDS-PAGE under reducing and non-reducing conditions and Western blot analysis (using Goat Anti-Human IgG-HRP (GenScript, Cat. No.A00166), which showed that proteins and constituent chains had the desired molecular weights. Purity of the proteins was assessed using size-exclusion HPLC

Example 2

Nine of the sixteen different bispecific antibodies described in Example 1 were analyzed for binding to PD-1 on the surface of cells using flow cytometry. Cell lines used were CHO-K1 cells (negative control) and a CHO-K1/PD-1 expressing. Antibodies used were an irrelevant human IgG (negative control), and commercially available anti-PD-1 (positive controls). The secondary antibody used was a goat anti Human IgG(H+L) iFluor 647 (1 μg/ml) (data not shown). In order to demonstrate concurrent binding of the bispecific molecules to PD1 and IL-1β, a sandwich FACS assay was performed. The binding curves are shown in Figure 7. CHO cells expressing PD1 were bound by bispecific antibodies at the indicated concentrations. Unbound antibody was washed away and detection was performed using biotinylated IL-1β (2 ug/ml) followed by SA-iFluor 647 (1 ug/ml). Fluorescence intensity is indicative of both BiSAb immobilization on PD1+ cells and ability to concurrently bind IL-1β. Various combinations of anti-IL-1β VH/VL binding domains with two different pairs of anti-PD-1 VH/VL binding domains were formatted as BiS2, BiS3, FIT-IG or FAT-IG molecules. Of the constructs tested, the four binding molecules having anti-PD-1 VH/VL binding regions containing the CDR regions defined by the sequences of SEQ ID Nos: 1-6 all achieved a higher mean fluorescence intensity compared to the five binding

molecules having anti-PD-1 VH/VL binding regions containing the CDR regions defined by the sequences of SEQ ID Nos: 6-12.

Figures 8-12 show additional binding data for additional binding proteins. Figure 8 shows binding curves for binding of bispecific antibodies to cell membrane-bound PD-1 and soluble IL-1β simultaneously in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of the ITC, ITD and ITE series of bispecific antibodies are shown. All the binding affinities are substantially higher than control human IgG.

Figure 9 shows binding curves for binding to cell membrane-bound PD-1 in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of the ITB and ITF series of bispecific antibodies are shown. All the binding affinities are substantially higher than control human IgG.

Figure 10 shows binding curves for binding to cell membrane-bound PD-1 and soluble IL-1β simultaneously in a multiple-dose sandwich assay, as detected by flow cytometry. Variable binding affinities of ITB and ITF series of bispecific antibodies are shown. All the binding affinities are substantially higher than control human IgG.

Figure 11 shows that bispecific antibodies block PD-1 activity in a PD-1/PD-L1 reporter assay. Variable blockade activities are shown for the ITA series of bispecific antibodies. All the blockade activies are substantially higher than control human IgG.

Figure 12 shows that bispecific antibodies block IL-1β activity in a IL-1β functional assay. Variable blockade activities are demonstrated of the ITA series of bispecific antibodies. All the blockade activies are substantially higher than control human IgG.

Claims

What is claimed is:

1. A binding protein comprising a first binding domain and a second binding domain, wherein said first binding domain specifically binds and inhibits activation of PD-1 or PD-L1, and wherein said second binding domain specifically binds and inhibits the activity of IL-β or IL-1R.

2. The binding protein according to claim1 wherein said first and second binding domains comprise immunoglobulin binding domains.

3. The binding protein according to claim 2 wherein said immunoglobulin binding domains are human immunoglobulin binding domains.

4. The binding protein according to any previous claim wherein said binding protein comprises a third binding domain that specifically binds and inhibits activation of PD-1 or PD-L1 and a fourth binding domain that specifically binds and inhibits the activity of IL-β or IL-1R.

5. The binding protein according to claim 4 where said first and said third binding domains comprise the same CDR regions and wherein said second and said fourth binding domains comprise the same CDR regions.

6. The binding protein according to any previous claim wherein said first binding domain comprises a) a heavy chain CDR1 of SEQ ID NO.:1; (b) a heavy chain CDR2 of SEQ ID NO.:2; (c) a heavy chain CDR3 of SEQ ID NO.:3; (d) a light chain CDR1 of SEQ ID NO.:4; (e) a light chain CDR2 of SEQ ID NO.:5; and (f) a light chain CDR3 of SEQ ID NO.:6.

7. The binding protein according to any of claims 1-5 wherein said first binding domain comprises a) a heavy chain CDR1 of SEQ ID NO.:7; (b) a heavy chain CDR2 of SEQ ID NO.8; (c) a heavy chain CDR3 of SEQ ID NO.:9; (d) a light chain CDR1 of SEQ ID NO.:10; (e) a light chain CDR2 of SEQ ID NO.:11; and (f) a light chain CDR3 of SEQ ID NO.:12.

8. The binding protein according to any of claims 1-5 wherein said first binding domain comprises a) a heavy chain CDR1 of SEQ ID NO.:13; (b) a heavy chain CDR2 of SEQ ID NO.14; (c) a heavy chain CDR3 of SEQ ID NO.:15; (d) a light chain CDR1 of SEQ ID NO.:16; (e) a light chain CDR2 of SEQ ID NO.:17; and (f) a light chain CDR3 of SEQ ID NO.:18.

9. The binding protein according to any of claims 1-5 wherein said first binding domain comprises (a) a heavy chain CDR1 of SEQ ID NO.:19; (b) a heavy chain CDR2 of

SEQ ID NO.:20; (c) a heavy chain CDR3 of SEQ ID NO.:21; (d) a light chain CDR1 of SEQ ID NO.:22; (e) a light chain CDR2 of SEQ ID NO.:23; and (f) a light chain CDR3 of SEQ ID NO.:24.

10. The binding protein according to any of claims 1-5 wherein said first binding domain comprises (a) a heavy chain CDR1 of SEQ ID NO.:25; (b) a heavy chain CDR2 of SEQ ID NO.:26; (c) a heavy chain CDR3 of SEQ ID NO.:27; (d) a light chain CDR1 of SEQ ID NO.:28; (e) a light chain CDR2 of SEQ ID NO.:29; and (f) a light chain CDR3 of SEQ ID NO.:30.

11. The binding protein according to any of claims 1-5, wherein said first binding domain comprises (a) a heavy chain CDR1 of SEQ ID NO.:31; (b) a heavy chain CDR2 of SEQ ID NO.:32; (c) a heavy chain CDR3 of SEQ ID NO.:33; (d) a light chain CDR1 of SEQ ID NO.:34; (e) a light chain CDR2 of SEQ ID NO.:35; and (f) a light chain CDR3 of SEQ ID NO.:36.

12. The binding protein according to claim 6, wherein said first binding domain comprises the heavy chain variable region of SEQ ID NO: 37 and the light chain variable region of SEQ ID NO:38.

13. The binding protein according to claim 7, wherein said first binding domain comprises the heavy chain variable region of SEQ ID NO: 39 and the light chain variable region of SEQ ID NO:40.

14. The binding protein according to claim 8, wherein said first binding domain comprises the heavy chain variable region of SEQ ID NO: 41 and the light chain variable region of SEQ ID NO:42.

15. The binding protein according to claim 9, wherein said first binding domain comprises the heavy chain variable region of SEQ ID NO: 43 and the light chain variable region of SEQ ID NO:44.

16. The binding protein according to claim 10, wherein said first binding domains comprises the heavy chain variable region of SEQ ID NO: 45 and the light chain variable region of SEQ ID NO:46.

17. The binding protein according to claim 11, wherein said first binding domains comprises the heavy chain variable region of SEQ ID NO: 47 and the light chain variable region of SEQ ID NO:48.

18. The binding protein according to any preceding claim wherein said second binding domain contain (a) a heavy chain CDR1 of SEQ ID NO.:49; (b) a heavy chain CDR2 of SEQ ID NO.:50; (c) a heavy chain CDR3 of SEQ ID NO.:51; (d) a light chain CDR1 of

SEQ ID NO.:52; (e) a light chain CDR2 of SEQ ID NO.:53; and (f) a light chain CDR3 of SEQ ID NO.:54.

19. The binding protein according to claim 18, wherein said first binding domain comprises the heavy chain variable region of SEQ ID NO: 55 and the light chain variable region of SEQ ID NO:56.

20. The binding protein according to any of claims 1-17, wherein said second binding domain contain (a) a heavy chain CDR1 of SEQ ID NO.:62 (b) a heavy chain CDR2 of SEQ ID NO.:63; (c) a heavy chain CDR3 of SEQ ID NO.:64; (d) a light chain CDR1 of SEQ ID NO.:65; (e) a light chain CDR2 of SEQ ID NO.:66; and (f) a light chain CDR3 of SEQ ID NO.:67.

21. The binding protein according to claim 21, wherein said first binding domain comprises the heavy chain variable region of SEQ ID NO: 68 and the light chain variable region of SEQ ID NO:69.

22. The binding protein according to claim 1,wherein said first binding domain is an antibody binding domain that specifically binds and inhibits activation of PD-1 or PD-L1, and wherein said second binding domain comprises an interleukin-1β-binding domain of interleukin-1 receptor type 1 (IL-1R1) or interleukin-1 receptor type 2 (IL-1R2).

23. The binding protein according to claim 22 comprising; a first protein chain comprising (a) an interleukin-1β-binding domain of IL-1R1 linked to (b) a VH domain of an immunoglobulin that binds and inhibits PD-1 or PD-L1 linked to (c) an immunoglobulin Fc domain; and a second protein chain comprising a VL domain of said immunoglobulin that binds and inhibits PD-1 or PD-L1.

24. The binding protein according to claim 22 comprising; a first protein chain comprising (a) a VH domain of an immunoglobulin that binds that binds and inhibits PD-1 or PD-L1 linked to (b) an interleukin-1β-binding domain of IL-1R1 linked to (c) an immunoglobulin Fc domain; and a second protein chain comprising a VL domain of said immunoglobulin that binds and inhibits PD-1 or PD-L1.

25. The binding protein according to claim 22 comprising two identical protein chains wherein each protein chain comprises (a) an interleukin-1β-binding domain of IL-1R1 linked to (b) an immunoglobulin Fc domain linked to (c) an scFV domain that binds and inhibits activation of PD-1 or PD-L1.

26. The binding protein according to claim 22 comprising; a first protein chain comprising (a) an interleukin-1β-binding domain of IL-1R1 linked to (b) VL and CL domains of an immunoglobulin that binds and inhibits PD-1 or PD-L1 linked to (c) an

immunoglobulin Fc domain; and a second protein chain comprising VH and CH1 domains of said immunoglobulin that binds and inhibits PD-1 or PD-L1.

27. The binding protein according to claim 22 comprising; a first protein chain comprising (a) Vl and CL domains of an immunoglobulin that binds that binds and inhibits PD-1 or PD-L1 linked to (b) an interleukin-1β-binding domain of IL-1R1 linked to (c) an immunoglobulin Fc domain; and a second protein chain comprising VH and CH1 domains of said immunoglobulin that binds and inhibits PD-1 or PD-L1.

28. The binding protein according to any of claims 22-27, wherein said interleukin-1β-binding domain of IL-1R1 further comprises IL-1R accessory protein.

29. A method for the preparation of a binding protein according to any one of claims 1 to 28 comprising the steps of a) transforming a host cell with vectors comprising nucleic acid molecules encoding said first binding domains and said second binding domain; b) culturing the host cell under conditions that allow synthesis of said binding protein; and c) recovering said binding protein from said culture.

30. A host cell comprising vectors comprising nucleic acid molecules encoding the first binding domain and second binding domain according to any one of claims 1 to 28.

31. A pharmaceutical composition comprising a binding protein according to any one of claims 1 to 28 and a pharmaceutically acceptable excipient.

32. A method of treating cancer in a subject comprising administering a binding protein according to any of claims 1-28 or a composition according to claim 31 to a subject in need thereof.

33. The method of claim 32, further comprising administering to said subject an antitumor agent.

34. A bispecific binding protein comprising a first protein chain, a second protein chain and a third protein chain,

wherein said first protein chain comprises a heavy chain comprising VH, CH1, CH2, and CH3 domains, and further comprises a first Fab domain (Fab1) at a solvent exposed loop in the CH2 domain, the CH3 domain, or at the interface of the CH2 and CH3 domains;

wherein said second chain comprises a second Fab domain and

wherein said third chain comprises a third Fab domain;

wherein said second chain Fab domain associates with the VH and CH1 domains of said first protein chain to form a first binding domain, and

wherein said third chain Fab domain associates with the first Fab domain at the solvent exposed loop in said first protein to form a second binding domain.

35. The protein of claim 34, wherein the solvent exposed loop comprises an amino acid sequence from the CH2 domain.

36. The protein of claim 35, wherein the solvent exposed loop comprises the amino acid sequence ISRTP (SEQ ID NO:57).

37. The protein of claim 34, wherein the solvent exposed loop comprises an amino acid sequence from the CH3 domain.

38. The protein of claim 37, wherein the solvent exposed loop comprises the amino acid sequence SNG.

39. The protein of claim 34, wherein the solvent exposed loop comprises an amino acid sequence from the interface of the CH2 domain and the CH3 domain.

40. The protein of claim 39, wherein the solvent exposed loop comprises the amino acid sequence AKGQP (SEQ ID NO:58).

41. The protein of claim 34, wherein the CH1 domain is connected to the CH2 domain via an antibody hinge region.

42. The protein of any of claims 34-41, wherein the CH2 and CH3 domains comprises an Fc region selected from the group consisting of an Fc region from an IgGl, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

43. The protein of any of claims 34-41, wherein said first protein chain further comprises a first peptide linker between a first terminus of said first Fab domain and said CH2 domain, CH3 domain, or interface of the CH2 and CH3 domains.

44. The protein of claim 43 further comprising a second peptide linker between the second terminus of said first Fab domain and said CH2 domain, CH3 domain, or interface of the CH2 and CH3 domains.

45. The protein of claim 43 or claim 44 wherein said first and said second peptide linker are independently selected from the group consisting of $(G4S)_2$ (SEQ ID NO:59), $(G4S)_3$, (SEQ ID NO:60), and $(G4S)_4$ (SEQ ID NO:61).

46. The protein of any of claims 34-45, wherein said first protein chain comprises the following polypeptide domains, from N-terminus to C-terminus:

VH1-CH1-hinge-CH2(N-term)-Fab1-CH2(C-term)-CH3.

47. The protein of any of claims 34-45, wherein said first protein chain comprises the following polypeptide domains, from N-terminus to C-terminus:

VH1-CH1-hinge-CH2-CH3(N-term)-Fab1-CH3(C-term).

48. The protein of any of claims 34-45, wherein said first protein chain comprises the following polypeptide domains, from N-terminus to C-terminus:

VH1-CH1-CH2-Fab1-CH3.

49. A bispecific binding protein according to any of claims 34-48, wherein said first binding domain binds specifically to PD-1 or PD-L1 and said second binding domain binds specifically to IL-1β or IL-1R.

50. A bispecific binding protein according to any of claims 34-48, wherein said first binding domain binds specifically to IL-1β or IL-1R, and said second binding domain binds specifically to PD-1 or PD-L1.

51. A bispecific binding protein according to claim 49, wherein the CDR regions of said first binding domain are selected from the group consisting of the CDR domains of SEQ ID NO:1-6: the CDR domains of SEQ ID NO:7-12; the CDR domains of SEQ ID NO:13-18; the CDR domains of SEQ ID NO:19-24, the CDR domains of SEQ ID NO:25-30; and the CDR domains of SEQ ID NO:31-36 and wherein the CDR regions of said second binding domain are the CDR domains of SEQ ID NO:49-54.

52. A bispecific binding protein according to claim 50, wherein the CDR regions of said first binding domain are the CDR domains of SEQ ID NO:49-54 and wherein the CDR regions of said second binding domain are selected from the group consisting of: CDR domains of SEQ ID NO:1-6: the CDR domains of SEQ ID NO:7-12; the CDR domains of SEQ ID NO:13-18; the CDR domains of SEQ ID NO:19-24, the CDR domains of SEQ ID NO:25-30; and the CDR domains of SEQ ID NO:31-36.

53. A pharmaceutical composition comprising the binding protein of any one of claims 34-52 and a pharmaceutically acceptable carrier.

54. A method for the preparation of a binding protein according to any one of claims 34-52 comprising the steps of a) transforming a host cell with vectors comprising nucleic acid molecules encoding said first, second and third protein chains; b) culturing the host cell under conditions that allow synthesis of said binding protein; and c) recovering said binding protein from said culture.

55. A host cell comprising vectors comprising nucleic acid molecules encoding said first, second and protein chain according to any one of claims 34 to 52.

56. A method of treating cancer in a subject comprising administering a binding protein according to any of claims 34-52 or a composition according to claim 53 to a subject in need thereof.

57. The method of claim 56, further comprising administering to said subject an antitumor agent.

58. The method of claim 32 or claim 56 wherein said cancer is lung cancer.

59. The method of claim 58, wherein said lung cancer if small cell lung cancer.

60. The method of claim 59, wherein said small cell lung cancer is combined small-cell lung carcinoma.

61. The method of claim 59, wherein said lung cancer is non-small cell lung cancer.

62. The method of claim 61, wherein said non-small cell lung cancer is selected from the group consisting of squamous cell lung carcinoma, large cell lung carcinoma, lung adenocarcinoma, pulmonary pleomorphic carcinoma, lung carcinoid tumor, salivary gland carcinoma, or carcinoma NOS (not otherwise specified).

63. The method of claim 32 or claim 56 wherein said cancer is combined small-cell lung carcinoma, extrapulmonary small-cell carcinoma, extrapulmonary small-cell carcinoma localized in the lymph nodes or small-cell carcinoma of the prostate.

64. The method of claim 32 or claim 56 wherein said cancer is a cancer with microsatellite instability

65. The method of claim 32 or claim 56 wherein said subject has previously been treated with cancer immune therapy or has been found to be resistant to said therapy.

66. The method of claim 32 or 56 wherein said subject has previously been treated with cancer immune therapy or has been found to be refractory to cancer immune therapy.

67. The method of claim 65 or 66 wherein said cancer immune therapy is treatment with at least one immune checkpoint inhibitor.)

68. The method of claim 32 or 56 further comprising administering to said subject an additional anti-tumor therapy.

69. The method of claim 68, wherein said anti-tumor therapy is chemotherapy, immune therapy, treatment with biologics or small molecules, vaccination, or a cell therapy.

70. A method of preventing or reducing the risk of cancer in a subject at risk thereof, comprising administering to said subject an effective amount of a binding protein according to any of claims 1-28 or 34-52 or a composition according to claim 31 or 53.

71. The method of claim 70, wherein said cancer is lung cancer.

72. The method of claim 70 wherein said subject previously was diagnosed with cancer and is in remission.

73. The method of claim 72 wherein said subject was previously treated for cancer.

74. The method of claim 70, wherein said subject is considered to be at risk of cancer due to environmental exposure, tobacco use or exposure, genetic mutation, or a family history of cancer.

75. A nucleic acid molecule encoding a binding domain of a binding protein according to any of claims 1-28 or 34-52.

76. The method of claim 32 or 56, wherein said cancer is selected from the group consisting of esophageal, pancreatic, hepatic, colorectal, breast, and ovarian cancer, or multiple myeloma or precancerous conditions.

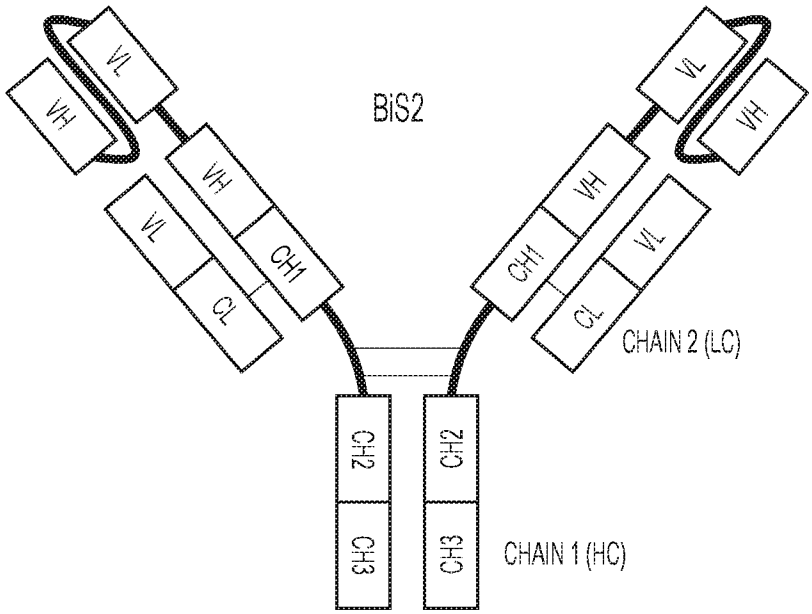

BiS2
VL
VH
VH
VL
CH1
CL
VL
VH
CH1
VL
CL
CHAIN 2 (LC)
CH2
CH3
CH2
CH3
CHAIN 1 (HC)

FIG.1

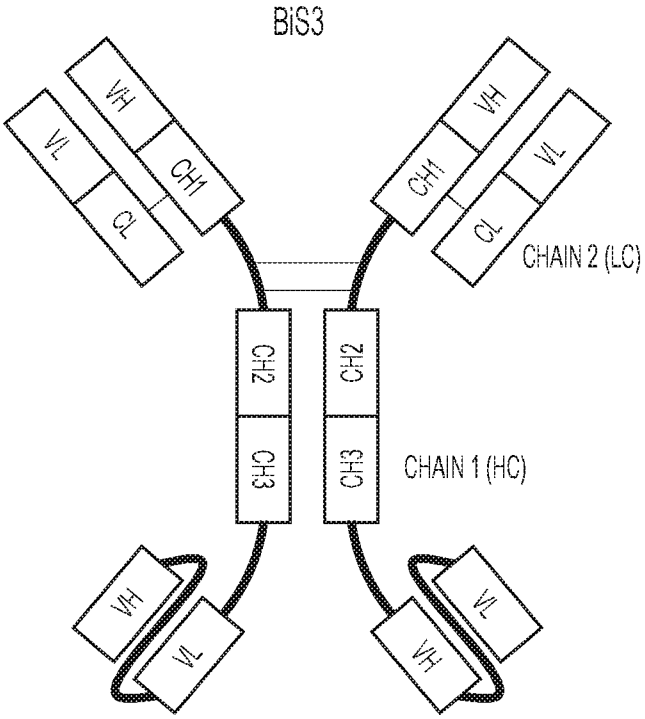

BiS3
VH
VL
CH1
CL
VH
CH1
VL
CL
CHAIN 2 (LC)
CH2
CH3
CH2
CH3
CHAIN 1 (HC)
VH
VL
VL
VH

FIG.2A

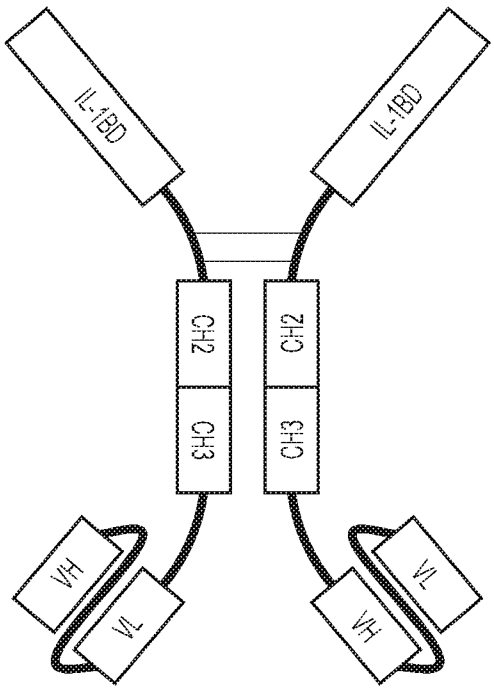
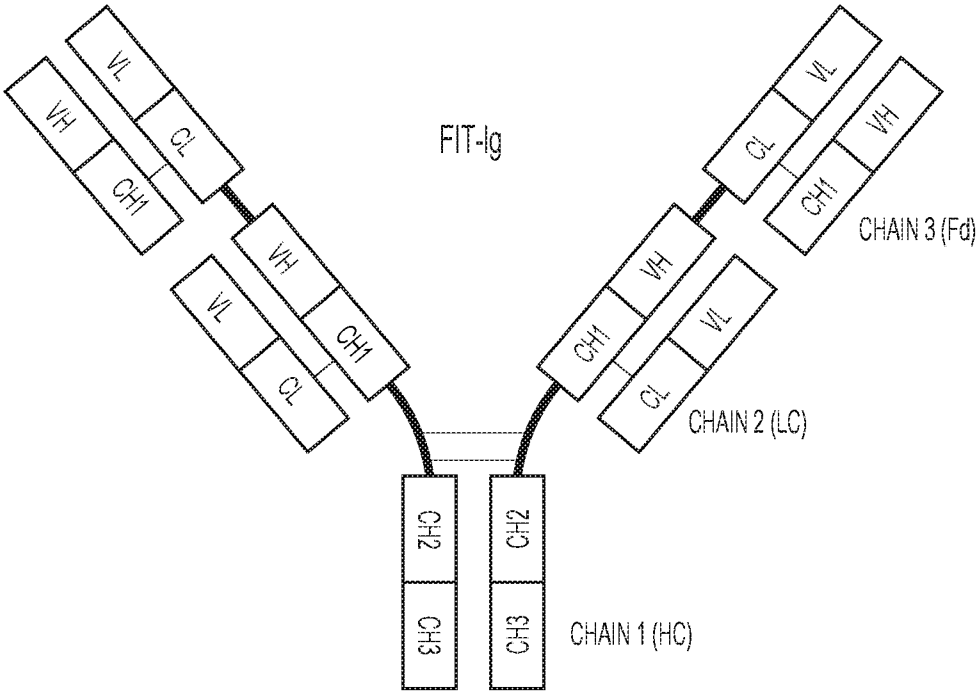


FIG.2B


FIT-Ig
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CL
VH
CH1
VH
CH1
VL
CL
VL
CL
VH
CH1
CHAIN 3 (Fd)
CH1
VH
VL
CL
CHAIN 2 (LC)
CH2
CH3
CH2
CH3
CHAIN 1 (HC)

FIG.3A

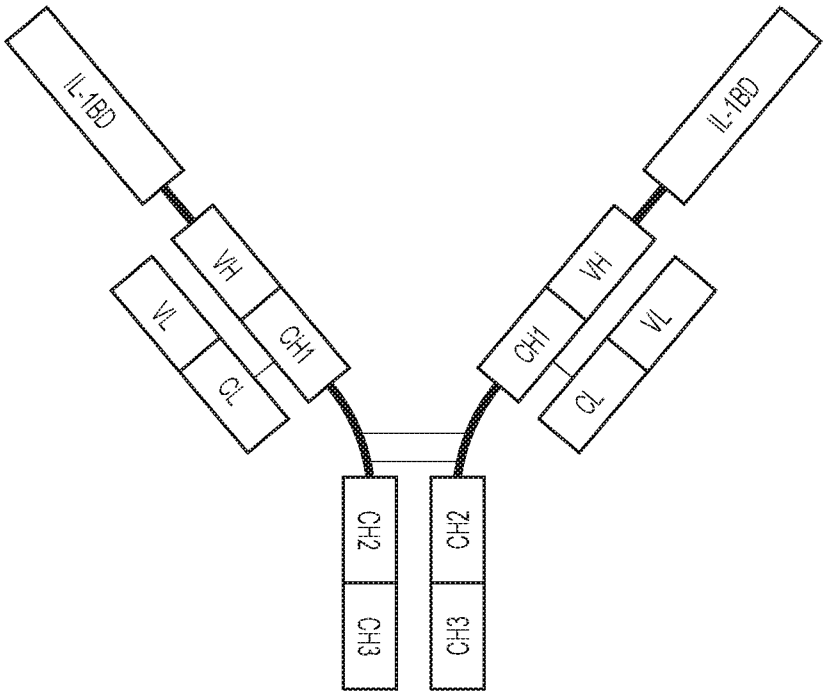

FIG.3B

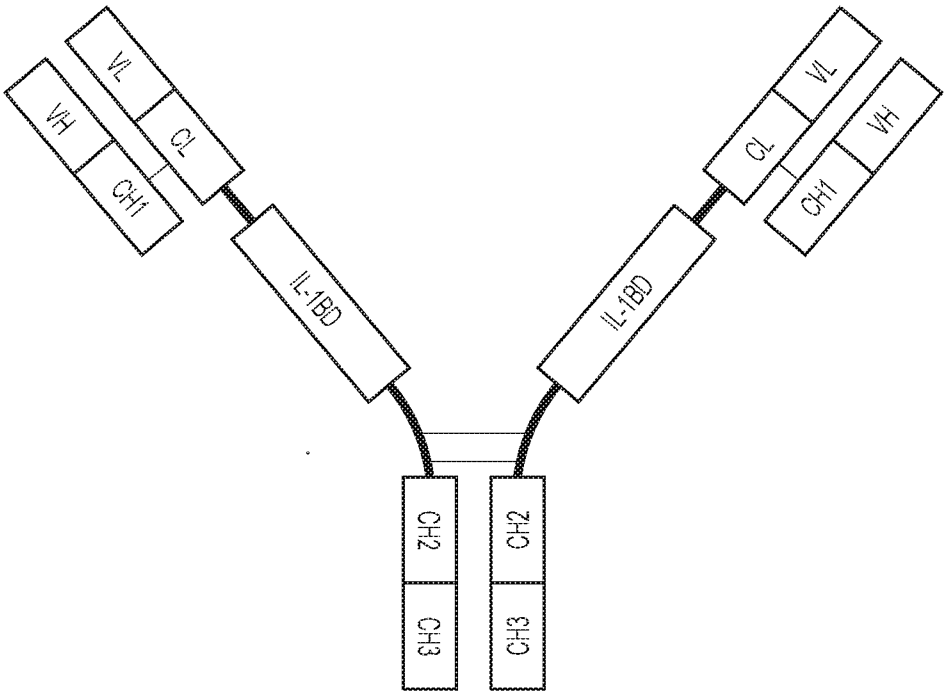

FIG.3C

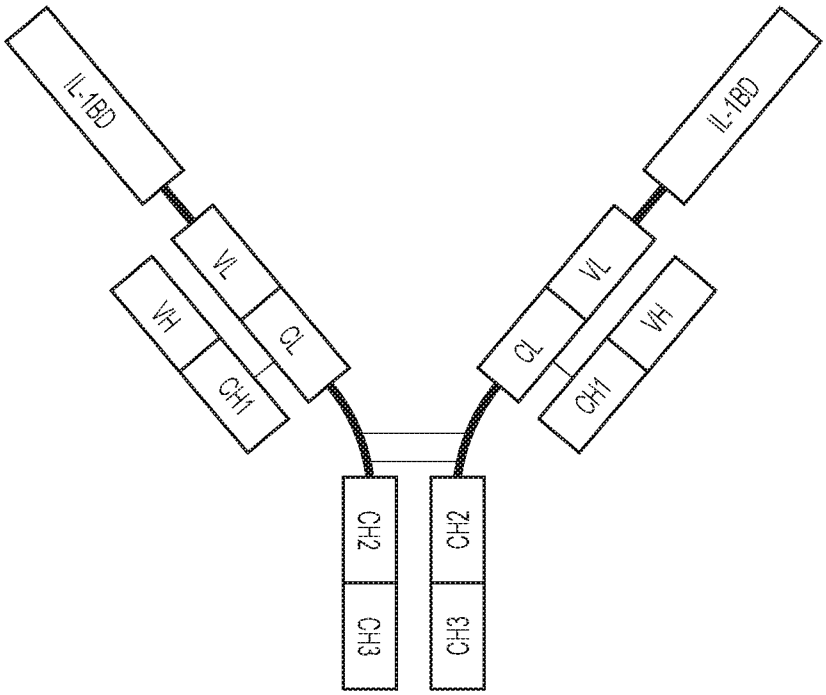

FIG.3D

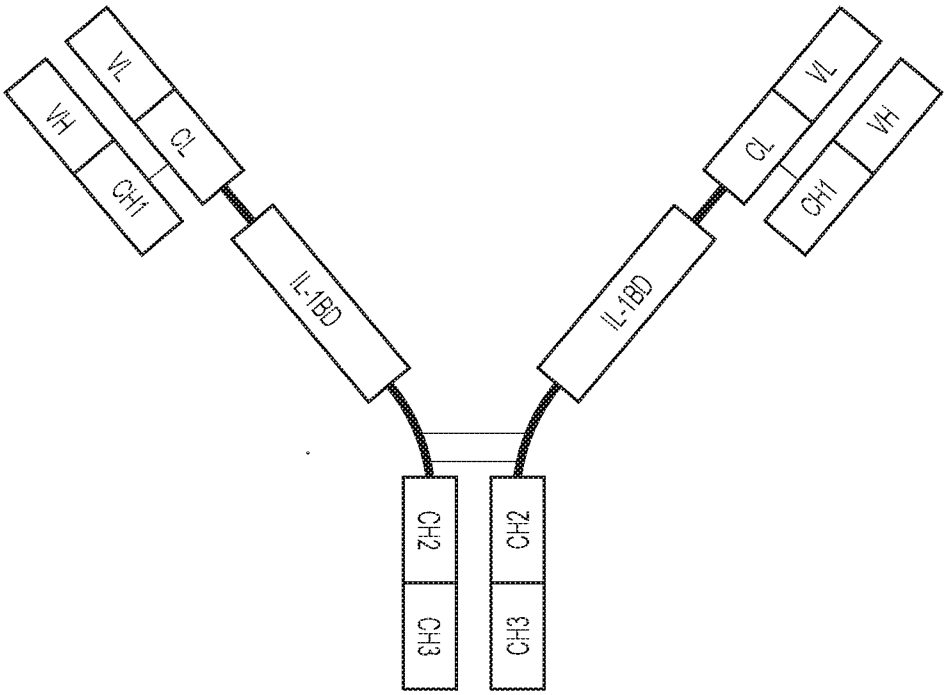

FIG.3E

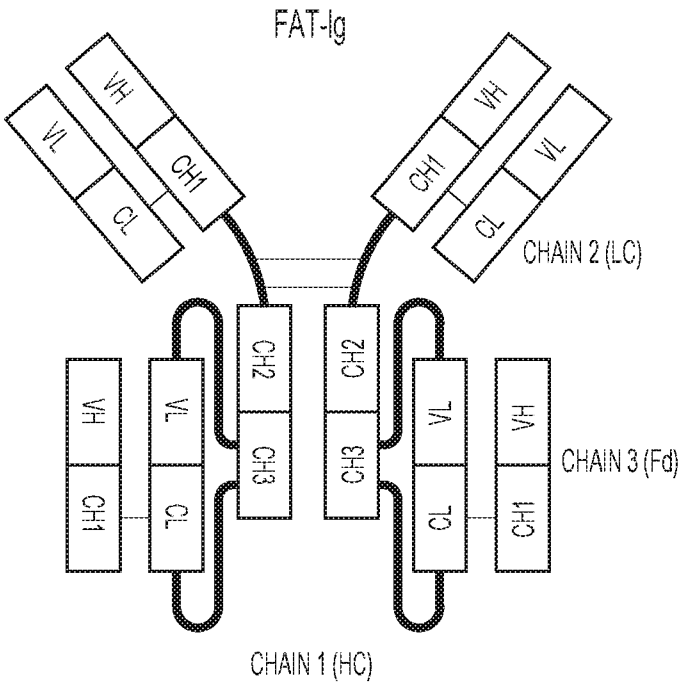

FIG.4

Figure 5: Amino acid sequences of binding proteins:

ITA101

Chain 1

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQS
PATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKGGGGSGGGGSQ
VQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWVAIIWYDGD
NQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPFDYWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPC
PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2

MGWSCIILFLVATATGVHSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQK
PDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTF
GPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

ITA102

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSP
GERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTL
TISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKGGGGSGGGGSQVQLVESGGGVV
QPGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWVAIIWYDGDNQYYADSVKGR
FTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPFDYWGQGTLVTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA101

ITA103

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPDF
QSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGT
DFTLTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDIKGGGGSGGGGSQVQLVQSGV
EVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKF
KNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV
YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHW
YQQKPGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRD
LPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC

ITA104

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPDF
QSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGT
DFTLTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDIKGGGGSGGGGSQVQLVESGG
GVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSV
KGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQK
PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTF
GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

ITA201

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHK
PSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH
YTQKSLSLSLGKGGGGSGGGGSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYY
MYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQF
DDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEI
VLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLES
GVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIK

Chain 2 same as chain 2 of ITA101

ITA202

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHK
PSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH
YTQKSLSLSLGKGGGGSGGGGSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGM
HWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAE
DTAVYYCATNDDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATL
SLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGT
DFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIK

Chain 2 same as chain 2 of ITA101

ITA203

Chain 1

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLGKGGGGSGGGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSV
YGMNWVRQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNG
LRAEDTAVYYCARDLRTGPFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEI
VLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSR
FSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDIK

Chain 2 same as chain 2 of ITA103

ITA204

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
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KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK
SLSLSLGKGGGGSGGGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWVR
QAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTAV
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SVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTD
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Chain 2 same as chain 2 of ITA 104

ITA301

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHW
YQQKPGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRD
LPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGECQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWV
AIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGP
FDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as Chain 2 of ITA101

Chain 3:

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKRVEPKSC

ITA302

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQK
PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTF
GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
ECQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWVAIIWY
DGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPFDYW
GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPP
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV
EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA
KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA101

Chain 3:

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKRVEPKSC

ITA303

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQK
PDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTF
GPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
ECQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGIN
PSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFD
YWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKY
GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA103

Chain 3:

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
PSNTKVDKRVEPKSC

ITA304

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQK
PDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTF
GPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
ECQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWY

DGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTL
VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPA
PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA104

Chain 3 same as chain 3 of ITA303

ITA401: X=2, Y=4; ITC401: X=1, Y=5; ITC402: X=3, Y=3; ITC403: X=4, Y=2;

ITC404: X=5, Y=1; ITC405: X=4, Y=4; ITC406: X=2, Y=2; ITD401: X=5, Y=5;

ITD402: X=4, Y=5; ITD403: X=5, Y=4

Chain 1:

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHK
PSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESN(GGGGS)$_X$EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPG
QAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGG
GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(
GGGGS)$_Y$QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH
YTQKSLSLSLGK

Chain 2 same as chain 2 of ITA101

Chain 3:

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKRVEPKSC

ITA402

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWV
RQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA
VYYCARDLRTGPFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHK
PSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQ
APRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG
GGGSGGGGSGGGGSGGGGSGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA101

Chain 3 same as chain 3 of ITA302

TA403

Chain 1

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD
IAVEWESNGGGGSGGGGSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKP
DQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTFG
PGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
CGGGGSGGGGSGGGGSGGGGSGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
GNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA103

Chain 3 same as chain 3 of ITA303

ITA404

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGGGGSGGGGSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLL
IKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDI
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV
TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSG
GGGSGGGGSGGGGSGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA104

Chain 3 same as chain 3 of ITA303

ITB101

Chain 1

MGWSCIILFLVATATGVHSSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNY
STAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCM
LRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKP
TITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTV
KVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK
PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKV
KQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVS
TEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNA
QAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDR
LIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEV
DLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITV
LNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNGGGGSGGGGSQVQLVQSGVE
VKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKF
KNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV

YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA103

ITB102

Chain 1

MGWSCIILFLVATATGVHSSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNY
STAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCM
LRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKP
TITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTV
KVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK
PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKV
KQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVS
TEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNA
QAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDR
LIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEV
DLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITV
LNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNGGGGSGGGGSQVQLVESGGG
VVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSVK
GRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSV
FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA104

ITB103

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGGGGSGGGGSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNY
STAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCM
LRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKP
TITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTV
KVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK

PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKV
KQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVS
TEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNA
QAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDR
LIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEV
DLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITV
LNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNSGDKTHTCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA104

ITB104

Chain 1

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNV
DHKPSNTKVDKRVESKYGGGGSGGGGSERCDDWGLDTMRQIQVFEDEPARIKCPLF
EHFLKFNYSTAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLND
TGNYTCMLRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDG
YFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTF
HLTRTLTVKVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEV
WWTIDGKKPDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKG
EVAKAAKVKQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWY
KDDSKTPVSTEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVE
NEPNLCYNAQAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLD
NIHFSGVKDRLIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVI
VSPANETMEVDLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENP
ANKRRSTLITVLNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNSGDKTHTCPPC
PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA103

ITB105

MGWSCIILFLVATATGVHSSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNY
STAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCM
LRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKP
TITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTV
KVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK
PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKV
KQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVS
TEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNA
QAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDR
LIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEV
DLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITV
LNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNSGDKTHTCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGSQVQLVESG
GGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADS
VKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSGGGGS
GGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQ
APRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQG
TKVEIK

ITB106

Chain 1

MGWSCIILFLVATATGVHSSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNY
STAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCM
LRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKP
TITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTV
KVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK
PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKV
KQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVS
TEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNA
QAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDR
LIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEV
DLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITV
LNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNGGGGSGGGGSEIVLTQSPATLS
LSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARFSGS
GSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGPPCPPCPAPEFLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA301

ITB107

Chain 1

MGWSCIILFLVATATGVHSSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNY
STAHSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCM
LRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKP
TITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTV
KVVGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKK
PDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKV
KQKVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVS
TEQASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNA
QAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDR
LIVMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEV
DLGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITV
LNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNGGGGSGGGGSEIVLTQSPATLS
LSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD
FTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGECGPPCPPCPAPEFLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA302

ITB108

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQK
PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTF
GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
ECGGGGSGGGGSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGL
TLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCS

KVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMG
CYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPK
NAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDV
TINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQKVPAP
RYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVSTEQASRIH
QHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNAQAIFKQKL
PVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDRLIVMNVAE
KHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEVDLGSQIQLI
CNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITVLNISEIESRF
YKHPFTCFAKNTHGIDAAYIQLIYPVTNSGDKTHTCPPCPAPEFLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
GNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA302

ITB109

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHW
YQQKPGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRD
LPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGECGGGGSGGGGSERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTA
HSAGLTLIWYWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRN
TTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTIT
WYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTYPENGRTFHLTRTLTVKV
VGSPKNAVPPVIHSPNDHVVYEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPD
DITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKQ
KVPAPRYTVEKCKEREEKIILVSSANEIDVRPCPLNPNEHKGTITWYKDDSKTPVSTE
QASRIHQHKEKLWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNAQ
AIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPLLLDNIHFSGVKDRLI
VMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEVD
LGSQIQLICNVTGQLSDIAYWKWNGSVIDEDDPVLGEDYYSVENPANKRRSTLITVL
NISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTNSGDKTHTCPPCPAPEFLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA301

ITE101

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKCLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSP
GERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTL
TISSLEPEDFAVYYCQQSSNWPRTFGCGTKVEIKGGGGSGGGGSQVQLVESGGGVV
QPGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWVAIIWYDGDNQYYADSVKGR
FTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPFDYWGQGTLVTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA101

ITE102

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQK
PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTF
GCGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLRLDCKASGI
TFSNSGMHWVRQAPGKCLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQ
MNSLRAEDTAVYYCATNDDYWGQGTLVTVSSGGGGSGGGGSQVQLVESGGGVVQ
PGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWVAIIWYDGDNQYYADSVKGRF
TISRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPFDYWGQGTLVTVSSASTKGP
SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA101

ITE201

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP

VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK
SLSLSLGKGGGGSGGGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWVR
QAPGKCLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTAV
YYCARDLRTGPFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPDFQ
SVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTD
FTLTINSLEAEDAAAYYCHQSSSLPFTFGCGTKVDIK

Chain 2 same as chain 2 of ITA104

ITE202

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK
SLSLSLGKGGGGSGGGGSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKP
DQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTFG
CGTKVDIKGGGGSGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLRLSCAASGFT
FSVYGMNWVRQAPGKCLEWVAIIWYDGDNQYYADSVKGRFTISRDNSKNTLYLQM
NGLRAEDTAVYYCARDLRTGPFDYWGQGTLVTVSS

Chain 2 same as chain 2 of ITA104

ITE301

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQK
PDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTF
GPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
ECGSGQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMG
GINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDM
GFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE

SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

Chain 2 and chain 3 same as chain 2 and chain 3 of ITA301

ITE302

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQK
PDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTF
GPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
ECGGGGSGSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGL
EWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDY
RFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT
VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV
QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGK

Chain 2 and chain 3 same as chain 2 and chain 3 of ITA301

ITF101

Chain 1

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQK
PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTF
GCGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLRLDCKASGI
TFSNSGMHWVRQAPGKCLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQ
MNSLRAEDTAVYYCATNDDYWGQGTLVTVSSGGGGSGGGGSEVQLVESGGGVVQ
PGRSLRLSCSSSGFIFSSYDMSWVRQAPGKGLEWVAYISSGGGGTYYPDTVKGRFTIS
RDNSKNTLFLQMDSLRPEDTGVYFCARGGVTKGYFDVWGQGTPVTVSSASTKGPSV
FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2

MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASGNIHNYLTWYQQ
TPGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHFWSIPYT
FGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

ITF102

Chain 1

MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASGNIHNYLTWYQQ
TPGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHFWSIPYT
FGCGTKLEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCSSSGF
IFSSYDMSWVRQAPGKCLEWVAYISSGGGGTYYPDTVKGRFTISRDNSKNTLFLQM
DSLRPEDTGVYFCARGGVTKGYFDVWGQGTPVTVSSGGGGSGGGGSQVQLVESGG
GVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSV
KGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain2 of ITA104

ITF103

Chain 1

MGWSCIILFLVATATGVHSEVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYDMSWVR
QAPGKCLEWVAYISSGGGGTYYPDTVKGRFTISRDNSKNTLFLQMDSLRPEDTGVYF
CARGGVTKGYFDVWGQGTPVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSL
SASVGDRVTITCRASGNIHNYLTWYQQTPGKAPKLLIYNAKTLADGVPSRFSGSGSG
TDYTFTISSLQPEDIATYYCQHFWSIPYTFGCGTKLEIKGGGGSGGGGSQVQLVESGG
GVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSV
KGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain2 of ITA104

ITF201

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK
SLSLSLGKGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASGNIHNYLTWYQQT
PGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHFWSIPYTF
GCGTKLEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCSSSGFI
FSSYDMSWVRQAPGKCLEWVAYISSGGGGTYYPDTVKGRFTISRDNSKNTLFLQMD
SLRPEDTGVYFCARGGVTKGYFDVWGQGTPVTVSS

Chain 2 same as ITA104

ITF202

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK
SLSLSLGKGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYDMSWVRQ
APGKCLEWVAYISSGGGGTYYPDTVKGRFTISRDNSKNTLFLQMDSLRPEDTGVYFC
ARGGVTKGYFDVWGQGTPVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLS
ASVGDRVTITCRASGNIHNYLTWYQQTPGKAPKLLIYNAKTLADGVPSRFSGSGSGT
DYTFTISSLQPEDIATYYCQHFWSIPYTFGCGTKLEIK

Chain 2 same as ITA104

ITF301

Chain 1

MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASGNIHNYLTWYQQ
TPGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHFWSIPYT
FGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GECGSGQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWM
GGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFD
MGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS
IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL
GK

Chain 2 same as chain 2 of ITA103

Chain 3

MGWSCIILFLVATATGVHSEVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYDMSWVR
QAPGKGLEWVAYISSGGGGTYYPDTVKGRFTISRDNSKNTLFLQMDSLRPEDTGVYF
CARGGVTKGYFDVWGQGTPVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSC

ITF302

Chain 1

MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASGNIHNYLTWYQQ
TPGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHFWSIPYT
FGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GECGGGGSGSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQG
LEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRD
YRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV
DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS
LSLSLGK

Chain 2 and Chain 3 same as chain 2 and chain 3 of ITF301

ITF401

Chain 1

MGWSCIILFLVATATGVHSEVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYDMSWVR
QAPGKGLEWVAYISSGGGGTYYPDTVKGRFTISRDNSKNTLFLQMDSLRPEDTGVYF
CARGGVTKGYFDVWGQGTPVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS
NTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGGGGSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKG
VSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDF
AVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT
HQGLSSPVTKSFNRGECGGGGSGGGGSGGGGSGGGGSGGGGSGQPENNYKTTPPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITF101

Chain 3 same as chain 3 of ITA301

ITF402

Chain 1

MGWSCIILFLVATATGVHSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYW
VRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTA
VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD
IAVEWESNGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCR
ASGNIHNYLTWYQQTPGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPED
IATYYCQHFWSIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT
HQGLSSPVTKSFNRGECGGGGSGGGGSGGGGSGGGGSGGGGSGQPENNYKTTPPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 and Chain 3 same as chain 2 and chain 3 of ITF302

ITF403

Chain 1

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWV
RQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTA
VYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASGNIHN
YLTWYQQTPGKAPKLLIYNAKTLADGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQ
HFWSIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGECGGGGSGGGGSGGGGSGGGGSGGGGSGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Chain 2 same as chain 2 of ITA104

Chain 3 same as chain 3 of ITF402

Binding protein	Summary
ABT-981 (Abbvie)	ABT-981 is an anti IL-1 alpha/ß dual variable domain immunoglobulin (DVD-Ig), under development for the treatment of osteoarthritis (OA)
Anakinra (Biovitrum)	Anakinra is a recombinant non-glycosylated, human interleukin-1 receptor antagonist
Avelumab (Merck KGaA)	Avelumab (MSB-0010718C) is an MAb against PD-L1 (programmed cell death ligand), developed for the treatment of solid tumours
BCD-100, (Biocad)	BCD-100 is an anti-PD1 monoclonal antibody, for the treatment of melanoma, non-small cell lung cancer and renal cell carcinoma
Bermekimab (XBiotech)	Bermekimab (MABp1) is a human IgG1 monoclonal antibody specific for human interleukin-1 alpha, for the treatment of peripheral vascular disease, type 2 diabetes, pyoderma gangrenosum, hiradentis suppurtiva, colorectal cancer, NSCLC, pancreatic cancer, psoriasis, acne, atopic dermatitis and cachexia.
BGB-A333 (BeiGene)	BGB-A333 is a humanized IgG1-variant monoclonal antibody against programmed cell death 1-ligand 1 (PD-L1), under development by for the treatment of cancer
Camrelizumab (Jiangsu Hengrui Medicine)	Camrelizumab (SHR-1210) is a humanized anti-PD1 IgG4 monoclonal antibody, under development by for the treatment of cancer
Canakinumab (Novartis)	Canakinumab (ACZ-885) is an anti-interleukin-1ß human MAb, developed for the treatment of cryopirin-associated periodic syndromes (CAPS) (familial cold urticaria syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID) (chronic infertile neurological cutaneous articular syndrome (CINCA))

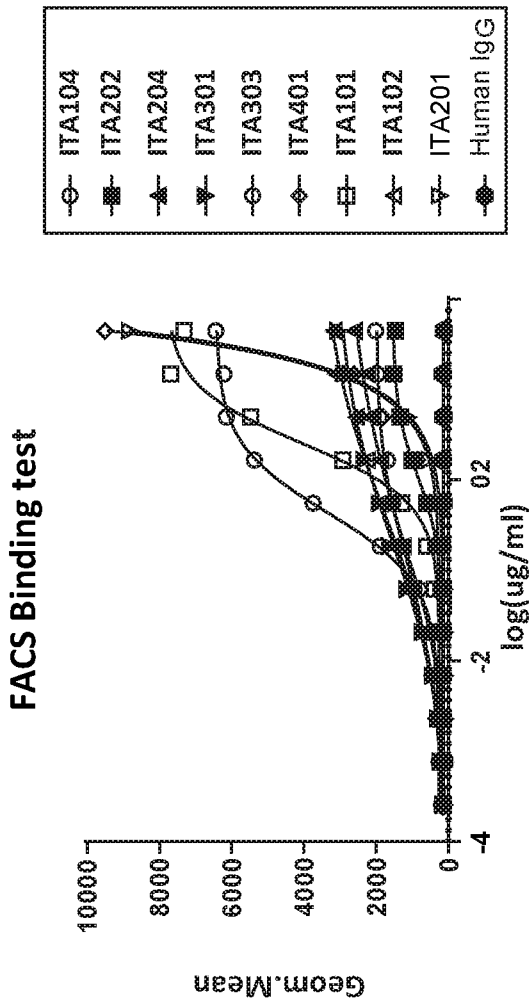
FIG.6A

Cemiplimab (Regeneron)	Cemiplimab (REGN-2810) is a fully-humanized monoclonal antibody targeting PD-1, developed for the treatment of cancer
CS-1001 (CStone Pharmaceuticals)	CS-1001 (WBP3155) is a fully humanized, recombinant anti PD-L1 IgG monoclonal antibody, under development for the treatment of cancer
Durvalumab (MedImmune)	Durvalumab (MEDI-4736) is an anti-PDL1 MAb, developed for the treatment of cancer.
envafolimab (Alphamab)	Envafolimab (KN-035) is a nanobody targeting PDL1, under development for the treatment of cancer
Gevokizumab (Xoma)	Gevokizumab (XOMA-052) is an anti-inflammatory humanized IgG2 MAb that binds to interleukin-1ß (IL-1ß), for the treatment of pyoderma gangrenosum.
JNJ-63723283 (Johnson & Johnson)	JNJ-3283 (JNJ-63723283) is an anti-PD-1 monoclonal antibody, under development for the treatment of cancer
M-7824 (Merck KGaA)	M-7824 (MSB0011359C) is a bi-functional fusion protein targeting PD-L1 monoclonal antibody and TGFß, for treatment of cancer
MEDI-0680 (MedImmune)	MEDI-0680 (AMP-514) is an anti-PD-1 monoclonal antibody, under development for the treatment of cancer and infectious disease
MGA-012 (MacroGenics)	MGA-012 is a humanized anti-PD-1 monoclonal antibody, under development for the treatment of solid tumours
Nivolumab (BMS)	Nivolumab (MDX-1106; ONO-4538) is a fully-human IgG4 MAb against PD-1, for the treatment of cancer and chronic viral infections
Rilonacept (Regeneron)	Rilonacept (RGN-303; Arcalyst) is a human Cytokine Trap protein which blocks the activity of interleukin-1a and interleukin-1b, for the treatment of cryopyrin-associated periodic syndrome (CAPS), a spectrum of rare inflammatory disorders including Muckle-Wells syndrome (MWS), familial cold

FIG.6B

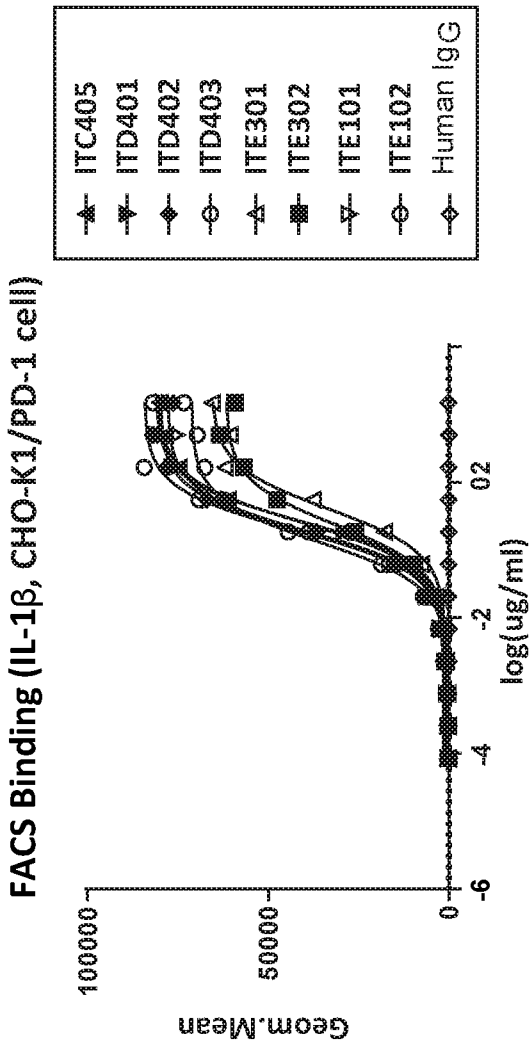
	inflammatory syndrome (FCAS) and certain other conditions.
Sintilimab (Innovent Biologics)	Sintilimab is a fully human anti-PD-1 monoclonal antibody, developed for the treatment of cancer
SOBI-006 (Affibody)	SOBI-006 is an interleukin-1 (IL-1) inhibitor, under development for the treatment of inflammatory and autoimmune diseases
Spartalizumab (Novartis)	Spartalizumab (PDR-001) is a PD-1 humanized IgG4 antibody, under development for the treatment of cancer

FIG.6C



	ITA104	ITA202	ITA204	ITA301	ITA303	ITA401	ITA101	ITA102
EC50	0.1268	1.090	0.3688	0.2153	0.4375	~ 124541	2.541	0.4724

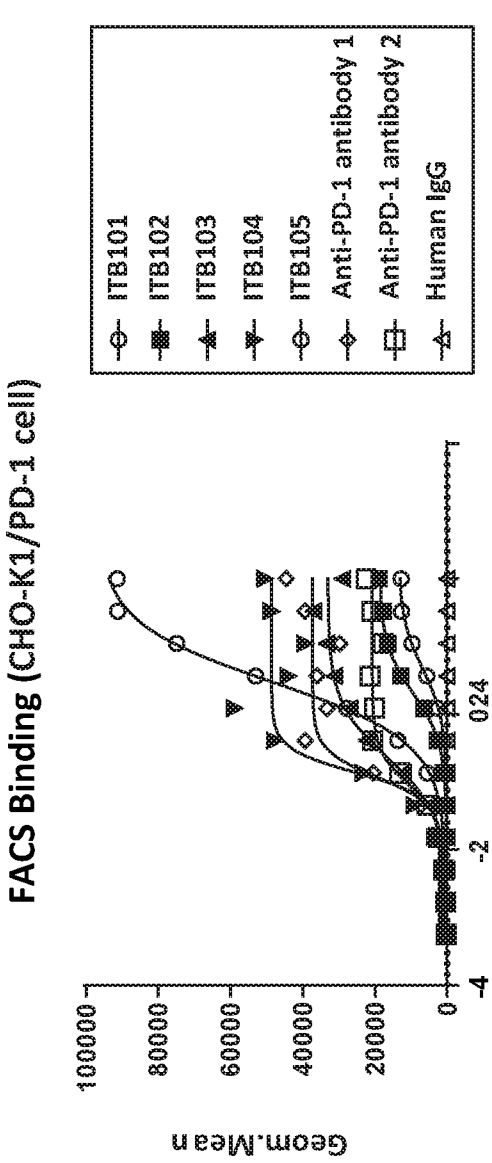
FIG.7



	ITC405	ITD401	ITD402	ITD403
EC50	0.2576	0.2680	0.1984	0.1977

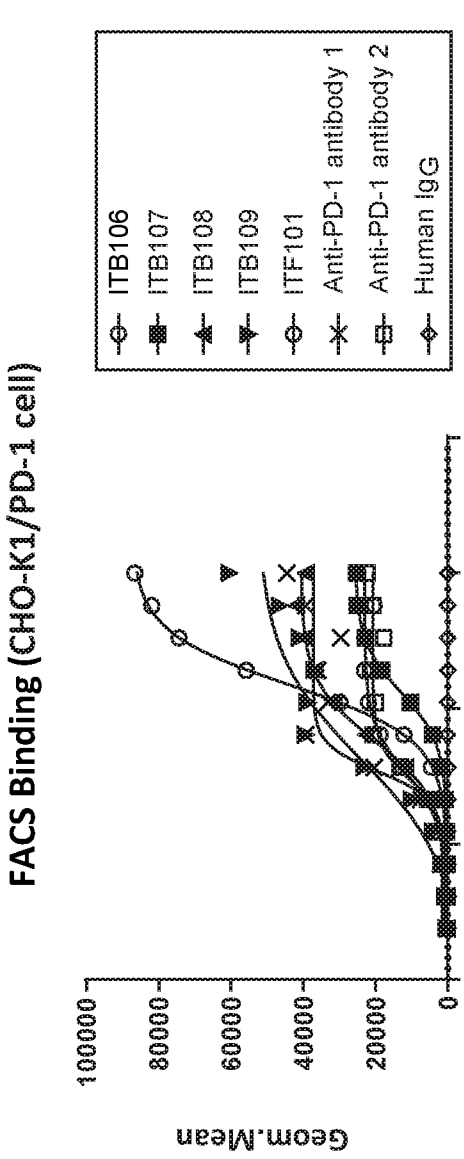
	ITE301	ITE302	ITE101	ITE102
EC50	0.4063	0.2365	0.1893	0.1314

FIG.8



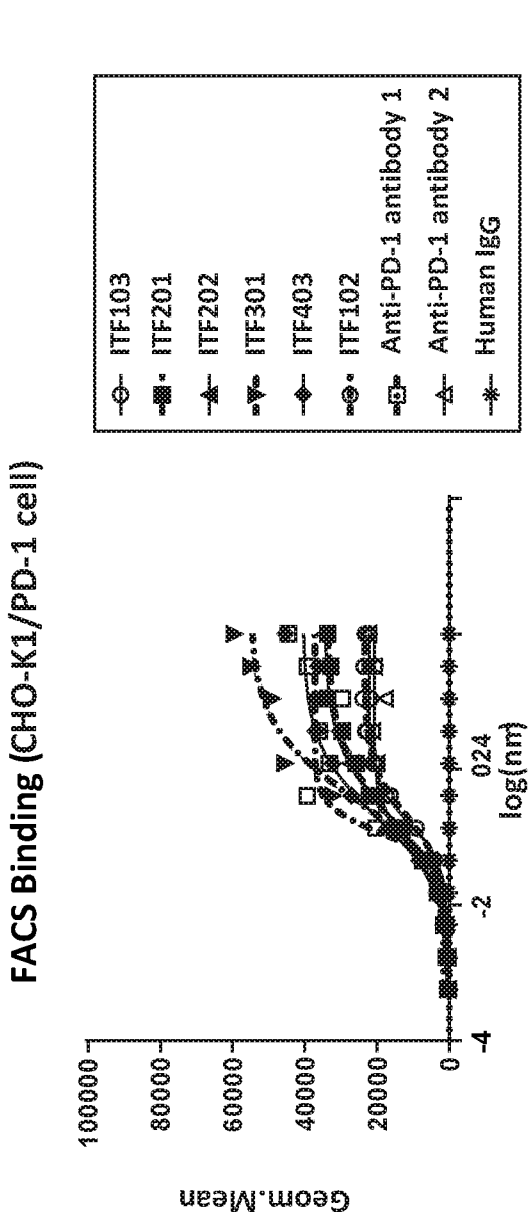
	ITB101	ITB102	ITB103	ITB104	ITB105	Anti-PD-1 antibody 1	Anti-PD-1 antibody 2	Human IgG
EC50	3.024	2.068	0.2304	0.1394	4.739	0.1188	0.09180	26.15

FIG.9A



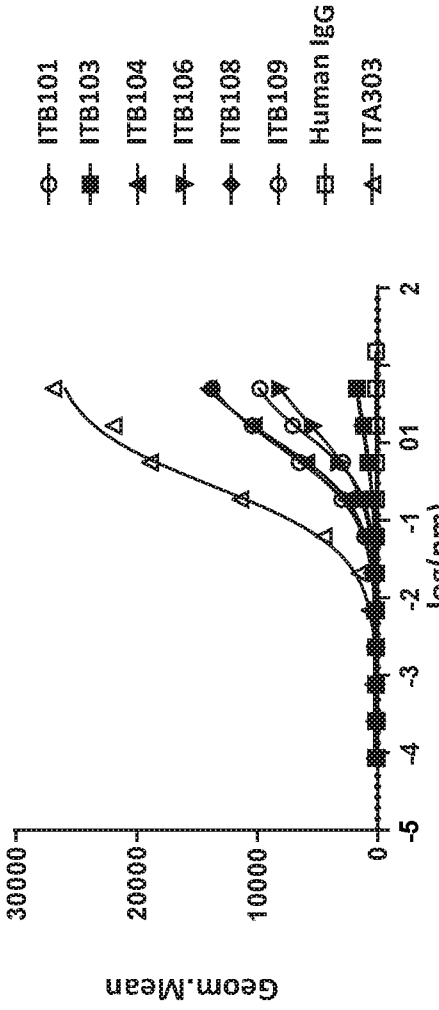
	ITB106	ITB107	ITB108	ITB109	ITF101	Anti-PD-1 antibody 1	Anti-PD-1 antibody 2	Human IgG
EC50	2.244	1.752	0.3413	0.1852	0.1149	0.1188	0.09180	26.15

FIG.9B



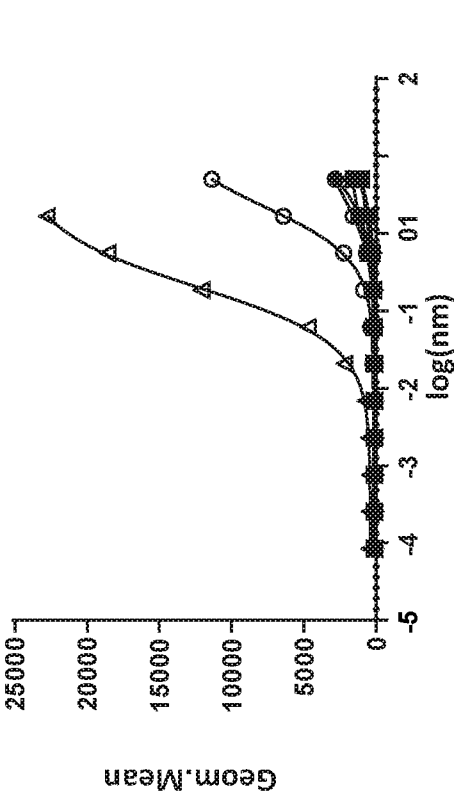
	ITF103	ITF201	ITF202	ITF301	ITF403	ITF102	Anti-PD-1 antibody 1	Anti-PD-1 antibody 2	Human IgG
EC50	0.1716	0.2061	0.2088	0.3939	0.2014	0.2090	0.1188	0.09180	26.15

FIG.9C



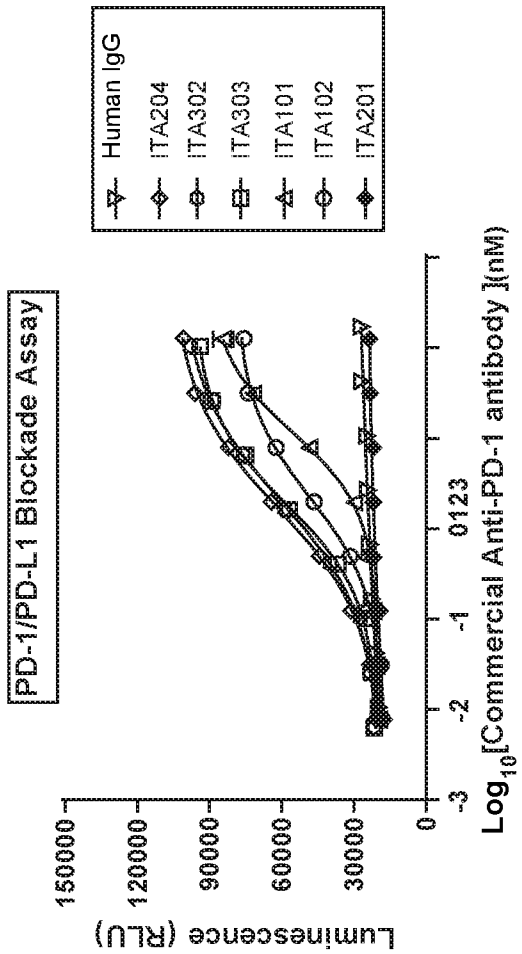
	ITB101	ITB103	ITB104	ITB106	ITB108	ITB109	Human IgG	ITA303
EC50	1.260	2.893	1.092	1.715	1.472	0.8741	~ 3.022e+019	0.2762

FIG.10A



	ITF101	ITF102	ITF103	ITF201	ITF202	ITF301	ITF403	ITA303
EC50	2.620	1.122	2.344	~ 11565	6.566	2.174	~ 55712	0.2049

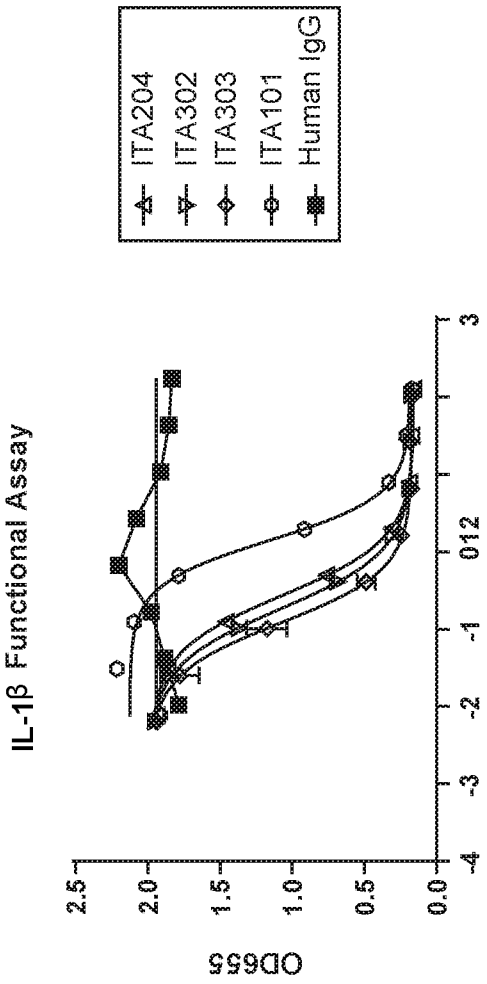
FIG.10B



	Human IgG
EC50	~ 1.065e-019

ITA204	ITA302	ITA303	ITA101	ITA102	ITA201
1.751	2.118	1.927	11.81	2.239	0.01105

FIG.11



	ITA204	ITA302	ITA303
EC50	0.2719	0.1817	0.1201

FIG.12A

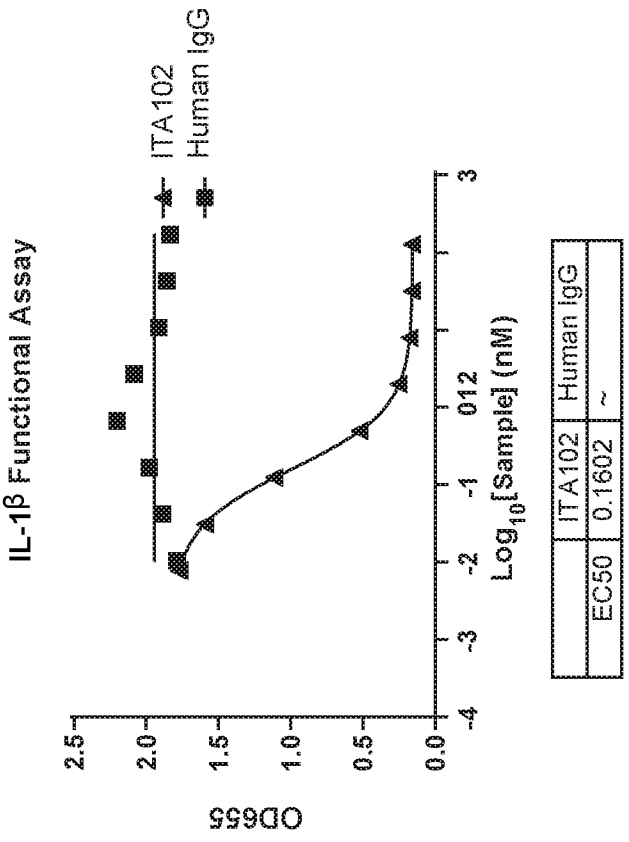

IL-1β Functional Assay
ITA102
Human IgG
OD655
2.5
2.0
1.5
1.0
0.5
0.0
-4
-3
-2
-1
012
3
Log10[Sample] (nM)
ITA102
Human IgG
EC50
0.1602
~

FIG.12B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025979

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/24; C07K 16/28 (2020.01)

CPC - C07K 16/245; C07K 16/2803; C07K 16/2866; C07K 2317/31; C07K 2317/76; C07K 2319/30; C07K 2319/32 (2020.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017/0298106 A1 (ZYNGENIA, INC.) 19 October 2017 (19.10.2017) entire document	1-5
--		--------
Y		22-28
Y	WO 2018/119001 A1 (FRED HUTCHINSON CANCER RESEARCH CENTER) 28 June 2018 (28.06.2018) entire document	22-28
Y	US 2018/0022807 A1 (MEDIMMUNE, LLC) 25 January 2018 (25.01.2018) entire document	34-44
Y	US 2016/0009824 A1 (MERCK PATENT GMBH) 14 January 2016 (14.01.2016) entire document	34-44
A	US 2017/0037131 A1 (BERNETT et al) 09 February 2017 (09.02.2017) entire document	1-5, 22-28, 34-44
A	US 2018/0264130 A1 (THE JOHNS HOPKINS UNIVERSITY) 20 September 2018 (20.09.2018) entire document	1-5, 22-28, 34-44

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 July 2020	12 AUG 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025979

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1-30, 37-48, 57, 58, 68, and 69 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/025979

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-21, 29-33, 45-76
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.