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(54) **Title:** COMBINATION THERAPY

(57) **Abstract:** The present invention relates to a method of treating cancer in a human in need thereof, the method comprising administering to the human a TLR4 agonist at a dose of about 5 ng to about 1000 ng, and administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

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Combination Therapy

FIELD OF THE INVENTION

The present invention relates to a method of treating cancer in a mammal and to combinations useful in such treatment. In particular, the present invention relates to combinations of TLR4 agonists and anti-ICOS antibodies.

BACKGROUND OF 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.

ICOS is a co-stimulatory T cell receptor involved in multiple processes of the immune system. Antigen binding proteins and antibodies that bind ICOS receptor and modulate ICOS signaling are known in the art and are disclosed as immunotherapy, for example, for cancer.

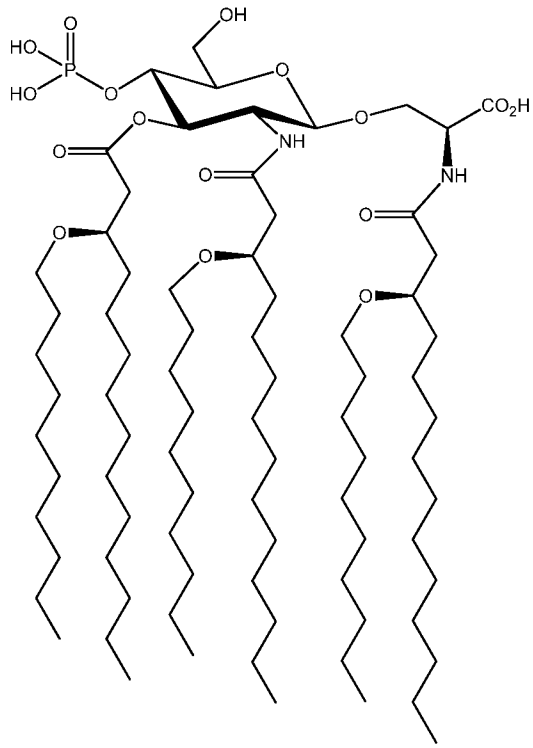
Aminoalkyl glucosaminide phosphates (AGPs) are synthetic ligands of Toll-like Receptor 4 (TLR4). AGPs are known to be useful as vaccine adjuvants and for stimulating cytokine production, activating macrophages, promoting innate immune response, and augmenting antibody production in immunized animals.

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 combinations and methods herein that relate to combining therapeutic approaches for enhancing anti-tumor immunity address this need.

SUMMARY OF THE INVENTION

In one aspect, a method of treating cancer in a human in need thereof is provided, the method comprising administering to the human a TLR4 agonist at a dose of about 5 ng to about 1000 ng, and administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

In another aspect, a method of treating cancer in a human in need thereof is provided, the method comprising administering to the human a TLR4 agonist at a dose of 50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng, 350 ng, 400 ng, 450 ng, 500 ng, 550 ng, or 600 ng and administering to the human an ICOS binding protein or antigen binding portion thereof at a dose of 80 mg or 24 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, and wherein the TLR agonist is CRX-601



(CRX-601).

In still another aspect, a TLR4 agonist and an agonist ICOS binding protein for simultaneous or sequential use in treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng, and the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 24 mg to about 240 mg.

In another aspect, a TLR4 agonist for use in treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 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 to be administered at a dose of about 24 mg to about 240 mg and is to be administered simultaneously or sequentially with a TLR4 agonist at a dose of about 5 ng to about 1000 ng.

In one aspect, use of a TLR4 agonist in the manufacture of a medicament for treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of about 5 ng

to about 1000 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

In another aspect, use of an agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament for treating cancer is provided, wherein the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 24 mg to about 240 mg and is to be administered simultaneously or sequentially with a TLR4 agonist at a dose of about 5 ng to about 1000 ng.

In one aspect, a pharmaceutical kit is provided, the pharmaceutical kit comprising about 5 ng to about 1000 ng of a TLR4 agonist and about 24 mg to about 240 mg of an agonist ICOS binding protein or antigen binding portion thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plot showing CT-26 tumor growth in Balb/c Mice Treated with CRX-601 (TLR4 agonist) and/or ICOS agonist antibody (7E.17G9, mouse surrogate for H2L5 IgG4PE)

FIG. 2 is a plot showing survival of Balb/c mice implanted with CT-26 tumors and treated with CRX-601 (TLR4 agonist) and/or ICOS agonist antibody (7E.17G9, mouse surrogate for H2L5 IgG4PE).

DETAILED DESCRIPTION OF THE INVENTION

Antigen Binding Proteins and Antibodies that bind ICOS

"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 VH

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," and "binding protein" are used interchangeably herein.

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:9.

MKSGLWYFFLFCLRIKVLTGEINGSANYEMFIFHNGGVQILCKYPDIVQQFKMQLLKGGQILCDLT
KTKGSGNTVSIKSLKFCHSQLSNNSVSFFLYNLDHSHANYYFCNLSIFDPPPFKVTLTGGYLHIYE
SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKM (SEQ ID NO:9)

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

MKSGLWYFFL FCLRIKVLTG EINGSANYEM FIFHNGGVQI LCKYPDIVQQ
FKMQLLKGGQ ILCDLTKTKG SGNTVSIKSL KFCHSQLSNN SVSFFLYNLD
HSHANYYFCN LSIFDPPPFK VTLTGGYLHI YESQLCCQLK FWLPIGCAAF
VVVCILGCIL ICWLTKKKYS SSVHDPNGEY MFMRAVNTAK KSRLTDVTL
(SEQ ID NO: 10)

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 S 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 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 T_H1 and T_H2 effector T cell populations (Paulos CM 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 through TCR engagement (Wakamatsu E, 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 T_H1 and T_H2 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)). Antibodies to ICOS and methods of using in the treatment of disease are described, for instance, in WO 2012/131004, 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 (WO 2012/131004). Antibodies to ICOS are also disclosed in WO 2008/137915, WO 2010/056804, EP 1374902, EP1374901, and EP1125585. Agonist antibodies to ICOS or ICOS binding proteins are disclosed in WO2012/13004, WO2014/033327, WO2016/120789, US20160215059, US20160304610, US2018/0289790, and WO2018/029474. Exemplary antibodies in US2016/0304610 include 37A10S713. Sequences of 37A10S713 are reproduced below as SEQ ID NOS: 11-18.

37A10S713 heavy chain variable region:

EVQLVESGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDEDGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS (SEQ. ID NO:11)

37A10S713 light chain variable region:

DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRHT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K (SEQ. ID NO:12)

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

37A10S713 V_H CDR2: NIDEDGSITEYSPFVKG (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)

Exemplary antibodies in US2018/0289790 include ICOS.33 IgG1f S267E. Sequences of ICOS.33 IgG1f S267E are reproduced below as SEQ ID NOS: 19-20:

ICOS.33 IgG1f S267E Heavy Chain Variable Domain

EVQLVESGGG LVKPGGSLRL SCAASGFTFS DYFMHWVRQAPGKGLEWVGV IDTKSFNYAT YYSDLVKGRF TISRDDSKNT LYLQMNSLKT EDTAVYYCTA TIAVPYYFDY WGQGTLVTVS S (SEQ ID NO: 19)

ICOS.33 IgG1f S267E Light Chain Variable Domain

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLSWYQQKP GKAPKLLIYY TNLLAEGVPS RFSGSGSGTD FTFTISSLQP EDIATYYCQQ YYNYRTFGPG TKVDIK (SEQ ID NO: 20)

Exemplary antibodies in WO2018/029474 include STIM003. Sequences of STIM003 are reproduced below as SEQ ID NOS: 21-22.

STIM003 Heavy chain variable domain

EVQLVESGGGVVRPGGSLRLSCVASGVTFDDYGMSWVRQAPGKGLEWVSGINWNGGDT DYSDSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCARDFYGSGSYYHVPFDYWGQG ILVTVSS (SEQ ID NO: 21)

STIM003 Light chain variable domain

EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKRGQAPRLLIYGASSRATGIPDR FSGDGSGTDFTLSISRLEPEDFAVYYCHQYDMSPFTFGPGTKVDIK (SEQ ID NO: 22)

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 ICOS binding protein. In some other embodiments, the agent directed to ICOS is an ICOS agonist.

The term "ICOS binding protein" as used herein refers to antibodies and other protein constructs, such as domains, which are capable of binding to ICOS. In some instances, the ICOS is human ICOS. The term "ICOS binding protein" can be used interchangeably with "ICOS antigen binding protein." Thus, as is understood in the art, anti-ICOS antibodies and/or ICOS antigen binding proteins would be considered ICOS binding proteins. As used herein, "antigen binding protein" is any protein, including but not limited to antibodies, domains and other constructs described herein, that binds to an antigen, such as ICOS. 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 ICOS antibodies 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 some embodiments, the anti-ICOS antibodies of the present invention comprise a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:7. Suitably, the 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.

Humanized Heavy Chain (V_H) Variable Region (H2):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DYAMHWVRQA PGQGLEWMGL ISIYSDHTNY NQKFQGRVTI TADKSTSTAY MELSSLRSED TAVYYCGRNN YGNYGWYFDV WGQGTTVTVS S
(SEQ ID NO:7)

In one embodiment of the present invention the ICOS antibody 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. 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 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 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 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)

EIVLTQSPAT LSLSPGERAT LSCSASSSVS YMHWYQQKPG QAPRLLIYDT SKLASGIPAR FSGSGSGTDY TLTISSLEPE DFAVYYCFQG SGYPYTFGQG TKLEIK (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, for example as shown in Table 1 below.

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 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 resulting antibody with both mutations is referred to as IgG4PE. The numbering of the hIgG4 amino acids was derived from EU numbering reference: Edelman, G.M. 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 may have the designation IgG4PE.

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

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 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 (Opdivo/nivolumab and Keytruda/pembrolizumab), anti-CTLA-4 antibodies such as ipilimumab (YERVOY), and anti-ICOS antibodies.

As used herein the term "agonist" refers to an antigen binding protein including but not limited to an antibody, which 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 know 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.

As used herein the term "antagonist" refers to an antigen binding protein including but not limited to an antibody, which 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 its natural ligand, (2) reduces, decreases or shortens the activity, function or presence of the receptor and/or (3) reduces, descrease, 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 end points such as, but not limited to the measurement of T cell proliferation or cytokine production.

The term "antibody" is used herein in the broadest sense to refer to molecules with 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}, VL, 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).

Alternative antibody formats include alternative scaffolds in which the one or more CDRs of the antigen binding protein 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.

The term "domain" refers to a folded protein structure which retains its tertiary structure independent of the rest of the protein. Generally domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins 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.

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.

Affinity is the strength of binding of one molecule, e.g. an antigen binding protein of the invention, to another, 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 is the sum total of the strength of binding of two molecules to one another at multiple sites, e.g. taking into account the valency of the interaction.

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 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 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 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 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 extrachromasomal or stably integrated into the chromosome of the cell.

A "chimeric antibody" refers to a type of engineered antibody which 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.

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, 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 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 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 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 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 "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.

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 full length antibody sequences 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 antibody may mean that other residues are considered part of the CDR sequence and would be understood to be so by a skilled person.

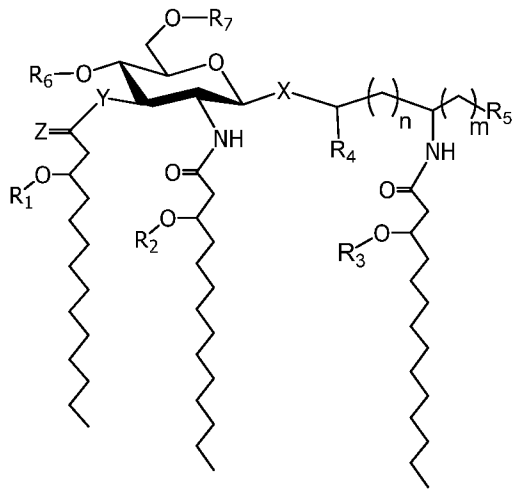
Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods. The minimum overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the "minimum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

TLR4 modulators

The combinations of the invention comprise TLR4 "modulators", that is, molecules that modulate TLR4, for example, by binding and initiating conformational changes or signaling by engaging TLR4, molecules that block binding with a TLR4 ligand.

In one embodiment, TLR4 modulators are aminoalkyl glucosaminide phosphate compounds (AGPs). TLR4 recognizes bacterial LPS (lipopolysaccharide) and when activated initiates an innate immune response. AGPs are a monosaccharide mimetic of the lipid A protein of bacterial LPS and have been developed with ether and ester linkages on the "acyl chains" of the compound. Processes for making these compounds are known and disclosed, for example, in WO 2006/016997, U.S. Patent Nos. 7,288,640 and 6,113,918, and WO 01/90129. Other AGPs and related processes are disclosed in U.S. Patent No. 7,129,219, U.S. Patent No. 6,525,028 and U.S. Patent No 6,911,434. AGPs with ether linkages on the acyl chains employed in the composition of the invention are known and disclosed in WO 2006/016997. The AGP compounds set forth and described according to Formula (III) at paragraphs [0019] through [0021] in WO 2006/016997 may be employed in the presently claimed methods and combinations.

AGP compounds employed in the present invention have the structure set forth in Formula 1 as follows:



(Formula 1)

wherein

m is 0 to 6

n is 0 to 4;

X is O or S, preferably O;

Y is O or NH;

Z is O or H;

each R1, R2, R3 is selected independently from the group consisting of a C1-20 acyl and a C1-20 alkyl;

R4 is H or Me;

R5 is selected independently from the group consisting of -H, -OH, -(Cl-C4) alkoxy, -PO3R8R9, -OPO3R8R9, -SO3R8, -OSO3R8, -NR8R9, -SR8, -CN, -NO2, -CHO, -CO2R8, and –CONR8R9, wherein R8 and R9 are each independently selected from H and (Cl-C4) alkyl; and

each R6 and R7 is independently H or PO3H2.

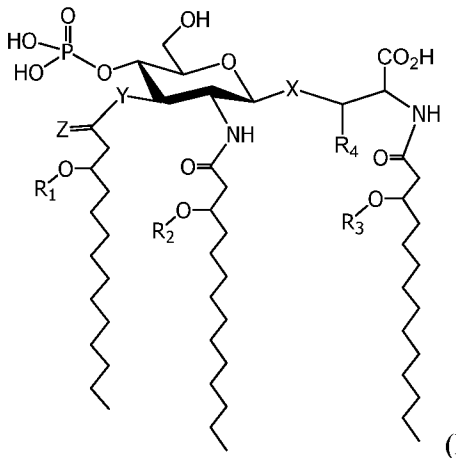
In Formula 1 the configuration of the 3' stereogenic centers to which the normal fatty acyl residues (that is, the secondary acyloxy or alkoxy residues, *e.g.*, R1O, R2O, and R3O) are attached is R or S, preferably R (as designated by Cahn-Ingold-Prelog priority rules). Configuration of aglycon stereogenic centers to which R4 and R5 are attached can be R or S. All stereoisomers, both enantiomers and diastereomers, and mixtures thereof, are considered to fall within the scope of the present invention.

The number of carbon atoms between heteroatom X and the aglycon nitrogen atom is determined by the variable "n", which can be an integer from 0 to 4, or an integer from 0 to 2.

The chain length of normal fatty acids R1, R2, and R3 can be from about 6 to about 16 carbons, or from about 9 to about 14 carbons. The chain lengths can be the same or different. Some embodiments include chain lengths where R1, R2 and R3 are 6 or 10 or 12 or 14.

Formula 1 encompasses L/D-seryl, -threonyl, -cysteinyl ether and ester lipid AGPs, both agonists and antagonists and their homologs (n=1-4), as well as various carboxylic acid bioisosteres (*i.e,* R5 is an acidic group capable of salt formation; the phosphate can be either on 4- or 6- position of the glucosamine unit, preferably, is in the 4-position).

In a one embodiment of the invention employing an AGP compound of Formula 1, n is 0, R5 is CO2H, R6 is PO3H2, and R7 is H. This AGP compound is set forth as the structure in Formula 1a as follows:



(Formula 1a)

wherein X is O or S; Y is O or NH; Z is O or H; each R1, R2, R3 is selected independently from the group consisting of a C1-20 acyl and a C1-20 alkyl; and R4 is H or methyl.

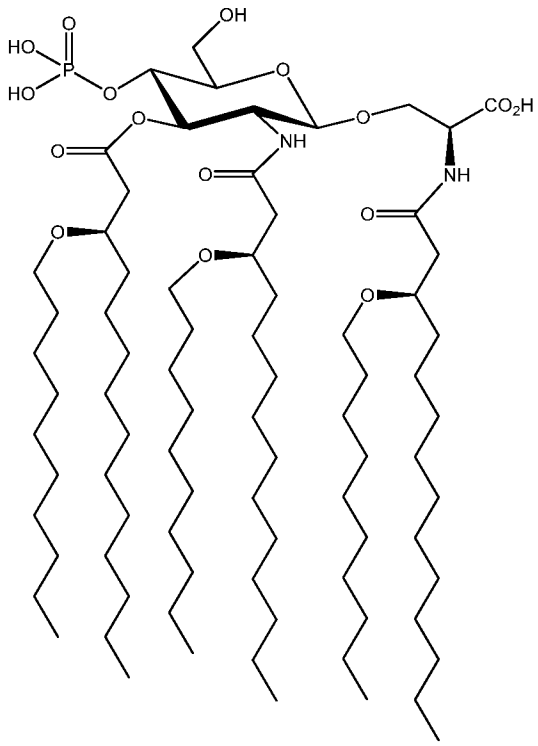
In Formula 1a the configuration of the 3' stereogenic centers to which the normal fatty acyl residues (that is, the secondary acyloxy or alkoxy residues, *e.g.*, R1O, R2O, and R3O) are attached as R or S, preferably R (as designated by Cahn-Ingold-Prelog priority rules).

Configuration of aglycon stereogenic centers to which R4 and CO2H are attached can be R or S. All stereoisomers, both enantiomers and diastereomers, and mixtures thereof, are considered to fall within the scope of the present invention.

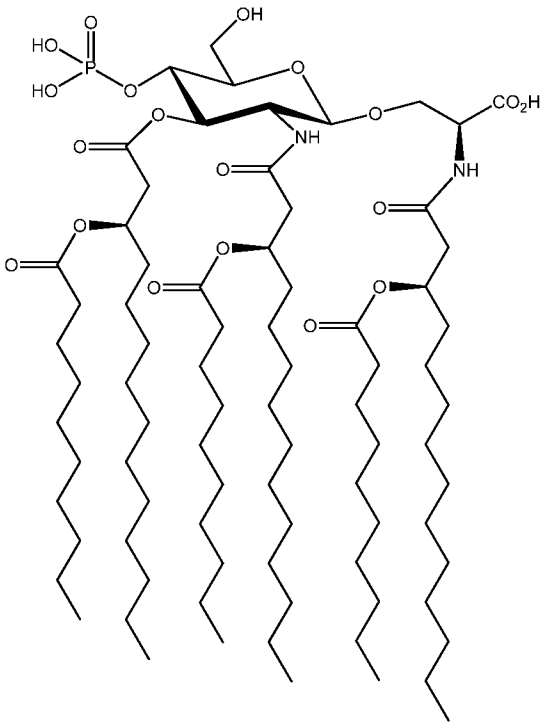
Formula 1a encompasses L/D-seryl, -threonyl, -cysteinyl ether or ester lipid AGPs, both agonists and antagonists.

In both Formula 1 and Formula 1a, Z is O attached by a double bond or two hydrogen atoms which are each attached by a single bond. That is, the compound is ester-linked when Z=Y=O; amide-linked when Z =O and Y=NH; and ether-linked when Z=H/H and Y=O.

Compounds of Formula 1 are referred to as CRX-601 and CRX-527. Their structures are set forth as follows:

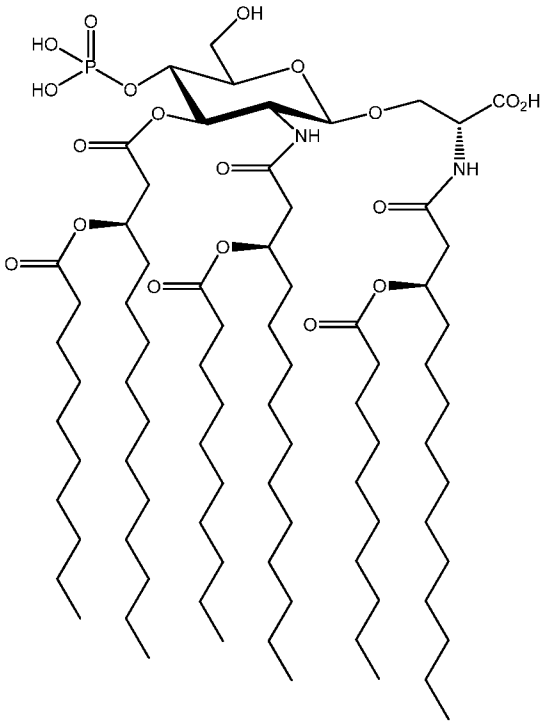


CRX-601



CRX-527

Additionally, another preferred embodiment employs CRX-547 having the structure shown. CRX-547



CRX-547

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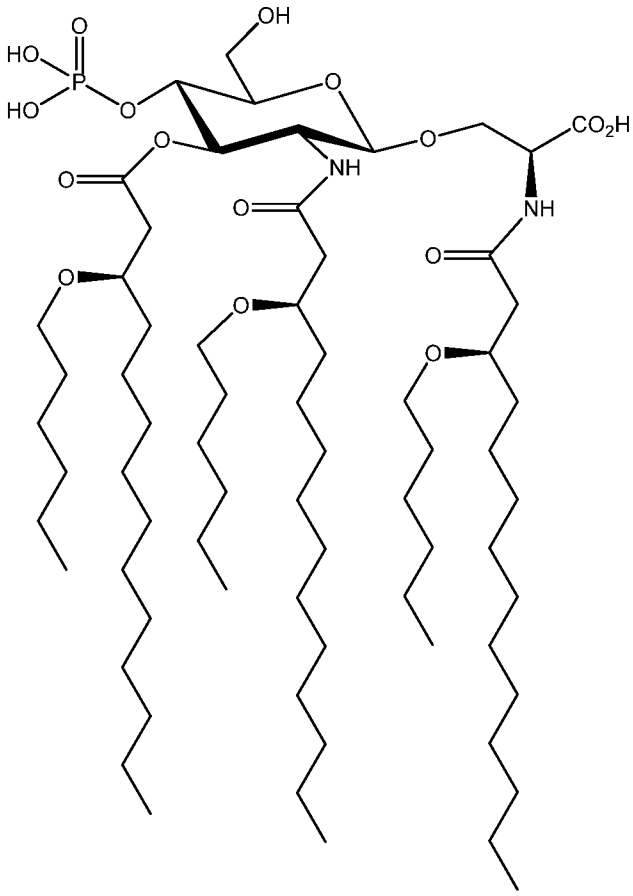
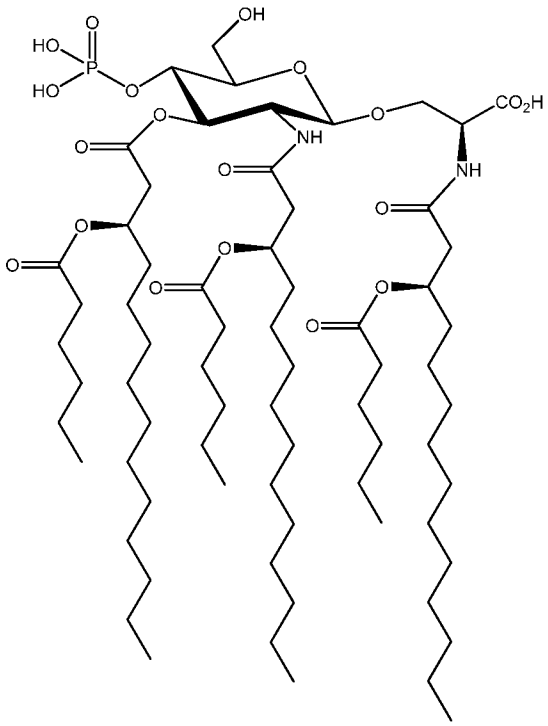
Still other embodiments include AGPs, such as CRX-602 or CRX-526 providing increased stability to AGPs having shorter secondary acyl or alkyl chains.



CRX-602



CRX-526

In a further embodiment of the invention, the TLR4 modulator is an agonist. In a further embodiment, the TLR4 modulator that is an agonist is selected from the group consisting of: CRX-601, CRX-547, and CRX-527.

AGP Buffers

In one embodiment of the present invention, the composition comprising a TLR4 modulator, such as an AGP, is buffered using a zwitterionoic buffer. In one embodiment of the invention, the zwitterionic buffer is an aminoalkanesulfonic acid or suitable salt. Examples of amninoalkanesulfonic buffers include, but are not limited, to HEPES, HEPPS/EPPS, MOPS, MOBS and PIPES. In one embodiment of the invention, the buffer is a pharmaceutically acceptable buffer, suitable for use in humans, such as in for use in a commercial injection product. In one embodiment of the invention, the buffer is HEPES.

Methods of Treatment

The combinations of the invention are believed to have utility in disorders wherein the engagement of ICOS and/or TLR4, is beneficial.

The present invention thus also provides a combination of the invention, for use in therapy, particularly, in the treatment of disorders wherein the engagement of ICOS and/or TLR4, is beneficial, particularly cancer.

In one aspect, a method of treating cancer in a human in need thereof is provided, the method comprising administering to the human a TLR4 agonist at a dose of about 5 ng to about 1000 ng, and administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg. In one embodiment, the TLR4 agonist is administered at a dose of 50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng, 350 ng, 400 ng, 450 ng, 500 ng, 550 ng, or 600 ng. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of 24 mg, 80 mg, or 240 mg. In one embodiment, the ICOS binding protein or antigen binding portion thereof is administered at a dose of 80 mg.

In one aspect, a TLR4 agonist and an agonist ICOS binding protein for simultaneous or sequential use in treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng, and the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 24 mg to about 240 mg.

In another aspect, a TLR4 agonist for use in treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

In one aspect, an agonist ICOS binding protein or antigen binding portion thereof for use in treating cancer is provided, wherein the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 24 mg to about 240 mg and is to be administered simultaneously or sequentially with a TLR4 agonist at a dose of about 5 ng to about 1000 ng.

In another aspect, use of a TLR4 agonist in the manufacture of a medicament for treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

In one aspect, use of an agonist ICOS binding protein or antigen binding portion thereof in the manufacture of a medicament for treating cancer is provided, wherein the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 24 mg to about 240 mg and is to be administered simultaneously or sequentially with a TLR4 agonist at a dose of about 5 ng to about 1000 ng.

In one aspect a pharmaceutical kit is provided, the kit comprising about 5 ng to about 1000 ng of a TLR4 agonist and about 24 mg to about 240 mg of an agonist ICOS binding protein or antigen binding portion thereof.

In one embodiment, the dose of the TLR4 agonist is in the range of about 5 ng to about 1000 ng. In another embodiment, the dose of the TLR4 agonist is in the range of about 50 ng to about 1000 ng. In one embodiment, the dose of the TLR4 agonist is in the range of about 50 ng to about 800 ng. In one embodiment, dose of the TLR4 agonist is in the range of about 100 ng to about 600 ng. In one embodiment, dose of the TLR4 agonist is in the range of about 150 ng to about 600 ng. In one embodiment, the TLR4 agonist is to be administered at a dose of about 5 ng, about 50 ng, about 100 ng, about 150 ng, about 200 ng, about 250 ng, about 300 ng, about 350 ng, about 400 ng, about 450 ng, about 500 ng, about 550 ng, about 600 ng, about 650 ng, about 700 ng, about 750 ng, about 800 ng, about 850 ng, about 900 ng, about 950 ng, or about 1000 ng.

In one embodiment, the dose of the ICOS binding protein or antigen binding portion thereof is in the range of about 0.04 mg to about 480 mg. In another embodiment, the dose of the ICOS binding protein or antigen binding portion thereof is in the range of about 0.08 mg to about 240 mg. In another embodiment, the dose of the ICOS binding protein or antigen binding portion thereof is 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.

In one embodiment, the TLR4 agonist is administered via IV injection. In one embodiment, the TLR4 agonist is administered intravenously, intratumorally, or

subcutaneously. In another embodiment, the ICOS binding protein or antigen binding portion thereof is administered via IV infusion.

In one embodiment, the TLR4 agonist is 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.

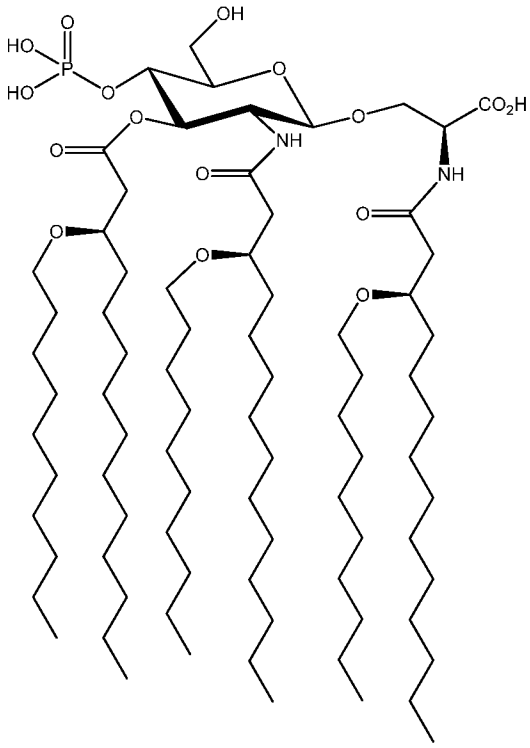
In one embodiment, the ICOS binding protein is administered once every 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 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 another embodiment, the TLR4 agonist is administered once every week, once every two weeks, or once every three weeks. In one embodiment, the ICOS binding protein is administered once every week, once every two weeks, or once every three weeks.

In one embodiment, a TLR4 agonist is administered in a run-in period prior to administering the combination of TLR4 agonist and agonist ICOS binding protein or antigen binding portion thereof. In one embodiment, the TLR4 agonist is administered in a run-in period of between about 1 week to about 3 weeks. In another embodiment, the run-in period is 1 week, 2 weeks, or 3 weeks. In another embodiment, the run-in period is 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.

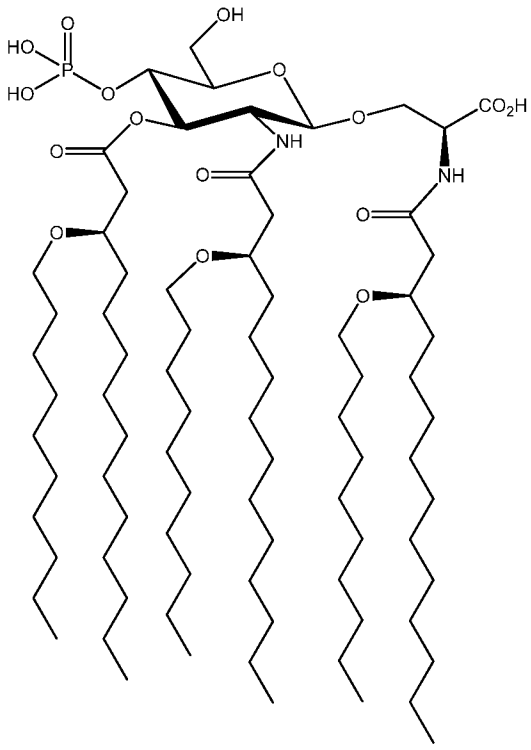
In one aspect, there is provided a method of treating cancer in a human in need thereof, the method comprising administering to the human a TLR4 agonist at a dose of 50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng, 350 ng, 400 ng, 450 ng, 500 ng, 550 ng, or 600 ng and administering to the human an ICOS binding protein or antigen binding portion thereof at a dose of 80 mg or 24 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, and wherein the TLR agonist is CRX-601



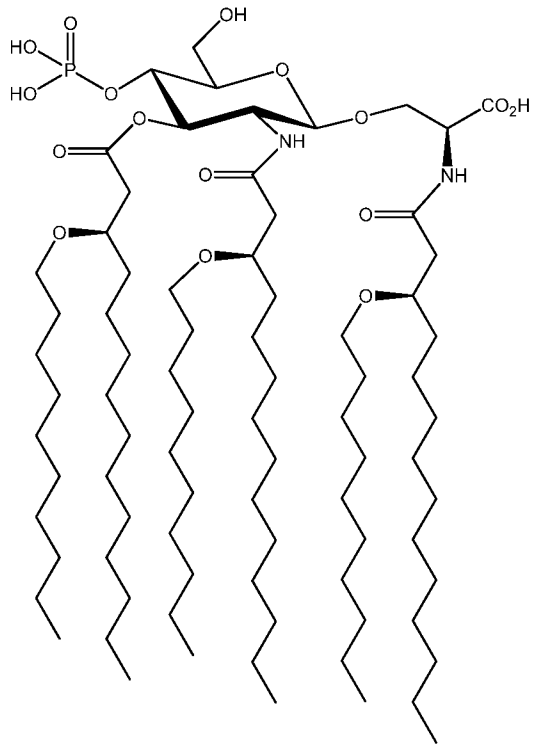
(CRX-601).

In another aspect, a TLR4 agonist and an agonist ICOS binding protein for simultaneous or sequential use in treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of 50 ng, 100 ng, 150 ng, 200 ng, or 250 ng, and the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of 24 mg or 80 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, and wherein the TLR agonist is CRX-601



(CRX-601).

In another aspect, use of a TLR4 agonist in the manufacture of a medicament for treating cancer is provided, wherein the TLR4 agonist is to be administered at a dose of dose of 50 ng, 100 ng, 150 ng, 200 ng, or 250 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of 24 mg or 80 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, and wherein the TLR agonist is CRX-601



(CRX-601).

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), gastric cancer, melanoma, renal cell carcinoma (RCC), esophageal cancer, non-small cell lung carcinoma, prostate cancer, colorectal cancer, ovarian cancer and pancreatic cancer. In one aspect the human has one or more of the following: SCCHN, colorectal cancer (CRC), esophageal, cervical, bladder, breast, head and neck, ovarian, melanoma, renal cell carcinoma (RCC), EC squamous cell, non-small cell lung carcinoma, mesothelioma, and prostate cancer. In another aspect the human has a liquid tumor such as diffuse large B cell lymphoma (DLBCL), multiple myeloma, chronic lyphomblastic leukemia (CLL), follicular lymphoma, acute myeloid leukemia and chronic myelogenous leukemia.

The present disclosure also relates to a method for treating or lessening the severity of a cancer selected from: brain (gliomas), glioblastomas, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast, inflammatory breast cancer, Wilm's tumor, Ewing's sarcoma, Rhabdomyosarcoma, ependymoma, medulloblastoma, colon, head and neck, kidney, lung, liver, melanoma, ovarian, pancreatic, prostate, sarcoma, osteosarcoma, giant cell tumor of bone, thyroid, 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, Hodgkins lymphoma, non-hodgkins lymphoma, lymphoblastic T cell lymphoma, Burkitt's lymphoma, follicular lymphoma, neuroblastoma, bladder cancer, urothelial cancer, lung cancer, vulval cancer, cervical cancer, endometrial cancer, renal cancer, mesothelioma, esophageal cancer, salivary gland cancer, hepatocellular cancer, gastric cancer, nasopharangeal cancer, buccal cancer, cancer of the mouth, GIST (gastrointestinal stromal tumor) and testicular 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 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, effects or side effects associated with the condition or treatment thereof, (4) to slow the progression of the condition or one or more of the biological 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. 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 has been exposed to a carcinogen.

As used herein, the terms "cancer," "neoplasm," 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 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 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 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 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 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 polcythemia vera (PCV). Myeloid malignancies also include

myelodysplasia (or 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 (SNCCL) 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 one embodiment, the methods of the present invention further comprise administering at least one neo-plastic agent to said human. The methods of the present invention may also be employed with other therapeutic methods of cancer treatment.

Typically, any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be co-administered in the treatment of cancer in the present invention. Examples of such agents can be found in Cancer Principles and Practice of Oncology by V.T. Devita, T.S. Lawrence, and S.A. Rosenberg (editors), 10th edition (December 5, 2014), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Typical anti-neoplastic agents useful in the present invention include, but are not limited to, anti-microtubule or anti-mitotic agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as actinomycins, anthracyclins, and bleomycins; topoisomerase I inhibitors such as camptothecins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and anti-folate compounds; hormones and hormonal analogues; signal transduction pathway inhibitors; non-receptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; cell cycle signalling inhibitors;

proteasome inhibitors; heat shock protein inhibitors; inhibitors of cancer metabolism; and cancer gene therapy agents such as genetically modified T cells.

Examples of a further active ingredient or ingredients for use in combination or co-administered with the present methods or combinations are anti-neoplastic agents. Examples of anti-neoplastic agents include, but are not limited to, chemotherapeutic agents; immuno-modulatory agents; immuno-modulators; and immunostimulatory adjuvants.

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: In vivo studies

CRX-601 and H2L5 IgG4PE combination was evaluated in BALB/c mice implanted with syngeneic CT-26 tumors. Four groups of 10 BALB/c mice with intact immune systems were implanted with CT-26 tumors. The mice received one of the following treatments: placebo, CRX-601 (TLR4 agonist), 7E.17G9 agonist (mouse surrogate ICOS agonist antibody), or the combination of CRX-601 and 7E.17G9 agonist. While the monotherapies had very modest effects on tumor growth (FIG. 1), the combination treatment of CRX-601 and 7E.17G9 produced greater activity and durable responses. CRX-601 and 7E.17G9 monotherapies resulted in 10% and 40% of mice that survived more than 100 days respectively, while the combination therapy resulted in 60% tumor free mice that survived more than 100 days (FIG. 2).

Example 2: In vitro studies

The combination of CRX-601 and H2L5 IgG4PE was also evaluated for cytokine release in PBMCs isolated from 10 donors under resting or pre-stimulation (anti-CD3) conditions. Cytokines (IL-2, IL-6, IL-10, IFN-γ, and TNF-α) were measured following 24 hours incubation with CRX-601 (10, 100 or 1000 pg/mL) and/or H2L5 IgG4PE (0.01, 0.1, 1 or 10 μg/mL). Positive cytokine induction by CRX-601 and H2L5 IgG4PE combination was defined as $\geq$3-fold increase above that of CRX-601 alone. No increases greater than 3-fold over single treatment arm in IL-2, IL-6, IL-10, IFN-γ, or TNF-α were detected in PBMCs treated with H2L5 IgG4PE in combination with CRX-601 (at concentrations up to

1000 pg/mL and 10 μg/mL, respectively) compared to H2L5 IgG4PE alone, either in the presence or absence of anti-CD3 stimulation. These results suggest that the potential for enhanced cytokine release when these agents are administered in combination in patients appears to be low.

Example 3: Overall Design

This is a study designed to evaluate the safety, tolerability, PK, pharmacodynamic, and preliminary clinical activity of CRX-601 administered in combination with other immunotherapies to participants with advanced solid tumors.

In Part 1, the safety and tolerability of escalating doses of CRX-601 and a single dose level of a monoclonal antibody (mAb) combination partner H2L5 IGG4PE will be evaluated in separate cohorts of participants with advanced solid tumor cancers according to an Neuenschwander-Continual Reassessment Method (N-CRM) design to identify doses for evaluation in Part (Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. Statistics Med. 2008;27:2420-2439). Part 1 will include a CRX-601 run-in period of 2 weeks (i.e., CRX-601 administration on Days 1 and 8) prior to administration of the combination partner beginning at Day 15. Approximately 5 dose levels of CRX-601 in combination with a single fixed dose level of the combination partner are planned to be evaluated in Part 1. Following protocol amendment, CRX-601 may also be further evaluated by additional routes of administration.

In Part 2, expansion cohorts of approximately 6 to 15 participants with squamous cell carcinoma of the head and neck (SCCHN) will be enrolled in each combination treatment arm to further evaluate the safety and activity of dose(s) identified in Part 1. The dose(s) of CRX-601 administered in combination with 80 mg H2L5 IGG4PE will be determined based on data from Part 1 and may differ for each combination treatment. Following protocol amendment, additional expansion cohorts in other tumor types may be enrolled, based on emerging nonclinical and clinical data.

In addition, PK/Pharmacodynamic cohorts for each combination will be opened to enrollment during Part 1 to obtain additional PK and pharmacodynamic data, with an emphasis to obtain insight on the potential impact of the combination treatments on the immune cells and status of the tumor microenvironment, in conjunction with PK and

pharmacodynamic markers obtained from blood. Tumor biopsies are required for enrollment to the PK/Pharmacodynamic cohorts, whereas biopsies are strongly encouraged but not mandatory for Part 1 dose escalation cohorts. For each combination, participants in the PK/Pharmacodynamic cohorts may be enrolled to any dose level which has already been completed and supported by adequate safety and tolerability from dose escalation for that combination. Up to a maximum of 45 participants may be enrolled into the PK/Pharmacodynamic cohorts with up to approximately 6 per dose level for each combination.

Number of Participants:

The number of dose levels which will be needed to identify the target dose level(s) for Part 2 is not known in advance, consequently, the number of participants in the study can only be estimated. It is estimated that up to approximately 162 total participants will be enrolled into the study. Up to approximately 72 participants will be enrolled into Part 1 (dose-escalation) of the study. Additionally, for each combination, up to 6 participants in each dose cohort, and a maximum of approximately 45 participants in total, may be enrolled into the PK/Pharmacodynamic cohort of Part 1. Additional cohorts (up to a maximum of 12 total participants) may be enrolled in Part 1 to allow for evaluation of more dose levels. Up to approximately 45 participants will be enrolled in Part 2 (expansion cohorts) of the study.

Treatment Groups and Duration:

Participants will receive the combination of CRX-601 with H2L5 IGG4PE. In Part 1, escalating doses of CRX-601 will be evaluated as guided by the N-CRM approach. In Part 2, participants will receive a single dose level of CRX-601 as identified based on data from Part 1, in combination with H2L5 IGG4PE.

The study includes a screening period, a treatment period, and a follow-up period. Participants will be screened for eligibility beginning 4 weeks before the start of treatment. The duration of study treatment is expected to be up to 2 years. For participants that discontinue study treatment prior to a determination of progressive disease (PD), the follow-up period will include disease assessments every 12 weeks until documented PD occurs (PFS Follow Up [FU]). Following PD or for participants that discontinue study

treatment for PD, participants will be contacted every 12 weeks to assess survival status (Survival FU [SFU]) for up to 2 years from the start of the study treatment.

Example 4: Study design

Overall Design

This study is designed to evaluate the safety, tolerability, PK, pharmacodynamic, and preliminary clinical activity of CRX-601 administered in combination with other immunotherapies to participants with advanced solid tumors.

The study will be conducted in two parts. Part 1 is divided into 3 treatment arms based on the CRX-601 combination partner; Part 1a, Part 1b, or Part 1c. Each of the three treatment arms may have up to 5 dose escalation cohorts to investigate the safety and tolerability of escalating doses of CRX-601with a single dose level of the combination partner. In Part1b and Part2b, the CRX-601 combination partner is H2L5 IgG4PE 80 mg (Part 1b, Part 2b)

Part 1 will include a CRX-601 run-in period of 2 weeks (i.e., CRX-601 administration on days 1 and 8) prior to administration of the combinations beginning on day 15 (Week 3). Following protocol amendment, CRX-601 may also be evaluated by additional routes of administration. Safety data will be evaluated according to a Neuenschwander-Continual Reassessment Method (N-CRM) design (Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. Statistics Med. 2008;27:2420-2439) to help identify a dose for investigation in Part 2.

Part 2 is also divided into 3 treatment arms for the expansion cohorts; Part 2a, Part 2b, or Part 2c. Expansion cohorts of approximately 6 to 15 participants with SCCHN will be enrolled to each combination to further evaluate safety and activity of the dose regimen(s) identified in Part 1. The dose(s) of CRX-601 administered with 80 mg H2L5 IgG4PE will be determined based on data from Part 1 and may differ for each combination treatment. Additional expansion cohorts in other tumor types may be enrolled based on emerging nonclinical and/or clinical data.

For each combination of CRX-601 in Part 1, PK/Pharmacodynamic cohorts will be opened at cleared dose levels for that combination (i.e. the most recent investigated dose level that

supported dose escalation) to explore the potential relationships between dose, biological effects in the tumor microenvironment, and tumor response. A particular emphasis in the PK/Pharmacodynamic cohort is placed on evaluating the possible effects of the combination on the immune cells and immune status within the tumor microenvironment. Thus, to be eligible for the PK/Pharmacodynamic cohort, participants must consent to mandatory fresh biopsy collection at baseline and on treatment. An additional radiographic disease assessment will support exploratory investigation of tumor growth kinetics in this cohort. Note that while consent to fresh tumor biopsy is not required for participation in the dose escalation cohorts in Part 1, it is strongly encouraged. Up to 6 participants per dose level may be enrolled into the PK/Pharmacodynamic cohort for each combination.

The study includes a screening period, a treatment period, and a follow-up period. Participants will be screened for eligibility beginning 4 weeks before the start of treatment. The duration of study treatment will be up to 2 years. For participants that discontinue study treatment prior to a determination of PD, the follow-up period includes disease assessments every 12 weeks until documented PD. Following PD or for participants that discontinue study treatment for PD, participants will be contacted every 12 weeks to assess survival status for 2 years from the start of the study.

Participants with confirmed PR or CR will be followed for response duration and may be eligible (outside of Canada) for continued study treatment at the time of relapse/progression. The decision whether a participant will receive additional treatment will be discussed and agreed upon by the treating investigator and the Sponsor/Medical Monitor on a case-by-case basis.

Additional participants may be enrolled to evaluate additional routes of study treatment administration (e.g., intratumoral administration), additional agents to be used in combination with CRX-601, or additional indications, based on emerging nonclinical and/or clinical data.

Part 1: Dose Escalation of CRX-601 administered in combination with H2L5 IgG4PE

In Part 1, dose escalation will be performed to identify combination dose levels comprising CRX-601 with 80 mg H2L5 IgG4PE (Part 1b). One (1) dose level of H2L5 IgG4PE with up to 5 dose levels of CRX-601 are planned for evaluation, pending emerging safety and tolerability information as dose escalation proceeds.

Part 1 will include a run-in period of 2 weeks in which CRX-601 is administered once-weekly [i.e., administration on day 1 (Week 1) and day 8 (Week 2)] prior to initiation of combination treatment with H2L5 IGG4PE beginning on day 15 (Week 3). During the run-in period, participants that experience a DLT, unacceptable toxicity, or an increase in ALT (1.5x ULN and 1.5x baseline) and not attributable to another cause will be discontinued from the study and will not receive CRX-601 in combination.

The starting schedule for CRX-601 will be at every 1-week intervals (Q1W) from Week 1 through Week 12 including the 2-week monotherapy run in period (Week 1 and Week 2). Subsequently, CRX-601 will be administered at every 3-week intervals (Q3W) to coincide with H2L5 IgG4PE dosing. Thus, beginning with Week 12 for Part 1 and Week 13 for Part 2, both CRX-601 and the combination partner will be administered on the same study day at a frequency of Q3W.

Cohorts will be opened beginning with 50 ng CRX-601 administered in combination with 80 mg H2L5 IgG4PE. Three (3) or more participants will be enrolled in each cohort. The total number of participants enrolled into each cohort and dose assignments will be guided by safety information from participants receiving the study treatment combinations according to N-CRM modelling (Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. Statistics Med. 2008;27:2420-2439).

Sequential cohorts will be enrolled and dose escalation (or de-escalation) will proceed guided by an N-CRM design. Dose escalation for each cohort will proceed independently of the other cohorts, e.g. dose escalation for Part 1a is not required prior to dose escalation for Part 1b or Part 1c. The first 3 participants at each dose level will receive study treatment at least 3 days apart (e.g., if the first participant in a cohort were dosed on Monday, the earliest the next participant could be dosed is Thursday). Once the 6-week DLT evaluation period has been completed, N-CRM analysis will be performed to guide the dose level to which the next 3 participants will be assigned based on DLT frequency. The number of participants allocated to any cohort is an estimate; participants may also be allocated to PK/Pharmacodynamic cohorts at a previous dose level that supported dose escalation.

Dose levels -1 are available for H2L5 IgG4PE (24 mg + 50 ng CRX-601) if the target toxicity level is exceeded in Cohort 1 and a dose reduction is needed below planned doses.

Description of the Continual Reassessment Method

The N-CRM model-based design is a Bayesian adaptive dose escalation scheme that assumes a 2-parameter logistic model for the toxicity rate as a function of dose. It is a modified version of the original Continual Reassessment Method proposed by O'Quigley J, Pepe M, Fisher L. Continual Reassessment Method: A Practical Design for Phase I Clinical Trials in Cancer. Biometrics. 1990;46:33-48. The N-CRM method is fully adaptive and makes use of all DLT information, therefore is expected to locate the target dose level efficiently. In this case, the model will be applied to the dose escalation decision for CRX-601, which will be performed independently for each combination.

Dose escalation decisions will be held after participants within any given cohort have been observed for at least 6 weeks after starting the study treatment. At the time of each dose escalation decision, the Fixed and Adaptive Clinical Trial Simulator (FACTS [Tessella, Abington, United Kingdom]) will be used to obtain the posterior probabilities for the DLT rate. The N-CRM estimates for each potential dose will provide the posterior probabilities that the DLT rate lies in each of four toxicity ranges:

- [0%, 16%] Underdosing
- [16%, 33%] Target toxicity
- [33%, 60%] Excessive toxicity
- [60%, 100%] Unacceptable toxicity

The recommended dose for dose escalation, based on the N-CRM model, will be the dose with the highest posterior probability of lying in the target toxicity interval with the additional requirement that the sum of the posterior probabilities of the DLT rate lying in the excessive toxicity or unacceptable toxicity range is less than 25%. An updated estimate of the toxicity curve will be provided at the time of each dose escalation meeting. Note that de-escalation as well as escalation is possible using this method. Dose escalation will continue until conditions for either scenario (i) or (ii) are met:

i) Six participants have been treated at the current target dose

AND

For the current dose level, the posterior probabilities of the DLT rate lying within the excessive toxicity interval or within the unacceptable toxicity interval sum to less than 25%

AND

For the next higher dose, the posterior probabilities of the DLT rate lying within the excessive toxicity interval or within the unacceptable toxicity interval sum to greater than 25%.

ii) No doses are usable (i.e., for all doses, the posterior probabilities of the DLT rate lying within the excessive toxicity interval or within the unacceptable toxicity interval sum to more than 25%)

AND

At least 2 DLTs have been observed.

Dose recommendations based on the N-CRM analysis will be used as guidance. To ensure safety of participants, additional participants may be enrolled at a current dose level at the discretion of the study investigators and sponsor, even though a higher dose is recommended by N-CRM analysis.

Logistic Model for N-CRM

A two-parameter logistic model will be used for N-CRM analysis for dose level selection during the dose escalation phase. This model will estimate the probability of observing a DLT at each dose level in the study as DLT information becomes available.

The logistic model that used for describing the dose-toxicity relationship is:

$$ln\left(p_d/(1-p_d)\right) = \alpha + \beta * ln\left(d/d_m\right),$$

where p_d is the probability of DLT at dose d, and d_m is a reference dose, and α and β are Bayesian priors.

PK/Pharmacodynamic Cohort(s)

Characterizing the effects of treatment on the tumor microenvironment is essential to the understanding the mechanism of action of CRX-601 and its combination partners at the site of action. Thus, for each combination of CRX-601 in Part 1, PK/Pharmacodynamic cohorts will be opened to characterize the biological effects in the tumor microenvironment and explore the potential relationships between dose and tumor response. PK/Pharmacodynamic cohorts, with up to 6 participants per dose level, will be opened for CRX-601 dose levels previously cleared for dose escalation.

Pre- and on-treatment tumor biopsies are required for enrollment to this cohort. PK, pharmacodynamic markers, and safety samples will be drawn according to Section **Error! Reference source not found.** to obtain additional PK and pharmacodynamic data. Participants in the PK/Pharmacodynamic cohort may have the dose escalated to a higher completed dose level (not exceeding the target toxicity level) after Week 9 once the necessary PK/Pharmacodynamic procedures and tissue biopsies have been completed.

Dose-Limiting Toxicity

All toxicities will be graded using National Cancer Institute - Common Toxicity Criteria for AEs (NCI-CTCAE), version 4.0.

An AE is considered to be a DLT if it is considered by the investigator to be clinically relevant and attributed (definitely, probably, or possibly) to the study treatment and meets at least 1 of the criteria listed in Table . If an AE is considered related to the underlying disease, it is not a DLT.

Table 2 Dose-Limiting Toxicity Criteria

Toxicity	DLT Definition
Hematologic	• Grade 4 neutropenia of >7 days' duration or febrile neutropenia • Grade 4 anemia • Grade 4 thrombocytopenia or Grade 3 thrombocytopenia with bleeding
Non-hematologic	• Grade 4 toxicity

	• Grade 3 toxicity that does not resolve to ≤Grade 1 or baseline within 14 days despite optimal supportive care • Immune-related toxicity requiring >10 mg prednisone (or equivalent) per day after 12 weeks from the last dose of study treatment • Cardiopulmonary or hemodynamic toxicity starting within 24 hours of study treatment injection that requires >40% fraction inspired oxygen (FiO2), vasopressor administration, antiarrhythmic agent or other significant medical intervention. • ECG changes showing asystole or bradycardia that is symptomatic and requires medical intervention. • Liver toxicity (see **Error! Reference source not found.**) including o ALT ≥ 5xULN o ALT ≥ 3xULN persists for ≥4 weeks o ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) o ALT ≥ 3xULN and INR >1.5, if INR measured o ALT ≥ 3xULN associated with symptoms (new or worsening) believed to be related to liver injury or hypersensitivity • Following events are **not** considered DLTs o Changes in leukocyte parameters within 48 hours of CRX-601 administration o Grade 1 changes in ALT o ≥ Grade 3 electrolyte abnormalities that are corrected within 24 hours without clinical sequelae o Grade 3 tumor flare (defined as local pain, irritation, or rash localized at sites of known or suspected tumor) o Grade 3 fatigue
Other (not addressed above)	• Toxicity that results in permanent discontinuation or dose reduction of CRX-601 during the first 6 weeks of treatment • Any other event which in the judgment of the investigator and GSK Medical Monitor is considered to be a DLT

For participants to be considered evaluable for dose escalation decisions, missed study treatments must be limited during the first 6 weeks of dosing. Participants may not miss any study treatment administered Q3W and may not miss more than 1 study treatment administered Q1W.

Participants who withdraw from the study before completing 6 weeks of treatment for reasons other than DLT may be replaced. If a participant experiences a DLT during this period, the participant will be discontinued from the study.

Part 2: Expansion Cohort

Part 2 of the study will further characterize the safety and tolerability of CRX-601 administered in combination with H2L5 IgG4PE (Part 2b) in participants with recurrent, locally advanced, or metastatic SCCHN as determined by safety and tolerability results from the respective cohorts in Part 1. Part 2 will also characterize antitumor activity, PK, and pharmacodynamics effects, including effects measured from tumor biopsy. Part 2 may be opened for a given combination before Part 1 has been completed provided a tolerable dose level within or below the target toxicity range has been identified for that combination. The dose of CRX-601 to be administered in the expansion cohort will be based on all available data and may have a DLT frequency within or below the target toxicity range

Between approximately 6 and 15 participants with SCCHN will be enrolled in the Part 2b expansion cohort. No more than 6 participants will be enrolled in an expansion cohort without meeting eligibility requirements for mandatory biopsy requirement.

Interim analysis for futility will be performed on an on-going basis for Part 2b, and cohort(s) may be stopped if interim analysis reveals futility. Actual decisions will depend on the totality of the data.

Oversight

The active study investigators, GSK medical monitor, GSK pharmacovigilance physician, GSK statistician, GSK physician(s) independent of the study team and contract organization physicians will be responsible for decisions to escalate doses and to progress from Part 1 of the study to Part 2. Decisions will be based on safety information and other available data from ongoing and prior cohorts.

The dose-determination decision and rationale for each cohort will be discussed with investigators during teleconference(s) and documented in writing, with copies maintained at each study site and in the study master file.

Although the N-CRM will be used to recommend the next dosing level, clinical judgment by the study investigators and GSK study team can override this recommendation or halt enrollment into any cohorts as deemed appropriate at any time during the trial.

Tumor Types Enrolled During Parts 1 and 2

In Part 1, participants with advanced solid tumors will be enrolled. In Part 2, only participants with SCCHN will be enrolled.

Intra-Participant Dose Escalation

Following the selection of a recommended combination dose for Part 2, participants in respective cohorts in Part 1 may be considered for escalation to the Part 2 dose level. Intra-participant dose escalation will be considered on a case-by-case basis provided the participant has completed at least 6 weeks of study treatment without the occurrence of a SAE or ≥Grade 2 drug-related toxicity. Approval by the Sponsor is required for intra-participant dose escalation.

Study Treatment

Study Part	**Study Treatment: CRX-601 in combination with H2L5 IgG4PE**
Part 1: Dose Escalation	
1b	H2L5 IgG4PE 80 mg IV and CRX-601 IV
Part 2: Expansion	
2b	H2L5 IgG4PE 80 mg IV and CRX-601 IV

Number of Participants

The number of dose levels which will be needed to identify the target dose level(s) for Part 2 is not known in advance, consequently, the number of participants in the study can only be estimated. It is estimated that up to approximately 162 total participants will be

enrolled into the study. Up to approximately 72 participants will be enrolled into Part 1 (dose-escalation) of the study with approximately 24 participants in each combination. Up to approximately 45 participants will be enrolled in Part 2 (expansion cohorts) of the study with up to 15 participants in Part 2a and 2b and 15 participants in Part 2c.

Additionally, for each combination, up to 6 participants in each dose cohort and a maximum of approximately 45 participants in total may be enrolled into the PK/Pharmacodynamic cohorts of Part 1. Additional cohorts (up to a maximum of 12 total participants) may be enrolled in Part 1 to allow for evaluation of additional dose levels of CRX-601.

In Part 1, if a participant prematurely discontinues before the completion of 6-weeks treatment for reasons other than DLT, a replacement participant may be enrolled at the discretion of the Sponsor in consultation with the investigator. Participants who are dosed with study treatment will not be replaced in Part 2 (expansion cohort) of the study.

Participant and Study Completion

In Part 1, participants will be considered to have completed the study if they complete screening assessments and receive at least 1 study treatment and experience a DLT or complete the 6-week DLT observation period, and the treatment discontinuation visit (TDV).

In Part 2, a participant will be considered to have completed the study if they meet the study completion criteria for Part 1 and:

- they are followed for 2 years from start of study treatment, or
- they die while receiving study treatment or in follow up

Cause of death must be documented in the case report form (CRF)/electronic CRF (eCRF). A participant will be considered to have withdrawn from the study if the participant has not died and is lost to follow-up, has withdrawn consent, at the investigator's discretion is no longer being followed or if the study is closed/terminated.

Disease progression, discontinuation of study treatment, and AEs, are not by themselves reasons for withdrawal from the study as follow-up for OS is desired. The end of the study is defined as the last participant's last visit.

Scientific Rationale for Study Design

The combination of CRX-601 with H2L5 IgG4PE was selected based on complementary mechanisms of action and robust antitumor activity in preclinical models.

Eligibility criteria require that participants have progressed after standard therapies or are otherwise unsuitable for standard therapies, and the criteria are intended to minimize the risk of adverse reactions to treatment with immunotherapies.

In Part 1, dose escalation of CRX-601, with a fixed dose of the combination partners, will be performed using an N-CRM model to optimize the allocation of participants to dose levels with a 16-33% DLT frequency. The DLT criteria are based on typical oncology rules with additional modifications for toxicities expected for the study treatments.

In Part 1, a 2-week run-in period for CRX-601 precedes the administration of the combination therapy. The run-in provides an evaluation of monotherapy CRX-601 safety and tolerability in participants with cancer and prevents the administration of combination therapy to participants that experience, with CRX-601 alone, a DLT, unacceptable tolerability, or an increase in ALT to 1.5x ULN and 1.5x baseline.

In Part 2, expansion cohorts will be opened to evaluate safety and tolerability of the combinations as well as preliminary activity in participants with SCCHN. SCCHN was chosen for further study based on observations of responses to other immunotherapies and recognition of the considerable unmet need for this indication. Additionally, since TLR agonists are being developed by different routes of administration, including intratumoral injection, SCCHN is a possible indication for future exploration of alternative approaches to dosing.

Dose Justification

Overview

CRX-601 and H2L5 IgG4PE have been previously administered as monotherapies in studies.

The selection of starting combination doses has taken into consideration all available data, including the safety, tolerability, and pharmacology data of monotherapy CRX-601 and monotherapy H2L5 IgG4PE observed in the respective FTIH studies, together with

pharmacology and safety data from animal models and human ex vivo (peripheral blood mononuclear cell [PBMC]) assays, conducted under monotherapy and combination conditions.

Starting dose for TLR4 agonist CRX-601

The starting dose of CRX-601 is 50 ng administered once-weekly IV. Previously CRX-601 was administered at doses up to 100 ng IV to healthy participants in the FTIH Study. Based on data from the FTIH study, the starting dose in the current study (50 ng) is expected to produce low level pharmacological effects consistent with TLR4 agonism based on data from the FTIH study.

Because robust TLR receptor saturation assays are not available, target engagement by CRX-601 in the FTIH study (204685) was monitored using representative inflammatory cytokine biomarkers. Based on review of available preliminary data (dose levels up to 100 ng), post-dose elevations of cytokines following administration of CRX-601 in the FTIH study were of a low magnitude compared to historical clinical studies of TLR agonists administered to cancer patients. For example, the peak levels of inflammatory cytokines at 2h, such as TNFα (median:12 pg/ml; min: 6 pg/ml; max: 23 pg/ml) and IL-6 (median: 132 pg/ml; min: 81 pg/ml; max: 184 pg/ml pg/ml, respectively), associated with administration of 100 ng CRX-601 are below levels reported in previous studies of TLR agonists in cancer patients (>1000 pg/ml) (Chow L, Morishima C, Eaton K, Baik C, Goulart BH, Anderson L, et al. Phase Ib Trial of the Toll-like Receptor 8 Agonist, Motolimod (VTX-2337), Combined with Cetuximab in Patients with Recurrent or Metastatic SCCHN. Clin Cancer Res. 2017 May 15;23(10):2442-2450.; Engelhardt R, Mackensen A, Galanos C. Phase I trial of intravenously administered endotoxin (Salmonella abortus equi) in cancer patients. Cancer Res. 1991 May 15;51(10):2524-30). These differences are likely not a function of differences in study populations, given that prior comparisons of TLR agonists in healthy participants and cancer participants have shown similar cytokine responses between populations (Schmidt M, Kapp K, Oswald D, Matthias S, Wittig B, Zurlo A. Pharmacokinetic and pharmacodynamic data of the immunotherapeutic TLR-9 agonist MGN1703 from healthy volunteers and cancer patients. Journal of Clinical Oncology. 2015;33(15_suppl):abstract e14015; Dietsch G, Whiting S, Northfelt D, Ramanathan R, Cohen P, Manjarrez K, et al. Comparison of immune modulation by TLR8 agonist vtx-2337 (motolimod) in cancer patients and healthy

volunteers. Journal for ImmunoTherapy of Cancer. 2014;2(Suppl 3):P165). Even acknowledging possible differences in pharmacodynamic assay performance, the greater than 10–100 fold margin between cytokine concentrations associated with 100 ng doses of CRX-601 versus concentrations reported in other studies of cancer patients provides reassurance that a significant margin separates the starting dose of CRX-601 and maximum tolerated dose of other TLR agonists.

Consistent with the mechanism of action of CRX-601, body temperature and heart rate increased with dose in Study **Error! Reference source not found.**. Mean maximum change with 95% CI in body temperature in the participants that received placebo was 0.4 ± 0.2°C. For CRX-601 dose levels, 7 ng, 21 ng, 60 ng, and 100 ng, mean maximum change with 95% CI in body temperature was 0.5 ± 0.3, 0.6 ± 0.4, 0.8±.5, and 1.3 ± 0.3 °C, respectively. Mean maximum change with 95% CI in heart rate in the participants that received placebo was 6 ± 5 beats per minute. For CRX-601 dose levels, 7 ng, 21 ng, 60 ng, and 100 ng, mean maximum change with 95% CI in heart rate was 8 ± 9, 10 ± 18, 18 ± 15, 21 ± 5 beats per minute, respectively. Thus, a 50 ng starting dose is expected to be associated with modest changes in body temperature and heart rate.

Based on preliminary, unblinded safety data, the most common clinical findings were influenza-like symptoms and increased body temperature. Predominantly mild AEs were reported when doses up to 60 ng were administered. Three (3) participants experienced moderate AEs following administration of 100 ng CRX-601.

In addition to the aforementioned dose-related AEs, 1 out of the 12 participants that received a 60 ng dose of CRX-601 experienced a 12-fold ULN increase in ALT, 5-fold ULN increase in AST, and 1.4-fold ULN increase in total bilirubin on day 35. As only 1 event was observed and the dose administered was below the maximum administered, 100 ng, the data are too limited to relate elevations in hepatic laboratories to dose. Because of the possible risk of infrequent or idiosyncratic transaminase elevations, a 2-week CRX-601 run-in period will be performed in Part 1, including monitoring and study treatment discontinuation criteria for ALT elevations, before CRX-601 and combination partners are administered.

In summary, at the CRX-601 starting dose of 50 ng, minimal pharmacodynamic effects and dose-related cytokine-associated clinical effects are expected. The risk of infrequent

events of uncertain relationship to CRX-601 dose will be mitigated by a 2-week monotherapy run-in period.

Dose for ICOS agonist H2L5 IgG4PE

The starting dose of H2L5 IgG4PE is 80 mg (~1 mg/kg) IV administered Q3W. In the FTIH study of H2L5 IgG4PE, doses up to 3mg/kg (~240 mg) Q3W were evaluated as monotherapy. Based on the available clinical exposure and safety data, H2L5 IgG4PE can be dosed up to 3 mg/kg (~240 mg). One DLT was reported in one out of 10 participants treated at this dose. The planned dose of H2L5 IgG4PE for evaluation in the present study is 80 mg, or approximately 1/3 the top dose studied to date.

H2L5 IgG4PE systemic concentrations at different dose levels have been simulated with a population pharmacokinetic model developed with available clinical data.

ICOS receptor occupancy (RO) in the central circulation has been estimated based on predicted H2L5 IgG4PE systemic exposures and potency values for the functional effects of H2L5 IgG4PE characterized from *in vitro* binding/activation assays. Potency values generated from three different binding/activation assays (Kd, T-cell binding, IFN-γ release) were in the range of 0.09 to 4.14 μg/mL. The corresponding ICOS RO levels with predicted H2L5 IgG4PE systemic exposures at different dose levels are listed in Table . The predicted RO demonstrates that the 80 mg dose (~1 mg/kg) provides adequate level of target engagement across all three approaches. The currently planned 80mg H2L5 IgG4PE dose may be adjusted lower to 24mg based on emerging safety, exposure and/or pharmacodynamic data if agreed by the study investigators and GSK study team.

Table 3 Projected CD4+ receptor occupancy in central circulation from population PK predicted median steady-state peak and trough exposures of H2L5 IgG4PE (Q3W regimen) based on in vitro potency estimates

Dose (mg)	C_{max} (μg/mL)	Cτ (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 Cτ	At C_{max}	At Cτ	At C_{max}	At Cτ

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

Note: 8 (~0.01mg/kg) to 240mg (~3mg/kg) Q3W regimen evaluated as monotherapy and combination

Combination considerations

At the 50 ng starting dose of CRX-601, only minimal clinical and pharmacodynamic effects are expected. Therefore, CRX-601 is not expected to significantly alter the safety and tolerability profile of H2L5 IgG4PE.

Meanwhile, H2L5 IgG4PE did not enhance cytokine induction by CRX-601 in vitro. Based on the nonclinical data, H2L5 IgG4PE is not expected to significantly alter the safety and tolerability profile of CRX-601.

The anti-ICOS 7E.17G9 clone was evaluated in the CT26 murine syngeneic tumor model in combination with the TLR4 agonist, CRX-601 in two separate *in vivo* studies. Female BALB/c mice bearing CT26 mouse colon carcinoma tumors (n=10/group), were given twice weekly IP doses of 7E.17G9 at 10 or 100 μg/mouse for 3 weeks alone and in combination with CRX-601 at 5, 10 or 25 μg/mouse. The treatments were well tolerated as no significant loss of body weight was observed in both studies.

Overall, the clinical and nonclinical data for CRX-601 and H2L5 IgG4PE administered as monotherapies support the starting combination of low doses of CRX-601 with H2L5 IgG4PE.

Dose Escalation and Top Dose

Based on available clinical data, the tolerability of CRX-601 approximates that of LPS. Therefore, the top dose of CRX-601 for study participants with cancer is expected to be similar to doses of LPS studied in similar populations, namely 2 to 4 ng/kg (i.e., 160 to 320 ng). The top dose of CRX-601 will not exceed approximately 250 ng, which would represent a less than 3-fold escalation beyond the 100 ng dose which has been studied in the FTIH healthy volunteer study. The dose escalation step size of 50 ng increments

results in a dose escalation scheme with progressively more conservative relative increases (e.g., 50 ng to 100 ng = 100% increase; 100 to 150 ng = 50% increase; 150 ng to 200 ng = 33% increase; 200 ng to 250 ng = 25% increase).

Example 5: Study population

Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant must be ≥18 years of at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Histological documentation of advanced solid tumor.
3. Archival tumor tissue obtained at any time from the initial diagnosis to study entry. Although a fresh biopsy obtained during screening is preferred, archival tumor specimen is acceptable if it is not feasible to obtain a fresh biopsy.

   Note: Participants enrolled in a PK/Pharmacodynamic Cohort must provide a fresh biopsy of a tumour lesion not previously irradiated during the screening period and must agree to provide at least one additional on-treatment biopsy.
4. Disease that has progressed after standard therapies or for which standard therapy is otherwise unsuitable (e.g., intolerance).
5. Measurable disease, i.e., presenting with at least 1 measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).
6. Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-1.
7. Life expectancy of at least 12 weeks.
8. Adequate organ function (see Table):
9. In France, a participant will be eligible for inclusion in this study only if either affiliated to or a beneficiary of a social security category.

Table 4 Organ Function

System	Laboratory Values
Hematologic	
ANC	≥1.5x10^9/L
Hemoglobin[a]	≥9 g/dL
Platelets[a]	≥100x10^9/L
PT/INR and PTT (unless participant is receiving anticoagulant)	<1.5xULN
Hepatic	
Total bilirubin *For participants with Gilbert's Syndrome (only if direct bilirubin ≤35%)*	≤1.5xULN *≤3.0xULN*
ALT	≤1.5xULN
Renal	
Calculated CrCl[b]	> 50 mL/min
Endocrine	
TSH[c]	WNL
Cardiac	
Ejection fraction	≥ 50% by echocardiogram [d]

ANC = Absolute neutrophil count; ALT = alanine aminotransferase; CrCl = creatinine clearance; INR = International Normalized Ratio; TSH = thyroid-stimulating hormone; ULN = upper limit of normal; WNL = within normal limits; PT = prothrombin time; PTT = partial thromboplastin time

a. Participants must maintain hemoglobin and/or platelet values for at least 2 weeks without transfusion or growth factor support.
b. Estimated CrCl should be calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (see **Error! Reference source not found.)**
c. If TSH is not within normal limits at baseline, the participant may still be eligible if total T3 or free T3 and free T4 are within the normal limits
d. Multigated acquisition scan (MUGA) is acceptable if echocardiography is not available

Sex

10. Male or female

a. Female participants:

A female participant is eligible to participate if she is not pregnant, not breastfeeding, and at least 1 of the following conditions applies:

i. Not a woman of childbearing potential (WOCBP)

OR

ii. A WOCBP who agrees to follow contraceptive guidance during the treatment period and for at least 120 days after the last dose of study treatment.

Informed Consent

11. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

Additional Inclusion Criteria for Patients in Part 2b (H2L5 IgG4PE expansion)

12. Histological or cytological documentation of SCCHN (oral cavity, oropharynx, hypopharynx, or larynx) that is recurrent, locally advanced, or metastatic and is not amenable to curative treatment options, surgery or definitive chemoradiation therapy.
13. Received or ineligible for platinum-based therapy and PD-1/PD-L1 therapy.
14. Received no more than 3 prior lines of systemic therapy for metastatic disease.

Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Malignancy other than disease under study with the exception of those from which the participant has been disease-free for more than 2 years and not expected to affect the safety of the participant or the endpoints of the trial.
2. Symptomatic central nervous system (CNS) metastases or asymptomatic CNS metastases that have required steroids within 2 weeks prior to first dose of study treatment.
3. Active autoimmune disease that has required systemic disease modifying or immunosuppressive treatment within the last 2 years.

Note: Replacement therapy (e.g., thyroxine or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is permitted.

4. Concurrent medical condition requiring the use of systemic immunosuppressive treatment within 28 days before the first dose of study treatment.
5. Known human immunodeficiency virus infection.
6. Current unstable liver or biliary disease per investigator assessment defined by the presence of ascites, encephalopathy, coagulopathy, hypoalbuminaemia, oesophageal or gastric varices, persistent jaundice, or cirrhosis.

NOTE: Stable chronic liver disease (including Gilbert's syndrome or asymptomatic gallstones) or hepatobiliary involvement of malignancy is acceptable if participant otherwise meets entry criteria.

7. Presence of Hepatitis B surface antigen (HBsAg) at screening or within 3 months prior to first dose of study treatment

8. Positive Hepatitis C test result at screening or within 3 months prior to first dose of study treatment.

NOTE: Participants with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA test is obtained. Participants with negative Hepatitis C antibody test are not required to also undergo Hepatitis C RNA testing

9. QTcF >450 msec or QTcF >480 msec for participants with bundle branch block

The QTcF is the QT interval corrected for heart rate according to Fridericia's formula, machine-read or manually over-read.

10. Recent history (within the past 6 months) of acute diverticulitis, inflammatory bowel disease, intra-abdominal abscess, or gastrointestinal obstruction.

11. Recent history of allergen desensitization therapy within 4 weeks of starting study treatment.

12. History of severe hypersensitivity to mAbs.

13. History or evidence of cardiovascular (CV) risk including any of the following:

- Recent (within the past 6 months) history of serious uncontrolled cardiac arrhythmia or clinically significant ECG abnormalities including second degree (Type II) or third degree atrioventricular block.
- Cardiomyopathy, myocardial infarction, acute coronary syndromes (including unstable angina pectoris), coronary angioplasty, stenting, or bypass grafting within the past 6 months before enrollment.
- Congestive heart failure (Class II, III, or IV) as defined by the New York Heart Association functional classification system (NYHA: The Criteria Committee of the New York Heart Association (NYHA). Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th Ed. Boston, Mass: Little, Brown & Co. 1994:253-256).

- Recent (within the past 6 months) history of symptomatic pericarditis.

14. History of idiopathic pulmonary fibrosis, pneumonitis, interstitial lung disease, or organizing pneumonia, or evidence of active, non-infectious pneumonitis. Note: post-radiation changes in the lung related to prior radiotherapy and/or asymptomatic radiation-induced pneumonitis not requiring treatment may be permitted if agreed by the investigator and Sponsor.
15. Recent history (within 6 months) of uncontrolled symptomatic ascites or pleural effusions.
16. Any serious and/or unstable pre-existing medical, psychiatric disorder, or other condition that could interfere with the participant's safety, obtaining informed consent, or compliance to the study procedures.
17. Is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling or child) who is investigational site or sponsor staff directly involved with this trial, unless prospective Institutional Review Board (IRB) approval (by chair or designee) is given allowing exception to this criterion for a specific participant.

Prior/Concomitant Therapy

18. Prior treatment with the following agents:

- Tumor necrosis factor receptor (TNFR) agonists, including OX40, CD27, CD137 (4-1BB), CD357 (glucocorticoid-induced TNFR family-related gene) at any time.
- Prior systemic or intratumoral therapy with TLR agonist.
- Anticancer therapy or investigational therapy within 30 days or 5 half-lives of the drug, whichever is shorter.
- Prior radiation therapy: permissible if at least 1 non-irradiated measurable lesion is available for assessment according to RECIST version 1.1 or if a solitary measurable lesion was irradiated, objective progression is documented. A wash out of at least 14 days before start of study treatment for radiation of any intended use to the extremities for bone metastases and 28 days for radiation to the chest, brain, or visceral organs is required.

19. Prior allogeneic or autologous bone marrow transplantation or another solid organ transplantation.
20. Toxicity from previous treatment including:
    - Toxicity Grade ≥3 related to prior immunotherapy and that lead to study treatment discontinuation.
    - Toxicity related to prior treatment has not resolved to Grade ≤1 (except alopecia, or endocrinopathy managed with replacement therapy).
21. Received transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including G-CSF, granulocyte-macrophage colony-stimulating factor, and recombinant erythropoietin) within 2 weeks before the first dose of study treatment.

Other Exclusions

22. Major surgery ≤4 weeks before the first dose of study treatment. Participants must have also fully recovered from any surgery (major or minor) and/or its complications before initiating study treatment.
23. Known drug or alcohol abuse.
24. Receipt of any live vaccine within 4 weeks.

Lifestyle Restrictions

Meals and Dietary Restrictions

No dietary restrictions are required. Note that participants should be well-hydrated before receiving study treatment (see Section 0)

Caffeine, Alcohol, and Tobacco

Participants who use products containing caffeine, alcohol, or tobacco are not required to change their habits of using these products during the study treatment.

Activity

Participants may experience orthostatic dizziness following administration of CRX-601. Precautions should be taken to avoid falls after rising from a lying or seated position for several hours after administration of study treatment. In addition, participants will abstain from strenuous exercise for 8 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (e.g., watching television, reading).

Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAEs.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened once. This includes retesting specific vital sign measurements, laboratory assessments, etc. that may not have met eligibility criteria.

Example 6: Treatments

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol. The term 'study treatment' is used throughout the protocol to describe any combination of products received by the participant as per the protocol design.

Treatments Administered

Participants receiving study treatment should be well-hydrated (TLR agonists have rarely been associated with severe bradycardia or asystole in clinical trials, attributed to poor hydration and/or history of syncope) (van Eijk LT, Pickkers P, Smits P, Bouw MP, van der Hoeven JG. Severe vagal response after endotoxin administration in humans. Intensive Care Med. 2004 Dec;30(12):2279-81. Epub 2004 Oct 26). Oral hydration should be

encouraged in the days prior to study treatment and/or IV fluids (e.g., 1 L or as clinically indicated) administered before CRX-601. Participants with a history of syncope and/or uncertain compliance with hydration recommendations should receive additional pre-dose and/or post-dose fluids at the discretion of the investigator.

Following administration of CRX-601, assessments must be performed. Cytokine-related AEs including changes in vital signs commonly begin within several hours of administration of CRX-601. Participants must be monitored for 6 hours after administration of the first dose of CRX-601 or longer as clinically indicated. Similarly, participants must be monitored for 6 hours after administration of the first 2 study treatments of CRX-601 and combination partners. Participants that tolerate CRX-601 without adverse changes in heart rate or blood pressure may have the duration of observation with subsequent study treatment reduced to 2 hours, provided the dose and schedule has not been changed.

CRX-601 and mAb combination partner H2L5 IgG4PE will be administered to participants at each study site under medical supervision of an investigator or designee. H2L5 IgG4PE will be administered first, and CRX-601 will be administered at least 1 hour after the completion of the mAb infusion. The date and time of administration will be recorded in the source documents and reported in the eCRF.

If a participant experiences an infusion reaction with the administration of the mAb combination partner, associated AEs should resolve before CRX-601 is administered. If AEs associated with the mAb are slow to resolve, it is acceptable to administer CRX-601 on the following day. Should further delay be required, the participant will be discontinued from study treatment. Any participant who experiences an infusion reaction attributable to the mAb may receive CRX-601 on the following day for all subsequent study treatments.

The specific time of study treatment administration (e.g., time of the week for first administration; time of the day for each administration) should take into consideration PK sampling time points and study visit procedures.

Table 5 Investigational Product Dosage/Administration

Study Treatment Name:	CRX-601	H2L5 IgG4PE
Dosage formulation:	Solution for injection	Solution for infusion
Unit dose strength(s)/ Dosage level(s):	0.001 mg/mL (1000 ng/mL) Dose Level: 50-250 ng	10 mg/mL solution Dose Level: 80 mg
Route of Administration:	IV injection	IV infusion - 30 min[a]
Packaging and Labeling	Study Treatment will be provided in container. Each container will be labeled as required per country requirement.	Study Treatment will be provided in container. Each container will be labeled as required per country requirement.
Dosing instructions:	Slow IV push	Administer over 30 min. -5 min/+10 min (or 25 to 40 min)
Manufacturer:	GSK	GSK

Note: Refer to the SRM for detailed information on the investigational products

a. Infusions may be prolonged in the event of an infusion reaction. If multiple participants experience clinically significant infusion reactions, the infusion rate may be slowed for all future administrations of study treatment(s) for all participants. Should this global change in infusion rate be required, it will be communicated to the sites in writing.

Dose Modification

Safety management guidelines, including dose modification algorithms, are provided below.

An overview of the dose modification guidelines is presented in Table6.

All AEs are to be graded according to NCI-CTCAE, version 4.0 (http://ctep.cancer.gov). All dose modifications and the reason(s) for the dose modification must be documented in the eCRF.

The major classes of toxicity include "cytokine-related AEs and infusion reactions" and "immune-related AEs". Even though both cytokine production and immune activity play roles in both categories of events, the nomenclature is intended to describe distinct classes of AEs, as described below.

In case a dose reduction is necessary, the dose level of CRX-601, the mAb or both may be changed as determined by the investigator and sponsor. Participants may not discontinue only 1 study treatment. If either study treatment is deemed intolerable and requires discontinuation despite optimal management, as described below, the participant must be discontinued from both study treatments. CRX-601 may be restarted at the next lower dose level, and/or the mAb (H2L5 IgG4PE) at the next lower dose level.

Table 6 General Dose Modification and Management Guidelines for Drug-Related Non-Hematologic Adverse Events Not Otherwise Specified

Severity	Management	Follow-up
Grade 1	• Administer symptomatic treatment as appropriate • Continue study treatment	*Symptoms resolve to baseline within 7 days:* • Provide close follow-up to evaluate for increased severity *Symptoms ongoing >7 days*: • Consider following algorithm for Grade 2 events
Grade 2	• Administer symptomatic treatment • Investigate etiology • Consider consulting subspecialist, biopsy, and/or diagnostic procedure • Discuss with Sponsor/Medical Monitor	*Symptoms ongoing >7 days or worsening* • Consider interruption of study treatment o Resume study treatment at the same dose if symptoms have improved to Grade 1 and steroid dose is 10 mg prednisone/day or less • For immune-related events, consider starting moderate dose systemic corticosteroids (e.g., 0.5 mg/kg/day of prednisone or equivalent) o Continue steroids until improvement to Grade 1 or resolution; taper steroids as medically appropriate • If symptoms continue or worsen to Grade 3-4, follow algorithm for Grade 3-4 events
Grade 3-4	• Interrupt or discontinue study treatment • Consult subspecialist • For possible immune-related events, administer 1-2 mg/kg/day IV methylprednisolone • Discuss with Sponsor/Medical Monitor	*Symptoms improve to Grade ≤2:* • For possible immune-related events, continue steroids until improvement to Grade ≤1 or baseline; taper steroids over at least 1 month, then if symptoms have improved to Grade 1 and steroid dose is 10 mg prednisone/day or less, consider resumption of study treatment at the next lower dose level of CRX-601 and/or mAb *Symptoms ongoing:* • Discuss further management with consultant and Sponsor/Medical Monitor • Consider alternative immunosuppressive therapy

Example 7: Study Assessments and Procedures

- Protocol waivers or exemptions are not allowed
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame as defined in the Schedule of Activities.

If assessments are scheduled for the same nominal time, it is recommended that the assessments should occur in the following order: 12-lead ECG, vital signs, and blood draws. The timing of the assessments should allow the blood draw to occur at the exact nominal time.

The timing and number of planned study assessments may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring for the following assessments: safety, PK, pharmacodynamics/biomarker, or other assessments (not applicable for participants in Canada).

- The change in timing or addition of time points for any planned study assessments must be approved by the relevant GSK study team member and then archived in the study Sponsor and site study files, but this will not constitute a protocol amendment.

The IRB/ IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the informed consent form.

No more than 550 mL of blood for the purposes of this study, will be collected over the first 12 weeks of the study. No more than 30 mL of blood will be collected at each dosing

visit, thereafter. The total volume will depend on how long the participant remains on treatment. There may be additional blood collection performed for non-study reasons.

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Schedule of Activities, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment.

Efficacy Assessments

- Lesion assessment method and timing, evaluation of disease, disease progression and response criteria will be conducted according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1) (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247) as outlined below and in **Error! Reference source not found.** of this protocol.
- Disease assessment modalities may include imaging (e.g., computed tomography [CT] scan, magnetic resonance imaging [MRI], bone scan, plain radiography) and physical examination (as indicated for palpable/superficial lesions). Scans will be collected centrally during the study and may be reviewed or analyzed by an independent central reviewer. Details will be provided in the SRM.
- The baseline disease assessment will be completed up to 28 days prior to the first dose of study treatment.
- Assessments must be performed on a calendar schedule and should not be affected by dose interruptions/delays.
- For post-baseline assessments, a window of [±7 days] is permitted to allow for flexible scheduling. If the last radiographic assessment was 6 weeks or more prior to the participant's withdrawal from study treatment and PD has not been documented, a disease assessment should be obtained at the time of withdrawal from study treatment.
- To ensure comparability between the baseline and subsequent assessments, the same method of assessment and the same technique will be used when assessing response.

Evaluation of Anti-Cancer Activity

- RECIST version 1.1 guidelines will be used to determine the overall tumor burden at baseline, select target and non-target lesions, and in the disease assessments throughout the duration of the study (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247). irRECIST assessments will be evaluated as well. Treatment decisions according to irRECIST are encouraged, including confirmatory disease assessments at least 4 weeks after the date disease progression was declared. Similarly, new lesions should be measured, as feasible, and may be incorporated into assessments of tumor burden according to irRECIST guidelines.
- Lymph nodes that have a short axis of <10 mm are considered non-pathological and should not be recorded or followed.
- Pathological lymph nodes with <15 mm and but ≥10 mm short axis are considered non-measurable.
- Pathological lymph nodes with ≥15 mm short axis are considered measurable and can be selected as target lesions, however lymph nodes should not be selected as target lesions when other suitable target lesions are available.
- Measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions, and recorded and measured at baseline. These lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

Note: Cystic lesions thought to represent cystic metastases should not be selected as target lesions when other suitable target lesions are available.

Note: Measurable lesions that have been previously irradiated and have not been shown to be progressing following irradiation should not be considered as target lesions.

- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by CT or MRI can be considered measurable.

Bone scans, fluorodeoxyglucose (FDG)-positron-emission tomography (PET) scans or X-rays are not considered adequate imaging techniques to measure bone lesions.

- All other lesions (or sites of disease) should be identified as non-target and should also be recorded at baseline. Non-target lesions will be grouped by organ. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

- The following are required at baseline (up to 28 days before first dose, see Section **Error! Reference source not found.**): CT scan with contrast of the chest, abdomen, and pelvis is required. For participants with SCCHN, a scan of the head and neck area is required. Other areas should be evaluated as indicated by the participant's underlying disease prior to screening, including clinical disease assessment for palpable/visible lesions. Although CT scan is preferred, MRI may be used as an alternative method of baseline disease assessment, especially for those participants where a CT scan is contraindicated due to allergy to contrast, provided that the method used to document baseline status is used consistently throughout study treatment to facilitate direct comparison. At each post baseline assessment, evaluations of the sites of disease identified by these scans are required. Refer to RECIST version 1.1 guidelines for use of FDG-PET/CT (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247).

Example 8: Guidelines for Assessment of Disease, Disease Progression and Response Criteria – adapted from RECIST version 1.1

Assessment Guidelines

Please note the following:

- The same diagnostic method, including use of contrast when applicable, must be used throughout the study to evaluate a lesion. Contrast agents must be used in accordance with the Image Acquisition Guidelines.
- All measurements should be taken and recorded in millimeters (mm), using a ruler or calipers.
- Ultrasound is not a suitable modality of disease assessment. If new lesions are identified by ultrasound, confirmation by CT or MRI is required.
- Fluorodeoxyglucose (FDG)-PET is generally not suitable for ongoing assessments of disease. However, FDG-PET can be useful in confirming new sites of disease where a positive FDG-PET scans correlates with the new site of disease present on CT/MRI or when a baseline FDG-PET was previously negative for the site of the new lesion. FDG-PET may also be used in lieu of a standard bone scan providing coverage allows interrogation of all likely sites of bone disease and FDG-PET is performed at all assessments.
- If PET/CT is performed then the CT component can only be used for standard response assessments if performed to diagnostic quality, which includes the required anatomical coverage and prescribed use of contrast. The method of assessment should be noted as CT on the CRF.

Clinical Examination: Clinically detected lesions will only be considered measurable when they are superficial (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler/calipers to measure the size of the lesion, is required (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247).

CT and MRI: Contrast enhanced CT with 5mm contiguous slices is recommended. Minimum size of a measurable baseline lesion should be twice the slice thickness, with a minimum lesion size of 10 mm when the slice thickness is 5 mm. MRI is acceptable, but when used, the technical specification of the scanning sequences should be optimized for the evaluation of the type and site of disease and lesions must be measured in the same anatomic plane by use of the same imaging examinations. Whenever possible the same scanner should be used (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247).

X-ray: In general, X-ray should not be used for target lesion measurements owing to poor lesion definition. Lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung; however, chest CT is preferred over chest X-ray (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247).

Guidelines for Evaluation of Disease

Measurable and Non-Measurable Definitions

Measurable lesion:

A non-nodal lesion that can be accurately measured in at least 1 dimension (longest dimension) of

- ≥10 mm with MRI or CT when the scan slice thickness is no greater than 5mm. If the slice thickness is greater than 5mm, the minimum size of a measurable lesion must be at least double the slice thickness (e.g., if the slice thickness is 10 mm, a measurable lesion must be ≥20 mm).
- ≥10 mm caliper/ruler measurement by clinical exam or medical photography.
- ≥20 mm by chest x-ray.

Additionally, lymph nodes can be considered pathologically enlarged and measurable if

- ≥15mm in the short axis when assessed by CT or MRI (slice thickness recommended to be no more than 5mm). At baseline and follow-up, only the short axis will be measured (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247).

Non-measurable lesion:

All other lesions including lesions too small to be considered measurable (longest diameter <10 mm or pathological lymph nodes with ≥ 10 mm and <15 mm short axis) as well as truly non-measurable lesions, which include: leptomeningeal disease, ascites, pleural or pericardial effusions, inflammatory breast disease, lymphangitic involvement of the skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques (Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guidelines (version 1.1). Eur J Cancer. 2009;45:228-247).

Measurable disease: The presence of at least 1 measurable lesion. Palpable lesions that are not measurable by radiologic or photographic evaluations may not be utilized as the only measurable lesion.

Non-Measurable only disease: The presence of only non-measurable lesions. Note: non-measurable only disease is not allowed per protocol.

Immune-Related RECIST Response Criteria

Table 7 Evaluation of Target Lesions

New, measurable[a] lesions	Incorporated into tumor burden
New, non-measurable lesions	Do not define progression (but preclude CR)
irCR	Disappearance of all lesions in 2 consecutive observations not less than 4 weeks apart. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
irPR	≥30% decrease in tumor burden compared with baseline in 2 observations at least 4 weeks apart
irSD	30% decrease in tumor burden compared with baseline cannot be established nor 20% increase compared with nadir
irPD[b]	At least 20% increase in tumor burden compared with nadir (at any single time point) in 2 consecutive observations at least 4 weeks apart. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

a. Measurable according to RECIST version 1.1.

b. Treatment decisions may be based upon the immune-related RECIST guidelines.

Antitumor response based on total measurable tumor burden

For Modified RECIST based on RECIST version 1.1 and irRECIST (Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Clin Cancer Res. 2009;15(23):7412-20; Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements. Clin Cancer Res. 2013;19:3936-3943), the initial target ('index") and measurable new lesions are taken into account. At the baseline tumor assessment, the sum of the diameters in the plane of

measurement of all target lesions (maximum of 5 lesions in total and a maximum of 2 lesions per organ representative of all involved organs) is calculated.

Note: If pathological lymph nodes are included in the sum of diameters, the short axis of the lymph node(s) is added into the sum. The short axis is the longest perpendicular diameter to the longest diameter of a lymph node or nodal mass. At each subsequent tumor assessment, the sum of diameters of the baseline target lesions and of new, measurable nodal and non-nodal lesions (≥10 mm), up to 2 new lesions per organ are added together to provide the total tumor burden:

Tumor Burden = Sum of diameters$_{\text{target lesions}}$ + sum of diameters$_{\text{new, measurable lesions}}$

Time-point response assessment using the Immune-Related RECIST criteria

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the sum of diameters of all target lesions at screening).

Response Criteria

Evaluation of target lesions

Definitions for assessment of response for target lesion(s) are as follows:

- CR: Disappearance of all target lesions. Any pathological lymph nodes must be <10mm in the short axis.
- PR: At least a 30% decrease in the sum of the diameters of target lesions, taking as a reference, the baseline sum of the diameters (e.g., percent change from baseline).
- SD: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.
- PD: At least a 20% increase in the sum of the diameters of target lesions, taking as a reference, the smallest sum of diameters recorded since the treatment started (e.g., percent change from nadir, where nadir is defined as the smallest sum of diameters

recorded since treatment start). In addition, the sum must have an absolute increase from nadir of 5mm.

- Not Applicable (NA): No target lesions at baseline.
- Not Evaluable (NE): Cannot be classified by 1 of the 5 preceding definitions.

Note:

- If lymph nodes are documented as target lesions the short axis is added into the sum of the diameters (e.g., sum of diameters is the sum of the longest diameters for non-nodal lesions and the short axis for nodal lesions). When lymph nodes decrease to non-pathological size (short axis <10mm) they should still have a measurement reported in order not to overstate progression.
- If at a given assessment time point all target lesions identified at baseline are not assessed, sum of the diameters cannot be calculated for purposes of assessing CR, PR, or SD, or for use as the nadir for future assessments. However, the sum of the diameters of the assessed lesions and the percent change from nadir should be calculated to ensure that progression has not been documented. If an assessment of PD cannot be made, the response assessment should be NE.
- All lesions (nodal and non-nodal) should have their measurements recorded even when very small (e.g., 2 mm). If lesions are present but too small to measure, 5 mm should be recorded and should contribute to the sum of the diameters, unless it is likely that the lesion has disappeared in which case 0 mm should be reported.
- If a lesion disappears and reappears at a subsequent time point it should continue to be measured. The response at the time when the lesion reappears will depend upon the status of the other lesions. For example, if the disease had reached a CR status then PD would be documented at the time of reappearance. However, if the response status was PR or SD, the diameter of the reappearing lesion should be added to the remaining diameters and response determined based on percent change from baseline and percent change from nadir.

Evaluation of non-target lesions

Definitions for assessment of response for non-target lesions are as follows:

- Complete Response: The disappearance of all non-target lesions. All lymph nodes identified as a site of disease at baseline must be non-pathological (e.g., <10 mm short axis).
- Non-CR/Non-PD: The persistence of 1 or more non-target lesion(s) or lymph nodes identified as a site of disease at baseline ≥ 10 mm short axis.
- Progressive Disease: Unequivocal progression of existing non-target lesions.
- Not Applicable (NA): No non-target lesions at baseline.
- Not Evaluable (NE): Cannot be classified by 1 of the 4 preceding definitions.

Note:

- In the presence of measurable disease, progression on the basis of solely non-target disease requires substantial worsening such that even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy.
- Sites of non-target lesions, which are not assessed at a particular timepoint based on the assessment schedule, should be excluded from the response determination (e.g., non-target response does not have to be "Not Evaluable").

New lesions

New malignancies denoting disease progression must be unequivocal. Lesions identified in follow-up in an anatomical location not scanned at baseline are considered new lesions.

Any equivocal new lesions should continue to be followed. Treatment can continue at the discretion of the investigator until the next scheduled assessment. If at the next assessment the new lesion is considered to be unequivocal, progression should be documented.

Evaluation of overall response

Table presents the overall response at an individual time point for all possible combinations of tumor responses in target and non-target lesions with or without the appearance of new lesions for participants with measurable disease at baseline.

Table 8 Evaluation of Overall Response for Participants with Measurable Disease at Baseline

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR or NA	No	CR
CR	Non-CR/Non-PD or NE	No	PR
PR	Non-PD or NA or NE	No	PR
SD	Non-PD or NA or NE	No	SD
NE	Non-PD or NA or NE	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR=complete response, PR = partial response, SD=stable disease, PD=progressive disease, NA= Not applicable, and NE=Not Evaluable

Note:

- Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Objective response status is determined by evaluations of disease burden. Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of CR depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the CR.

Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence and will be determined programmatically by GSK based on the investigators assessment of response at each time point.

- To be assigned a status of SD, follow-up disease assessment must have met the SD criteria at least once after the first dose at a minimum interval of the first scheduled tumor evaluation.
- If the minimum time for SD is not met, best response will depend on the subsequent assessments. For example, if an assessment of PD follows the assessment of SD and SD does not meet the minimum time requirement the best response will be PD. Alternatively, participants lost to follow-up after an SD assessment not meeting the minimum time criteria will be considered not evaluable.

Confirmation Criteria:

To be assigned a status of PR or CR, a confirmatory disease assessment should be performed no less than 4 weeks (28 days) after the criteria for response are first met.

CKD-EPI Formula

Chronic Kidney Disease (CKD) stage: Kidney Disease Outcomes Quality Initiative CKD stages 3/4/5 defined by estimated glomular filtration rate (GFR) using the CKD Epidemiology Collaboration formula (Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med. 2009;150:604-612).

$GFR = 141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times$ 1.159 [if black]

where:

S_{cr} is serum creatinine in mg/dL,

κ is 0.7 for females and 0.9 for males,

α is -0.329 for females and -0.411 for males,

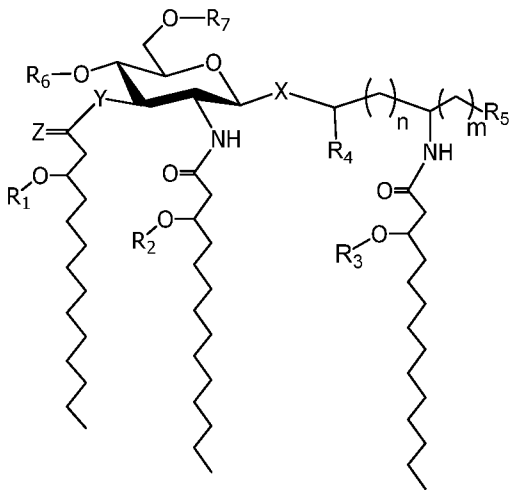
min indicates the minimum of S_{cr}/κ or 1, and

max indicates the maximum of S_{cr}/κ or 1.

Claims:

1. A method of treating cancer in a human in need thereof, the method comprising administering to the human a TLR4 agonist at a dose of about 5 ng to about 1000 ng, and administering to the human an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

2. The method of claim 1, wherein the TLR4 agonist is a compound according to Formula I:



(Formula I)

wherein

m is 0 to 6;

n is 0 to 4;

X is O or S;

Y is O or NH;

Z is O;

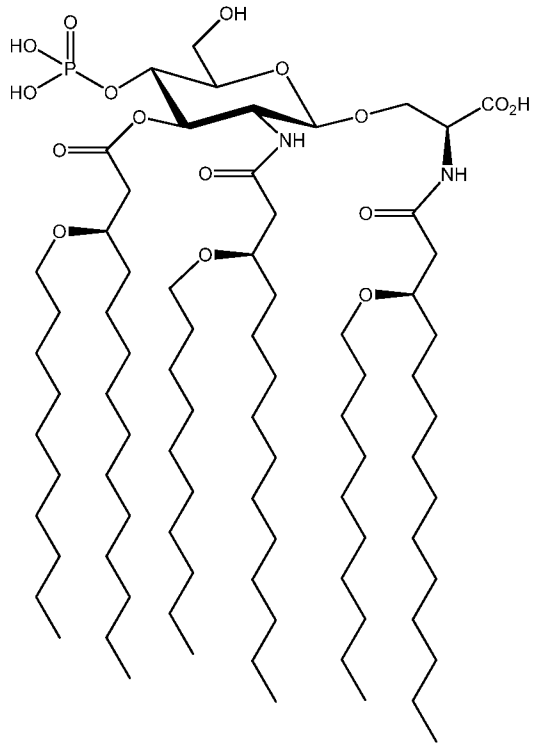
each R_1, R_2, R_3 is selected independently from the group consisting of a C_{1-20} acyl and a C_{1-20} alkyl;

R_4 is H or methyl;

R_5 is selected independently from the group consisting of -H, -OH, -(C_1-C_4) alkoxy, -$PO_3R_8R_9$, -$OPO_3R_8R_9$, -SO_3R_8, -OSO_3R_8, -NR_8R_9, -SR_8, -CN, -NO_2, -

CHO, -CO_2R_8, and –$CONR_8R_9$, wherein R_8 and R_9 are each independently selected from H and (C_1-C_4) alkyl; and
each R_6 and R_7 is independently H or PO_3H_2.

3. The method of claim 1 or 2, wherein the TLR4 agonist is CRX-601



(CRX-601).

4. The method of any one of claims 1-3, wherein the 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.

5. The method of any one of claims 1-4, wherein the 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 ICOS binding protein specifically binds to human ICOS.

6. The method of any one of claims 1-5, wherein the ICOS binding protein comprises a heavy chain variable region comprising SEQ ID NO:1; SEQ ID NO:2; and SEQ ID NO:3 and wherein said ICOS binding protein comprises a light chain variable region comprising SEQ ID NO:4; SEQ ID NO:5, and SEQ ID NO:6.

7. The method of any one of claims 1-6, wherein the ICOS binding protein comprises a V_H domain comprising the amino acid sequence set forth in SEQ ID NO:7 and a V_L domain comprising the amino acid sequence as set forth in SEQ ID NO:8.

8. The method of any one of claims 1-7, wherein the ICOS binding protein or antigen binding portion thereof comprises an hIgG4PE scaffold.

9. The method of any one of claims 1-8, wherein the ICOS binding protein is a monoclonal antibody.

10. The method of any one of claims 1-9, wherein the ICOS binding protein is a humanized monoclonal antibody.

11. The method of any one of claims 1-10, wherein the TLR4 agonist is administered at a dose of 50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng, 350 ng, 400 ng, 450 ng, 500 ng, 550 ng, or 600 ng.

12. The method of any one of claims 1-11, wherein the ICOS binding protein is administered at a dose of 24 mg or 80 mg.

13. The method of any one of claims 1-12, wherein the ICOS binding protein is administered at a dose of 80 mg.

14. The method of any one of claims 1-13, wherein the TLR4 agonist is administered via IV injection.

15. The method of any one of claims 1-14, wherein the ICOS binding protein is administered via IV infusion.

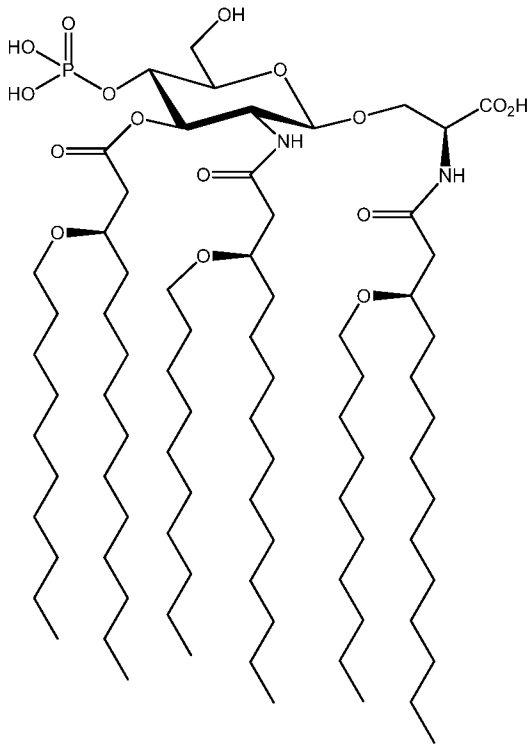
16. The method of any one of claims 1-15, wherein the cancer is an advanced solid tumor.

17. The method of any one of claims 1-16, wherein the cancer is squamous cell carcinoma of the head and neck (SCCHN).

18. The method of any one of claims 1-17, wherein the TLR4 agonist is administered once every week, once every two weeks, or once every three weeks.

19. The method of any one of claims 1-17, wherein the ICOS binding protein is administered once every week, once every two weeks, or once every three weeks.

20. A method of treating cancer in a human in need thereof, the method comprising administering to the human a TLR4 agonist at a dose of 150 ng, 200 ng, 250 ng, 300 ng, 350 ng, 400 ng, 450 ng, 500 ng, 550 ng, or 600 ng and administering to the human an ICOS binding protein or antigen binding portion thereof at a dose of 80 mg or 24 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, and wherein the TLR agonist is CRX-601



(CRX-601).

21. A TLR4 agonist and an agonist ICOS binding protein for simultaneous or sequential use in treating cancer, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng, and the agonist ICOS binding protein or antigen binding portion thereof is to be administered at a dose of about 24 mg to about 240 mg.

22. A TLR4 agonist for use in treating cancer, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

23. 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 to be administered at a dose of about 24 mg to about 240 mg and is to be administered simultaneously or sequentially with a TLR4 agonist at a dose of about 5 ng to about 1000 ng.

24. Use of a TLR4 agonist in the manufacture of a medicament for treating cancer, wherein the TLR4 agonist is to be administered at a dose of about 5 ng to about 1000 ng and is to be administered simultaneously or sequentially with an agonist ICOS binding protein or antigen binding portion thereof at a dose of about 24 mg to about 240 mg.

25. 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 24 mg to about 240 mg and is to be administered simultaneously or sequentially with a TLR4 agonist at a dose of about 5 ng to about 1000 ng.

26. A pharmaceutical kit comprising about 5 ng to about 1000 ng of a TLR4 agonist and about 24 mg to about 240 mg of an agonist ICOS binding protein or antigen binding portion thereof.

FIG. 1

CT-26 Tumor Growth in Balb/c Mice Treated with CRX-601 (TLR4 agonist) and/or ICOS agonist antibody (7E.17G9, mouse surrogate for H2L5 IgG4PE)

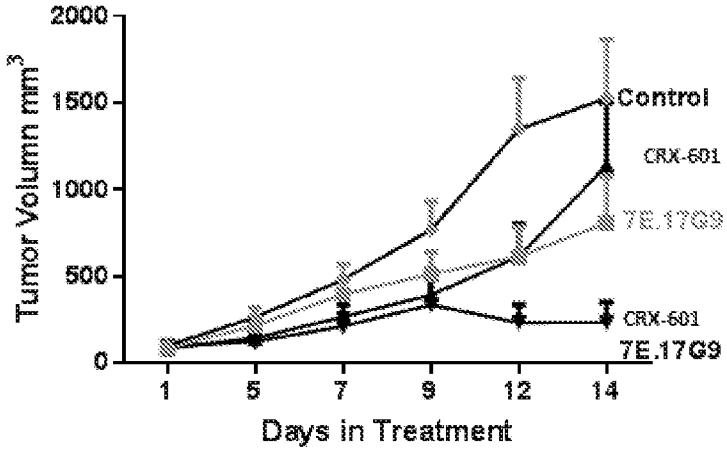
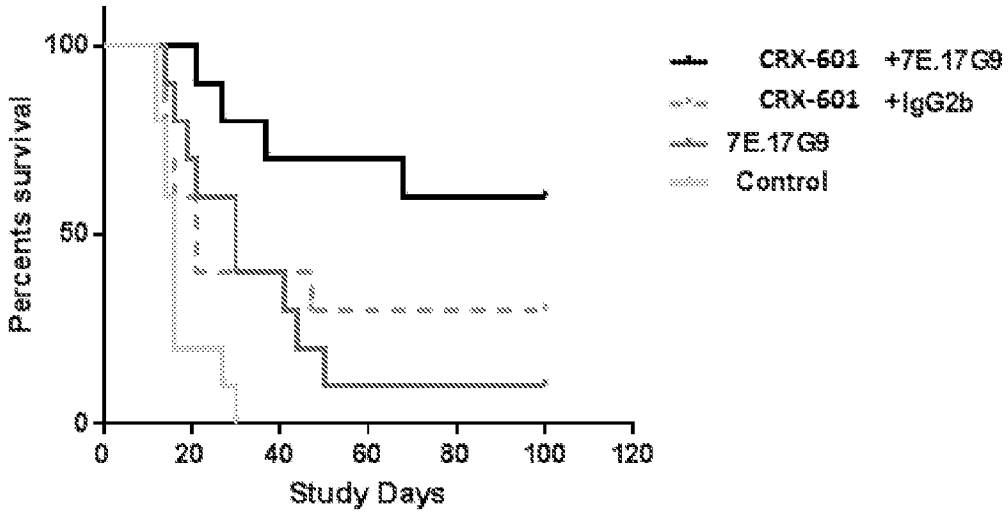


FIG. 2

Survival of Balb/c Mice Implanted with CT-26 Tumors and Treated with CRX-601 (TLR4 agonist) and/or ICOS agonist antibody (7E.17G9, mouse surrogate for H2L5 IgG4PE)



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2019/056689

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/28
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)
A61K C07K

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/215059 A1 (LIU YAO-BIN [US] ET AL) 28 July 2016 (2016-07-28) figure 20 page 28, paragraph 336 - paragraph 339 -----	1-26
A	WO 2017/021792 A1 (GLAXOSMITHKLINE BIOLOGICALS SA [BE]) 9 February 2017 (2017-02-09) page 41 - page 46; example 10 -----	1

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2019	02/12/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sitch, David

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/056689

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

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

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

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

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

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

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

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2019/056689

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2016215059 A1	28-07-2016	AU 2016210867 A1	03-08-2017
		AU 2019200751 A1	21-02-2019
		BR 112017016336 A2	27-03-2018
		CA 2974910 A1	04-08-2016
		CL 2017001921 A1	16-03-2018
		CN 107667117 A	06-02-2018
		CR 20170351 A	22-01-2018
		DO P2017000171 A	31-10-2017
		EA 201791706 A1	30-04-2018
		EP 3250603 A1	06-12-2017
		EP 3572432 A1	27-11-2019
		EP 3575324 A1	04-12-2019
		JP 6553197 B2	31-07-2019
		JP 2018505177 A	22-02-2018
		JP 2019167355 A	03-10-2019
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