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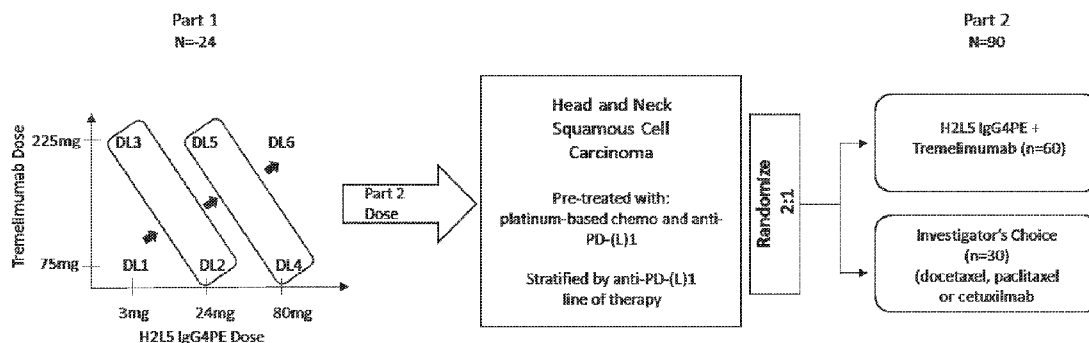
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(54) Title: DOSING REGIMEN FOR THE TREATMENT OF CANCER WITH AN ANTI ICOS AGONISTIC ANTIBODY AND TREMELIMUMAB

FIG. 5



(57) Abstract: The present disclosure relates to a method of treating cancer in a human in need thereof, the method comprising administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 0.08 mg to about 240 mg and administering to the human tremelimumab.

Dosing

FIELD OF THE INVENTION

The present invention relates to a method of treating cancer in a human. In particular, the present invention relates to dosing of a combination of an agonist anti-ICOS antibody and tremelimumab.

BACKGROUND TO THE INVENTION

Effective treatment of hyperproliferative disorders, including cancer, is a continuing goal in the oncology field. Generally, cancer results from the deregulation of the normal processes that control cell division, differentiation and apoptotic cell death and is characterized by the proliferation of malignant cells which have the potential for unlimited growth, local expansion and systemic metastasis. Deregulation of normal processes includes abnormalities in signal transduction pathways and response to factors that differ from those found in normal cells.

Immunotherapies are one approach to treat hyperproliferative disorders. A major hurdle that scientists and clinicians have encountered in the development of various types of cancer immunotherapies has been to break tolerance to self antigen (cancer) in order to mount a robust anti-tumor response leading to tumor regression. Unlike traditional development of small and large molecule agents that target the tumor, cancer immunotherapies target cells of the immune system that have the potential to generate a memory pool of effector cells to induce more durable effects and minimize recurrences.

Though there have been many recent advances in the treatment of cancer, there remains a need for more effective and/or enhanced treatment of an individual suffering the effects of cancer. The methods herein that relate to combining therapeutic approaches for enhancing anti-tumor immunity address this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, there is provided a method of treating cancer in a human in need thereof, the method comprising administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 0.08 mg to about 240 mg and administering to the human tremelimumab.

In another aspect of the invention, there is provided an agonist ICOS binding protein or antigen binding portion thereof for use in treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered with tremelimumab.

In a further aspect of the invention, there is provided a combination of an agonist ICOS binding protein or antigen binding portion thereof and tremelimumab for use in treating cancer for use in treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 0.08 mg to about 240 mg.

5 In yet another aspect of the invention, there is provided an agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament for treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered with tremelimumab.

10 In another aspect of the invention, there is provided a pharmaceutical kit comprising an ICOS binding protein or an antigen binding portion thereof at about 10 mg/ml and tremelimumab at about 20 mg/ml.

In one aspect of the invention, there is provided a composition comprising an agonist ICOS binding protein or antigen binding portion thereof at about 0.08 mg to about 240 mg and tremelimumab at about 8 mg to about 1200 mg.

15 In one embodiment of the method, agonist ICOS binding protein, combination, use, or kit of the invention, the agonist ICOS binding protein or antigen binding portion thereof comprises one or more of: CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and/or CDRL3 as set forth in SEQ ID NO:6 or a direct equivalent of each CDR, wherein a direct equivalent has no
20 more than two amino acid substitutions in said CDR.

In another embodiment of the method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of the invention, the agonist ICOS binding protein or antigen binding portion thereof comprises a VH domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a VL domain comprising an amino acid sequence at least
25 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said agonist ICOS binding protein specifically binds to human ICOS.

In one embodiment of the method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of the invention, the agonist ICOS binding protein is a monoclonal antibody.

30 In another embodiment of the method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of the invention, the agonist ICOS binding protein is a humanized or fully human monoclonal antibody.

In one embodiment of the method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of the invention, the agonist ICOS binding protein comprises an hIgG4PE scaffold.

35 In some embodiments of the method, agonist ICOS binding protein, combination or use, any one of the invention, the agonist ICOS binding protein or antigen binding portion thereof is

administered at a dose of about 0.08 mg, about 0.24 mg, about 0.8 mg, about 2.4 mg, about 8 mg, about 24 mg, about 80 mg, or about 240 mg.

5 In another embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 24 mg.

In one embodiment of the method, agonist ICOS binding protein, a combination or use of the invention, the agonist ICOS binding protein or antigen binding portion thereof is administered once every three weeks or every six weeks.

10 In another embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the agonist ICOS binding protein or antigen binding portion thereof and/or tremelimumab is administered via IV infusion.

In a further embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the cancer is a solid tumor. In another embodiment, of the method, agonist ICOS binding protein, combination or use of the invention, the cancer is selected from NSCLC, HNSCC, urothelial cancer, cervical cancer and melanoma. In one embodiment, the cancer is NSCLC.

In another embodiment of the method, agonist ICOS binding protein, combination or use of the invention, tremelimumab is administered at a dose of about 8 mg to about 1200 mg.

In a further embodiment of the method, agonist ICOS binding protein, combination or use of the invention, tremelimumab is administered at a dose of about 75 mg to about 225 mg.

20 In one embodiment of the method, agonist ICOS binding protein, combination or use of the invention, tremelimumab is administered once every three weeks or 12 weeks.

In another embodiment of the method, agonist ICOS binding protein, combination or use of the invention, tremelimumab is administered once every three weeks for 6 doses and every 12 weeks thereafter.

25 In a further embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the agonist ICOS binding protein is administered at a dose of about 0.08 mg, about 0.24 mg, about 0.8 mg, about 2.4 mg, about 8 mg, about 24 mg, about 80 mg, or about 240 mg every three weeks, and tremelimumab is administered at a dose of about 7.5 mg, about 75 mg, about 225 mg, about 750 mg or about 1125 mg every three weeks for 6 doses and every 12 weeks thereafter.

30 In another embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the agonist ICOS binding protein or antigen binding portion thereof comprises CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and CDRL3 as set forth in SEQ ID NO:6, and tremelimumab is administered at a dose of about 75 mg or about 225 mg every three weeks for 6 doses and every 12 weeks thereafter.

In yet a further embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the agonist ICOS binding protein or antigen binding portion thereof comprises CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and CDRL3 as set forth in SEQ ID NO:6, and tremelimumab is administered at a dose of about 75 mg or about 225 mg every three weeks for 6 doses and every 12 weeks thereafter; and tremelimumab is administered first as an IV infusion and the agonist ICOS binding protein or antigen binding portion thereof is administered as an IV infusion beginning at least 1 hour and no more than 2 hours following the end of the administration of tremelimumab.

In another embodiment of the method, agonist ICOS binding protein, combination or use of the invention, the agonist ICOS binding protein or antigen binding portion thereof and tremelimumab is administered concurrently and/or sequentially. It is to be understood that the embodiments described in the application relate to the method of treatment, the agonist ICOS binding protein or antigen binding portion thereof for use, combination for use, use of the agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament, the composition and the pharmaceutical kit of the invention.

DESCRIPTION OF DRAWINGS/FIGURES

FIG. 1 is the result from a patient case study showing a set of scans of Patient 1 (H2L5 IgG4PE monotherapy treatment).

FIGS. 2A-2B are plots showing duration of H2L5 IgG4PE monotherapy treatment: individual patient data. FIG. 2A shows monotherapy dose escalation cohort. FIG. 2B shows PK/PD cohort.

FIG. 3A-3D are plots showing PK and receptor occupancy. FIG 3A shows dose-proportional PK from 0.01 mg/kg to 3 mg/kg; FIG. 3B shows peak receptor occupancy corresponding to maximum plasma concentration; similar relationship for CD8+ receptor occupancy (data not shown). FIG. 3C shows CD4⁺RO with H2L5 IgG4PE 0.3 mg/kg and 1.0 mg/kg monotherapy (Part 1A) FIG. 3D is a plot showing receptor occupancy (RO) H2L5 IgG4PE concentration.

FIGS. 4A-4C show PK/PD and immunofluorescence data characterising immune phenotype of TIL. FIG. 4A shows cytotoxic T cell to Treg ratio across H2L5 IgG4PE concentrations. FIG. 4B shows MultiOmyxTM dose-response curves. FIG 4C. shows ratio of cytotoxic T cell proliferation:Treg proliferation.

FIG. 5 shows diagram showing the study design of H2L5 IgG4PE and tremelimumab combination study.

DETAILED DESCRIPTION OF THE INVENTION

5 DEFINITIONS

Antigen Binding Protein (ABP)" means a protein that binds an antigen, including antibodies or engineered molecules that function in similar ways to antibodies. Such alternative antibody formats include triabody, tetrabody, miniantibody, and a minibody. Also included are alternative scaffolds in which the one or more CDRs of any molecules in accordance with the disclosure can be arranged onto a suitable non-immunoglobulin protein scaffold or skeleton, such as an affibody, a SpA scaffold, an LDL receptor class A domain, an avimer (see, e.g., U.S. Patent Application Publication Nos. 2005/0053973, 2005/0089932, 2005/0164301) or an EGF domain. An ABP also includes antigen binding fragments of such antibodies or other molecules. Further, an ABP may comprise the V_H regions of the invention formatted into a full length antibody, a (Fab')₂ fragment, a Fab fragment, a bi-specific or biparatopic molecule or equivalent thereof (such as scFv, bi- tri- or tetra-bodies, TANDABS etc.), when paired with an appropriate light chain. The ABP may comprise an antibody that is an IgG1, IgG2, IgG3, or IgG4; or IgM; IgA, IgE or IgD or a modified variant thereof. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain. The ABP may also be a chimeric antibody of the type described in WO86/01533, which comprises an antigen binding region and a non-immunoglobulin region. The terms "ABP", "antigen binding protein", "binding protein", "antigen binding agent" and "binding agent" are used interchangeably herein. For example, disclosed herein are agonist ICOS binding proteins.

"Antigen binding site" refers to a site on an antigen binding protein that is capable of specifically binding to an antigen, this may be a single variable domain, or it may be paired V_H/V_L domains as can be found on a standard antibody. Single-chain Fv (scFv) domains can also provide antigen-binding sites.

The term "antibody" is used herein in the broadest sense to refer to molecules comprising an immunoglobulin-like domain (for example IgG, IgM, IgA, IgD or IgE) and includes monoclonal, recombinant, polyclonal, chimeric, human, humanized, multispecific antibodies, including bispecific antibodies, and heteroconjugate antibodies; a single variable domain (e.g. V_H, V_{HH}, V_L, domain antibody (DAB)), antigen binding antibody fragments, Fab, F(ab')₂, Fv, disulphide linked Fv, single chain Fv, disulphide-linked scFv, diabodies, TANDABS, etc. and modified versions of any of the foregoing (for a summary of alternative "antibody" formats see, e.g. Holliger and Hudson, Nature Biotechnology, 2005, Vol 23, No. 9, 1126-1136).

A "chimeric antibody" refers to a type of engineered antibody that contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

5 A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g. Queen et al. Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al. Bio/Technology, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g. the KABAT database, Los Alamos database, 10 and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar 15 manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanized antibodies – see, for example, EP-A-0239400 and EP-A-054951.

The term "fully human antibody" includes antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies 20 of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g. mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). Fully human antibodies comprise amino acid sequences encoded only by polynucleotides that are ultimately of human origin or amino acid sequences that are identical to such sequences. As meant herein, antibodies encoded by human immunoglobulin-encoding DNA inserted 25 into a mouse genome produced in a transgenic mouse are fully human antibodies since they are encoded by DNA that is ultimately of human origin. In this situation, human immunoglobulin-encoding DNA can be rearranged (to encode an antibody) within the mouse, and somatic mutations may also occur. Antibodies encoded by originally human DNA that has undergone such changes in a mouse are fully human antibodies as meant herein. The use of such transgenic mice makes it possible to select 30 fully human antibodies against a human antigen. As is understood in the art, fully human antibodies can be made using phage display technology wherein a human DNA library is inserted in phage for generation of antibodies comprising human germline DNA sequence.

The term full, whole or intact antibody, used interchangeably herein, refers to a heterotetrameric glycoprotein with an approximate molecular weight of 150,000 daltons. An intact 35 antibody is composed of two identical heavy chains (HCs) and two identical light chains (LCs) linked by covalent disulphide bonds. This H2L2 structure folds to form three functional domains comprising

two antigen-binding fragments, known as 'Fab' fragments, and a 'Fc' crystallisable fragment. The Fab fragment is composed of the variable domain at the amino-terminus, variable heavy (VH) or variable light (VL), and the constant domain at the carboxyl terminus, CH1 (heavy) and CL (light). The Fc fragment is composed of two domains formed by dimerization of paired CH2 and CH3 regions. The Fc may elicit effector functions by binding to receptors on immune cells or by binding C1q, the first component of the classical complement pathway. The five classes of antibodies IgM, IgA, IgG, IgE and IgD are defined by distinct heavy chain amino acid sequences which are called μ , α , γ , ϵ and δ respectively, each heavy chain can pair with either a κ or λ light chain. The majority of antibodies in the serum belong to the IgG class, there are four isotypes of human IgG, IgG1, IgG2, IgG3 and IgG4, the sequences of which differ mainly in their hinge region.

Fully human antibodies can be obtained using a variety of methods, for example using yeast-based libraries or transgenic animals (e.g. mice) which are capable of producing repertoires of human antibodies. Yeast presenting human antibodies on their surface which bind to an antigen of interest can be selected using FACS (Fluorescence-Activated Cell Sorting) based methods or by capture on beads using labelled antigens. Transgenic animals that have been modified to express human immunoglobulin genes can be immunised with an antigen of interest and antigen-specific human antibodies isolated using B-cell sorting techniques. Human antibodies produced using these techniques can then be characterised for desired properties such as affinity, developability and selectivity.

The term "domain" refers to a folded polypeptide structure that retains its tertiary structure independent of the rest of the polypeptide. Generally domains are responsible for discrete functional properties of polypeptides and in many cases may be added, removed or transferred to other polypeptides without loss of function of the remainder of the protein and/or of the domain.

The term "single variable domain" refers to a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains such as V_H , V_{HH} and V_L and modified antibody variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain. A single variable domain is capable of binding an antigen or epitope independently of a different variable region or domain. A "domain antibody" or "DAB" may be considered the same as a "single variable domain". A single variable domain may be a human single variable domain, but also includes single variable domains from other species such as rodent, nurse shark and Camelid V_{HH} DABS. Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanized

according to standard techniques available in the art, and such domains are considered to be "single variable domains". As used herein V_H includes camelid V_{HH} domains.

The terms "V_H" and "V_L" are used herein to refer to the heavy chain variable region and light chain variable region respectively of an antigen binding protein.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antigen binding protein. These are the hypervariable regions of immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, all three light chain CDRs, all heavy and light chain CDRs, or at least two CDRs.

Throughout this specification, amino acid residues in variable domain sequences and variable domain regions within full length antigen binding sequences, *e.g.* within an antibody heavy chain sequence or antibody light chain sequence, are numbered according to the Kabat numbering convention. Similarly, the terms "CDR", "CDRL1", "CDRL2", "CDRL3", "CDRH1", "CDRH2", "CDRH3" used in the Examples follow the Kabat numbering convention. For further information, see Kabat *et al.* Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1991).

It will be apparent to those skilled in the art that there are alternative numbering conventions for amino acid residues in variable domain sequences and full length antibody sequences. There are also alternative numbering conventions for CDR sequences, for example those set out in Chothia *et al.* (1989) Nature 342: 877-883. The structure and protein folding of the antigen binding protein may mean that other residues are considered part of the CDR sequence and would be understood to be so by a skilled person.

Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods. The minimum overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the "minimum binding unit". The minimum binding unit may be a sub-portion of a CDR.

CDRs or minimum binding units may be modified by at least one amino acid substitution, deletion or addition, wherein the variant antigen binding protein substantially retains the biological characteristics of the unmodified protein, such as an antibody comprising SEQ ID NO:7 and SEQ ID NO:8.

CDRs or minimum binding units may be modified by at least one amino acid substitution, deletion or addition, wherein the variant antigen binding protein substantially retains the biological characteristics of the unmodified protein, such as an antibody comprising SEQ ID NO:7 and SEQ ID NO:8.

It will be appreciated that each of CDR H1, H2, H3, L1, L2, L3 may be modified alone or in combination with any other CDR, in any permutation or combination. In one embodiment, a CDR is

modified by the substitution, deletion or addition of up to 3 amino acids, for example 1 or 2 amino acids, for example 1 amino acid. Typically, the modification is a substitution, particularly a conservative substitution (referred herein also as a direct equivalent), for example as shown in Table 1 below.

5 **Table 1**

Side chain	Members
Hydrophobic	Met, Ala, Val, Leu, Ile
Neutral hydrophilic	Cys, Ser, Thr
Acidic	Asp, Glu
Basic	Asn, Gln, His, Lys, Arg
Residues that influence chain orientation	Gly, Pro
Aromatic	Trp, Tyr, Phe

The VH or VL (or HC or LC) sequence may be a variant sequence with up to 10 amino acid substitutions, additions or deletions. For example, the variant sequence may have up to 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitution(s), addition(s) or deletion(s). The sequence variation may exclude one or more or all of the CDRs, for example the CDRs are the same as the VH or VL (or HC or LC) sequence and the variation is in the remaining portion of the VH or VL (or HC or LC) sequence, so that the CDR sequences are fixed and intact. Typically, the variation is a substitution, particularly a conservative substitution, for example as shown in Table 1.

“Percent identity” between a query amino acid sequence and a subject amino acid sequence is the “Identities” value, expressed as a percentage, that is calculated using a suitable algorithm or software, such as BLASTP, FASTA, DNASTAR Lasergene, GeneDoc, Bioedit, EMBOSS needle or EMBOSS infoalign, over the entire length of the query sequence after a pair-wise global sequence alignment has been performed using a suitable algorithm/software such as BLASTP, FASTA, ClustalW, MUSCLE, MAFFT, EMBOSS Needle, T-Coffee, and DNASTAR Lasergene. Importantly, a query amino acid sequence may be described by an amino acid sequence identified in one or more claims herein.

The query sequence may be 100% identical to the subject sequence, or it may include up to a certain integer number of amino acid or nucleotide alterations as compared to the subject sequence such that the % identity is less than 100%. For example, the query sequence is at least 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identical to the subject sequence. Such alterations include at least one amino acid deletion, substitution (including conservative and non-conservative substitution), or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the query sequence or anywhere between those terminal positions, interspersed either individually

among the amino acids or nucleotides in the query sequence or in one or more contiguous groups within the query sequence.

The % identity may be determined across the entire length of the query sequence, including the CDRs. Alternatively, the % identity may exclude one or more or all of the CDRs, for example all of the CDRs are 100% identical to the subject sequence and the % identity variation is in the remaining portion of the query sequence, *e.g.* the framework sequence, so that the CDR sequences are fixed and intact.

The variant sequence substantially retains the biological characteristics of the unmodified protein, such as an agonist for ICOS.

An antigen binding fragment may be provided by means of arrangement of one or more CDRs on non-antibody protein scaffolds. "Protein Scaffold" as used herein includes, but is not limited to, an immunoglobulin (Ig) scaffold, for example an IgG scaffold, which may be a four chain or two chain antibody, or which may comprise only the Fc region of an antibody, or which may comprise one or more constant regions from an antibody, which constant regions may be of human or primate origin, or which may be an artificial chimera of human and primate constant regions.

The protein scaffold may be an Ig scaffold, for example an IgG, or IgA scaffold. The IgG scaffold may comprise some or all the domains of an antibody (*i.e.* CH1, CH2, CH3, V_H, V_L). The antigen binding protein may comprise an IgG scaffold selected from IgG1, IgG2, IgG3, IgG4 or IgG4PE. For example, the scaffold may be IgG1. The scaffold may consist of, or comprise, the Fc region of an antibody, or is a part thereof.

The subclass of an antibody in part determines secondary effector functions, such as complement activation or Fc receptor (FcR) binding and antibody dependent cell cytotoxicity (ADCC) (Huber *et al.* Nature 229(5284): 419-20 (1971); Brunhouse *et al.* Mol Immunol 16(11): 907-17 (1979)). In identifying the optimal type of antibody for a particular application, the effector functions of the antibodies can be taken into account. For example, hIgG1 antibodies have a relatively long half life, are very effective at fixing complement, and they bind to both FcγRI and FcγRII. In contrast, human IgG4 antibodies have a shorter half life, do not fix complement and have a lower affinity for the FcRs. Replacement of serine 228 with a proline (S228P) in the Fc region of IgG4 reduces heterogeneity observed with hIgG4 and extends the serum half life (Kabat *et al.* "Sequences of proteins of immunological interest" 5.sup.th Edition (1991); Angal *et al.* Mol Immunol 30(1): 105-8 (1993)). A second mutation that replaces leucine 235 with a glutamic acid (L235E) eliminates the residual FcR binding and complement binding activities (Alegre *et al.* J Immunol 148(11): 3461-8 (1992)). The numbering of the hIgG4 amino acids was derived from EU numbering reference: Edelman *et al.* Proc. Natl. Acad. USA, 63, 78-85 (1969). PMID: 5257969. In one embodiment of the present invention the ICOS antibody is an IgG4 isotype. In one embodiment, the ICOS antibody comprises an IgG4 Fc region comprising the replacement S228P and L235E or a functional variant thereof. Such

an antibody may have the designation IgG4PE. In a preferred embodiment, the agonist ICOS binding protein is H2L5 IgG4PE.

The term "donor antibody" refers to an antibody that contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner. The donor, therefore, provides the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralising activity characteristic of the donor antibody.

The term "acceptor antibody" refers to an antibody that is heterologous to the donor antibody, which contributes all (or any portion) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. A human antibody may be the acceptor antibody.

Affinity, also referred to as "binding affinity", is the strength of binding at a single interaction site, *i.e.* of one molecule, *e.g.* an antigen binding protein of the invention, to another molecule, *e.g.* its target antigen, at a single binding site. The binding affinity of an antigen binding protein to its target may be determined by equilibrium methods (*e.g.* enzyme-linked immunoabsorbent assay (ELISA) or radioimmunoassay (RIA)), or kinetics (*e.g.* BIACORE analysis).

Avidity, also referred to as functional affinity, is the cumulative strength of binding at multiple interaction sites, *e.g.* the sum total of the strength of binding of two molecules (or more, *e.g.* in the case of a bispecific or multispecific molecule) to one another at multiple sites, *e.g.* taking into account the valency of the interaction.

As used herein an "immuno-modulator" or "immuno-modulatory agent" refers to any substance including monoclonal antibodies that affects the immune system. In some embodiments, the immuno-modulator or immuno-modulatory agent upregulates an aspect of the immune system. Immuno-modulators can be used as anti-neoplastic agents for the treatment of cancer. For example, immuno-modulators include, but are not limited to, anti-PD-1 antibodies (*e.g.* dostarlimab, OPDIVO/nivolumab, KEYTRUDA/pembrolizumab and LIBTAYO/cemiplimab), anti-CTLA-4 antibodies and anti-ICOS antibodies.

As used herein the term "agonist" refers to an antigen binding protein including, but not limited to, an antibody, that is capable of activating the antigen to which it binds to induce a full or partial antigen-mediated response that is above the response measured in the absence of the antigen binding protein. Examples of agonistic responses include but are not limited to transduction of a survival, growth, proliferation, differentiation and/or maturation signal. In one embodiment, the agonist upon contact with a co-signalling receptor causes one or more of the following (1) stimulates or activates the receptor, (2) enhances, increases or promotes, induces or prolongs an activity, function or presence of the receptor and/or (3) enhances, increases, promotes or induces the expression of the receptor. Agonist activity can be measured *in vitro* by various assays known in the

art such as, but not limited to, measurement of cell signalling, cell proliferation, immune cell activation markers, cytokine production. Agonist activity can also be measured *in vivo* by various assays that measure surrogate end points such as, but not limited to the measurement of T cell proliferation or cytokine production.

5 As used herein the term "antagonist" refers to an antigen binding protein including, but not limited to, an antibody, that is capable of fully or partially inhibiting the biological activity of the antigen to which it binds for example by fully or partially blocking binding or neutralising activity. In one embodiment, the antagonist upon contact with a co-signalling receptor causes one or more of the following (1) attenuates, blocks or inactivates the receptor and/or blocks activation of a receptor by
10 its natural ligand, (2) reduces, decreases or shortens the activity, function or presence of the receptor and/or (3) reduces, decrease, abrogates the expression of the receptor. Antagonist activity can be measured *in vitro* by various assays know in the art such as, but not limited to, measurement of an increase or decrease in cell signalling, cell proliferation, immune cell activation markers, cytokine production. Antagonist activity can also be measured *in vivo* by various assays that measure surrogate
15 end points such as, but not limited to the measurement of T cell proliferation or cytokine production. In one embodiment, the PD-1 binding protein is an antagonist PD-1 binding protein.

By "isolated" it is intended that the molecule, such as an antigen binding protein or nucleic acid, is removed from the environment in which it may be found in nature. For example, the molecule may be purified away from substances with which it would normally exist in nature. For example, the
20 mass of the molecule in a sample may be 95% of the total mass.

The term "expression vector" as used herein means an isolated nucleic acid, which can be used to introduce a nucleic acid of interest into a cell, such as a eukaryotic cell or prokaryotic cell, or a cell free expression system, where the nucleic acid sequence of interest is expressed as a peptide chain such as a protein. Such expression vectors may be, for example, cosmids, plasmids, viral
25 sequences, transposons, and linear nucleic acids comprising a nucleic acid of interest. Once the expression vector is introduced into a cell or cell free expression system (*e.g.* reticulocyte lysate) the protein encoded by the nucleic acid of interest is produced by the transcription/translation machinery. Expression vectors within the scope of the disclosure may provide necessary elements for eukaryotic or prokaryotic expression and include viral promoter driven vectors, such as CMV promoter driven
30 vectors, *e.g.* pcDNA3.1, pCEP4, and their derivatives, Baculovirus expression vectors, *Drosophila* expression vectors, and expression vectors that are driven by mammalian gene promoters, such as human Ig gene promoters. Other examples include prokaryotic expression vectors, such as T7 promoter driven vectors, *e.g.* pET41, lactose promoter driven vectors and arabinose gene promoter driven vectors. Those of ordinary skill in the art will recognize many other suitable expression vectors
35 and expression systems.

The term "recombinant host cell" as used herein means a cell that comprises a nucleic acid sequence of interest that was isolated prior to its introduction into the cell. For example, the nucleic acid sequence of interest may be in an expression vector while the cell may be prokaryotic or eukaryotic. Exemplary eukaryotic cells are mammalian cells, such as but not limited to, COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, HepG2, 653, SP2/0, NS0, 293, HeLa, myeloma, lymphoma cells or any derivative thereof. Most preferably, the eukaryotic cell is a HEK293, NS0, SP2/0, or CHO cell. *E. coli* is an exemplary prokaryotic cell. A recombinant cell according to the disclosure may be generated by transfection, cell fusion, immortalization, or other procedures well known in the art. A nucleic acid sequence of interest, such as an expression vector, transfected into a cell may be extrachromosomal or stably integrated into the chromosome of the cell.

As used herein, the term "effective dose" means that dose of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. Furthermore, the term "therapeutically effective dose" means any dose that, as compared to a corresponding subject who has not received such dose, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope doses effective to enhance normal physiological function. Therapeutically effective amounts and treatment regimes are generally determined empirically and may be dependent on factors, such as the age, weight, and health status of the patient and disease or disorder to be treated. Such factors are within the purview of the attending physician.

Ranges provided herein, of any type, include all values within a particular range described and values about an endpoint for a particular range.

The term "therapeutic agents" refers to therapeutic agents of the invention. In some embodiments, the therapeutic agents are agonist ICOS binding proteins, tremelimumab and immunomodulatory agents. In other embodiments, one or more additional agents may be administered in addition to agonist ICOS binding proteins and tremelimumab. Examples of one or more additional agents include, but are not limited to additional immunomodulators and chemotherapeutic agents. Examples of additional immunomodulators such as a PD1 binding protein or antigen binding portion thereof or a PDL-1 binding protein or antigen binding portion thereof.

It will be understood that references to "therapeutic agents" include embodiments where the two therapeutic agents are administered in any temporal order, such as concurrently or sequentially. The terms concurrent and sequential administration of therapeutic agents are well understood in the art. The individual therapeutic agents, and pharmaceutical compositions comprising such therapeutic agents may be administered together or separately. When administered separately, this may occur concurrently or sequentially in any order (by the same or by different routes of administration). Such sequential administration may be close in time or remote in time. The dose of a therapeutic agents or

pharmaceutically acceptable salt thereof and the further therapeutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

The administration of the therapeutic agents may be advantageous over the individual therapeutic agents in that the combination of the therapeutic agents may provide one or more of the following improved properties when compared to the individual administration of a single therapeutic agent alone: i) a greater anticancer effect than the most active single agent, ii) synergistic or highly synergistic anticancer activity, iii) a dosing protocol that provides enhanced anticancer activity with reduced side effect profile, iv) a reduction in the toxic effect profile, v) an increase in the therapeutic window, and/or vi) an increase in the bioavailability of one or both of the therapeutic agents.

In one embodiment, each therapeutic agent is formulated into its own pharmaceutical composition and each of the pharmaceutical compositions are administered to treat cancer. In this embodiment, each of the pharmaceutical compositions may have the same or different carriers, diluents or excipients. For example, in one embodiment, a first pharmaceutical composition contains an agonist ICOS binding protein, a second pharmaceutical composition contains tremelimumab, and the first and second pharmaceutical compositions are both administered to treat cancer.

In one embodiment, the combination comprising an agonist ICOS binding protein and tremelimumab is formulated together into a single pharmaceutical composition and administered to treat cancer. For example, in one embodiment, a single pharmaceutical composition contains both an agonist ICOS binding protein and tremelimumab and is administered as a single pharmaceutical composition to treat cancer.

ICOS and CTLA-4 binding proteins

Agents directed to ICOS in any of the aspects or embodiments of the present invention include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to ICOS. In some embodiments, the mAb to ICOS specifically binds to human ICOS. In one embodiment, the agonist ICOS binding protein is a monoclonal antibody or antigen binding fragment thereof. The mAb may be a human antibody, a humanized antibody or a chimeric antibody, and may include a human constant region. The human constant region is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4 constant regions, and in preferred embodiments, the human constant region is an IgG1 or IgG4 constant region. The antigen binding fragment may be selected from the group consisting of Fab, Fab'-SH, F(ab')₂, scFv and Fv fragments.

As used herein "ICOS" means any Inducible T-cell costimulator protein. Pseudonyms for ICOS (Inducible T-cell COStimulator) include AILIM; CD278; CVID1, JTT-1 or JTT-2, MGC39850, or 8F4. ICOS is a CD28-superfamily costimulatory molecule that is expressed on activated T cells. The protein encoded by this gene belongs to the CD28 and CTLA-4 cell-surface receptor family. It forms

homodimers and plays an important role in cell-cell signaling, immune responses, and regulation of cell proliferation. The amino acid sequence of human ICOS (isoform 2) (Accession No.: UniProtKB - Q9Y6W8-2) is shown below as SEQ ID NO:11.

5 MKSGLWYFFLFCLRIKVLGTGEINGSANYEMFIFHNGGVQILCKYPDIVQQFKMQLLKGGQILCDLTKTKGSGNTV
SIKSLKFCHSLSNNSVSFFLYNLDSHANYFCNLSIFDPPPFKVTLTGGYLHIYESQLCCQLKFWLPIGCAAFV
VCILGCILICWLTKKM (SEQ ID NO:11)

10 The amino acid sequence of human ICOS (isoform 1) (Accession No.: UniProtKB - Q9Y6W8-1) is shown below as SEQ ID NO:12.

MKSGLWYFFLFCLRIKVLGTGEINGSANYEMFIFHNGGVQILCKYPDIVQQFKMQLLKGGQILCDLTKTKGSGNTV
SIKSLKFCHSLSNNSVSFFLYNLDSHANYFCNLSIFDPPPFKVTLTGGYLHIYESQLCCQLKFWLPIGCAAFV
VCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL (SEQ ID NO:12)

15
Activation of ICOS occurs through binding by ICOS-L (B7RP-1/B7-H2). Neither B7-1 nor B7-2 (ligands for CD28 and CTLA4) bind or activate ICOS. However, ICOS-L has been shown to bind weakly to both CD28 and CTLA-4 (Yao *et al.* "B7-H2 is a costimulatory ligand for CD28 in human", *Immunity*, 34(5); 729-40 (2011)). Expression of ICOS appears to be restricted to T cells. ICOS expression levels
20 vary between different T cell subsets and on T cell activation status. ICOS expression has been shown on resting TH17, T follicular helper (TFH) and regulatory T (Treg) cells; however, unlike CD28; it is not highly expressed on naïve TH1 and TH2 effector T cell populations (Paulos *et al.* "The inducible costimulator (ICOS) is critical for the development of human Th17 cells", *Sci Transl Med*, 2(55); 55ra78 (2010)). ICOS expression is highly induced on CD4+ and CD8+ effector T cells following activation
25 through TCR engagement (Wakamatsu *et al.* "Convergent and divergent effects of costimulatory molecules in conventional and regulatory CD4+ T cells", *Proc Natl Acad Sci USA*, 110(3); 1023-8 (2013)). Co-stimulatory signalling through ICOS receptor only occurs in T cells receiving a concurrent TCR activation signal (Sharpe AH and Freeman GJ. "The B7-CD28 Superfamily", *Nat. Rev Immunol*, 2(2); 116-26 (2002)). In activated antigen specific T cells, ICOS regulates the production of both TH1
30 and TH2 cytokines including IFN- γ , TNF- α , IL-10, IL-4, IL-13 and others. ICOS also stimulates effector T cell proliferation, albeit to a lesser extent than CD28 (Sharpe AH and Freeman GJ. "The B7-CD28 Superfamily", *Nat. Rev Immunol*, 2(2); 116-26 (2002)).

By "agent directed to ICOS" is meant any chemical compound or biological molecule capable of binding to ICOS. In some embodiments, the agent directed to ICOS is an agonist ICOS binding
35 protein or antigen binding portion thereof.

The term "ICOS binding protein" as used herein refers to a protein that binds to ICOS, including an antibody or an antigen binding fragment thereof, or engineered molecules that function in similar ways to antibodies that are capable of binding to ICOS. In one embodiment, the antibody is a monoclonal antibody. In some instances, the ICOS is human ICOS. The term "ICOS binding protein" can be used interchangeably with "ICOS binding protein", "ICOS binding agent", "ICOS antigen binding protein" or "ICOS antigen binding agent". Thus, as is understood in the art, anti-ICOS antibodies and/or ICOS antigen binding proteins would be considered ICOS binding proteins. This definition does not include the natural cognate ligand or receptor. References to ICOS binding proteins, in particular anti-ICOS antibodies, includes antigen binding portions or fragments thereof. As used herein "antigen binding portion" of an ICOS binding protein would include any portion of the ICOS binding protein capable of binding to ICOS, including but not limited to, an antigen binding antibody fragment.

In one embodiment, the agonist ICOS binding proteins of the present invention comprise any one or a combination of the following CDRs:

CDRH1: DYAMH (SEQ ID NO:1)
 CDRH2: LISIYSDHTNYNQKFQG (SEQ ID NO:2)
 CDRH3: NNYGNYGWYFDV (SEQ ID NO:3)
 CDRL1: SASSSVSYMH (SEQ ID NO:4)
 CDRL2: DTSKLAS (SEQ ID NO:5)
 CDRL3: FQGSGYPYT (SEQ ID NO:6)

In one embodiment, the agonist ICOS binding protein comprises a heavy chain variable region CDR1 ("CDRH1") comprising an amino acid sequence with one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:1.

In one embodiment, the agonist ICOS binding protein comprises a heavy chain variable region CDR2 ("CDRH2") comprising an amino acid sequence with five or fewer, such as four or fewer, three or fewer, two or fewer, or one amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:2. In a further embodiment, the CDRH2 comprises an amino acid sequence with one or two amino acid variation(s) to the amino acid sequence set forth in SEQ ID NO:2.

In one embodiment, the agonist ICOS binding protein comprises a heavy chain variable region CDR3 ("CDRH3") comprising an amino acid sequence with one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:3.

In one embodiment, the agonist ICOS binding protein comprises a light chain variable region CDRL1 ("CDRL1") comprising an amino acid sequence with three or fewer, such as one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:4.

In one embodiment, the agonist ICOS binding protein comprises a light chain variable region CDR2 ("CDRL2") comprising an amino acid sequence with one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:5.

5 In one embodiment, the agonist ICOS binding protein comprises a light chain variable region CDR3 ("CDRL3") comprising an amino acid sequence with three or fewer, such as one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:6.

10 In one embodiment, the agonist ICOS binding protein comprises a CDRH1 comprising an amino acid sequence with up to one amino acid variation to the amino acid sequence set forth in SEQ ID NO:1; a CDRH2 comprising an amino acid sequence with up to five amino acid variations to the amino acid sequence set forth in SEQ ID NO:2; a CDRH3 comprising an amino acid sequence with up to one amino acid variation to the amino acid sequence set forth in SEQ ID NO:3; a CDRL1 comprising an amino acid sequence with up to three amino acid variations to the amino acid sequence set forth in SEQ ID NO:4; a CDRL2 comprising an amino acid sequence with up to one amino acid variation to the amino acid sequence set forth in SEQ ID NO:5; and/or a CDRL3 comprising an amino acid
15 sequence with up to three amino acid variations to the amino acid sequence set forth in SEQ ID NO:6. In one embodiment, the agonist ICOS binding protein binding protein comprises any one or a combination of the CDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6. In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof comprises one or more of : CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ
20 ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and/or CDRL3 as set forth in SEQ ID NO:6 or a direct equivalent of each CDR wherein a direct equivalent has no more than two amino acid substitutions in said CDR. In one embodiment, the ICOS binding protein binding protein comprises the CDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

25 In one embodiment of the present invention the agonist ICOS binding protein comprises CDRH1 (SEQ ID NO:1), CDRH2 (SEQ ID NO:2), and CDRH3 (SEQ ID NO:3) in the heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:7. The agonist ICOS binding proteins of the present invention comprising the humanized heavy chain variable region set forth in SEQ ID NO:7 are designated as "H2." In some embodiments, the anti-ICOS antibodies of the present invention
30 comprise a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:7. Suitably, the agonist ICOS binding proteins of the present invention may comprise a heavy chain variable region having about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:7. In one embodiment, the anti-ICOS antibodies of the present invention comprise a heavy chain variable region having at least 90%
35 sequence identity to SEQ ID NO:7. In one embodiment, the anti-ICOS antibodies of the present invention comprise a heavy chain variable region as set forth in SEQ ID NO:7.

Humanized heavy chain (V_H) variable region (H2):

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYAMHWVRQAPGQGLEWMGLISYSDHTNYNQKFQGRVTITA
DKSTSTAYMELSSLRSEDTAVYYCGRNNYGNYGWYFDVWGQGTITVTVSS (SEQ ID NO:7; underlined
5 amino acid residues correspond to the positions of CDRs).

In one embodiment, the agonist ICOS binding protein comprises a heavy chain variable region ("V_H") comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:7. In one embodiment, the V_H comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:7, such as between 1 and 5, such as between 1 and 3, in particular up to 2 amino acid variations to the amino acid sequence set forth in SEQ ID NO:7.

In one embodiment of the present invention the agonist ICOS binding protein comprises CDRL1 (SEQ ID NO:4), CDRL2 (SEQ ID NO:5), and CDRL3 (SEQ ID NO:6) in the light chain variable region having the amino acid sequence set forth in SEQ ID NO:8. Agonist ICOS binding proteins of the present invention comprising the humanized light chain variable region set forth in SEQ ID NO:8 are designated as "L5." Thus, an agonist ICOS binding protein of the present invention comprising the heavy chain variable region of SEQ ID NO:7 and the light chain variable region of SEQ ID NO:8 can be designated as H2L5 herein.

In some embodiments, the agonist ICOS binding proteins of the present invention comprise a light chain variable region having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:8. Suitably, the agonist ICOS binding proteins of the present invention may comprise a light chain variable region having about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:8.

Humanized light chain (V_L) variable region (L5):

EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGQAPRLLIYDTSKLASGIPARFSGSGSGTDYTLTISS
LEPEDFAVYYCFQGSQYPYTFGQGTKLEIK (SEQ ID NO:8; underlined amino acid residues correspond to
the positions of CDRs).

In one embodiment, the agonist ICOS binding protein comprises a light chain variable region ("V_L") comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID

NO:8. In one embodiment, the V_L comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:8, such as between 1 and 5, such as between 1 and 3, in particular up to 2 amino acid variations to the amino acid sequence set forth in SEQ ID NO:8. In one embodiment, the anti-ICOS antibody comprises a light chain variable region
 5 having at least 90% sequence identity to SEQ ID NO:8. In one embodiment, the anti-ICOS antibody comprises a light chain variable region as set forth in SEQ ID NO:8.

In one embodiment, the agonist ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid
 10 sequence as set forth in SEQ ID NO:8. In one embodiment, the agonist ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8, wherein the agonist ICOS binding protein specifically binds to human ICOS. In one embodiment, the agonist ICOS binding protein comprises a
 15 V_H with the amino acid sequence set forth in SEQ ID NO:7; and a V_L with the amino acid sequence set forth in SEQ ID NO:8.

In one embodiment, the agonist ICOS binding protein comprises a V_H comprising an amino acid sequence of SEQ ID NO:7 and a V_L comprising an amino acid sequence of SEQ ID NO:8. In one embodiment, the agonist ICOS binding protein specifically binds to human ICOS.

In one embodiment, the agonist ICOS binding protein comprises a V_H comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:7; and a V_L comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:8.

In one embodiment, the agonist ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8, and further comprises any one or a combination of the CDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

In one embodiment, the agonist ICOS binding protein is a humanized monoclonal antibody comprising a heavy chain (HC) amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:9.

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYAMHWVRQAPGQGLEWMGLISISDHTNYNQKFQGRVTITA
 DKSTSTAYMELSSLRSEDVAVYYCGRNNYGNYGWYFDVWGQGTTVTSSASTKGPSVFPLAPCSRSTSESTAAL

GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRVES
 KYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREE
 QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV
 KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL
 5 SLGK (SEQ ID NO:9)

In one embodiment, the HC comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:9, such as between 1 and 10, such as between 1 and 7, in particular up to 6 amino acid variations to the amino acid sequence set forth in
 10 SEQ ID NO:9. In a further embodiment, the HC comprises one, two, three, four, five, six or seven amino acid variations to the amino acid sequence set forth in SEQ ID NO:9.

In one embodiment, the agonist ICOS binding protein is a humanized monoclonal antibody comprising a light chain (LC) amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID
 15 NO:10.

EIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGQAPRLLIYDTSKLASGIPARFSGSGSGTDYTLTISS
 LEPEDFAVYYCFQGSQPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:10)

20 In one embodiment, the LC comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:10, such as between 1 and 10, such as between 1 and 5, in particular up to 3 amino acid variations to the amino acid sequence set forth in SEQ ID NO:10. In a further embodiment, the LC comprises one, two or three amino acid variations to
 25 the amino acid sequence set forth in SEQ ID NO:10.

In one embodiment, the agonist ICOS binding protein comprises a HC comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:9; and a LC comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%
 30 sequence identity to the amino acid sequence set forth in SEQ ID NO:10. Therefore, the antibody is an antibody with a heavy chain at least about 90% identical to the heavy chain amino acid sequence of SEQ ID NO:9 and/or with a light chain at least about 90% identical to the light chain amino acid sequence of SEQ ID NO:10.

In one embodiment, the agonist ICOS binding protein comprises a heavy chain amino acid
 35 sequence at least about 90% identical to the amino acid sequence of SEQ ID NO:9 and/or a light chain amino acid sequence at least about 90% identical to the amino acid sequence of SEQ ID NO:10.

In one embodiment, the agonist ICOS binding protein comprises a heavy chain sequence of SEQ ID NO:9 and a light chain sequence of SEQ ID NO:10.

In one embodiment there is provided an agonist ICOS binding protein comprising a heavy chain constant region that has reduced ADCC and/or complement activation or effector functionality as compared to IgG1. In one embodiment, the IgG1 is wild type (WT) IgG1. In one such embodiment the heavy chain constant region may comprise a naturally disabled constant region of IgG2 or IgG4 isotype or a mutated or disabled IgG1 constant region.

In one embodiment, the agonist ICOS binding protein comprises an IgG4 Fc region comprising the amino acid substitutions S228P and L235E or functional equivalents thereof. In one embodiment, the agonist ICOS binding protein comprises an IgG4 Fc region comprising amino acid substitutions S229P and L236E. Such embodiments may have the designation IgG4PE. Thus, an agonist ICOS binding protein having the heavy chain variable region H2 and the light chain variable region L5 and an IgG4PE Fc region will be designated as H2L5 IgG4PE or synonymously as H2L5 hIgG4PE.

Antibodies to ICOS and methods of using in the treatment of disease are described, for instance, in WO2012131004, US20110243929, and US20160215059. US20160215059 is incorporated by reference herein. CDRs for murine antibodies to human ICOS having agonist activity are shown in PCT/EP2012/055735 (WO2012131004). Antibodies to ICOS are also disclosed in WO2008137915, WO2010056804, EP1374902, EP1374901, and EP1125585. Agonist antibodies to ICOS or ICOS binding proteins are disclosed in WO2012/13004, WO2014033327, WO2016120789, US20160215059, and US20160304610. Exemplary antibodies in US20160304610 include 37A10S713. Sequences of 37A10S713 are reproduced below as SEQ ID NOS:13-20.

37A10S713 V_H CDR1: GFTFSDYWMD (SEQ ID NO:13)

37A10S713 V_H CDR2: NIDEDGSITEYSPFVK (SEQ ID NO:14)

37A10S713 V_H CDR3: WGRFGFDS (SEQ ID NO:15)

37A10S713 V_L CDR1: KSSQSLLSGSFNYLT (SEQ ID NO:16)

37A10S713 V_L CDR2: YASTRHT (SEQ ID NO:17)

37A10S713 V_L CDR3: HHHYNAPPT (SEQ ID NO:18)

37A10S713 heavy chain variable region:

EVQLVESGGLVQPGGSLRLSCAASGFTFSDYWMDWVRQAPGKGLVWVSNIDEDGSITEYSPFVKGRFTISRDN
AKNTLYLQMNSLRAEDTAVYYCTRWGRFGFDSWGQGTLLTVSS (SEQ ID NO:19; underlined amino acid
residues correspond to the positions of CDRs)

37A10S713 light chain variable region:

DIVMTQSPDSLAVSLGERATINCKSSQSLLSGSFNYLTWYQQKPGQPPKLLIFYASTRHTGVPDRFSGSGSGTDF
TLTISSLQAEDVAVYYCHHHYNAPPTFGPGTKVDIK (SEQ ID NO:20; underlined amino acid residues
correspond to the positions of CDRs)

5 In an embodiment, the agonist ICOS binding protein is vopratelimab. In one embodiment, the
agonist ICOS binding protein is JTX-2011.

Exemplary antibodies in US2018/0289790 include ICOS.33 IgG1f S267E. Sequences of
ICOS.33 IgG1f S267E are reproduced below as SEQ ID NOS:21-22.

10

ICOS.33 IgG1f S267E heavy chain variable domain:

EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYFMHWVRQAPGKGLEWVGVIDTKSFNYATYYSDLVKGRFTISR
DDSKNTLYLQMNSLKTEDTAVYYCTATIAVPYYFDYWGQGTLTVSS (SEQ ID NO:21)

15 ICOS.33 IgG1f S267E light chain variable domain:

DIQMTQSPSSLSASVGDRVTITCQASQDISNYLSWYQQKPGKAPKLLIYYTNLLAEGVPSRFSGSGSGTDFFTI
SSLQPEDATYYCQYYNYRTFGPGTKVDIK (SEQ ID NO:22)

In one embodiment, the agonist ICOS binding protein is BMS-986226.

20

Exemplary antibodies in WO2018/029474 include STIM003. Sequences of STIM003 are
reproduced below as SEQ ID NOS: 23-24.

STIM003 heavy chain variable domain:

25 EVQLVESGGGVVRPGGSLRLSCVASGVTFDYGMWVRQAPGKGLEWVSGINWNGGDTDYSDSVKGRFTISR
DNAKNSLYLQMNSLRAEDTALYYCARDYFGSGSYHVPFDYWGQGILTVSS (SEQ ID NO:23)

STIM003 light chain variable domain:

30 EIVLTQSPGTLSPGERATLSCRASQSVRSYLAWYQQKRGQAPRLIYGASSRATGIPDRFSGDGS GTDFTLSI
SRLEPEDFAVYYCHQYDMSPFTFGPGTKVDIK (SEQ ID NO:24)

In one embodiment, the agonist ICOS binding protein is KY1044.

Exemplary antibodies in WO2018/045110 include XENP23104. Sequences of the ICOS binding Fab side ([ICOS]_H0.66_L0) of XENP23104 are reproduced below as SEQ ID NOS:25-32.

XENP23104 [ICOS]_H0.66_L0 heavy chain variable domain:

5 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVRQAPGQGLEWMGWINPHSGETIYAQKFQGRVTMT
 RDTISISTAYMELSSLRSEDTAVYYCARTYYYDTSGYYHDAFDVWGQGTMTVSS (SEQ ID NO:25;
 underlined amino acid residues correspond to the positions of CDRs).

XENP23104 [ICOS]_H0.66_L0 V_H CDR1: GYMH (SEQ ID NO:26)

10 XENP23104 [ICOS]_H0.66_L0 V_H CDR2: WINPHSGETIYAQKFQG (SEQ ID NO:27)

XENP23104 [ICOS]_H0.66_L0 V_H CDR3: TYYDTSGYYHDAFDV (SEQ ID NO:28)

XENP23104 [ICOS]_H0.66_L0 light chain variable domain:

DIQMTQSPSSVSASVGDRVTITCRASQGISRLLAWYQQKPGKAPKLLIYVASSLQSGVPSRFSSGSGSGTDFTLTI
 15 SSLQPEDFATYYCQQANSFPWTFGQGTKVEIK (SEQ ID NO:29; underlined amino acid residues
 correspond to the positions of CDRs).

XENP23104 [ICOS]_H0.66_L0 V_L CDR1: RASQGISRLLA (SEQ ID NO:30)

XENP23104 [ICOS]_H0.66_L0 V_L CDR2: VASSLQS (SEQ ID NO:31)

20 XENP23104 [ICOS]_H0.66_L0 V_L CDR3: QQANSFPWT (SEQ ID NO:32)

As used herein "ICOS-L" and "ICOS Ligand" are used interchangeably and refer to the membrane bound natural ligand of human ICOS. ICOS ligand is a protein that in humans is encoded by the *ICOSLG* gene. ICOSLG has also been designated as CD275 (cluster of differentiation 275).

25 Pseudonyms for ICOS-L include B7RP-1 and B7-H2.

Antagonistic anti-CTLA4 antibodies that block the CTLA4 signaling pathway have been reported to enhance T cell activation. By "an anti-CTLA4 antibody" is meant an antibody that selectively binds a CTLA-4 polypeptide. An example of such an antibody is tremelimumab.
 30 Tremelimumab (also known as Ticilimumab, CP-675 and CP-675,206) is a humanised IgG2 monoclonal antibody specific for human cytotoxic T lymphocyte-associated antigen 4 (CTLA-4, CD152) expressed on the surface of activated T lymphocytes. Blocking the CTLA-4 negative costimulatory receptor with the antagonistic antibody tremelimumab results in immune activation and fostering cancer cell death.

Tremelimumab is described for example in US Patent Nos. 6,682,736 (Tremelimumab is 11.2.1, therein); 7,109,003; 7,123,281; 7,411,057; 7,824,679; 8,143,379; 7,807,797; and 8,491,895, which are herein incorporated by reference.

5 In one aspect of the invention, the CTLA-4 binding protein or antigen binding portion thereof comprises any one or a combination of the following CDRs:

CDRH1: GFTFSSYGMH (SEQ ID NO:33)

CDRH2: VIWYDGSNKYYADSV (SEQ ID NO:34)

10 CDRH3: DPRGATLYYYYYGMDV (SEQ ID NO:35)

CDRL1: RASQSINSYLD (SEQ ID NO:36)

CDRL2: AASSLQS (SEQ ID NO:37)

CDRL3: QQYYSTPFT (SEQ ID NO:38)

15 In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a heavy chain variable region CDR1 ("CDRH1") comprising an amino acid sequence with one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:33.

In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a heavy chain variable region CDR2 ("CDRH2") comprising an amino acid sequence with five or fewer, such as four or fewer, three or fewer, two or fewer, or one amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:34. In a further embodiment, the CDRH2 comprises an amino acid sequence with one or two amino acid variation(s) to the amino acid sequence set forth in SEQ ID NO:34.

25 In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a heavy chain variable region CDR3 ("CDRH3") comprising an amino acid sequence with one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:35.

In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a light chain variable region CDR1 ("CDRL1") comprising an amino acid sequence with three or fewer, such as one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:36.

30 In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a light chain variable region CDR2 ("CDRL2") comprising an amino acid sequence with one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:37.

In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a light chain variable region CDR3 ("CDRL3") comprising an amino acid sequence with three or fewer,

such as one or two amino acid variation(s) ("CDR variant") to the amino acid sequence set forth in SEQ ID NO:38.

In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises a CDRH1 comprising an amino acid sequence with up to one amino acid variation to the amino acid sequence set forth in SEQ ID NO:33; a CDRH2 comprising an amino acid sequence with up to five amino acid variations to the amino acid sequence set forth in SEQ ID NO:34; a CDRH3 comprising an amino acid sequence with up to one amino acid variation to the amino acid sequence set forth in SEQ ID NO:35; a CDRL1 comprising an amino acid sequence with up to three amino acid variations to the amino acid sequence set forth in SEQ ID NO:36; a CDRL2 comprising an amino acid sequence with up to one amino acid variation to the amino acid sequence set forth in SEQ ID NO:37; and/or a CDRL3 comprising an amino acid sequence with up to three amino acid variations to the amino acid sequence set forth in SEQ ID NO:38. In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises any one or a combination of the CDRs of SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38. In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises one or more of : CDRH1 as set forth in SEQ ID NO:33; CDRH2 as set forth in SEQ ID NO:34; CDRH3 as set forth in SEQ ID NO:35; CDRL1 as set forth in SEQ ID NO:36; CDRL2 as set forth in SEQ ID NO:37 and/or CDRL3 as set forth in SEQ ID NO:38 or a direct equivalent of each CDR wherein a direct equivalent has no more than two amino acid substitutions in said CDR. In one embodiment, the CTLA-4 binding protein or antigen binding portion thereof comprises the CDRs of SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38.

In one embodiment, the CTLA-4 binding protein comprises CDRH1 (SEQ ID NO:33), CDRH2 (SEQ ID NO:34), and CDRH3 (SEQ ID NO:35) in the heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:39. In some embodiments, the CTLA-4 binding proteins comprise a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:39. Suitably, the CTLA-4 binding proteins may comprise a heavy chain variable region having about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:39. In one embodiment, the CTLA-4 binding proteins comprise a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:39. In one embodiment, the anti-CTLA-4 antibodies comprise a heavy chain variable region as set forth in SEQ ID NO:39.

CTLA-4 binding protein heavy chain (V_H) variable region :

GVVQPGRLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWDGSGNKKYADSVKGRFTISRDNKNTLYLQ
MNSLRAEDTAVYYCARDPRGATLYYYYYGMDVWGQGTITVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTWSWNSGALTSGVH (SEQ ID NO:39).

In one embodiment, the CTLA-4 binding protein comprises a heavy chain variable region ("V_H") comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:39. In one embodiment, the V_H comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:39, such as between 1 and 5, such as between 1 and 3, in particular up to 2 amino acid variations to the amino acid sequence set forth in SEQ ID NO:39.

In one embodiment of the present invention the CTLA-4 binding protein comprises CDRL1 (SEQ ID NO:36), CDRL2 (SEQ ID NO:37), and CDRL3 (SEQ ID NO:38) in the light chain variable region having the amino acid sequence set forth in SEQ ID NO:40.

In some embodiments, the CTLA-4 binding proteins comprise a light chain variable region having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:40. Suitably, the CTLA-4 binding proteins may comprise a light chain variable region having about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:40.

CTLA-4 binding protein light chain (V_L) variable region:

PSSLSASVGDRVITTCRASQSINSYLDWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF
ATYYCQQYYSTPFTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPPREAKV (SEQ ID NO:40).

In one embodiment, the CTLA-4 binding protein comprises a light chain variable region ("V_L") comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:40.

In one embodiment, the V_L comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:40, such as between 1 and 5, such as between 1 and 3, in particular up to 2 amino acid variations to the amino acid sequence set forth in SEQ ID NO:8. In one embodiment, the anti-CTLA-4 antibody comprises a light chain variable region having at least 90% sequence identity to SEQ ID NO:40. In one embodiment, the anti-CTLA-4 antibody comprises a light chain variable region as set forth in SEQ ID NO:40.

In one embodiment, the CTLA-4 binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:39 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:40. In one embodiment, the CTLA-4 binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:39 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the

amino acid sequence as set forth in SEQ ID NO:40, wherein the CTLA-4 binding protein specifically binds to human CTLA-4. In one embodiment, the CTLA-4 binding protein comprises a V_H with the amino acid sequence set forth in SEQ ID NO:39; and a V_L with the amino acid sequence set forth in SEQ ID NO:40.

5 In one embodiment, the CTLA-4 binding protein comprises a V_H comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:39; and a V_L comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:40.

10 In one embodiment, the CTLA-4 binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:39 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:40, and further comprises any one or a combination of the CDRs of SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38.

15 In one embodiment, the CTLA-4 binding protein is a humanized monoclonal antibody comprising a heavy chain (HC) amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:41.

20 CTLA-4 binding protein heavy chain (HC):

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWIYDGSNKYYADSVKGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCARDPRGATLYYYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSES
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDHKPSNTKVD
KTVKRCCKVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKP
25 REEQFNSTFRVSVLTVVHQDWLNGKEYCKKVSNGKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK (SEQ ID NO:41)

In one embodiment, the HC comprises an amino acid sequence with at least one amino acid
30 variation to the amino acid sequence set forth in SEQ ID NO:41, such as between 1 and 10, such as between 1 and 7, in particular up to 6 amino acid variations to the amino acid sequence set forth in SEQ ID NO:41. In a further embodiment, the HC comprises one, two, three, four, five, six or seven amino acid variations to the amino acid sequence set forth in SEQ ID NO:41.

In one embodiment, the CTLA-4 binding protein is a humanized monoclonal antibody
35 comprising a light chain (LC) amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:42.

CTLA-4 binding protein light chain (LC):

5 DIQMTQSPSSLSASVGDRVTITCRASQSINSYLDWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQYYSTPFTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:42)

10 In one embodiment, the LC comprises an amino acid sequence with at least one amino acid variation to the amino acid sequence set forth in SEQ ID NO:42, such as between 1 and 10, such as between 1 and 5, in particular up to 3 amino acid variations to the amino acid sequence set forth in SEQ ID NO:42. In a further embodiment, the LC comprises one, two or three amino acid variations to the amino acid sequence set forth in SEQ ID NO:42.

15 In one embodiment, the CTLA-4 binding protein comprises a HC comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:41; and a LC comprising an amino acid sequence with at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO:42. Therefore, the
20 antibody is an antibody with a heavy chain at least about 90% identical to the heavy chain amino acid sequence of SEQ ID NO:41 and/or with a light chain at least about 90% identical to the light chain amino acid sequence of SEQ ID NO:42.

In one embodiment, the CTLA-4 binding protein comprises a heavy chain amino acid sequence at least about 90% identical to the amino acid sequence of SEQ ID NO:41 and/or a light chain amino
25 acid sequence at least about 90% identical to the amino acid sequence of SEQ ID NO:42.

In one embodiment, the CTLA-4 binding protein comprises a heavy chain sequence of SEQ ID NO:41 and a light chain sequence of SEQ ID NO:42.

In one embodiment there is provided an CTLA-4 binding protein is type IgG1, IgG2, IgG3 or IgG4 constant region. In one embodiment, the CTLA-4 binding protein is type IgG1.

30 In one aspect of the invention, there is provided a method of treating cancer in a human in need thereof, the method comprising administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 0.08 mg to about 240 mg and administering to the human a CTLA-4 binding protein or antigen binding portion thereof, wherein the CTLA-4 binding protein or antigen binding portion thereof comprises one or more of CDRH1 as set out in SEQ ID
35 NO:33; CDRH2 as set out in SEQ ID NO:34; CDRH3 as set out in SEQ ID NO:35; CDRL1 as set out in

SEQ ID NO:36; CDRL2 as set out in SEQ ID NO:37 and/or CDRL3 as set out in SEQ ID NO: 38. In one embodiment, the CTLA-4 binding protein is tremelimumab.

It will be understood that aspects and embodiments of the invention pertaining to tremelimumab also apply to the CTLA-4 binding protein or antigen binding portion thereof disclosed herein.

Methods of Treatment

The therapeutic agents described herein may also be used in methods of treatment. It will be appreciated by those skilled in the art that references herein to treatment refer to the treatment of established conditions. However, compositions of the invention may, depending on the condition, also be useful in the prevention of certain diseases. The therapeutic agents described herein can be used in an effective amount for therapeutic, prophylactic or preventative treatment. A therapeutically effective amount of the therapeutic agents described herein is an amount effective to ameliorate or reduce one or more symptoms of, or to prevent or cure, the disease.

In one aspect, a method of treating cancer in a human in need thereof, the method comprising administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 0.08 mg to about 240 mg and administering to the human tremelimumab.

In another aspect, there is provided a combination comprising an agonist ICOS binding protein or antigen binding portion thereof and tremelimumab for use in treating cancer, wherein the ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 0.08 mg to about 240 mg.

In one aspect, there is provided an agonist ICOS binding protein or antigen binding portion thereof for use in treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered with tremelimumab.

In one aspect, there is provided use of an agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament for treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered with tremelimumab.

In one aspect, there is provided a composition comprising an agonist ICOS binding protein or antigen binding portion thereof at about 0.08 mg to about 240 mg and tremelimumab.

In another aspect, there is provided a pharmaceutical kit comprising about 0.08 mg to about 1000 mg of an ICOS binding protein or antigen binding portion thereof and tremelimumab.

Routes of administration and dosages

The doses provided in the present application are suitable for mammals, in particular a human. It is to be understood that where agonist ICOS binding protein is used herein, the antigen binding portion thereof is also implied.

In some embodiments, a therapeutically effective dose of the agonist ICOS binding protein is a dose of about 0.01 - 1000 mg (*e.g.* a dose about 0.01 mg; a dose about 0.08 mg; a dose about 0.1 mg; a dose about 0.24 mg; a dose about 0.8 mg; a dose about 1 mg; a dose about 2.4 mg; a dose about 7.2 mg; a dose about 8 mg; a dose about 10 mg; a dose about 20 mg; a dose about 24 mg; a dose about 30 mg; a dose about 40 mg; a dose about 48 mg; a dose about 50 mg; a dose about 60 mg; a dose about 70 mg; a dose about 72 mg; a dose about 80 mg; a dose about 90 mg; a dose about 100 mg; a dose about 160 mg; a dose about 200 mg; a dose about 240 mg; a dose about 300 mg; a dose about 320 mg; a dose about 400 mg; a dose about 480 mg; a dose about 500 mg; a dose about 600 mg; a dose about 700 mg; a dose about 720 mg; a dose about 800 mg; a dose about 900 mg; or a dose about 1000 mg).

In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg. In one embodiment, the agonist ICOS binding protein is administered at a dose of 0.08 mg, 0.24 mg, 0.8 mg, 2.4 mg, 8 mg, 24 mg, 48 mg, 80 mg, 160 mg or 240 mg in particular 24 mg, 48 mg, 80 mg or 160 mg.

It is to be understood that where mg/kg is used, this is mg/kg of body weight. In some embodiments, a therapeutically effective dose of the agonist ICOS binding protein is a dose of about 0.001 mg/kg to 10 mg/kg. In some embodiments, a therapeutically effective dose is about 0.001 mg/kg. In some embodiments, a therapeutically effective dose is about 0.003 mg/kg. In some embodiments, a therapeutically effective dose is about 0.01 mg/kg. In some embodiments, a therapeutically effective dose is about 0.03 mg/kg. In some embodiments, a therapeutically effective dose is about 0.1 mg/kg. In some embodiments, a therapeutically effective dose is about 0.3 mg/kg. In some embodiments, a therapeutically effective dose is about 0.6 mg/kg. In some embodiments, a therapeutically effective dose is about 1 mg/kg. In some embodiments, a therapeutically effective dose is about 2 mg/kg. In some embodiments, a therapeutically effective dose is about 3 mg/kg. In some embodiments, a therapeutically effective dose is about 4 mg/kg; about 5 mg/kg; about 6 mg/kg; about 7 mg/kg; about 8 mg/kg; about 9 mg/kg or about 10 mg/kg.

In one embodiment, the dose of the agonist ICOS binding protein is between about 0.001 mg/kg to about 3.0 mg/kg. In another embodiment, the dose of the agonist ICOS binding protein is about 0.001 mg/kg, about 0.003 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg, about 3.0 mg/kg, or about 10 mg/kg. In one embodiment, the dose of agonist ICOS binding protein is about 0.3 mg/kg. In another embodiment, the dose of the

agonist ICOS binding protein is at least 3.0 mg/kg. In one embodiment, the dose of the agonist ICOS binding protein is in the range of about 0.001 mg/kg to about 10 mg/kg. In one embodiment, the dose of the agonist ICOS protein is about 0.1 mg/kg to about 3 mg/kg. In one embodiment, the dose of the ICOS binding protein is about 0.1 mg/kg to about 1.0 mg/kg. In one embodiment, the dose of the agonist ICOS binding protein is about 0.1 mg/kg. In one embodiment, the dose of the ICOS binding protein is at least 0.1 mg/kg. In another embodiment, the dose of the agonist ICOS binding protein is about 0.3 mg/kg. In another embodiment, the dose of the agonist ICOS binding protein is about 1 mg/kg. In one embodiment, the dose of the agonist ICOS binding protein is about 3 mg/kg. In one embodiment, a fixed dose of agonist ICOS binding protein may be administered, assuming a typical median weight of 80 kg.

In one embodiment, the dose of agonist ICOS binding protein is increased during the treatment regimen. In one embodiment an initial dose of about 0.001 mg/kg, about 0.003 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg is increased to about 0.003 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg, about 3.0 mg/kg or at least 3.0 mg/kg. In one embodiment, an initial dose of 0.1 mg/kg is increased to 1 mg/kg. In one embodiment, an initial dose of 0.3 mg/kg is increased to 1 mg/kg. In one embodiment, the initial dose of 0.6 mg/kg is increased to 2 mg/kg.

In one embodiment, the agonist ICOS binding protein is administered at 0.1 mg/kg x 3 doses then 1 mg/kg. In one embodiment, the agonist ICOS binding protein is administered at about 0.001 mg/kg, about 0.003 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg, or about 3.0 mg/kg then increased to about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg, about 3.0 mg/kg or about 10 mg/kg.

In one embodiment, tremelimumab is administered at a dose of about 0.8 mg to about 1200 mg. In one embodiment, tremelimumab is administered at a dose of about 8 mg to about 1200 mg. In one embodiment, tremelimumab is administered at a dose of about 8 mg to about 800 mg. In one embodiment, tremelimumab is administered at a dose of about 75 mg to 1200 mg. In another embodiment, tremelimumab is administered at a dose of about 240 mg to 1200 mg. In one embodiment, tremelimumab is administered at a dose of about 75 mg to about 225 mg. In one embodiment, tremelimumab is administered at a dose of about 0.8 mg, about 5 mg, about 10 mg, about 20 mg, about 24 mg/kg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, 75 mg, about 80 mg, about 225 mg, about 300 mg, about 800 mg or about 1200 mg. In one embodiment, tremelimumab is administered at about 75 mg. In another embodiment, tremelimumab is administered at about 225 mg. In another embodiment, tremelimumab is administered at a dose of about 0.01 mg/kg to about 15 mg/kg. In another embodiment, tremelimumab is administered at a dose of about 1 mg/kg to about 10 mg/kg. In another embodiment, tremelimumab is administered at a dose of about 3 mg/kg to about 10 mg/kg. In another embodiment, tremelimumab is administered at a dose

of about 1 mg/kg to about 3 mg/kg. In another embodiment, tremelimumab is administered at a dose of about 0.1 mg/kg, about 1 mg/kg, about 3 mg/kg, about 6 mg/kg, about 10 mg/kg or about 15 mg/kg. In one embodiment, tremelimumab is administered at about 1 mg/kg. In one embodiment, tremelimumab is administered at 3 mg/kg.

5 In one embodiment, tremelimumab is administered once every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks. In one embodiment, tremelimumab is administered once every three weeks. In one embodiment, tremelimumab is administered once every twelve weeks. In one embodiment, tremelimumab is administered once every three weeks for 3, 4, 5, 6, 7, 8 or 9 doses and administered once every 12 weeks thereafter. In one embodiment, tremelimumab is administered once every three
10 weeks for 6 doses and then once every 12 weeks. In one embodiment, tremelimumab is administered at a dose of 75 mg every three weeks for 6 cycles followed by 75 mg every 12 weeks. In one embodiment, tremelimumab is administered at a dose of 225 mg every three weeks for 6 cycles followed by 225 mg every 12 weeks. In one embodiment, tremelimumab is administered at a dose of 75 mg every three weeks for 6 cycles followed by 225 mg every 12 weeks. In one embodiment,
15 tremelimumab is administered at a dose of 10 mg/kg every four weeks for 6 cycles followed by 10 mg/kg every 12 weeks.

 In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 8 mg to about 80 mg and tremelimumab is administered at a dose of about 75 mg to about 225 mg. In one embodiment, the ICOS binding protein or antigen binding
20 portion thereof is administered every 3 weeks and tremelimumab is administered every 3 weeks for 6 doses (i.e. cycles), followed by every 12 weeks. In one embodiment, tremelimumab is administered as an IV infusion over 60 minutes. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered as a 30-minute IV infusion. In one embodiment, tremelimumab is administered first as an IV infusion over 60 minutes and the ICOS binding protein or antigen binding
25 portion thereof is administered as an IV infusion over 30 minutes beginning at least 1 hour and no more than 2 hours following the end of the tremelimumab infusion.

 In some embodiments, tremelimumab is administered prior to administration of the agonist ICOS binding protein or antigen binding portion thereof. In some embodiments, the agonist ICOS
30 binding protein or antigen binding portion thereof is administered within 30 minutes and no longer than 3 hours following the end of administration of tremelimumab. In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered within 1 hour and no longer than 2 hours following the end of administration of tremelimumab.

 In some embodiments, the agonist ICOS binding protein or antigen binding portion thereof is
35 administered prior to tremelimumab. In some embodiments, tremelimumab is administered within at

least 30 minutes and no longer than one hour following administration of the agonist ICOS binding protein or antigen binding portion thereof.

In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 8 mg and the trememlimumab is administered at about 75 mg. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 8 mg and the trememlimumab is administered at about 225 mg. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 24 mg and the trememlimumab is administered at about 75 mg. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 24 mg and the trememlimumab is administered at about 225 mg. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 80 mg and the trememlimumab is administered at about 75 mg. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 80 mg and the trememlimumab is administered at about 225 mg.

In one embodiment, there is provided a method of treating cancer in a human in need thereof, the method comprising administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 0.08 mg to about 240 mg and administering to the human tremelimumab at a dose of about 75 mg to about 225 mg, wherein the ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said ICOS binding protein specifically binds to human ICOS.

In one embodiment, there is provided a combination of an agonist ICOS binding protein or antigen binding portion thereof and tremelimumab for use in treating cancer, wherein the ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and tremelimumab at a dose of about 75 mg to about 225 mg, wherein the ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said ICOS binding protein specifically binds to human ICOS.

In another embodiment, there is provided an agonist ICOS binding protein or antigen binding portion thereof for use in treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered concurrently and/or sequentially with tremelimumab at a dose of about 75 mg to about 225 mg, wherein the agonist ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain

comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said agonist ICOS binding protein specifically binds to human ICOS.

In another embodiment, there is provided use of an agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament for treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 0.08 mg to about 240 mg and is administered concurrently and/or sequentially with tremelimumab at a dose of about 75 mg to about 225 mg, wherein the ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said ICOS binding protein specifically binds to human ICOS.

In one embodiment, there is provided a pharmaceutical kit comprising about 0.08 mg to about 240 mg of an agonist ICOS binding protein or antigen binding portion thereof and tremelimumab at a dose of about 75 mg to about 225 mg, wherein the ICOS binding protein comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said ICOS binding protein specifically binds to human ICOS.

The therapeutic agents disclosed herein may be administered either in separate or combined form (*e.g.* as pharmaceutical formulations) by any convenient route. For some therapeutic agents (*i.e.* binding proteins), suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal, and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal, and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient of the combination and the cancer to be treated. It will also be appreciated that each of the agents administered may be administered by the same or different routes and that the therapeutic agents may be formulated together or in separate pharmaceutical compositions.

In one embodiment, the therapeutic agent(s) is administered intravenously. In a further embodiment, the therapeutic agent(s) is administered by intravenous infusion. In another embodiment, the therapeutic agent(s) administered intratumorally. In another embodiment, the therapeutic agent(s) is administered orally. In another embodiment, the therapeutic agent(s) is administered systemically, *e.g.* intravenously, and one or more other therapeutic agents of the invention are administered intratumorally. In another embodiment, all of the therapeutic agents are administered systemically, *e.g.* intravenously. In an alternative embodiment, all of the therapeutic

agents are administered intratumorally. In any of the embodiments, *e.g.* in this paragraph, the therapeutic agents of the invention may be administered as one or more pharmaceutical compositions.

In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered via intravenous (IV) infusion. In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered via intravenous (IV) infusion over 30 minutes. In one embodiment, tremelimumab is administered via IV infusion. In one embodiment, tremelimumab is administered via intravenous (IV) infusion over 60 minutes. In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof and tremelimumab are administered via IV infusion.

In one embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered concurrently or sequentially with tremelimumab. In another embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered concurrently with tremelimumab. In another embodiment, the agonist ICOS binding protein or antigen binding portion thereof is administered sequentially with tremelimumab.

In one embodiment, the therapeutic agent(s) are administered once every 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days, 36 days, 37 days, 38 days, 39 days, or 40 days.

In one embodiment, the therapeutic agent(s) are administered once every 1 to 12 weeks. In one embodiment, the therapeutic agent(s) are administered once every 1 week, once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks, once every 7 weeks, once every 8 weeks, once every 9 weeks, once every 10 weeks, once every 11 weeks or once every 12 weeks. In one embodiment, the therapeutic agent(s) are administered once every 3 weeks. In one embodiment, the therapeutic agent(s) are administered once every 6 weeks. In one embodiment the agonist ICOS binding protein or antigen binding fragment thereof is administered once every 3 weeks. In one embodiment, tremelimumab is administered once every three weeks. In one embodiment, tremelimumab is administered once every 3 weeks for 6 doses and once every 12 weeks thereafter.

In one embodiment, the combination is administered once every 3 weeks for 2-6 dosing cycles (*e.g.* the first 3, 4, or 5 dosing cycles, in particular, the first 4 dosing cycles). In one embodiment, the therapeutic agent(s) are administered for up to 2 years or unacceptable toxicity. In one embodiment, the therapeutic agent(s) are administered every three weeks up to 35 cycles or unacceptable toxicity.

If desired, the effective daily dose of a (therapeutic) combination may be administered as two, three, four, five, six or more doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

The present disclosure provides methods of treating cancer comprising administering to a patient in need of treatment one or both of the therapeutic agents at a first dose at a first interval for a first period; and administering to the patient one or both of the therapeutic agents at a second dose at a second interval for a second period. There may be a rest period between the first and second periods in which one or both of the binding proteins in the combination are not administered to the patient. In some embodiments, there is a rest period between the first period and second period. In some embodiments, the rest period is between 1 and 30 days. In some embodiments, the rest period is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or 31 days. In some embodiments, the rest period is 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks or 15 weeks.

In some embodiments, the first dose and second dose are the same. In some embodiments, the first interval and second interval are the same. In some embodiments, the first interval and the second interval are once every three weeks. In some embodiments, the first interval and the second interval are once every six weeks. In some embodiments, the first interval and the second interval are different. In some embodiments, the first interval is once every three weeks and the second interval is once every six weeks. In some embodiments, the first interval is once every three weeks and the second interval is once every twelve weeks.

In some embodiments, the first interval and the second interval are different. In some embodiments, the first interval is once every three weeks and the second interval is once every six weeks. In some embodiments, the therapeutic agent is administered at a first dose of 24 mg once every three weeks for the first period of 2-6 dosing cycles (e.g. the first 3, 4, or 5 dosing cycles, in particular, the first 4 dosing cycles), and at the second dose of 80 mg once every six weeks until therapy is discontinued (e.g. due to disease progression, an adverse event, or as determined by a physician).

In some embodiments, the therapeutic agent(s) described herein are administered according to dosing regimens demonstrated to achieve a clinical benefit for the patient. In some embodiments, a clinical benefit is stable disease ("SD"), a partial response ("PR") and/or a complete response ("CR"). In some embodiments, a clinical benefit is stable disease ("SD"). In some embodiments, a clinical benefit is a partial response ("PR"). In some embodiments, a clinical benefit is a complete response ("CR"). In some embodiments, PR or CR is determined in accordance with Response Evaluation Criteria in Solid Tumors (RECIST). In some embodiments, the combination is administered for a longer period to maintain clinical benefit.

In one aspect of the invention, there is provided a pharmaceutical kit comprising an ICOS binding protein or an antigen binding portion thereof and tremelimumab.

In one embodiment the pharmaceutical kit comprises about 0.08 mg to about 1000 mg of an agonist ICOS binding protein or antigen binding portion thereof and about 8 to 1200 mg of tremelimumab.

5 In one embodiment, the pharmaceutical kit comprises about 0.08 mg to about 240 mg of the agonist ICOS binding protein or antigen binding portion thereof and tremelimumab.

In some embodiments, the pharmaceutical kit comprises a further immunomodulatory agent. In one embodiment, the further immunomodulatory agent is a PD-1 binding protein or an antigen binding portion thereof, a PD-L1 binding protein or an antigen binding portion thereof.

10 In one embodiment, the pharmaceutical kit comprises the agonist ICOS binding protein at a concentration of 10 mg/ml. In one embodiment, the pharmaceutical kit comprises tremelimumab at a concentration of 20 mg/ml. In one embodiment, the pharmaceutical kit comprises the ICOS binding protein or an antigen binding portion thereof at about 10 mg/ml and tremelimumab at about 20 mg/ml.

15 In one embodiment, the pharmaceutical kit comprises the PD-1 binding protein at a concentration of about 20 mg/mL to about 125 mg/mL. In a further embodiment, the pharmaceutical kit comprises the PD-1 binding protein at a concentration of 20 mg/mL to 50 mg/mL. In one embodiment, the PD-1 binding protein is at a concentration of 20 mg/mL. In another embodiment, the PD-1 binding protein is at a concentration of 50 mg/mL.

20 Cancer

The therapeutic agent(s) and methods of the invention may be used in the treatment of cancer.

By the term "treating" and grammatical variations thereof as used herein, is meant therapeutic therapy. In reference to a particular condition, treating means: (1) to ameliorate, or lessen the severity
25 of, the condition of one or more of the biological manifestations of the condition, (2) to interfere with (a) one or more points in the biological cascade that leads to or is responsible for the condition or (b) one or more of the biological manifestations of the condition, (3) to alleviate one or more of the symptoms or signs, effects or side effects associated with the condition or treatment thereof, (4) to slow the progression of the condition, that is to say prolong survival, or one or more of the biological
30 manifestations of the condition and/or (5) to cure said condition or one or more of the biological manifestations of the condition by eliminating or reducing to undetectable levels one or more of the biological manifestations of the condition for a period of time considered to be a state of remission for that manifestation without additional treatment over the period of remission. One skilled in the art will understand the duration of time considered to be remission for a particular disease or condition.
35 Prophylactic therapy is also contemplated thereby. The skilled artisan will appreciate that "prevention" is not an absolute term. In medicine, "prevention" is understood to refer to the prophylactic

administration of a drug to substantially diminish the likelihood or severity of a condition or biological manifestation thereof, or to delay the onset of such condition or biological manifestation thereof. Prophylactic therapy is appropriate, for example, when a subject is considered at high risk for developing cancer, such as when a subject has a strong family history of cancer or when a subject
5 has been exposed to a carcinogen.

As used herein, the terms "cancer", "neoplasm", "malignancy", and "tumor" are used interchangeably and, in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells can be readily distinguished from non-cancerous cells by well-established techniques, particularly
10 histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and *in vitro* cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass; *e.g.* by procedures such as computed tomography (CT) scan, magnetic
15 resonance imaging (MRI), X-ray, ultrasound or palpation on physical examination, and/or which is detectable because of the expression of one or more cancer-specific antigens in a sample obtainable from a patient. Tumors may be a hematopoietic (or hematologic or hematological or blood-related) cancer, for example, cancers derived from blood cells or immune cells, which may be referred to as "liquid tumors." Specific examples of clinical conditions based on hematologic tumors include
20 leukemias such as chronic myelocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia and acute lymphocytic leukemia; plasma cell malignancies such as multiple myeloma, MGUS and Waldenstrom's macroglobulinemia; lymphomas such as non-Hodgkin's lymphoma, Hodgkin's lymphoma; and the like.

The cancer may be any cancer in which an abnormal number of blast cells or unwanted cell
25 proliferation is present or that is diagnosed as a hematological cancer, including both lymphoid and myeloid malignancies. Myeloid malignancies include, but are not limited to, acute myeloid (or myelocytic or myelogenous or myeloblastic) leukemia (undifferentiated or differentiated), acute promyeloid (or promyelocytic or promyelogenous or promyeloblastic) leukemia, acute myelomonocytic (or myelomonoblastic) leukemia, acute monocytic (or monoblastic) leukemia, erythroleukemia and
30 megakaryocytic (or megakaryoblastic) leukemia. These leukemias may be referred together as acute myeloid (or myelocytic or myelogenous) leukemia (AML). Myeloid malignancies also include myeloproliferative disorders (MPD) which include, but are not limited to, chronic myelogenous (or myeloid) leukemia (CML), chronic myelomonocytic leukemia (CMML), essential thrombocythemia (or thrombocytosis), and polycythemia vera (PCV). Myeloid malignancies also include myelodysplasia (or
35 myelodysplastic syndrome or MDS), which may be referred to as refractory anemia (RA), refractory

anemia with excess blasts (RAEB), and refractory anemia with excess blasts in transformation (RAEBT); as well as myelofibrosis (MFS) with or without agnogenic myeloid metaplasia.

Hematopoietic cancers also include lymphoid malignancies, which may affect the lymph nodes, spleens, bone marrow, peripheral blood, and/or extranodal sites. Lymphoid cancers include B-cell malignancies, which include, but are not limited to, B-cell non-Hodgkin's lymphomas (B-NHLs). B-NHLs may be indolent (or low-grade), intermediate-grade (or aggressive) or high-grade (very aggressive). Indolent Bcell lymphomas include follicular lymphoma (FL); small lymphocytic lymphoma (SLL); marginal zone lymphoma (MZL) including nodal MZL, extranodal MZL, splenic MZL and splenic MZL with villous lymphocytes; lymphoplasmacytic lymphoma (LPL); and mucosa-associated-lymphoid tissue (MALT or extranodal marginal zone) lymphoma. Intermediate-grade B-NHLs include mantle cell lymphoma (MCL) with or without leukemic involvement, diffuse large cell lymphoma (DLBCL), follicular large cell (or grade 3 or grade 3B) lymphoma, and primary mediastinal lymphoma (PML). High-grade B-NHLs include Burkitt's lymphoma (BL), Burkitt-like lymphoma, small non-cleaved cell lymphoma (SNCCCL) and lymphoblastic lymphoma. Other B-NHLs include immunoblastic lymphoma (or immunocytoma), primary effusion lymphoma, HIV associated (or AIDS related) lymphomas, and post-transplant lymphoproliferative disorder (PTLD) or lymphoma. B-cell malignancies also include, but are not limited to, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), Waldenstrom's macroglobulinemia (WM), hairy cell leukemia (HCL), large granular lymphocyte (LGL) leukemia, acute lymphoid (or lymphocytic or lymphoblastic) leukemia, and Castleman's disease. NHL may also include T-cell non-Hodgkin's lymphoma s(T-NHLs), which include, but are not limited to T-cell non-Hodgkin's lymphoma not otherwise specified (NOS), peripheral T-cell lymphoma (PTCL), anaplastic large cell lymphoma (ALCL), angioimmunoblastic lymphoid disorder (AILD), nasal natural killer (NK) cell / T-cell lymphoma, gamma/delta lymphoma, cutaneous T cell lymphoma, mycosis fungoides, and Sezary syndrome.

Hematopoietic cancers also include Hodgkin's lymphoma (or disease) including classical Hodgkin's lymphoma, nodular sclerosing Hodgkin's lymphoma, mixed cellularity Hodgkin's lymphoma, lymphocyte predominant (LP) Hodgkin's lymphoma, nodular LP Hodgkin's lymphoma, and lymphocyte depleted Hodgkin's lymphoma. Hematopoietic cancers also include plasma cell diseases or cancers such as multiple myeloma (MM) including smoldering MM, monoclonal gammopathy of undetermined (or unknown or unclear) significance (MGUS), plasmacytoma (bone, extramedullary), lymphoplasmacytic lymphoma (LPL), Waldenström's Macroglobulinemia, plasma cell leukemia, and primary amyloidosis (AL). Hematopoietic cancers may also include other cancers of additional hematopoietic cells, including polymorphonuclear leukocytes (or neutrophils), basophils, eosinophils, dendritic cells, platelets, erythrocytes and natural killer cells. Tissues which include hematopoietic cells referred herein to as "hematopoietic cell tissues" include bone marrow; peripheral blood; thymus; and peripheral lymphoid tissues, such as spleen, lymph nodes, lymphoid tissues associated with

mucosa (such as the gut-associated lymphoid tissues), tonsils, Peyer's patches and appendix, and lymphoid tissues associated with other mucosa, for example, the bronchial linings.

In some embodiments, the treatment of cancer is first-line treatment of cancer. In one embodiment, the treatment of cancer is second-line treatment of cancer. In some embodiments, the treatment is third-line treatment of cancer. In some embodiments, the treatment is fourth-line treatment of cancer. In some embodiments, the treatment is fifth-line treatment of cancer. In some embodiments, prior treatment to said second-line, third-line, fourth-line or fifth-line treatment of cancer comprises one or more of radiotherapy, chemotherapy, surgery or radiochemotherapy.

In one embodiment, the cancer is selected from: brain cancer, glioblastomas, glioma (such as diffuse intrinsic pontine glioma), Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast cancer (*e.g.* inflammatory breast cancer), Wilm's tumor, ependymoma, medulloblastoma, cardiac tumors, colon cancer, colorectal cancer, head and neck cancer (*e.g.* squamous cell carcinoma of the head and neck, cancer of the mouth (*i.e.* oral cancer), salivary gland cancer, buccal cancer, pharyngeal cancer, oropharyngeal cancer, nasopharyngeal cancer, hypopharyngeal cancer, laryngeal cancer), eye cancer (*e.g.* retinoblastoma), lung cancer (*e.g.* non-small cell lung cancer, small cell cancer), liver cancer (*i.e.* hepatocellular cancer), skin cancer (*e.g.* basal cell carcinoma, merkel cell carcinoma, squamous cell carcinoma), melanoma, ovarian cancer, pancreatic cancer, bile duct cancer, gallbladder cancer, prostate cancer, sarcoma (*e.g.* soft tissue sarcoma, Ewing's sarcoma, Kaposi sarcoma, rhabdomyosarcoma), bone cancer, osteosarcoma, giant cell tumor of bone, thyroid cancer, parathyroid cancer, thymoma, blood cancer (which may be broadly categorised as leukemias, lymphomas or myelomas, and include examples such as lymphoblastic T-cell leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, hairy-cell leukemia, acute lymphoblastic leukemia, acute myelogenous leukemia, chronic neutrophilic leukemia, acute lymphoblastic T-cell leukemia, plasmacytoma, immunoblastic large cell leukemia, mantle cell leukemia, multiple myeloma megakaryoblastic leukemia, multiple myeloma, acute megakaryocytic leukemia, promyelocytic leukemia, erythroleukemia, malignant lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, lymphoblastic T cell lymphoma, Burkitt's lymphoma, and follicular lymphoma), neuroblastoma, pituitary tumor, adrenocortical cancer, anal cancer (*i.e.* rectal cancer), bladder cancer, urothelial cancer, urethral cancer, vaginal cancer, vulvar cancer, cervical cancer, endometrial cancer, uterine cancer, fallopian tube cancer, renal cancer (*i.e.* kidney cancer, *e.g.* renal cell carcinoma), mesothelioma (*e.g.* malignant pleural mesothelioma), esophageal cancer (*e.g.* esophageal squamous cell carcinoma), gastric cancer (*i.e.* stomach cancer), gastrointestinal carcinoid tumor, GIST (gastrointestinal stromal tumor), appendicial cancer, penile cancer, testicular cancer, germ cell tumors.

In one embodiment, the cancer exhibits microsatellite instability (MSI). Microsatellite instability ("MSI") is or comprises a change that in the DNA of certain cells (such as tumor cells) in which the number of repeats of microsatellites (short, repeated sequences of DNA) is different than the number of repeats that was contained in the DNA from which it was inherited. Microsatellite instability arises from a failure to repair replication-associated errors due to a defective DNA mismatch repair (MMR) system. This failure allows persistence of mismatch mutations all over the genome, but especially in regions of repetitive DNA known as microsatellites, leading to increased mutational load. It has been demonstrated that at least some tumors characterized by MSI-H have improved responses to certain anti-PD-1 agents (Le *et al.* (2015) N. Engl. J. Med. 372(26):2509-2520; Westdorp *et al.* (2016) Cancer Immunol. Immunother. 65(10): 1249-1259).

In some embodiments, a cancer has a microsatellite instability status of high microsatellite instability (*e.g.* MSI-H status). In some embodiments, a cancer has a microsatellite instability status of low microsatellite instability (*e.g.* MSI-L status). In some embodiments, a cancer has a microsatellite instability status of microsatellite stable (*e.g.* MSS status). In some embodiments microsatellite instability status is assessed by a next generation sequencing (NGS)-based assay, an immunohistochemistry (IHC)-based assay, and/or a PCR-based assay. In some embodiments, microsatellite instability is detected by NGS. In some embodiments, microsatellite instability is detected by IHC. In some embodiments, microsatellite instability is detected by PCR.

In some embodiments, the cancer is associated with a high tumor mutation burden (TMB). In some embodiments, the cancer is associated with high TMB and MSI-H. In some embodiments, the cancer is associated with high TMB and MSI-L or MSS. In some embodiments, the cancer is endometrial cancer associated with high TMB. In some related embodiments, the endometrial cancer is associated with high TMB and MSI-H. In some related embodiments, the endometrial cancer is associated with high TMB and MSI-L or MSS.

In some embodiments, a cancer is a mismatch repair deficient (dMMR) cancer. Microsatellite instability may arise from a failure to repair replication-associated errors due to a defective DNA mismatch repair (MMR) system. This failure allows persistence of mismatch mutations all over the genome, but especially in regions of repetitive DNA known as microsatellites, leading to increased mutational load that may improve responses to certain therapeutic agents.

In some embodiments, a cancer is a hypermutated cancer. In some embodiments, a cancer harbors a mutation in polymerase epsilon (POLE). In some embodiments, a cancer harbors a mutation in polymerase delta (POLD).

In some embodiments, the cancer is an advanced cancer. In some embodiments, the cancer is a metastatic cancer. In some embodiments, the cancer is a recurrent cancer (*e.g.* a recurrent gynecological cancer such as recurrent epithelial ovarian cancer, recurrent fallopian tube cancer,

recurrent primary peritoneal cancer, or recurrent endometrial cancer). In one embodiment, the cancer is recurrent or advanced.

In some embodiments, the cancer is current/metastatic (R/M). In some embodiments, the cancer is recurring/refractory (R/R).

5 In another embodiment the human has a liquid tumor such as diffuse large B cell lymphoma (DLBCL), multiple myeloma, chronic lymphoblastic leukemia, follicular lymphoma, acute myeloid leukemia and chronic myelogenous leukemia.

In one embodiment, the human has a solid tumor. In one embodiment, the solid tumor is advanced solid tumor. In one embodiment, the cancer is selected from head and neck cancer, squamous cell carcinoma of the head and neck (SCCHN or HNSCC), gastric cancer, melanoma, mesothelioma, renal cell carcinoma (RCC), esophageal cancer, non-small cell lung carcinoma (NSCLC), prostate cancer, esophageal cancer, esophageal squamous cell carcinoma, colorectal cancer, cervical cancer, bladder cancer, urothelial cancer, ovarian cancer and pancreatic cancer. In one embodiment, the human has one or more of the following: HNSCC, colorectal cancer, esophageal cancer, cervical cancer, bladder cancer, breast cancer, head and neck cancer, ovarian cancer, melanoma, renal cell carcinoma (RCC), esophageal squamous cell carcinoma, non-small cell lung carcinoma, mesothelioma (*e.g.* pleural malignant mesothelioma), urothelial cancer and prostate cancer.

In one embodiment, the cancer is head and neck cancer. In one embodiment, the cancer is HNSCC. Squamous cell carcinoma is a cancer that arises from particular cells called squamous cells. Squamous cells are found in the outer layer of skin and in the mucous membranes, which are the moist tissues that line body cavities such as the airways and intestines. Head and neck squamous cell carcinoma (HNSCC) develops in the mucous membranes of the mouth, nose, and throat. HNSCC can occur in the mouth (oral cavity), the middle part of the throat near the mouth (oropharynx), the space behind the nose (nasal cavity and paranasal sinuses), the upper part of the throat near the nasal cavity (nasopharynx), the voicebox (larynx), or the lower part of the throat near the larynx (hypopharynx). Depending on the location, the cancer can cause abnormal patches or open sores (ulcers) in the mouth and throat, unusual bleeding or pain in the mouth, sinus congestion that does not clear, sore throat, earache, pain when swallowing or difficulty swallowing, a hoarse voice, difficulty breathing, or enlarged lymph nodes. HNSCC can metastasize to other parts of the body, such as the lymph nodes, lungs or liver.

Tobacco use and alcohol consumption are the two most important risk factors for the development of HNSCC, and their contributions to risk are synergistic. In addition, the human papillomavirus (HPV), especially HPV-16, is now a well-established independent risk factor. Patients with HNSCC have a relatively poor prognosis. Recurrent/metastatic (R/M) HNSCC is especially challenging, regardless of human papillomavirus (HPV) status, and currently, few effective treatment options are available in the art. HPV-negative HNSCC is associated with a locoregional relapse rate of

19–35% and a distant metastatic rate of 14–22% following standard of care, compared with rates of 9–18% and 5–12%, respectively, for HPV-positive HNSCC. The median overall survival for patients with R/M disease is 10–13 months in the setting of first-line chemotherapy and 6 months in the second-line setting. The current standard of care is platinum-based doublet chemotherapy with or without cetuximab. Second-line standard of care options include cetuximab, methotrexate, and taxanes. All of these chemotherapeutic agents are associated with significant side effects, and only 10–13% of patients respond to treatment. HNSCC regressions from existing systemic therapies are transient and do not add significantly increased longevity, and virtually all patients succumb to their malignancy.

In one embodiment, the cancer is recurrent/metastatic (R/M) HNSCC. In one embodiment, the cancer is recurring/refractory (R/R) HNSCC. In one embodiment, the cancer is HPV-negative or HPV-positive HNSCC. In one embodiment, the cancer is a locally advanced HNSCC. In one embodiment, the cancer is (R/M) HNSCC in PD-L1 CPS (Combined Positive Score) positive (CPS ≥ 1) patients. The combined positive score is as determined by an FDA-approved test. PD-L1 CPS is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. In one embodiment, PD-L1 CPS is determined using PharmDx 22C3. In one embodiment, the cancer is HNSCC in PD-1 binding protein/PD-L1 binding protein experienced or PD-1 binding protein/PD-L1 binding protein naïve patients. In one embodiment, the cancer is HNSCC in PD-1 binding protein/PD-L1 binding protein experienced or PD-1 binding protein/PD-L1 binding protein naïve patients.

In one embodiment, the head and neck cancer is oropharyngeal cancer. In one embodiment, the head and neck cancer is an oral cancer (*i.e.* a mouth cancer).

In one embodiment, the treatment is first-line or second line treatment of HNSCC. In one embodiment, the treatment is first-line or second line treatment of recurrent/metastatic HNSCC. In one embodiment the treatment is first line treatment of recurrent/metastatic (1L R/M) HNSCC. In one embodiment, the treatment is first line treatment of 1L R/M HNSCC in a PD-L1 CPS (combined positive score) positive (CPS ≥ 1) patients. In one embodiment the treatment is second line treatment of recurrent/metastatic (2L R/M) HNSCC.

In one embodiment, the treatment is first-line, second-line, third-line, fourth-line or fifth-line treatment of PD-1/PD-L1-naïve HNSCC. In one embodiment, the treatment first-line, second-line, third-line, fourth-line or fifth-line treatment of PD-1/PD-L1 experienced HNSCC.

In one embodiment, the cancer is lung cancer. In some embodiments, the lung cancer is a squamous cell carcinoma of the lung. In some embodiments, the lung cancer is small cell lung cancer (SCLC). In some embodiments, the lung cancer is non-small cell lung cancer (NSCLC), such as squamous NSCLC. In some embodiments, the lung cancer is an ALK-translocated lung cancer (*e.g.* ALK-translocated NSCLC). In some embodiments, the cancer is NSCLC with an identified ALK

translocation. In some embodiments, the lung cancer is an EGFR-mutant lung cancer (*e.g.* EGFR-mutant NSCLC). In some embodiments, the cancer is NSCLC with an identified EGFR mutation. In one embodiment, the cancer is advanced NSCLC. In another embodiment, the cancer is relapsed/refractory advanced NSCLC.

5 In one embodiment, the cancer is melanoma. In some embodiments, the melanoma is an advanced melanoma. In some embodiments, the melanoma is a metastatic melanoma. In some embodiments, the melanoma is a MSI-H melanoma. In some embodiments, the melanoma is a MSS melanoma. In some embodiments, the melanoma is a POLE-mutant melanoma. In some
10 embodiments, the melanoma is a POLD-mutant melanoma. In some embodiments, the melanoma is a high TMB melanoma.

In one embodiment, the cancer is urothelial cancer. In some embodiments, the urothelial cancer is an advanced urothelial cancer. In some embodiments, the urothelial cancer is a metastatic urothelial cancer. In some embodiments, the urothelial cancer is a MSI-H urothelial cancer. In some
15 embodiments, the urothelial cancer is a MSS urothelial cancer. In some embodiments, the urothelial cancer is a POLE-mutant urothelial cancer. In some embodiments, the urothelial cancer is a POLD-mutant urothelial cancer. In some embodiments, the urothelial cancer is a high TMB urothelial cancer.

The following examples are intended for illustration only, and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

The following study evaluated tumor growth inhibition efficacy of the surrogate murine ICOS IgG1 clone 7E.17G9 antibody in combination with an anti-mouse CTLA-4 clone 9H10 antibody in EMT-
25 6 syngeneic tumor model. EMT-6 murine breast carcinoma cell line readily form tumors in wild type BALB/c mice with intact immune systems and is routinely used as a model to evaluate anti-cancer immunotherapies.

All procedures on animals were reviewed and approved by the GSK Institutional Animal Care and Use Committee prior to initiation of the studies.

Animals

The mice were six week old female Balb/c mice (BALB/cAnNHsd, Envigo).

Cell line culture

EMT-6 were obtained from the American Type Culture Collection (ATCC) and cultured in flasks in a humidified incubator at 37°C with 5% CO₂. Cells were expanded and cryopreserved in multiple vials and stored at vapor phase of liquid nitrogen for future use. Cryopreserved cells stocks were negative for mouse pathogens. One vial of cells was thawed and cultured for additional 3 passages prior to tumor inoculation.

Tumor inoculation

Cells used for inoculation were harvested during log-phase and resuspended in cold PBS. Each mouse was injected subcutaneously in the right flank with 1 x 10⁵ EMT-6 cells (0.1 mL cell suspension). Tumor free mice resulting from treatments were re-challenged with 1 x 10⁵ EMT-6 cells at day 71 post the first dose.

Measurements

Tumor volume and body weight data were collected using the Study Director Software Package (Studylog Systems, South San Francisco, CA, USA). The volume was calculated using the formula: Tumor Volume (mm)³ = 0.52 * l * w² where w = width and l = length, in mm, of the tumor.

Randomization

When tumors reached approximately 100-150 mm³ approximately 7-8 days post EMT-6 tumor cell inoculation, mice were randomized into various groups based on tumor volume using stratified sampling method in the StudyLog software prior to initiation of treatment. ANOVA results were displayed to ensure similarity between groups (a p value >0.99).

Observations and Endpoints

Post EMT-6 inoculation, the animals were checked daily for any effects of tumor growth and treatments on behaviour such as mobility, food and water consumption, and general clinical observations. Tumor size and body weight was measured 2-3 times per week, and individual animals were euthanized when tumor reached a pre-determined endpoint (tumor volume of > 2000 mm³, ulceration, body weight loss >20%) or at the end of the study, whichever came first. Mortality and observed clinical signs were recorded for individual animals.

Statistical Analysis

Adjusted AUC: An integrated (across time) measure of tumor burden, adjusted for the number of days on study of tumor volume. Adjusted AUCs are analysed by a nonparametric ANOVA (ANOVA on the ranks), followed by a FDR multiplicity adjustment. Significance is defined as FDR ≤ 0.05.

Kaplan-Meier (KM) survival analysis: Method is carried out to estimate the survival probability of different treatment groups at a given time. The median time to endpoint and its corresponding 95% confidence interval is reported. Whether or not KM survival curves are statistically different between any two groups is then tested by log-rank test. p-values are adjusted for multiplicity using the FDR (false discovery rate) method. Significance is defined as $FDR \leq 0.05$. R analysis software is used.

Study design

α ICOS and α CTLA-4 were dosed at 5 μ g/mouse. Mouse IgG1 (Isotype for α ICOS)(clone MOP21 from Bioxcell) and hamster IgG (Isotype for α CTLA-4) were dosed at 5 μ g/mouse.

Result

Monotherapy treatment of EMT-6 tumor bearing mice with 5 μ g/mouse hamster anti-mouse CTLA-4 clone 9H10 antibody resulted in statistically significant tumor growth inhibition and survival advantage ($p < 0.05$) compared to isotype control. α ICOS in combination with α CTLA-4 resulted in statistically significant tumor growth inhibition and survival advantage ($p = 0.037$ and $p = 0.047$ respectively) compared to the α ICOS + isotype monotherapy as measured by AUC and Kaplan-Meier analysis. α ICOS in combination with α CTLA-4 did not result in statistically significant tumor growth inhibition or survival advantage compared to the efficacious α CTLA-4 monotherapy. A trend of combination enhanced survival was observed compared to CTLA-4 monotherapy however, in that the α ICOS + α CTLA-4 combination resulted in 9 tumor free mice compared to 4 for α ICOS + isotype and 7 for α CTLA-4 + isotype monotherapies. Isotype control treatment resulted in zero tumor free mice

Discussion

ICOS is a T-cell specific CD28-superfamily costimulatory molecule and immune checkpoint protein which is expressed on certain activated T cells and plays a key role in the proliferation and activation of T cells. ICOS mouse IgG1 clone 7E.17G9 is the surrogate agonistic antibody targeting and binding to ICOS expressed on murine T cells. The mouse IgG1 isotype corresponds to the ICOS human IgG4 (H2L5 IgG4PE) antibody in regard to Fc interaction in the mouse. 7E.17G9 expressed on a mouse IgG1 isotype interacts with Fc γ RIIb Fc receptors to enable ICOS receptor cross-linking without antibody-dependent cell-mediated cytotoxicity (ADCC).

The objective of this study was to evaluate tumor growth inhibition efficacy of the surrogate murine ICOS IgG1 clone 7E.17G9 antibody in combination with the anti-mouse CTLA-4 clone 9H10 antibody in the EMT-6 syngeneic tumor model.

ICOS in combination with anti-CTLA-4 showed a trend of combination benefit in that the total number of tumor free mice increased with the combinations, versus the most efficacious monotherapy. αICOS + αCTLA-4 resulted in 90% tumor free mice, compared to 70% for the CTLA-4 monotherapy. A statistically significant survival benefit ($p < 0.05$) was not achieved, however, due to the effective monotherapy treatments. The trend of greater numbers of tumor free mice with combination does support combination benefit potential for αICOS with αCTLA-4 therapy.

Example 2

H2L5 IgG4PE is a humanized IgG4 antibody selected for its potent binding, agonist activity against human ICOS and low/no depleting effects. The unique mechanistic profile of H2L5 IgG4PE offers an opportunity to investigate the antitumor potential of targeting a T cell co-stimulator alone and in combination with standard-of-care (SoC) agents. H2L5 IgG4PE comprises CDR sequences as shown in SEQ ID NOS: 1-6, variable heavy chain and variable light chain sequences as shown in SEQ ID NO:7 and SEQ ID NO: 8, respectively, and heavy chain and light chain sequences as shown in SEQ ID NO:9 and SEQ ID NO:9, respectively.

Described herein is a first-time in human study evaluating the safety, pharmacokinetics (PK), pharmacodynamics (PD), and antitumor activity of H2L5 IgG4PE in selected solid tumours. The study consists of dose escalation and cohort expansion phases; cohort expansion phases are ongoing in several tumor types.

The objectives of the study are as follows:

Primary

- Determine safety, tolerability, and maximum tolerated/administered dose of H2L5 IgG4PE.

Secondary

- Determine recommended H2L5 IgG4PE dose(s) for further exploration.
- Evaluate preliminary antitumor activity; characterize PK; evaluate immunogenicity.

Exploratory

- Evaluate PD effects.
- Explore associations between antitumor activity, PK and biomarkers in tissue and blood.

Methods

The Study is a dose escalation (DE) and ongoing expansion phase study of H2L5 IgG4PE.. Modified toxicity probability interval informed DE decisions with ≥ 3 patients enrolled per dose level (DL). H2L5 IgG4PE is administered as intravenous infusion every 3 weeks (Q3W); treatment continues up to 2

years or until progression or unacceptable toxicity. Patients must have metastatic or relapsed invasive malignancy, measurable disease, received ≤ 5 lines of prior therapy in the advanced setting, adequate organ function, and no active autoimmune disease requiring treatment; PK/PD cohorts require pre-treatment and Day 43 on-treatment tumor biopsies. Primary objective is to determine safety, tolerability, and maximum tolerated (MTD) H2L5 IgG4PE dose.

Patients – key inclusion criteria

- Histological or cytological documentation of advanced/metastatic or relapsed/refractory invasive malignancy and is one of the following tumour types:

- Bladder/urothelial cancer of the upper and lower urinary tract
- Cervical
- Colorectal (includes appendix) (microsatellite stability (MSS)/ high microsatellite instability (MSI-H))
- Esophageal squamous cell carcinoma
- Head and Neck Carcinoma (any histology)
- Melanoma (cutaneous/ocular)
- MPM
- NSCLC (including targeted mutations)
- Prostate
- MSI-H/dMMR tumor (Part 1B)
- HPV-positive or EBV-positive tumor (Part 1B)
- Malignant pleural mesothelioma

- Disease that has progressed after standard therapy for the specific tumor type, or for which standard therapy has proven to be ineffective, is intolerable, or is considered inappropriate, or if no further standard therapy exists

- ≤ 5 prior lines of therapy for advanced disease including both standard of care and investigational therapies.

- Measurable disease according to RECIST v1.1 guidelines; Eastern Cooperative Oncology Group performance status 0–1; adequate organ function.

- Agree to undergo a pre-treatment and on-treatment biopsy and have disease amenable to biopsy required in PK/PD dose expansion cohorts.

Patients – key exclusion criteria

- Prior anticancer or investigational therapy within 30 days or five half-lives, whichever is shorter.
- $\geq$ Grade 3 toxicity related to prior immunotherapy and led to treatment discontinuation.
- History of invasive malignancy other than disease under study unless disease-free for ≥ 2 years.

- Central nervous system (CNS) metastasis; exceptions include previously treated CNS metastasis that is asymptomatic and has no requirement for steroids at least 14 days prior to first dose of study treatment.
- Active autoimmune disease that required systemic treatment within the last 2 years.
- History of idiopathic pulmonary fibrosis, pneumonitis requiring steroids, interstitial lung disease, or organizing pneumonia.

Study Design

The study design involves

- Accelerated titration design for the first 3 dose levels; 1 patient enrolled at each dose level.
- Modified toxicity probability interval method informed subsequent dose escalation decisions (minimum 3 patients per dose level).
- Starting dose of 0.001 mg/kg: the projected human dose based on the minimally anticipated biologic effect observed in preclinical studies.

The study consists of a dose escalation phase followed by a cohort expansion phase.

This is a FTIH, open-label, multicenter study designed to investigate the safety, tolerability, pharmacology, PK, preliminary clinical activity, and establish a recommended dose of H2L5 IgG4PE for further exploration.

The study consists of a dose escalation (Part 1A) phase followed by a cohort expansion phase (Part 1B).

The dose escalation phase evaluates escalating weight-based dose levels of H2L5 IgG4PE administered intravenously once every three weeks (Q3W) to subjects with selected relapsed and/or refractory solid tumors. Based on safety and tolerability, and the PK/pharmacodynamic characteristics of the molecule, recommended monotherapy dose level or dose levels may be further investigated in expansion cohorts.

While expansion cohorts may initiate with H2L5 IgG4PE weight-based dosing, a transition to fixed dosing may be made.

Seamless design is implemented to combine dose escalation with dose expansion, based on toxicity and efficacy (Pan H, Fang X, Liu P, et al. A phase I/II seamless dose escalation/expansion with adaptive randomization scheme (SEARS). Clinical Trials. 2013; 0:1-11). The dose expansion phase may start before the dose escalation phase is completed. All available safety and tolerability data from subjects in dose expansion is incorporated into dose escalation decision making. The basis of the decision to initiate expansion of a dose level/dose will consider following graduation rules:

- Established safety and tolerability;
- Preliminary PK/pharmacodynamic characteristics (i.e., measures of target engagement and functional effects such as receptor occupancy and cytokine release) and/or
- Preliminary antitumor activity.

5

Once a dose level(s) passes the graduation rules the selected dose(s) may enter into the expansion phase for further investigation; alternate H2L5 IgG4PE schedules may be investigated in the expansion phase. In addition, dose levels under investigation in the ongoing dose escalation phase may incorporate information, such as safety data, from subjects who were accrued to the expansion phase. Randomization and/or futility rules may be incorporated if appropriate in expansion phase to optimize the dose allocation based on evaluations of safety and antitumor activity. The details of randomization schema for expansion cohorts will be documented before the initiation of expansion cohort; details of the futility rules will be documented in the RAP before initiation of interim analyses (Pan H, Fang X, Liu P, et al. A phase I/II seamless dose escalation/expansion with adaptive randomization scheme (SEARS). Clinical Trials. 2013; 0:1-11).

15

The study will enroll subjects diagnosed with solid tumor malignancies.

20

- In the dose escalation phases of the study, and in the PK/pharmacodynamic expansion cohorts the solid tumor types selected for inclusion include bladder/urothelial cancer, cervical cancer, colorectal cancer (CRC), esophageal cancer with squamous cell histology, head and neck (HN) cancer, melanoma, malignant pleural mesothelioma (MPM), non-small-cell lung cancer (NSCLC), and prostate cancer.

25

- In the cohort expansion phases of the study, several expansion cohorts have been defined by tumor histology or by a specific characteristic such as tumors exhibiting high microsatellite instability (MSI-H), deficiency in DNA mismatch repair (dMMR) processes, or viral-mediated pathology; enrollment in these cohorts is not limited to the tumor types/histologies in the aforementioned list (defined as tumor agnostic).

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- Additional expansion cohorts may enroll subjects with a specific tumor type selected from the aforementioned list or from a tumor type/histology not protocol-defined; the basis for the selection will be evidence-based and by an amendment to the protocol to define the cohorts.

The overall study size may extend beyond 500 by a protocol amendment if data from expansion phases support extended enrollment or additional combinations are investigated.

Assessment of disease status will be performed by the Investigator in accordance with Response Evaluation Criteria In Solid Tumors (RECIST) v1.1 and Immune Related (ir) RECIST. A decision to discontinue treatment due to disease progression will be based upon irRECIST; primary efficacy endpoint analysis will use irRECIST. Scans will be collected centrally and stored to allow for the option of central review.

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Table 2: Study Treatment

Product Name	H2L5 IgG4PE
Product Description	Humanized anti-ICOS IgG4 mAb
Dosage form /strength	10 mg/mL solution
Dosage level(s)	0.001 to 3 mg/kg
Route of Administration	IV infusion
Dosing instructions/ Frequency	Administer diluted product /once Q3W
Manufacturer	GSK

H2L5 IgG4PE Fixed Dose Rationale

5 H2L5 IgG4PE was administered on body weight-based dosing. Fixed doses may be tested in the expansion cohorts and in the safety run-in phase with chemotherapy combinations, assuming a typical median weight of 80 kg.

Therapeutic monoclonal antibodies are often dosed based on body-size due to the concept that this reduces inter-subject variability in drug exposure. However, body-weight dependency of PK parameters does not always explain the observed variability in the exposure of monoclonal antibodies (Zhao X, Suryawanshi, S; Hruska, M. Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3 mg/kg dosing regimen in patient with advanced tumors. Annals of Oncology. 2017;28:2002-2008). The advantage of body-weight based versus fixed dosing in this study was evaluated through population PK modelling and simulation efforts. A preliminary population PK model was developed from monotherapy dose escalation (data up to doses of 1 mg/kg; n=19 subjects). Simulations were performed by considering body weight distribution in the simulations based on the observed distribution in the preliminary dataset. At the 5th percentile of body weight (40–47 kg), there was a 70-100% increase in median steady-state AUC(0-); H2L5 IgG4PE exposures higher than these increases have been evaluated in the current Phase 1 study with the 3 mg/kg dose regimen. At the 95th percentile of body weight (107–118 kg), there was a 23-32% decrease in median steady-state AUC(0-) as compared to the median 80 kg exposure providing adequate RO with the minimal lowering of exposure. A similar outcome is expected for steady-state Cmax and trough concentrations between body weight-based and fixed dosing.

Overall, these preliminary population PK simulations indicate that using fixed dosing would result in a similar range of exposures as that of body weight-based dosing. Also, fixed dosing offers the advantage of reduced dosing errors, reduced drug wastage, shorten preparation time, and improve ease of administration. Thus, switching to a fixed dose based on a reference body weight of 80 kg is appropriate.

The fixed dose equivalents of the weight-based H2L5 IgG4PE dose levels using 80 kg weight are presented in Table 3.

Table 3 H2L5 IgG4PE Fixed Dose Calculations

Dose Level	H2L5 IgG4PE mg/kg	H2L5 IgG4PE mg
1	0.001	0.08
2	0.003	0.24
3	0.01	0.8
4	0.03	2.4
5	0.1	8.0
6	0.3	24.0
7	1.0	80.0
8	3.0	240.0

Results

In the DE phase and the PK/PD cohort, 62 patients enrolled: Part 1: 22 in DE and 40 in the PK/PD cohort. 22 patients (35%) had at least one treatment-related adverse event (TR-AE). The most frequent TR-AEs (≥ 3 patients) were fatigue (15%), AST elevations (5%) and diarrhea (3%); AST elevations were the most frequent Grade 3/4 TR-AE (N=2 [3%]). Approximate dose proportional increases in systemic H2L5 IgG4PE concentrations over 0.01 – 3mg/kg DLs were observed. At DLs ≥ 0.3 mg/kg, ICOS receptor occupancy was $\geq 75\%$ across the dosing interval. On-target PD effects in tumor infiltrating lymphocytes and clinical activity were observed in Part 1; including in anti-PD-1/L1 experienced patients.

Table 4 shows patient disposition by cohort and dose.

Table 4

H2L5 IgG4PE DOSE mg / kg	MONOTHERAPY DOSE ESCALATION	MONOTHERAPY PK / PD
0.001	1	NOT EVALUATED
0.003	1	NOT EVALUATED
0.01	2	NOT EVALUATED
0.03	3	5
0.1	5	10 (n-4 ONGOING)
0.3	3	14 (n-4 ONGOING)
1	4	3 (n-1 ONGOING)
3	3	8

Table 5 shows patient and disease characteristics.

Table 5

	MONOTHERAPY DOSE ESCALATION N = 22	MONOTHERAPY PK/PD N = 40
SEX, n (%), FEMALE/MALE	12 (55) / 10 (45)	17 (43) / 23 (58)
MEDIAN AGE, YEARS (MIN, MAX)	59 (36, 78)	59 (42, 81)
PRIMARY TUMOR TYPE, n (%)		
COLORECTAL, MSS/MSI-H*	8 (36)	10 (25)
CERVICAL	3 (14)	6 (15)
BLADDER/UROTHELIAL	2 (9)	1 (3)
HEAD/NECK, ANY HISTOLOGY/LOCATION	2 (9)	6 (15)
MELANOMA	2 (9)	8 (20)
MESOTHELIOMA	2 (9)	3 (8)
NSCLC	2 (9)	2 (5)
PROSTATE	1 (5)	2 (5)
ESOPHAGEAL, SCC	0	2 (5)
≥2 TARGET LESIONS LOCATIONS, n (%)	15 (68)	25 (63)
PRIOR SYSTEMIC REGIMENS IN ADVANCED/METASTATIC SETTING, n (%)		
<3	15 (68)	24 (60)
≥3	7 (32)	16 (40)
PRIOR PD-1/L1 INHIBITOR, n (%)	8 (36)	15 (38)

*MONOTHERAPY DOSE ESCALATION & PK /PD: 100% MSS; COMBINATION DOSE ESCALATION: 64% MSS, 36% MSI-H MSS, MICROSATELLITE STABILITY; MSI-H, HIGH MICROSATELLITE INSTABILITY; NSCLC, NON-SMALL-CELL LUNG CARCINOMA; PD-L1, PROGRAMMED DEATH-LIGAND 1; SCC, SQUAMOUS CELL CARCINOMA

5

Table 6 shows treatment-related AEs (in ≥3 patients).

Table 6

	MONOTHERAPY (DOSE ESCALATION AND PK/PD) N = 62	
AE BY PREFERRED TERM	ALL GRADES	GRADE 3/4
ANY TR-AE, n (%)	22 (35)	3 (5)
FATIGUE	9 (15)	0
AST INCREASED	3 (5)	2 (3)
DIARRHEA	3 (5)	0
PYREXIA	0	0

AE - Adverse Event; AST – Aspartate Aminotransferase; TR-AE – Treatment-related AE.

Treatment-related safety

- 5 • 1 patient in monotherapy dose escalation cohort experienced Grade 3/4 elevations in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, gamma-glutamyl transpeptidase, impaired liver function (serious) and Grade 1 amylase and G3 lipase.
- Serious adverse events (SAEs) in monotherapy group: 1 patient (3 mg/kg) had impaired liver function (Grade 3).
- 10 • Confounded by progression in liver metastases and biliary tract obstruction that required stenting.

Patient 1: H2L5 IgG4PE monotherapy (FIG. 1)

History:

- 53Y Male; Stage IIIC nodular melanoma [BRAF/cKIT mutation negative].
- 15 • Prior regimens: ipilimumab/nivolumab ~ 2 months; nivolumab ~ 1 year, SD best response.
- Disease Burden: 5 target lesions (LN, Lung, SubQ): SoD=225 mm. Multiple non target lesions.

Study treatment:

- 20 • H2L5 IgG4PE monotherapy to Week 48; 0.1 mg/kg x 3 doses, then 1 mg/kg.
-

Tumor biopsies collected after 43 days on-treatment showed greater number of T cells, granzyme-B expressing CD8 Tc cells, PD1 expressing T cells and proliferating T cells while fewer
25 proliferating tumor cells (data not shown).

Patient 2: H2L5 IgG4PE monotherapy

History:

- BRAF negative, N/KRAS mutation positive; Stage Ib superficial spreading melanoma.
- Prior regimens: nivolumab (advanced/metastatic) ~ 10 months; Electrochemotherapy.

5 *Study treatment:*

- H2L5 IgG4PE monotherapy at 1 mg/kg.

The post treatment sample (data not shown) showed:

- Higher TIL including cytotoxic, helper T cells and NK cells
- More Granzyme B+ T cells and less proliferating tumour cells
- Increase in activated T cells as observed with greater OX40 and HLADR expression
- Upregulation of PD1 and PD-L1 upon H2L5 IgG4PE treatment

Conclusions

- 15 • H2L5 IgG4PE was well tolerated in patients with advanced solid tumours at the 0.001–3 mg/kg dose range.
- Maximum tolerated dose was not reached; maximum administered dose was 3 mg/kg H2L5 IgG4PE.
- Majority of AEs were Grade 1/2 and not attributed to study treatment.
- 20 • AEs leading to discontinuation occurred in 1 patient (n=62 patients) at the highest dose level.
- Dose proportional increases in H2L5 IgG4PE concentrations.
- PK/PD analysis showed $\geq 75\%$ total ICOS receptor saturation across dosing interval at H2L5 IgG4PE dose levels ≥ 0.3 mg/kg.
- 25 • A range of doses (≥ 0.1 –1 mg/kg) have shown biological and clinical activity (including in patients with prior anti-PD-1/L1 exposure). These doses are being investigated further in expansion cohorts to establish the recommended H2L5 IgG4PE dose.
- Preliminary biological and clinical data support the mechanism of action of a non-depleting ICOS agonist as a clinical target.
- 30 • Doses over 0.1 mg/kg are being investigated further in expansion cohorts to establish the recommended H2L5 IgG4PE dose.

Example 3

Example 3 describes pharmacokinetics/pharmacodynamics (PK/PD) exposure-response characterization of H2L5 IgG4PE from the study described in Example 1. H2L5 IgG4PE is an agonist IgG4PE antibody against inducible co-stimulatory receptor (ICOS) with immune stimulating and anti-neoplastic activity. The study described Example 2 is the first in human study investigating H2L5 IgG4PE.

Methods

Safety, PK, PD, and preliminary antitumor activity of H2L5 IgG4PE were evaluated at doses from 0.001 to 3 mg/kg every 3 weeks (Q3W). Blood samples collected prior to dosing and select time points on-study were evaluated for PK and PD effects on lymphocytes and ICOS receptor occupancy (RO). Tumor tissue at Screening and Week 6 were evaluated for changes in tumor immune infiltrates (TIL) by a multiplexed immuno-fluorescence platform.

PK Analysis

- A preliminary population PK data set was constructed with all pooled concentration–time data
- Serial plasma samples were collected throughout; PK samples were assayed by validated ELISA assay and concentration–time data was modeled using nonlinear mixed effects, as implemented in NONMEM.

Pharmacodynamic (PD) Analysis

- Flow cytometry was performed instream throughout the study to evaluate ICOS receptor occupancy (RO) with H2L5 IgG4PE.
- For PK/PD and expansion cohorts, tumor tissue was collected at pre-dose and at Week 6 for evaluation of overall TIL, changes in activation, proliferation and gene expression changes.
- Exposure measure for PK/PD analyses defined as Week 6 pre-dose trough concentration derived from population PK model.
- Evaluation of gene expression changes in the tumour micro environment (TME) were performed using the Nanostring nCounter™ platform.
- Multiomyx™ multiplexed immunofluorescence was used to characterize the immune phenotype of the TIL.

Figures 2A and 2B are plots showing duration of H2L5 IgG4PE monotherapy treatment: individual patient data. Figure 2A shows monotherapy dose escalation cohort. Figure 2B shows PK/PD cohort.

Results

The PK disposition of H2L5 IgG4PE is consistent with that of other humanized mAbs, with low clearance and limited central volume of distribution. Dose and concentration-receptor occupancy (RO) analyses suggest $>\sim 0.1$ mg/kg H2L5 IgG4PE maintains high RO on CD4⁺ and CD8⁺ T cells. Quantitative TIL evaluation of paired tumor biopsies demonstrates potentially favorable immune microenvironment in the tumor at exposures observed in subjects treated with 0.3mg/kg dose. TIL and gene expression data from tumor RNA demonstrate non-linear, dose-dependent changes in select markers of immune activation. Clinical exposure-response assessments reveal no difference in baseline-to-Week 9 target lesion change across exposures in the 1L R/M HNSCC expansion cohort. Likewise, cross-cohort pooled exposure-response analysis of AEs of $\geq$ Grade 2 severity demonstrates similar safety outcomes across the exposures/doses. Population PK modeling suggests fixed doses maintain exposures within established safety bounds.

Pharmacokinetics and Target Engagement

- PK and target engagement characteristics of H2L5 IgG4PE are similar to prior reports, with a population clearance estimate of ~ 0.27 L/day and central volume estimate of ~ 3.6 L, and limited impact of bodyweight on systemic exposure.
- Plasma concentrations of H2L5 IgG4PE increase in a dose-proportional manner (Figure 3A), while ICOS RO was maintained above $\sim 70\%$ with H2L5 IgG4PE doses of 0.1 mg/kg and higher (Figure 3B).
- Minimal differences in RO are observed for CD4⁺ with H2L5 IgG4PE doses of 0.3 mg/kg and 1.0 mg/kg (Figure 3C), with similar results for CD8⁺ (data not shown). However, there was large variability in RO for doses < 1.0 mg/kg (Figure 3D)

MultiOmyx and Gene Expression Data

- Quantitative evaluation of TILs in paired tumor biopsies demonstrates on-study changes in TILs follow a non-linear, exposure/dose-dependent pattern.
- Changes in select immune activation markers favors a greater cytotoxic T cell to regulatory T cell ratio with H2L5 IgG4PE exposures of 1000-10000 ng/ml at C_{trough} which corresponds to doses between ~ 0.3 mg/kg to 1 mg/kg (Figure 4A).
- Non-monotone dose-dependent changes in total TIL as well as other activation and proliferating T cell phenotypes were detected in on-treatment biopsies when compared to baseline in MultiOmyxTM immunofluorescence data with H2L5 IgG4PE 0.3 mg/kg and higher doses (Figure 4B).

- Gene expression changes in the tumor show a non-linear dose response trend with the highest increases at ≥ 0.1 mg/kg and greatest reductions at < 1 mg/kg (data not shown).
- The ratio of cytotoxic T cell proliferation (CD3+CD8+Ki67+) over regulatory T cells proliferation (CD3+CD4+FOXP3+Ki67+) was higher in Week 6 on-treatment biopsies when compared to pre-treatment tumor samples for subjects at 0.3–1 mg/kg doses of H2L5 IgG4PE who experienced disease control (DC) benefit when compared to subjects that did not experience disease control (Figure 4C).

Conclusion

The current data provide preliminary evidence of H2L5 IgG4PE target engagement and biological activity at clinically tolerable doses and support further exploration of a 24 mg Q3W fixed dosage in R/M HNSCC.

Example 4

H2L5 IgG4PE is a humanized IgG4 antibody selected for its potent binding, agonist activity against human ICOS and low/no depleting effects. The unique mechanistic profile of H2L5 IgG4PE offers an opportunity to investigate the antitumor potential of targeting a T cell co-stimulator. H2L5 IgG4PE comprises CDR sequences as shown in SEQ ID NOS: 1-6, and variable heavy chain and variable light chain sequences as shown in SEQ ID NO:7 and SEQ ID NO: 8, respectively.

Described herein is a Phase I/II, Open-label, Two Part Study of H2L5 IgG4PE in combination with tremelimumab in participants with selected, advanced solid tumors.

The study is comprised of two parts: Part 1 - a dose escalation study of H2L5 IgG4PE in combination with tremelimumab; Part 2 - expansion study to evaluate the efficacy of the recommended phase 2 dose combination.

The objectives of the study are as follows:

Part 1

Primary

- Determine safety, tolerability and the recommended phase II dose of H2L5 IgG4PE in combination with tremelimumab

Secondary

- Evaluate clinical activity of H2L5 IgG4PE in combination with tremelimumab
- Characterise the PK properties of H2L5 IgG4PE and tremelimumab when administered in combination
- Determine immunogenicity of H2L5 IgG4PE and tremelimumab when administered in combination

Part 2*Primary*

- Evaluate clinical activity of H2L5 IgG4PE in combination with tremelimumab compared to SoC

Secondary

- 5 • Further evaluate the clinical activity of H2L5 IgG4PE in combination with tremelimumab compared to standard of care (SoC)
- Further evaluate the PK properties of H2L5 IgG4PE and tremelimumab when administered in combination
- 10 • Further evaluate immunogenicity of H2L5 IgG4PE and tremelimumab when administered in combination
- Further evaluate the safety and tolerability of H2L5 IgG4Peand tremelimumab when administered in combination

Patients – key inclusion criteria

- 15 • Histological or cytological documentation of advanced/metastatic or relapsed/refractory malignancy and is one of the following tumor types:
 - a. Part 1:
 - i. Cutaneous Melanoma
 - 20 ii. Head and Neck Squamous Cell Carcinoma (HNSCC) (oral cavity, larynx, oropharynx, hypopharynx, nasal cavity/paranasal sinuses)
 - iii. Non-Small Cell Lung Cancer (Squamous and Non-squamous)
 - iv. Urothelial carcinoma of the upper and lower urinary tract
 - v. Clear Cell Renal Carcinoma
 - vi. Castrate Resistant Prostate Adenocarcinoma
 - 25 b. Part 2:
 - Head and Neck Squamous Cell Carcinoma (oral cavity, larynx, pharynx, paranasal sinuses)
- Part 1 only: Disease that has progressed after standard therapy for the specific tumor type, or for which standard therapy has proven to be ineffective, is intolerable, or is considered
 - 30 inappropriate, if no further standard therapy exists, or where standard therapy is refused. May be anti-PD-1/anti-PD-L1 experienced or naïve.
- Part 2 only: Disease that has progressed after receiving platinum-based chemotherapy (unless medically contraindicated or discontinued due to toxicity) and anti-PD-1/anti-PD-L1 therapy (in combination or as separate lines of therapy in either sequence).
- 35 • Measurable disease according to RECIST v1.1 guidelines; Eastern Cooperative Oncology Group performance status 0 or 1; adequate organ function.

Patients – key exclusion criteria

- Received prior treatment with the following therapies; calculation is based on date of last therapy to date of first dose of study intervention or SOC:

- 5 a. CTLA-4 (including tremelimumab) or ICOS-directed therapies at any time.
- b. ≥ 4 lines of prior anticancer treatment:
 - i. In participants that relapse or progress within 1 year from the beginning of adjuvant or concurrent therapy, the adjuvant/concurrent therapy is considered first line therapy
- 10 c. Systemic anticancer therapy or investigational therapy within 30 days, or 5 half-lives, whichever is shorter; at least 14 days must have elapsed between the date of the last prior therapy to the date of first dose of study intervention or SOC.
- History of invasive malignancy other than disease under study unless disease-free for ≥ 2 years.
- 15 • Toxicity from previous anticancer treatment that includes:
 - a. $\geq$ Grade 3 toxicity considered related to prior immunotherapy and that led to treatment discontinuation.
 - b. Toxicity related to prior treatment that has not resolved to $\leq$ Grade 1 (except alopecia, vitiligo, hearing loss, endocrinopathy managed with replacement
 - 20 therapy, and peripheral neuropathy which must be $\leq$ Grade 2).
- Central nervous system (CNS) metastasis; exceptions include previously treated CNS metastasis that is asymptomatic and has no requirement for steroids at least 14 days prior to first dose of study intervention or SOC.
- Active autoimmune disease that required systemic treatment within the last 2 years.
- 25 Replacement therapies which include physiological doses of corticosteroids for treatment of endocrinopathies (i.e., adrenal insufficiency) are not considered systemic treatments.
- History of idiopathic pulmonary fibrosis, pneumonitis requiring steroids, interstitial lung disease, or organizing pneumonia.

30 Overall Design

Described herein is a Phase I/II, open-label, 2-part study of H2L5 IgG4PE in combination with tremelimumab. As shown in Figure 5, Part 1 is dose escalation and will enroll participants with advanced, selected solid tumors and Part 2 is randomized expansion and will enroll participants with relapsed/refractory (R/R) head and neck squamous cell carcinomas (HNSCC) who have disease

progression after receiving at least 1 platinum-based chemotherapy and at least 1 anti-PD-1/PD-L1 therapy, whether in combination or separately. In Part 2, participants will be stratified by line of anti-PD-(L)1 therapy (i.e., received in the first line or second line).

Part 1 dose escalation will initiate with 8 mg H2L5 IgG4PE and 75 mg tremelimumab (termed dose level [DL] 1), the planned lowest dose for each agent. The highest planned doses are 80 mg H2L5 IgG4PE and 225 mg tremelimumab. H2L5 IgG4PE will be administered every 3 weeks and tremelimumab will be administered every 3 weeks for 6 doses, followed by every 12 weeks. Tremelimumab is to be administered first as an IV infusion over 60 minutes. H2L5 IgG4PE will be administered as a 30-minute IV infusion beginning at least 1 hour and no more than 2 hours following the end of the tremelimumab infusion.

As shown in Figure 5, dosing will begin at DL1 and continue using zone-based dose escalation rules whereby each zone must be cleared for safety prior to opening the next zone of DLs.

Dose escalation will be guided by the bivariate Continuous Reassessment Method (CRM) model until the maximum tolerated dose (MTD) or maximum administered dose (MAD) dose combination(s) are determined. Unplanned dose combinations within the range of the planned doses of each agent may be investigated to support the identification of optimal doses to administer in combination. Additionally, doses lower than planned of either agent may be investigated. PK/pharmacodynamic cohort(s) may be initiated at any DL(s), once safety is cleared, with mandatory paired tumor sample collections to inform on dose selection for Part 2 of the study.

The severity of all toxicities will be graded using National Cancer Institute - Common Toxicity Criteria for Adverse Events (NCI-CTCAE) (version 5.0). The DLT observation period is 28 days in length and begins on the day H2L5 IgG4PE and tremelimumab are first administered to the participant.

The total number of participants to be enrolled in Part 1 will depend on the number of participants needed to characterize the individual dose cohorts for determination of the MTDs or MADs.

A single dose combination will be selected as the recommended Phase 2 dose (RP2D) and carried forward from Part 1 into Part 2. The totality of data will be used to determine whether to proceed to Part 2 and which dose combination will be chosen as the RP2D.

Part 2 expansion is randomized and open-label to evaluate the efficacy of the selected RP2D dose combination compared to the Investigator's choice of selected current standard of care (SOC) (to include paclitaxel, docetaxel or cetuximab) in the treatment of R/R HNSCC who have progressed after receiving at least 1 platinum-based chemotherapy and at least 1 anti-PD-1/PD-L1 therapy, whether in combination or separately. Randomization is 2:1 to the investigational and SOC arms, respectively. Part 2 will also characterize PK and pharmacodynamic effects. Additional tumor type cohorts may be added either as a single arm expansion or with an appropriate comparator if a signal is identified in Part 1, based on the totality of the evidence and would be the subject of a future amendment.

In Part 2, 90 participants will be enrolled. The primary objective of the Part 2 is to evaluate and compare overall survival (OS) in participants treated with study intervention with those treated with SOC. Part 2 is designed to provide clinical evidence whether the study intervention will graduate to a subsequent Phase 3 study based upon clinical efficacy.

5

Justification for Dose

H2L5 IgG4PE Dose Rationale

Preliminary PK data from H2L5 IgG4PE monotherapy dose escalation study was utilized to develop a population PK model and estimate median steady-state peak and trough exposures at different fixed doses. The 8, 24, 80, and 240 mg dose corresponds to an approximate 0.1, 0.3, 1, and 3 mg/kg dose assuming median body weight of 80 kg.

The functional effect of H2L5 IgG4PE has been characterized in several *in vitro* experiments yielding different activity coefficients depending on cell type, co-stimulation status, and cytokines analysed. The ICOS receptor occupancy (RO) based on CD4+ or CD8+ T cells at any given systemic exposure of H2L5 IgG4PE can be predicted by employing the *in vitro* potency values generated from different binding/activation assays in the range of 0.09 to 4.14 µg/mL as listed in Table 7

Table .

Sufficiently high CD4+ RO is expected at peak exposures (89% to >99% RO) as well as at trough exposures (69% to >99% RO) at steady-state with a H2L5 IgG4PE dose of 80 mg.

Collectively, based on the safety and exposure data from the Phase 1 study and the predicted target engagement, 8, 24, and 80 mg doses are proposed to be evaluated in combination with tremelimumab in this study. No drug-drug interaction related changes are expected in H2L5 IgG4PE PK with tremelimumab co-administration. Additionally, doses lower than the planned lowest 8 mg dose may be investigated based on emerging data.

Table 7 Projected CD4+ Receptor Occupancy from Population PK Predicted Median Steady-state Peak and Trough Exposures of H2L5 IgG4PE based on *in vitro* potency estimates.

Dose (mg)	C _{max} (µg/mL)	C _τ (µg/mL)	RO based on K _d [0.09 µg/mL] (%)		RO based on T-cell binding [0.989 µg/mL] (%)		RO based on IFNγ release (bound) [4.14 µg/mL] (%)	
			At C _{max}	At C _τ	At C _{max}	At C _τ	At C _{max}	At C _τ
8	3.47	0.919	97.5	91.1	77.8	48.2	45.6	18.2
24	10.3	2.73	99.1	96.8	91.3	73.4	71.4	39.8
80	34.7	9.23	99.7	99.0	97.2	90.3	89.3	69.0
240	103.6	27.3	99.9	99.7	99.1	96.5	96.2	86.8

H2L5 IgG4PE Dosing Frequency

The systemic half-life of H2L5 IgG4PE is approximately 25 days based on the preliminary population PK analysis of exposure data from ongoing study. Thus, H2L5 IgG4PE will be dosed Q3W in combination with tremelimumab.

H2L5 IgG4PE Rationale for Fixed Dose

Therapeutic monoclonal antibodies are often dosed based on body-size due to the concept that this reduces inter-participant variability in drug exposure. However, body-weight dependency of PK parameters does not always explain the observed variability in the exposure of monoclonal antibodies. The advantage of body-weight based versus fixed dosing in this study was evaluated through population PK modelling and simulation efforts. A preliminary population PK model was developed from monotherapy dose escalation (data up to doses of 1 mg/kg; n=19 participants).

Simulations were performed by considering body weight distribution similar to that observed in the preliminary dataset. At the 5th percentile of body weight (40–47 kg), there was a 70-100% increase in median steady-state AUC(0- τ); H2L5 IgG4PE exposures higher than these increases have been evaluated in the Phase 1 study with the 3 mg/kg dose regimen. At the 95th percentile of body weight (107–118 kg), there was a 23-32% decrease in median steady-state AUC(0- τ) as compared to the median 80 kg exposure providing adequate RO with the minimal lowering of exposure. A similar outcome is expected for steady-state C_{max} and trough concentrations between body weight-based and fixed dosing.

Overall, these preliminary population PK simulations indicate that using fixed dosing would result in a similar range of exposures as that of body weight-based dosing. Also, fixed dosing offers the advantage of reduced dosing errors, reduced drug wastage, shortened preparation time, and improved ease of administration. Thus, a fixed dose based on a reference body weight of 80 kg will be applied.

Tremelimumab Dose Rationale

Tremelimumab is an IgG2 mAb against human CTLA-4. In clinical studies, Tremelimumab exhibited dose-proportional PK following IV infusion following single IV doses of 0.1 to 15 mg/kg.

The current study will administer tremelimumab as a flat dose regimen of 75 mg and 225 mg approximating the 1 mg/kg and 3 mg/kg regimens already evaluated in multiple tremelimumab mono- and combination therapy studies. Tremelimumab has also been evaluated at a 10 mg/kg dose in combination with durvalumab, an anti-PD-L1 antibody, where tremelimumab was administered as 10 mg/kg Q4W for 6 cycles followed by 10 mg/kg Q12W [Antonia, 2016]. The dosing regimen for the

proposed doses in this study is Q3W for the first six doses and Q12W thereafter. This Q3W dosing frequency for tremelimumab matches the H2L5 IgG4PE Q3W dosing frequency providing greater patient convenience. The overall tremelimumab systemic exposures with the highest proposed 225 mg regimen (225 mg Q3W for 6 cycles followed by 225 mg Q12W) in the current study are expected to be well below the exposures observed with 10 mg/kg regimen (10 mg/kg Q4W for 6 cycles followed by 10 mg/kg Q12W) studied as monotherapy and combination therapy. No drug-drug interaction related changes are expected in H2L5 IgG4PE PK with tremelimumab co-administration.

Study Intervention

The route of administration for all agents is IV. Tremelimumab is to be administered first as an IV infusion over 60 minutes. H2L5 IgG4PE will be administered as a 30-minute IV infusion beginning at least 1 hour and no more than 2 hours following the end of the tremelimumab infusion. Infusion time of each may be adjusted based on infusion-related reactions.

Standard of care agents should be administered as per local and Institutional guidelines (Table 9). Participants may receive necessary premedication regimens according to the approved product label or standard practice (i.e., corticosteroids, antihistamines, etc).

Table 8 Description and Administration of Study Interventions

	Study Intervention	
Product Name:	H2L5 IgG4PE	Tremelimumab
Product Description	Humanized anti-ICOS agonist IgG4 mAb	Humanized anti-CTLA-4 IgG2 mAb
Dosage form /strength:	10 mg/mL solution	20 mg/mL solution
Planned dosage level(s):	Part 1: Dose escalation H2L5 IgG4PE /tremelimumab: 8 mg/75 mg; 8 mg/225 mg; 24 mg/75 mg; 24 mg/225 mg; 80 mg/75 mg; 80 mg/225 mg Part 2: RP2D determined from Part 1	
Route of Administration	IV infusion	IV infusion
Dosing instructions/ Frequency:	Administer diluted product/Q3W	Administer diluted product/Q3W for 6 doses then Q12W thereafter
Manufacturer	GSK	MedImmune

Table 9 Standard of Care Agents

Product Name:	Docetaxel	Paclitaxel	Cetuximab
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Description	Microtubule stabilizer/ small molecule	Microtubule stabilizer/ small molecule	Recombinant, human/mouse chimeric anti-EGFR mAb
Dosage form/strength	Refer to package insert	Refer to package insert	Refer to package insert
Dosage/frequency	75 mg/m ² administered once every 3 weeks	80 mg/m ² administered once weekly	Administer a loading dose of 400 mg/m ² followed by 250 mg/m ² once weekly
Route of administration	IV infusion	IV infusion	IV infusion

Efficacy Assessments

RECIST (Response Evaluation Criteria In Solid Tumors) 1.1 will be used in the assessment of disease burden (target and non-target lesions determination) at screening and as the primary measure of tumor response endpoints. iRECIST (Modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics) will be used by the Investigator to assess tumor response and progression and make treatment decisions.

Result (Safety)

As of June 2020, 34 patients have been screened and 18 patients dosed (Table 10).

Table 10

Dose Level, (tremelimumab/H2L5 IgG4PE in mg)	Dosed participants by tumour type (n)
1 (75/8)	1 - melanoma (1)
2 (75/24)	1 - melanoma (1)
3 (225/8)	5 - NSCLC (2), HNSCC (1), melanoma (2)
4 (75/80)	3 - NSCLC (2), urothelial (1)
5 (225/24)	8 - HNSCC (3), urothelial (2), melanoma (1), NSCLC (2)

The safety profile was consistent with known tremelimumab toxicities.

REFERENCES

Antonia S, Goldberg S, Bamanoukian A, Chافت J, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study; Lancet Oncology; 2016;17:299-308.

CLAIMS

1. A method of treating cancer in a human in need thereof, the method comprising administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 0.08 mg to about 240 mg and administering to the human tremelimumab.
2. An agonist ICOS binding protein or antigen binding portion thereof for use in treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered with tremelimumab.
3. A combination of an agonist ICOS binding protein or antigen binding portion thereof and tremelimumab for use in treating cancer for use in treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 0.08 mg to about 240 mg.
4. Use of an agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament for treating cancer, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg to about 240 mg and is administered with tremelimumab.
5. A pharmaceutical kit comprising an ICOS binding protein or an antigen binding portion thereof at 10 mg/ml and tremelimumab at 20 mg/ml.
6. The method, agonist ICOS binding protein, combination, use, or kit of any one of claims 1-5, wherein the agonist ICOS binding protein or antigen binding portion thereof comprises one or more of: CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and/or CDRL3 as set forth in SEQ ID NO:6 or a direct equivalent of each CDR wherein a direct equivalent has no more than two amino acid substitutions in said CDR.
7. The method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of any one of claims 1-6, wherein the agonist ICOS binding protein or antigen binding portion thereof comprises a V_H domain comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:7 and/or a V_L domain comprising an amino acid sequence at least 90% identical to the amino acid sequence as set forth in SEQ ID NO:8 wherein said agonist ICOS binding protein specifically binds to human ICOS.

8. The method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of any one of claims 1-7, wherein the agonist ICOS binding protein is a monoclonal antibody.
9. The method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of any one of claims 1-8, wherein the agonist ICOS binding protein is a humanized or fully human monoclonal antibody.
10. The method, agonist ICOS binding protein, combination, use, or pharmaceutical kit of any one of claims 1-9, wherein the agonist ICOS binding protein comprises an hIgG4PE scaffold.
11. The method, agonist ICOS binding protein, combination or use, any one of claims 1-4 and 6-10, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 0.08 mg, about 0.24 mg, about 0.8 mg, about 2.4 mg, about 8 mg, about 24 mg, about 80 mg, or about 240 mg.
12. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-11, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered at a dose of about 24 mg.
13. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-12, wherein the agonist ICOS binding protein or antigen binding portion thereof is administered once every three weeks or every six weeks.
14. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-13, wherein the agonist ICOS binding protein or antigen binding portion thereof and/or tremelimumab is administered via IV infusion.
15. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-14, wherein the cancer is a solid tumor.
16. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-15, wherein the cancer is selected from NSCLC, HNSCC, urothelial cancer, cervical cancer and melanoma.
17. The method, agonist ICOS binding protein, combination or use of claim 16, wherein the cancer is NSCLC.

18. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-17, wherein tremelimumab is administered at a dose of about 8 mg to about 1200 mg.
19. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-18, wherein tremelimumab is administered at a dose of about 75 mg to about 225 mg.
20. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-19, wherein tremelimumab is administered once every three weeks or 12 weeks.
21. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-20, wherein tremelimumab is administered once every three weeks for 6 doses and every 12 weeks thereafter.
22. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-21, wherein the agonist ICOS binding protein is administered at a dose of about 0.08 mg, about 0.24 mg, about 0.8 mg, about 2.4 mg, about 8 mg, about 24 mg, about 80 mg, or about 240 mg every three weeks, and tremelimumab is administered at a dose of about 7.5 mg, about 75 mg, about 225 mg, about 750 mg or about 1125 mg every three weeks for 6 doses and every 12 weeks thereafter.
23. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-22, wherein the agonist ICOS binding protein or antigen binding portion thereof comprises CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and CDRL3 as set forth in SEQ ID NO:6, and wherein tremelimumab is administered at a dose of about 75 mg or about 225 mg every three weeks for 6 doses and every 12 weeks thereafter.
24. The method, agonist ICOS binding protein, combination or use of any one of claims 1-4 and 6-24, wherein the agonist ICOS binding protein or antigen binding portion thereof comprises CDRH1 as set forth in SEQ ID NO:1; CDRH2 as set forth in SEQ ID NO:2; CDRH3 as set forth in SEQ ID NO:3; CDRL1 as set forth in SEQ ID NO:4; CDRL2 as set forth in SEQ ID NO:5 and CDRL3 as set forth in SEQ ID NO:6, and wherein tremelimumab is administered at a dose of about 75 mg or about 225 mg every three weeks for 6 doses and every 12 weeks thereafter; wherein tremelimumab is administered first as an IV infusion and the agonist ICOS binding protein or antigen binding portion thereof is administered as an IV infusion beginning at least 1 hour and no more than 2 hours following the end of the administration of tremelimumab.

FIG. 1

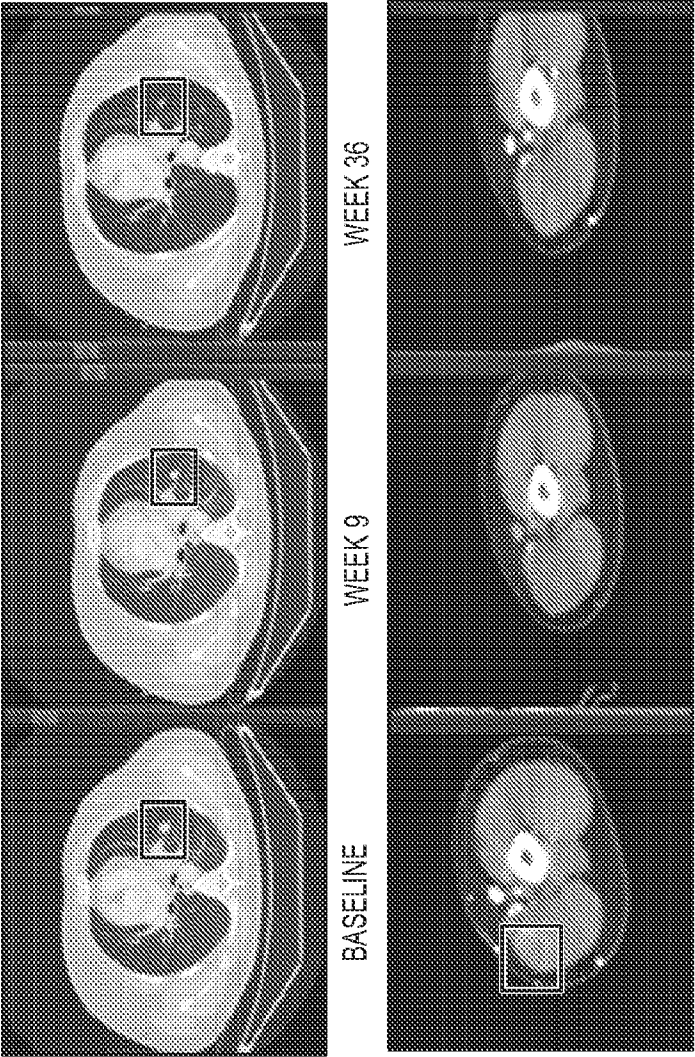


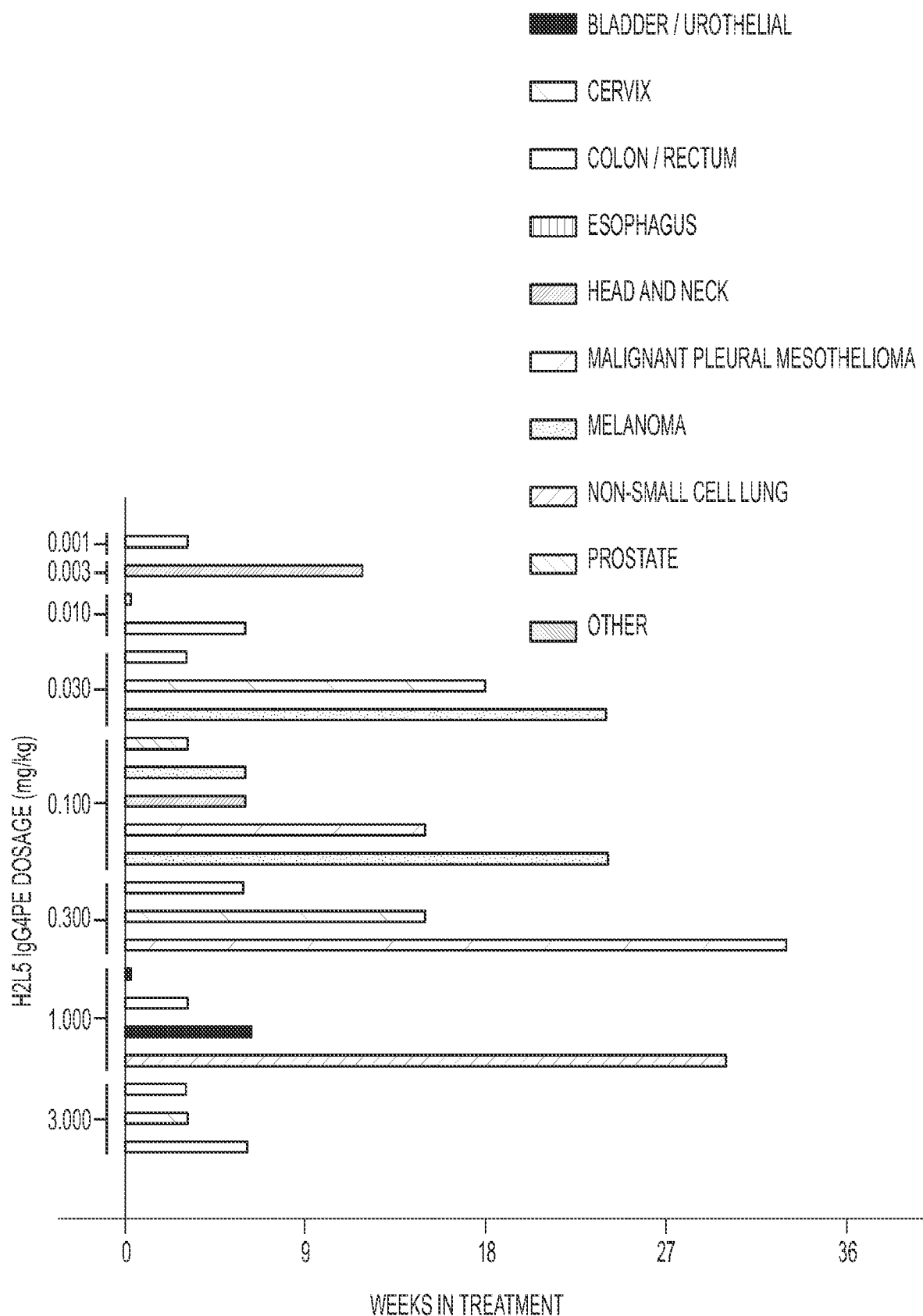
FIG. 2A

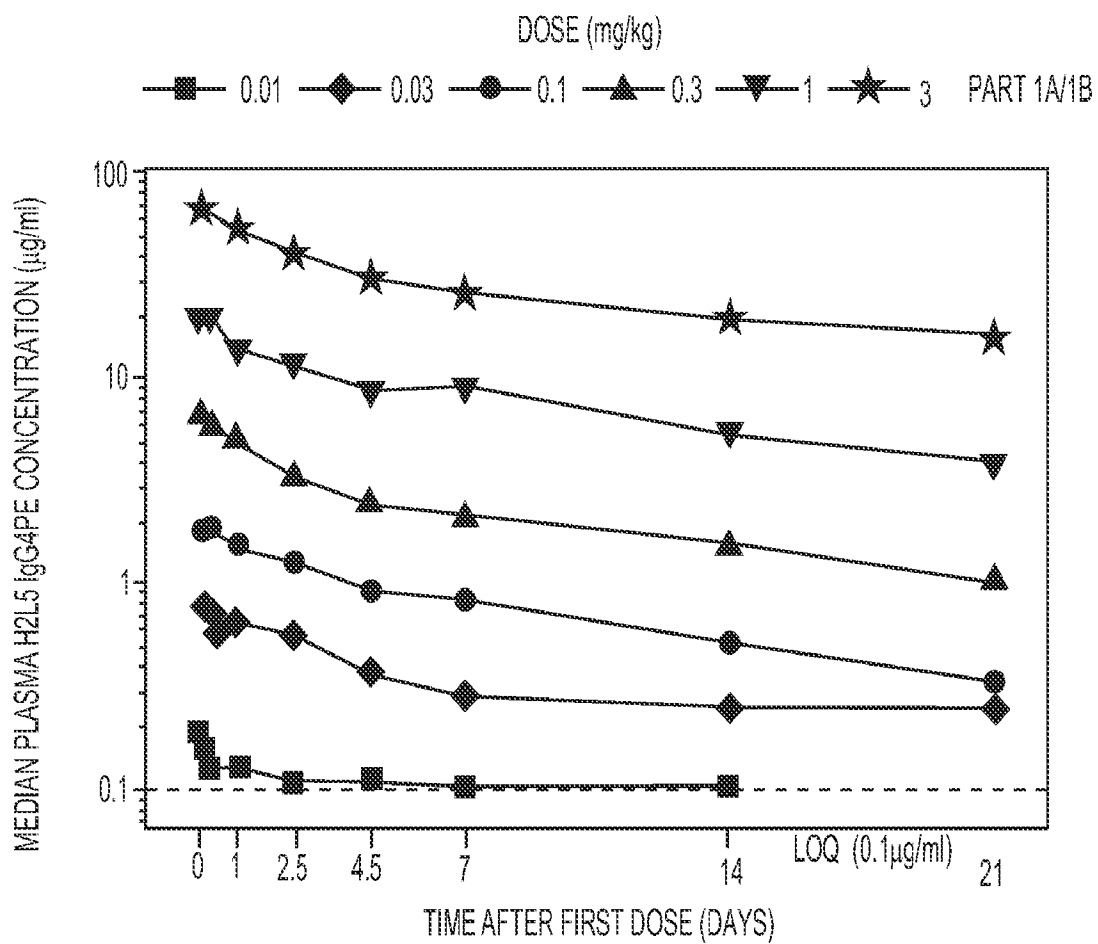
FIG. 3A

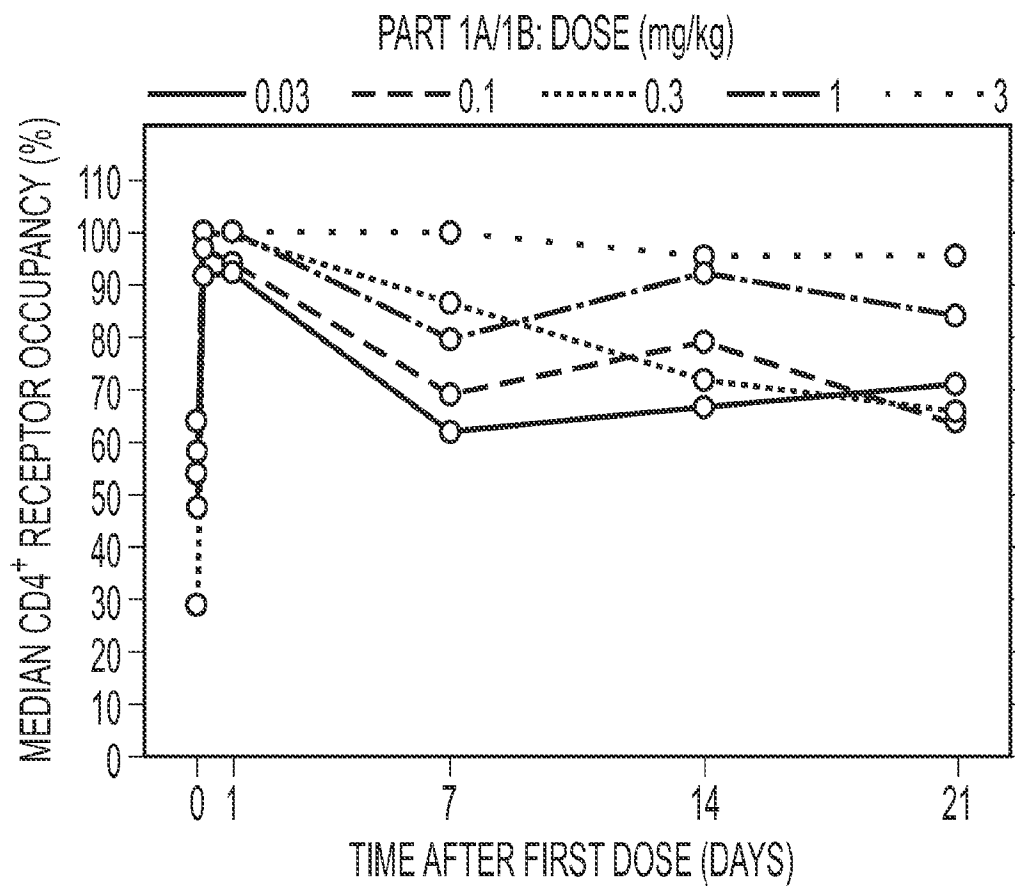
FIG. 3B

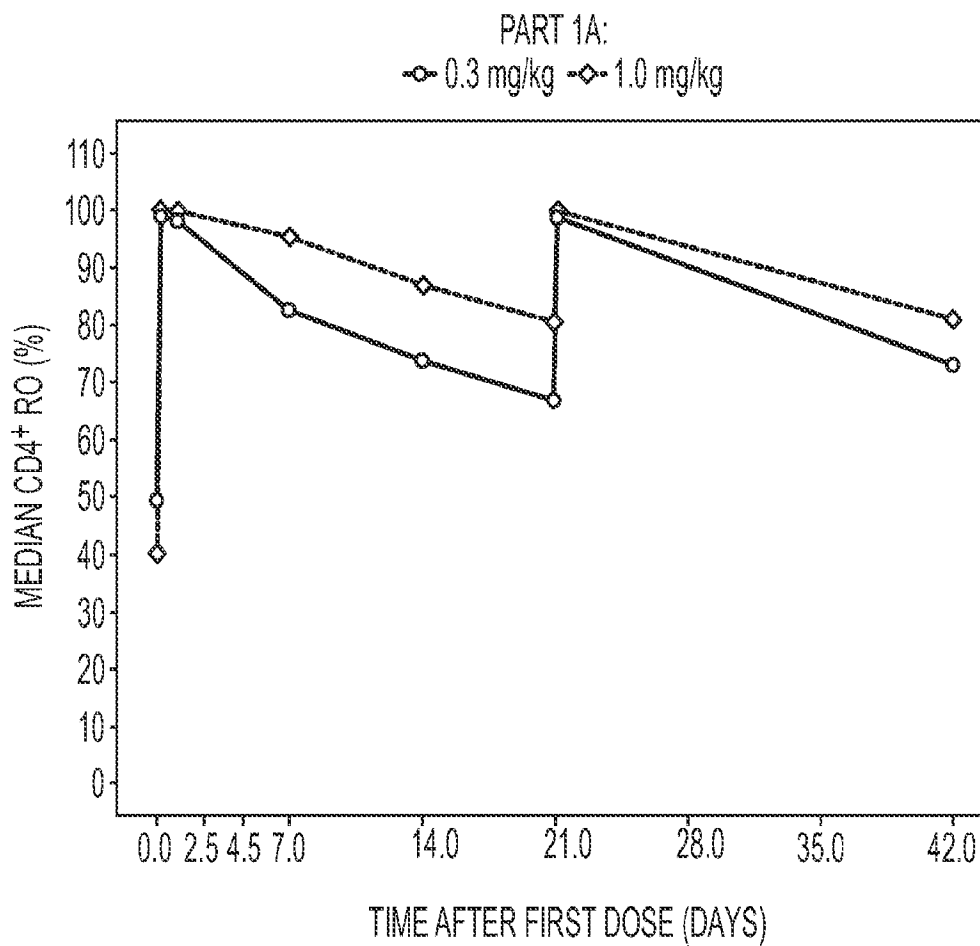
FIG. 3C

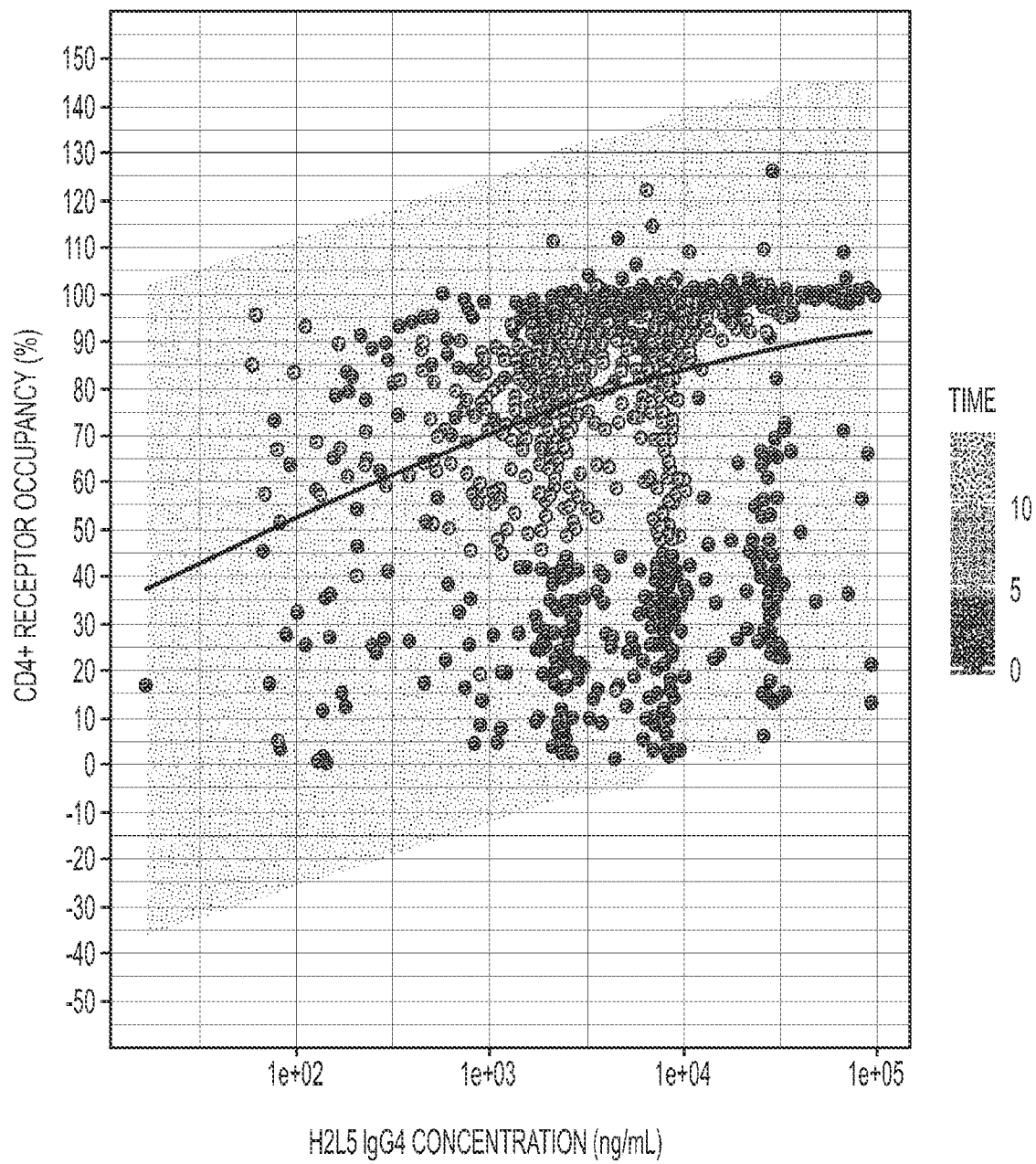
FIG. 3D

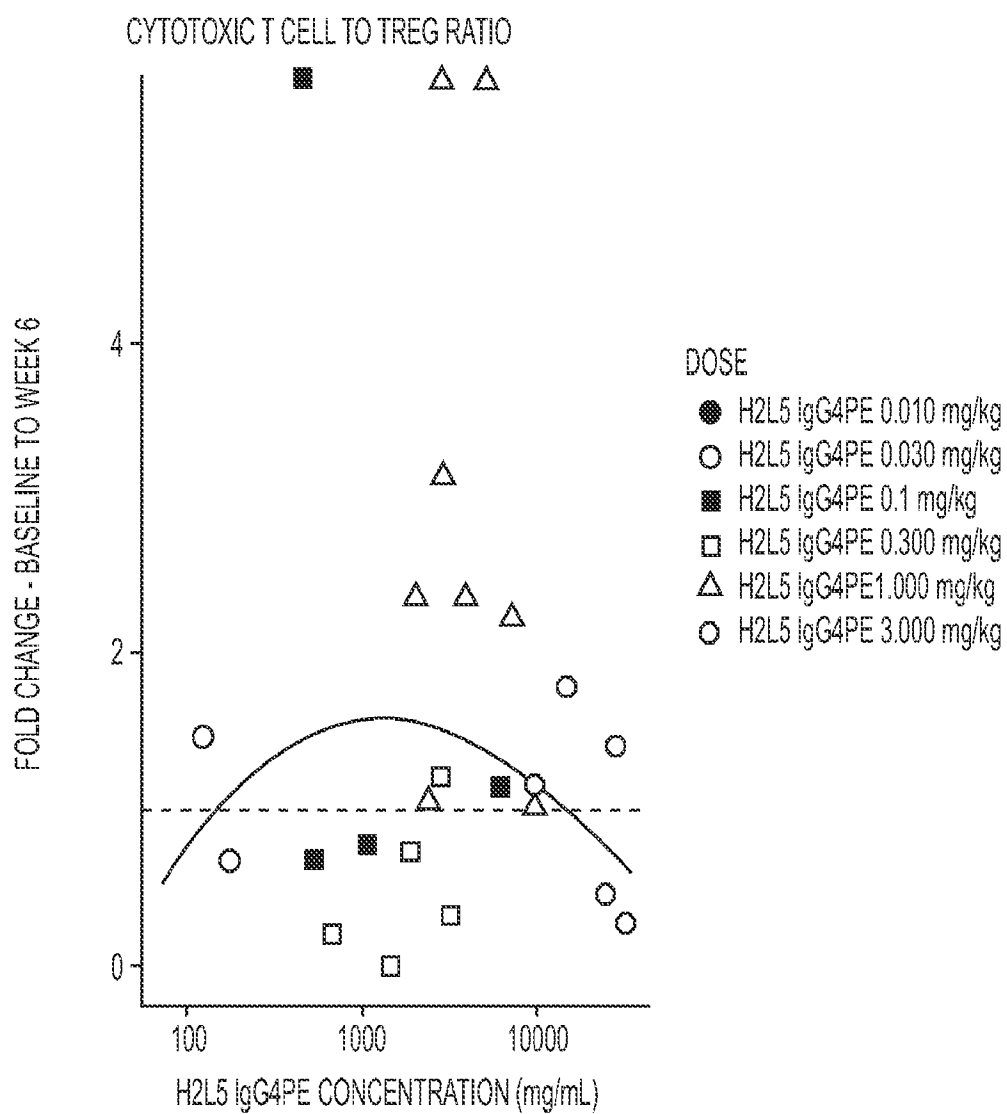
FIG. 4A

FIG. 4B

MULTIOMYX DOSE-RESPONSE CURVES (N=43
SUBJECTS, 40 PHENOTYPES)

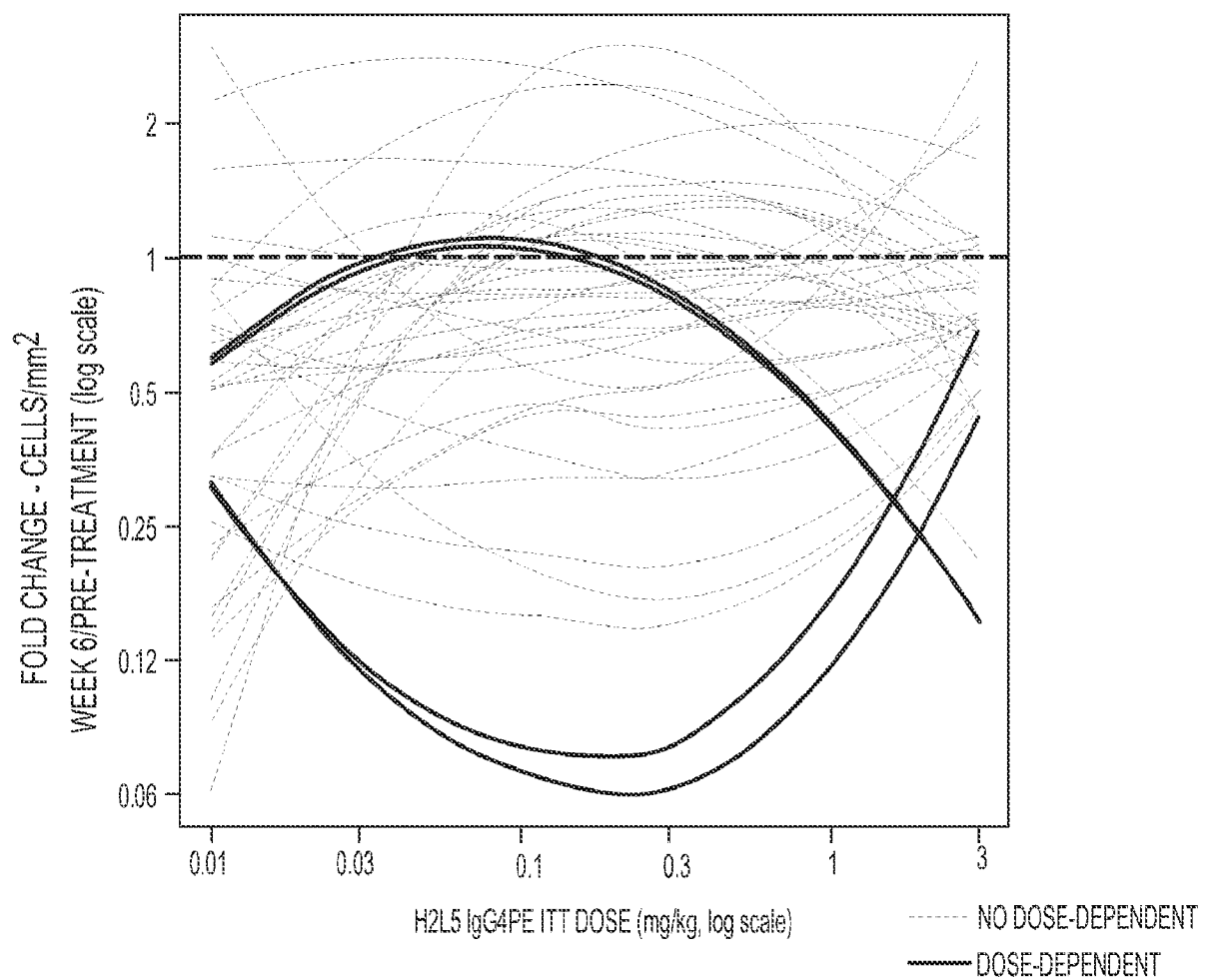


FIG. 4C

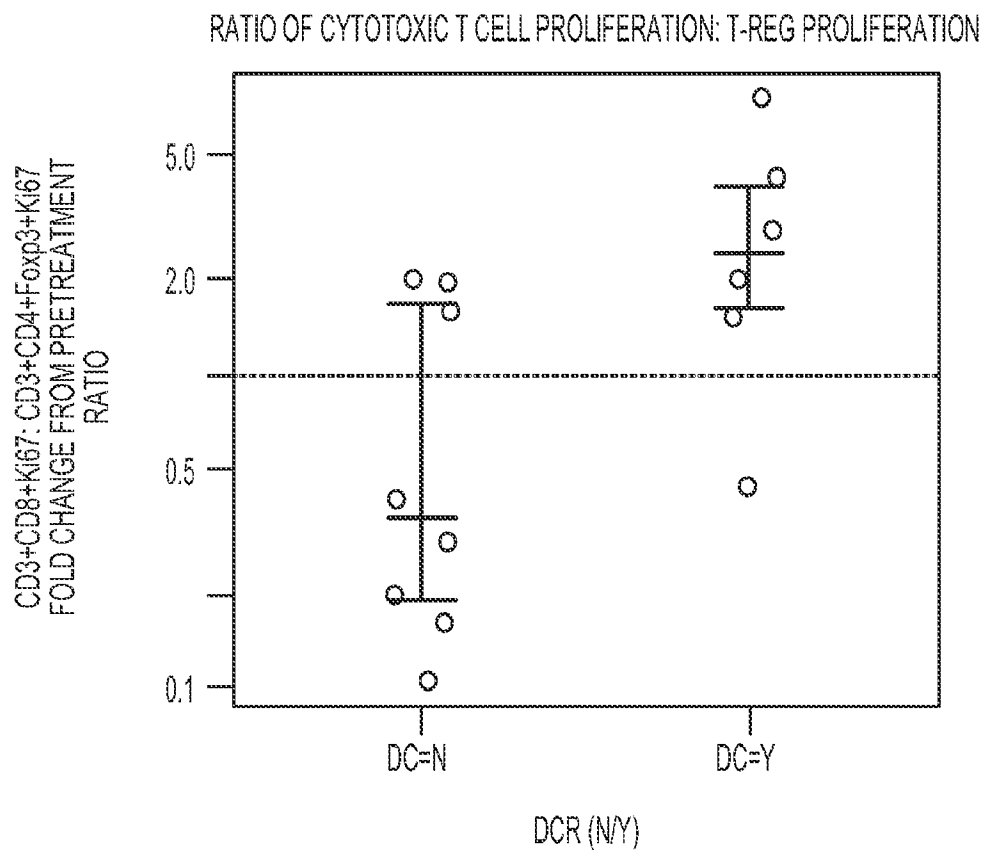
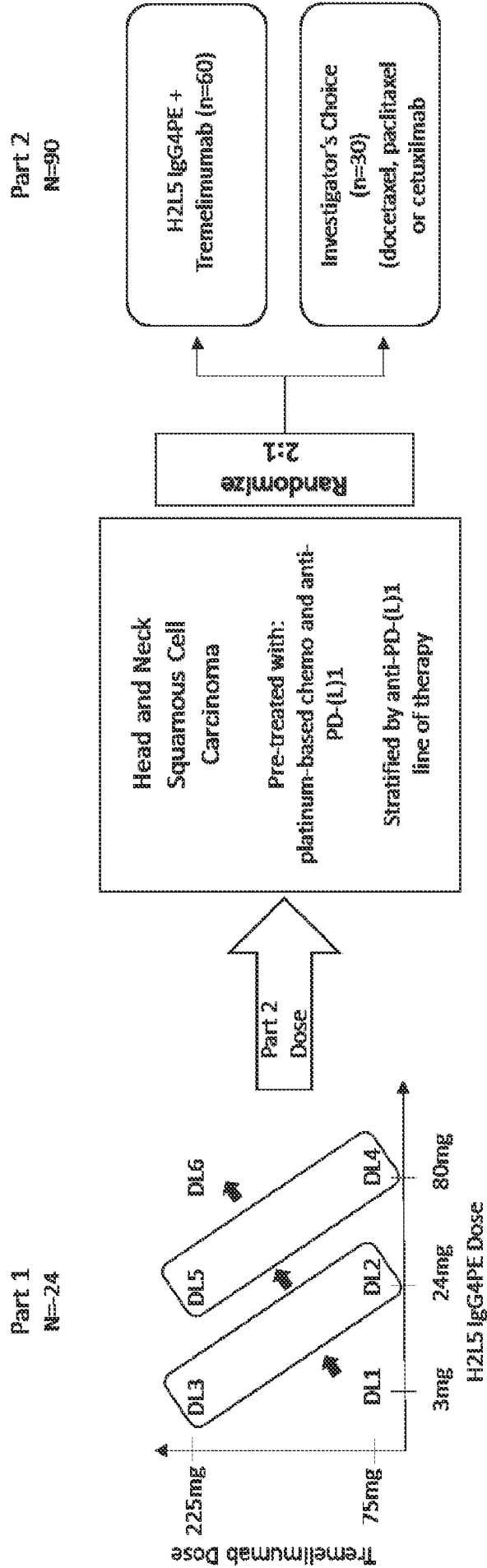


FIG. 5



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/049321

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00 A61K39/395
ADD.

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)
C07K A61K A61P

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HANSEN AABDUL-KARIM RRIZVI NRISCHEN DHILTON JLI ZOTT PKARPINICH-FEDORIW N ET AL: "A Phase I/II, open-label, two part study of GSK3359609 in combination with tremelimumab in participants with selected, advanced solid tumors", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 79, no. 13, Supplement, 1 July 2019 (2019-07-01), XP009520803, ISSN: 1538-7445, DOI: 10.1158/1538-7445.AM2019-CT166 the whole document paragraph [methods] ----- -/-	1-24



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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"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

6 November 2020

Date of mailing of the international search report

17/11/2020

Name and mailing address of the ISA/

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Authorized officer

Siaterli, Maria

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/049321

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/025871 A1 (GLAXOSMITHKLINE IP DEV LTD [GB]) 16 February 2017 (2017-02-16) page 9, paragraph 22; figure 7; examples 2,3; table 3 -----	1-24
X	WO 2016/120789 A1 (GLAXOSMITHKLINE IP DEV LTD [GB]; INST NAT SANTE RECH MED [FR] ET AL.) 4 August 2016 (2016-08-04) page 111, line 20 - line 25; examples 11,12; tables 12,13 -----	1-24
E	WO 2020/185739 A1 (JOUNCE THERAPEUTICS INC [US]) 17 September 2020 (2020-09-17) paragraph [0165]; claims 1-32; figure 1; example 3 -----	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/049321

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