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(54) **Title:** TARGETED IMMUNE ACTIVATION WITH IL-18 IMMUNOCYTOKINES

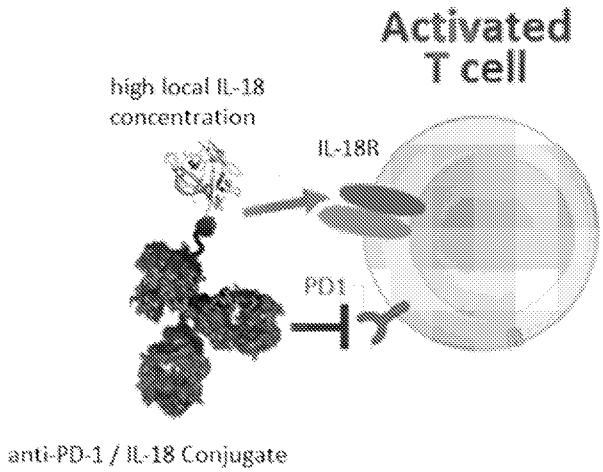


FIGURE 1A

(57) **Abstract:** The present disclosure relates to modified immunocytokine compositions comprising antibodies or antigen binding fragments specific for immune cell antigens (e.g., PD-1) and IL-18 polypeptides which are able to selectively stimulate the immune system at desired locations, such as within a tumor microenvironment. Such immunocytokines are in some instances useful for the expansion of CD8+ T cells in a subject, preferably in a tumor or tumor microenvironment of the subject. Also provided herein are methods of treatment with and methods of manufacture of immunocytokine compositions.

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[Continued on next page]

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TARGETED IMMUNE ACTIVATION WITH IL-18 IMMUNOCYTOKINES

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/534,298 filed August 23, 2023, and U.S. Provisional Application No. 63/535,496 filed August 30, 2023, each of which applications are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on August 22, 2024, is named 94917-0151_738601WO_SL.xml and is 250,783 bytes in size.

BRIEF SUMMARY

[0003] Described herein are immunocytokine compositions which contain an immune cell associated antigen specific antibody or antigen binding fragment thereof (e.g., and anti-PD-1 antibody or antigen binding fragment thereof) and an interleukin 18 (IL-18) polypeptide.

[0004] In one aspect, provided herein, is an immunocytokine composition, comprising: an IL-18 polypeptide; and an antibody or an antigen binding fragment thereof specific for an immune cell associated antigen (e.g., PD-1); and a linker, wherein the linker comprises: a first point of attachment to the IL-18 polypeptide; and a second point of attachment to the antibody or antigen binding fragment thereof.

[0005] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) stimulates IFNg secretion by a PD-1^+ cell more potently than PD-1^- cell. In some embodiments, the EC_{50} value is at least 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the EC_{50} value is at least 10-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the EC_{50} value is at least 50-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the EC_{50} value is at least 100-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the immunocytokine composition exhibits a reduced ability to be inhibited by IL-18BP when stimulating IFNg secretion of a PD-1^+ cell as compared to a PD-1^- cell. In some embodiments, the reduced ability to be inhibited by IL-18 BP is assessed as an IC_{50} value. In some embodiments, the IC_{50} value is at least 10-fold, 20-fold, 50-fold, 100-fold, 150-fold, 200-fold, or 250-fold higher for the PD-1^+ cell as compared to the PD-1^- cell. In some

embodiments, the IC_{50} value is at least 10-fold higher for the PD-1^+ cell as compared to the PD-1^- cell. In some embodiments, the IC_{50} value is at least 100-fold higher for the PD-1^+ cell as compared to the PD-1^- cell. In some embodiments, the IC_{50} value is at least 200-fold higher for the PD-1^+ cell as compared to the PD-1^- cell.

[0006] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) exhibits a reduced ability to be inhibited by IL-18 BP when stimulating IFNg secretion of a PD-1^+ cell compared to a corresponding IL-18 polypeptide not comprised in an immunocytokine composition. In some embodiments, the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold higher. In some embodiments, the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 5-fold higher. In some embodiments, the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 10-fold higher.

[0007] In some embodiments, an immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) described herein is used in a method of expanding a population of $CD8^+$ T cells in a subject in need thereof. In some embodiments, the $CD8^+$ T cells are PD-1^+/IL-18Rα^+ $CD8^+$ T cells. In some embodiments, the immunocytokine composition selectively expands $CD8^+$ T cells in a tumor microenvironment relative to $CD8^+$ cells in blood of a subject. In some embodiments, the $CD8^+$ T cells are effector memory T cells. In some embodiments, the expansion of $CD8^+$ T cells is assessed as the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes. In some embodiments, the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes increases by at least 2-fold, 3-fold, or 4-fold within a tumor after administration of the immunocytokine composition. In some embodiments, the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes reaches a fraction of at least 50%, 60%, 70%, 75%, or 80% within a tumor after administration of the immunocytokine composition. In some embodiments, the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes increases by at most 4-fold, 3-fold, or 2-fold in the blood of the subject after administration of the immunocytokine composition. In some embodiments, the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes reaches a fraction of at most 50%, 45%, 40%, 35%, 30%, or 20% within the blood of the subject after administration of the immunocytokine composition.

[0008] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) selectively suppresses macrophage cells in a tumor microenvironment relative to macrophage cells in blood of a subject. In some embodiments,

suppression of macrophage cells is assessed as the fraction of macrophages within $CD45^+$ leukocytes. In some embodiments, fraction of macrophage cells within $CD45^+$ leukocytes decreases by at least 2-fold, 3-fold, 4-fold, 5-fold, 7.5-fold, or 10-fold within a tumor after administration of the immunocytokine composition. In some embodiments, fraction of macrophage cells within $CD45^+$ leukocytes reaches a fraction of below 40%, 30%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% within a tumor after administration of the immunocytokine composition. In some embodiments, fraction of macrophage cells within $CD45^+$ leukocytes does not have any significant effect on macrophage levels in the blood of the subject (e.g., macrophage levels remain with ~5% of the total fraction of $CD45^+$ leukocytes in the blood).

[0009] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) has minimal effect on $CD4^+$ T cells or NK cells (e.g., $CD45^+$ leukocytes) in a tumor. In some embodiments, the immunocytokine composition expands expression of IL-18Ra on $CD4^+$ T cells within a tumor, optionally wherein the expression of IL-18Ra is increased by at least 2-fold, 3-fold, or 4-fold.

[0010] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) expands 1, 2, 3, or 4 cytokines selected from IFNg, GM-CSF, TNFa, and IL-6 within a tumor after administration of the immunocytokine composition. In some embodiments, the immunocytokine composition does not substantially expand the cytokine in blood of a subject (e.g., each of the 1, 2, 3, or 4 cytokines).

[0011] In some embodiments, the immunocytokine composition preferentially expands a population of IL-18 receptor alpha / PD-1 double-positive T cells after administration in vivo. The immunocytokine composition of claim 17, wherein the L-18 receptor alpha / PD-1 double-positive T cells are memory T cells.

[0012] In another aspect herein is a method of expanding a population of CD8+ T cells in a subject in need thereof, the method comprising administering to the subject the immunocytokine composition described herein.

[0013] Another aspect herein provides a method of expanding a population of IL-18 receptor alpha / PD-1 double-positive T cells in a subject in need thereof, the method comprising administering to the subject the immunocytokine composition described herein.

[0014] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative

embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0015] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIGURE 1A illustrates a non-limiting mechanism of action of an anti-PD-1 antibody / IL-18 immunocytokine provided herein, wherein an activated T cell shows enhanced activation through concurrent blockade of PD-1 and stimulation by IL-18.

[0017] FIGURE 1B illustrates a non-limiting mechanism of action of an anti-PD-L1 antibody / IL-18 immunocytokine provided herein, wherein the immunocytokine disrupts PD-L1/PD-1 interaction and effectuates IL-18R signaling.

[0018] FIGURE 1C illustrates a non-limiting mechanism of action of an anti-PD-L1 antibody / IL-18 immunocytokine acting on an immune cell expressing both IL-18R and PD-L1.

[0019] FIGURE 1D illustrates an immunocytokine composition provided herein with two different protein payloads (*e.g.,* IL-18 and IL-2) linked to an antibody (*e.g.,* PD-L1 antibody) acting on an immune cell with simultaneous activation of two different receptors (*e.g.,* IL-18R and IL-2R).

[0020] FIGURE 2 shows a representative immunocytokine composition with a drug antibody ratio of 1 (DAR1).

[0021] FIGURE 3 shows an illustration of a site selective introduction of a conjugation handle to an antibody as provided herein.

[0022] FIGURE 4 illustrates the mechanism of action of IL-18 on IFNγ and IL-18BP production, and IL-18 inhibitory activity by IL-18BP.

[0023] FIGURE 5 shows a schematic of a synthetic route which can be used to synthesize an IL-18 polypeptide for conjugation to an antibody.

[0024] FIGURE 6 shows a schematic representation of coupling of a bifunctional probe (also referred to as bifunctional linking reagent herein) to an IL-18 polypeptide provided herein.

[0025] FIGURE 7 shows exemplary reaction scheme which can be used to prepare antibody / IL-18 immunocytokine compositions provided herein with drug antibody ratio of 1 (top) or 2 (bottom).

[0026] FIGURE 8 shows a reverse phase HPLC chromatogram of a purified IL-18 / anti-PD-1 antibody (LZM009) as provided herein with a DAR of 1 attached at residue K248 of the Fc region of the antibody (EU numbering).

[0027] FIGURE 9 shows a size exclusion HPLC chromatogram of a purified IL-18 / anti-PD-1 antibody (LZM009) as provided herein with a DAR of 1 attached at residue K248 of the Fc region of the antibody (EU numbering).

[0028] FIGURE 10 shows a mass spectrometry trace (Q-TOF) of a purified IL-18 / anti-PD-1 antibody (LZM009) as provided herein with a DAR of 1 attached at residue K248 of the Fc region of the antibody (EU numbering).

[0029] FIGURE 11 shows the RP-HPLC chromatograms of purified composition A (detection: 280 nm).

[0030] FIGURE 12 shows the Analytical SEC-HPLC chromatograms of purified composition A (detection: 280 nm).

[0031] FIGURE 13 shows the Q-TOF mass spectrometry deconvolution chromatogram of purified composition A.

[0032] FIGURE 14A and FIGURE 14B show plots measuring ability of the unmodified and of conjugated anti-PD1 antibodies to bind with human PD1/CD279 ligand, with the figure showing ELISA signal on the y-axis and dosage of the biotinylated PD-1 protein on the x-axis.

[0033] FIGURE 14A Shows the unconjugated reference antibodies. Tested in this figure are Pembrolizumab, LZM-009, Nivolumab, Atezolizumab, Durvalumab, and Avelumab.

[0034] FIGURE 14B Shows the conjugated antibodies. Tested in this figure are compositions A and composition B.

[0035] FIGURE 15A and FIGURE 15B show plots measuring ability of the unmodified and of conjugated antibodies to bind with human PD-L1/B7-H1 ligand, with the figure showing ELISA signal on the y-axis and dosage of the biotinylated PD-L1 protein on the x-axis.

[0036] FIGURE 15A shows the unconjugated reference antibodies. Tested in this figure are Pembrolizumab, LZM-009, Nivolumab, Atezolizumab, Durvalumab, and Avelumab.

[0037] FIGURE 15B shows the conjugated antibodies. Tested in this figure are compositions A and composition B.

[0038] FIGURE 16 shows plots measuring ability of the unmodified and of conjugated antibodies to bind to human PD-L1/B7-H1 ligand, with the figure showing net BioLayer interferometry shift in nanometer on the y-axis and time of incubation dosage of the biotinylated PD-L1 protein on the x-axis.. The figure shows mean ELISA signal on the y-axis and dosage of the human Fc gamma receptors on the x-axis. The unconjugated reference antibodies are Pembrolizumab and LZM-009. The conjugated antibodies tested is Compositions A.

[0039] FIGURE 17A and **FIGURE 17B** show plots measuring ability of the unmodified and of conjugated anti-PD1 antibodies to interfere with PD1/PDL1 pathway, with the figure showing normalized luminescence intensity of effector cells NFAT-Lucia reporter on the y-axis and dosage of the unmodified and of conjugated anti-PD1 antibodies on the x-axis.

[0040] FIGURE 17A shows the unconjugated reference antibodies are Pembrolizumab, LZM-009, Nivolumab, Atezolizumab, Durvalumab, and Avelumab.

[0041] FIGURE 17B shows the conjugated antibodies tested .Tested in this figure are compositions A and composition B.

[0042] FIGURE 18A shows plots measuring ability of the unmodified and of conjugated antibodies to bind to human Fc gamma receptor I (CD64). The figure shows mean ELISA signal on the y-axis and dosage of the human Fc gamma receptors on the x-axis. The unconjugated reference antibodies are LZM-009 and Atezolizumab. The conjugated antibodies tested are Compositions A and B.

[0043] FIGURE 18B shows plots measuring ability of the unmodified and of conjugated antibodies to bind to human Fc gamma receptor IIIa (CD16). The figure shows mean ELISA signal on the y-axis and dosage of the human Fc gamma receptors on the x-axis. The unconjugated reference antibodies are LZM-009 and Atezolizumab. The conjugated antibodies tested are Compositions A and B.

[0044] FIGURE 19 shows plots measuring ability of the unmodified and of conjugated antibodies to bind to human Fc neonatal receptor. The figure shows mean AlphaLISA signal on the y-axis and dosage of the human Fc neonatal receptor (FcRn) on the x-axis. The unconjugated reference antibodies are LZM-009 and Atezolizumab. The conjugated antibodies tested are Compositions A and B.

[0045] FIGURE 20 shows plots measuring the levels PD-1 and PD-L1 surface expression on NK92 cells.

[0046] FIGURE 21 shows plots measuring ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by NK92 cells. The figure shows mean IFNg alphaLISA signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO: 1), SEQ ID NO: 30, and SEQ ID NO: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

[0047] FIGURE 22 shows plots measuring the levels PD-1 and PD-L1 surface expression on KG-1 cells.

[0048] FIGURE 23 shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by parental PD-$1^{negative}$ (round symbols, dotted lines) and by engineered PD-$1^{positive}$ KG-1 cells (squared symbols, solid lines). The figure shows mean IFNg legendplex signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID No: 1), SEQ ID No: 30, and SEQ ID No: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

[0049] FIGURE 24 shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to bind to the human IL-18 Binding Protein (IL-18BP). The figure shows mean free IL-18BP AlphaLISA signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO: 1), SEQ ID NO: 30, and SEQ ID NO: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

[0050] FIGURE 25 shows plots measuring the ability of the human IL-18 Binding Protein to inhibit the secretion of IFNgamma by NK92 cells stimulated with 2nM of unconjugated IL-18 variants and corresponding IL-18 immunocytokines. The figure shows mean IFNg alphaLISA

signal on the y-axis and dosage of the human IL-18 Binding Protein on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO: 1), SEQ ID NO: 30, and SEQ ID NO: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

[0051] FIGURE 26A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition A tested as a single agent at 0.3 and 1 mg/kg as two weekly *i.v.* injections. (n=9 ; mean ± SEM).

[0052] FIGURE 26B shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-L1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition B tested as a single agent at 1 and 3 mg/kg as two weekly *i.v.* injections. (n=9 ; mean ± SEM).

[0053] FIGURE 27A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the body weight of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition A tested as a single agent at 0.3 and 1 mg/kg as two weekly *i.v.* injections. (n=9 ; mean ± SEM).

[0054] FIGURE 27B shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-L1 antibody on the body weight of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition B tested as a single agent at 1 and 3 mg/kg as two weekly *i.v.* injections. (n=9 ; mean ± SEM).

[0055] FIGURE 28A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9 ; mean ± SEM).

[0056] FIGURE 28B shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows the mean tumor volume on day 17 post treatment initiation on the y-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; One-way Anova test *** P-value<0.001, ** P-value<0.01, * P-value<0.1, ns not significant, TGI: Tumor Growth Inhibition).

[0057] FIGURE 28C shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows the tumor volume of each individual animal on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9; CR: Complete Response).

[0058] FIGURE 29 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the on the body weight of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9 ; mean ± SEM).

[0059] FIGURE 30 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the survival of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice The immunocytokine tested in this figure are Composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9 ; mean ± SEM ; CR: Complete response).

[0060] FIGURE 31A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of B16F10 syngeneic melanoma tumors

in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly i.v. injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9 ; mean ± SEM).

[0061] FIGURE 31B shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of B16F10 syngeneic melanoma tumors in hPD1 C57BL/6 mice. The figure shows the mean tumor volume on day 10 post treatment initiation on the y-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; TGI: Tumor Growth Inhibition).

[0062] FIGURE 31C shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of B16F10 syngeneic melanoma tumors in hPD1 C57BL/6 mice. The figure shows the tumor volume of each individual animal on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; CR: Complete Response).

[0063] FIGURE 32 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the body weight of B16F10 syngeneic melanoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9 ; mean ± SEM).

[0064] FIGURE 33 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the survival of B16F10 syngeneic melanoma tumor-bearing hPD1 C57BL/6 mice. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted

immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9 ; mean ± SEM ; CR: Complete response).

[0065] FIGURE 34 shows plots measuring the levels PD-1 surface expression on wild type NK-92 cells and on NK-92 cells transduced with human PD-1.

[0066] FIGURE 35 (left panel) shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by parental PD-1^{negative} (round symbols, dotted lines) and by engineered PD-1^{positive} NK-92 cells (squared symbols, solid lines). The figure shows mean IFNg AlphaLISA signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO:1) and SEQ ID NO: 30. Corresponding IL-18 immunocytokines tested are Compositions A and C.

[0067] FIGURE 35 (right panel) shows plots measuring the ability of the human IL-18 Binding Protein to inhibit the secretion of IFNgamma by NK92 cells stimulated with 2nM of unconjugated IL-18 variants and corresponding IL-18 immunocytokines by parental PD-1^{negative} (round symbols, dotted lines) and by engineered PD-1^{positive} NK-92 cells (squared symbols, solid lines). The figure shows mean IFNg alphaLISA signal on the y-axis and dosage of the human IL-18 Binding Protein on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO:1) and SEQ ID NO: 30. Corresponding IL-18 immunocytokines tested are Compositions A and C.

[0068] FIGURE 36 shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by parental PD-1^{negative} (round symbols, dotted lines) and by engineered PD-1^{positive} NK-92 cells (squared symbols, solid lines) in the absence (black plain symbols) or presence (grey open symbols) of an excess of anti-PD-1 antibody. The figure shows mean IFNg legendplex signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO:1) and SEQ ID NO: 30. Corresponding IL-18 immunocytokines tested are Compositions A and C.

[0069] FIGURE 37A shows a plot describing plasma exposure of Total (bound and unbound to IL-18BP) and free (unbound to IL-18BP) composition A in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows plasma concentrations on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0070] FIGURE 37B shows a plot describing tumor exposure of Total (bound and unbound to IL-18BP) and free (unbound to IL-18BP) composition A in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows tumor concentrations on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0071] FIGURE 38 shows a plot describing the levels of surface expression of PD-1 and IL-18 receptor alpha in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice treated with vehicle. The figure shows the median fluorescence intensity of PD-1 (upper panel) and of IL-18 receptor alpha (bottom panel) surface staining the y-axis, and the subset of immune cell on the x-axis. (48h and 7 days after i.v. Injection of formulation buffer, n=5 per time point).

[0072] FIGURE 39 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expansion of CD4+ T-cells, CD8+ T-cells, macrophages, and Natural Killer (NK) cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the frequency of indicated immune cell subsets within CD45+ leukocytes on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0073] FIGURE 40 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expansion of naïve ($CD62L^{high}$ $CD44^{low}$), central memory ($CD62L^{high}$ $CD44^{high}$) effector memory ($CD62L^{negative}$ $CD44^{high}$) CD8+ T-cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the frequency of indicated immune cell subsets within CD45+ leukocytes on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0074] FIGURE 41 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expression of CD69 surface marker on CD4+ T-cells, CD8+ T-cells, and Natural Killer (NK) cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the median fluorescence intensity of CD69 surface staining the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0075] FIGURE 42 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expression of IL-18 receptor alpha on the surface of CD4+ T-cells, CD8+ T-cells, and

Natural Killer (NK) cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the median fluorescence intensity of IL-18 receptor alpha surface staining the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0076] FIGURE 43 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on levels of cytokines in blood and tumors of in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows plasma and tumor concentrations of IFNg, GM-CSF, TNFa, and IL-6 on the y-axis, and time on the x-axis. The IL-18 immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0077] FIGURE 44 shows a plot describing the effect of depleting antibody treatment on leukocytes populations of blood and tumors of in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows the frequency of indicated immune cell subsets within CD45+ leukocytes on the y-axis, and depleting antibody treatment on the x-axis. The IL-18 immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0078] FIGURE 45 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, Macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the volume of MC38 tumors on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group ; mean ± SEM).

[0079] FIGURE 46 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, Macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice 12 days after treatment. The figure shows the volume of MC38 tumors on the y-axis, and treatment received by mice on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group ; mean ± SEM ; TGI: Tumor Growth Inhibition).

[0080] FIGURE 47 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, Macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18

immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the volume of MC38 tumors on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group; CR: Complete Response).

[0081] FIGURE 48 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the percentage of surviving mice within each treatment group the y-axis, and time on the x-axis. The immunocytokine tested is Composition A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group).

[0082] FIGURE 49 shows plasma levels of Composition A at various time points in a non-human primate (NHP) study following the indicated single dose of Composition A. Results are shown in ng/mL.

[0083] FIGURE 50 shows plasma levels of both "free" Composition A (not bound to IL-18BP) and Composition A complexed with IL-18BP in an NHP study. The results show virtually no Composition A was complexed with IL-18BP.

[0084] FIGURE 51 shows quantification of anti-PD-1 antibody in plasma samples of NHPs measured by capturing material using PD-1 or anti-IL-18. The results from both capture methods are virtually identical, indicating all or nearly all of Composition A remained intact (e.g., no cleavage of the linker between the antibody and the IL-18 polypeptide).

[0085] FIGURE 52 shows levels of IL-18R / PD-1 double positive T cells following administration of Composition A at various dose levels in NHPs.

DETAILED DESCRIPTION

[0086] Disclosed herein are antibodies or antigen binding fragments specific for immune cell associated antigens (e.g., PD-1) linked to IL-18 polypeptides in immunocytokine compositions. In some instances, the immunocytokine compositions provided herein are useful as potent stimulators of one or more immune cell types (*e.g.,* T cells, macrophages, *etc.*). In some embodiments, the immunocytokine compositions can act by one or more modes of action.

[0087] In some embodiments, the antibody of the immunocytokine composition allows for targeting of the immunocytokine composition to an immune cell (e.g., a PD-1$^+$ immune cell, such

as a $CD8^+$ T cell). In some embodiments, the immunocytokine composition can inhibit an activity of the immune cell associated antigen (*e.g.,* inhibiting a checkpoint interaction such as a PD-1/PD-L1 interaction) through binding to the immune cell associated antigen. In some embodiments, the immunocytokine compositions induce IFNγ production in immune cells (*e.g.,* T cells or NK cells). The antibody or antigen binding fragment-IL-18 immunocytokine compositions of the disclosure can have synergistic efficacy and improved tolerability by a subject. In some embodiments, the antibody or antigen binding fragment-IL-18 immunocytokine compositions can significantly reduce the therapeutic dose of the antibody or antigen binding fragment, the IL-18 polypeptide, or both for a subject with a disease, such as a cancer, as compared to a treatment with one or both entities individually or in combination. In some embodiments, the immunocytokine compositions provided herein are associated with fewer side effects than administration of one or both entities individually or in combination, potentially due to the targeting nature of the antibodies for an immune cell.

[0088] An exemplary, non-limiting mechanism of action of an immunocytokine provided herein is shown in **FIGURE 1A**. In the exemplary embodiment, the immunocytokine composition comprises an anti-PD-1 antibody as the antibody or antigen binding fragment of the immunocytokine composition. In this embodiment, the anti-PD-1 antibody portion of the immunocytokine selectively binds to PD-1 present on the surface of an activated T cell (*e.g.,* a $CD8^+$ T cell). This binding prevents the checkpoint interaction of PD-1 and PD-L1/2, thus preventing attenuation of activity of the T cell. Concomitantly, the IL-18 portion of the immunocytokine, which is effectively in a high local concentration near the T-cell due to the linkage, further activates the T cell through IL-18R signaling. While the exemplary embodiment shows the mechanism of action of an anti-PD-1 antibody, antibodies or antigen binding fragments specific for other immune antigens provided herein can function according to a similar mechanism.

[0089] Another exemplary, non-limiting mechanism of action of an anti-PD-L1 antibody / IL-18 immunocytokine is shown in **FIGURE 1B**. In the exemplary embodiment, the anti-PD-L1 antibody portion of the immunocytokine binds to PD-L1 expressed on the surface of a tumor cell. When a T cell comes into contact with the tumor cell, an interaction between PD-1 on the T cell and PD-L1 on the cell is blocked, preventing attenuation of the activity of the T cell. Additionally, the IL-18 portion of the immunocytokine is free to signal through IL-18R on the immune cell, thereby inducing production of IFNγ and further activation of the immune cell. Though this

exemplary embodiment is demonstrated for PD-L1, other immune antigens provided herein in immunocytokines may display similar mechanisms of action. **FIGURE 1C** shows a comparable mechanism of action of an anti-PD-L1 / IL-18 immunocytokine acting on a T cell which expresses IL-18R and PD-L1. **FIGURE 1D** shows another mechanism of action of an additional embodiment described herein wherein the antibody of the immunocytokine contains two different protein payloads (e.g., two different cytokines) which act on different receptors of the immune cell.

[0090] Also disclosed herein are methods of manufacturing the immunocytokine compositions provided herein. In some embodiments, the immunocytokine compositions are prepared using chemical linkers which can attach the two moieties of immunocytokine composition to each other at pre-selected sites of each moiety with high fidelity. In some embodiments, the methods provided herein can be used on a wide variety of antibodies or antigen binding fragments in order to rapidly and easily generate a wide variety of immune antigen specific antibody or antigen binding fragments linked to IL-18 polypeptides as immunocytokines. In some embodiments, the methods can be used on readily commercially available antibodies to allow for rapid linking with IL-18 polypeptides provided herein. One non-limiting illustration of an immunocytokine as provided herein is shown in **FIGURE 2**, which depicts an IL-18 polypeptide linked to an antibody as provided herein with a point of attachment of the linker to the IL-18 polypeptide at a side chain of a residue and to a side chain of a residue in the Fc region.

[0091] The following description and examples illustrate embodiments of the present disclosure in detail. It is to be understood that this present disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this present disclosure, which are encompassed within its scope.

[0092] Although various features of the present disclosure may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the present disclosure may be described herein in the context of separate embodiments for clarity, the present disclosure may also be implemented in a single embodiment.

[0093] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Immune Antigen Specific Antibodies Linked to IL-18 Polypeptides as Immunocytokines

[0094] Provided herein are antibodies and antigen binding fragments which binds to immune cell associated antigens linked to IL-18 polypeptides as immunocytokine compositions. The immunocytokine compositions provided herein are effective for simultaneously delivering the IL-18 polypeptide and the antibody or antigen binding fragment to a target cell, such as an immune cell. This simultaneous delivery of both agents to the same cell has numerous benefits, including improved IL-18 polypeptide selectivity, enhanced therapeutic potential of the IL-18 polypeptide, and minimized risk of side effects from administering IL-18 therapies. In some embodiments, the immunocytokine compositions act through multiple modes of action, including without limitation disrupting an activity of the immune cell associated antigen (*e.g.*, immune checkpoint evasion) and/or enhanced activation of immune cells in or around a tumor microenvironment.

[0095] The immunocytokine compositions provided herein utilize linkers to attach the antibody or antigen binding fragment to the IL-18 polypeptide. In some embodiments, the linkers are attached to each moiety (*i.e.,* the antibody or antigen binding fragment and the IL-18 polypeptide) at specific residues or a specific subset of residues. In some embodiments, the linkers are attached to each moiety in a site-selective manner, such that a population of the immunocytokine compositions is substantially uniform. This can be accomplished in a variety of ways as provided herein, including by site-selectively adding reagents for a conjugation reaction to a moiety to be conjugated, synthesizing or otherwise preparing a moiety to be conjugated with a desired reagent for a conjugation reaction, or a combination of these two approaches. Using these approaches, the sites of attachment (such as specific amino acid residues) of the linker to each moiety can be selected with precision.

[0096] Additionally, these approaches allow a variety of linkers to be employed for the composition which are not limited to amino acid residues as is required for fusion proteins. This combination of linker choice and precision attachment to the moieties allows the linker to also, in some embodiments, perform the function of modulating the activity of one of the moieties, for example if the linker is attached to the IL-18 polypeptide at a position that interacts with a protein which binds to the IL-18 polypeptide (*e.g.*, IL-18 binding protein).

[0097] In one aspect, provided herein, is an immunocytokine composition, comprising: an IL-18 polypeptide and an antibody or an antigen binding fragment thereof specific for an immune cell associated antigen. In some embodiments, the immunocytokine composition comprises a linker.

In some embodiments, the linker comprises a first point of attachment to the IL-18 polypeptide. In some embodiments, the linker comprises a second point of attachment to the antibody or antigen binding fragment thereof.

Immune Cell Specific Antibodies

[0098] In some embodiments, an antibody or an antigen binding fragment of the disclosure specifically binds to an immune cell associated antigen. An immune cell associated antigen provided herein is an antigen expressed at elevated levels in cells of an immune cell relative to other cells. In some embodiments, the immune cell associated antigen is expressed at a level of at least 25% greater, at least 30% greater, at least 40% greater, at least 50% greater, at least 60% greater, at least 70% greater, at least 80% greater, at least 90% greater, or at least 100% greater in the immune cell than another cell. In some embodiments, the immune cell associated antigen is expressed at a level of at least 2-fold greater, at least 4-fold greater, at least 6-fold greater, at least 8-fold greater, or at least 10-fold greater in the immune cell than another cell.

[0099] An antibody selectively binds or preferentially binds to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to specific binding means preferential binding where the affinity of the antibody, or antigen binding fragment thereof, is at least at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater than the affinity of the antibody for unrelated amino acid sequences. In some embodiments, an antibody or an antigen binding fragment of the disclosure can inhibit the action/activity of the substance to which it binds. In some embodiments, an antibody or antigen binding fragment of the disclosure can agonize the action/activity of the substance to which it binds (*e.g.,* an immune cell agonist antibody or antigen binding fragment such as one specific for CD16A, NKG2D, NKp30, or other targets).

[0100] As used herein, the term "antibody" refers to an immunoglobulin (Ig), polypeptide, or a protein having a binding domain which is, or is homologous to, an antigen binding domain. The term further includes "antigen binding fragments" and other interchangeable terms for similar

binding fragments as described below. Native antibodies and native immunoglobulins (Igs) are generally heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light chains and two identical heavy chains. Each light chain is typically linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain ("V_H") followed by a number of constant domains ("C_H"). Each light chain has a variable domain at one end ("V_L") and a constant domain ("C_L") at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

[0101] In some instances, an antibody or an antigen binding fragment comprises an isolated antibody or antigen binding fragment, a purified antibody or antigen binding fragment, a recombinant antibody or antigen binding fragment, a modified antibody or antigen binding fragment, or a synthetic antibody or antigen binding fragment.

[0102] Antibodies and antigen binding fragments herein can be partly or wholly synthetically produced. An antibody or antigen binding fragment can be a polypeptide or protein having a binding domain which can be, or can be homologous to, an antigen binding domain. In one instance, an antibody or an antigen binding fragment can be produced in an appropriate *in vivo* animal model and then isolated and/or purified.

[0103] Depending on the amino acid sequence of the constant domain of its heavy chains, immunoglobulins (Igs) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. An Ig or portion thereof can, in some cases, be a human Ig. In some instances, a C_H3 domain can be from an immunoglobulin. In some cases, a chain or a part of an antibody or antigen binding fragment, a modified antibody or antigen binding fragment, or a binding agent can be from an Ig. In such cases, an Ig can be IgG, an IgA, an IgD, an IgE, or an IgM, or is derived therefrom. In cases where the Ig is an IgG, it can be a subtype of IgG, wherein subtypes of IgG can include IgG1, an IgG2a, an IgG2b, an IgG3, or an IgG4. In some cases, a C_H3 domain can be from an immunoglobulin selected from the group consisting of an IgG, an IgA, an IgD, an IgE, and an IgM, or is derived therefrom. In some

embodiments, an antibody or antigen binding fragment described herein comprises an IgG or is derived therefrom. In some instances, an antibody or antigen binding fragment comprises an IgG1 or is derived therefrom. In some instances, an antibody or antigen binding fragment comprises an IgG4 or is derived therefrom. In some instances, an antibody or antigen binding fragment comprises an IgG2 or is derived therefrom. In some embodiments, an antibody or antigen binding fragment described herein comprises an IgM, is derived therefrom, or is a monomeric form of IgM. In some embodiments, an antibody or antigen binding fragment described herein comprises an IgE or is derived therefrom. In some embodiments, an antibody or antigen binding fragment described herein comprises an IgD or is derived therefrom. In some embodiments, an antibody or antigen binding fragment described herein comprises an IgA or is derived therefrom.

[0104] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ("κ" or "K") or lambda ("λ"), based on the amino acid sequences of their constant domains.

[0105] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th *Ed.*, 1991, National Institutes of Health, Bethesda Md., pages 647-669; hereafter "Kabat"); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-Iazikani *et al.* (1997) *J. Molec. Biol.* 273:927-948)). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

[0106] With respect to antibodies, the term "variable domain" refers to the variable domains of antibodies that are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. Rather, it is concentrated in three segments called hypervariable regions (also known as CDRs) in both the light chain and the heavy chain variable domains. More highly conserved portions of variable domains are called the "framework regions" or "FRs." The variable domains

of unmodified heavy and light chains each contain four FRs (FR1, FR2, FR3, and FR4), largely adopting a β-sheet configuration interspersed with three CDRs which form loops connecting and, in some cases, part of the β-sheet structure. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (*see*, Kabat).

[0107] The terms "hypervariable region" and "CDR" when used herein, refer to the amino acid residues of an antibody which are responsible for antigen binding. The CDRs comprise amino acid residues from three sequence regions which bind in a complementary manner to an antigen and are known as CDR1, CDR2, and CDR3 for each of the V_H and V_L chains. In the light chain variable domain, the CDRs typically correspond to approximately residues 24-34 (CDRL1), 50-56 (CDRL2), and 89-97 (CDRL3), and in the heavy chain variable domain the CDRs typically correspond to approximately residues 31-35 (CDRH1), 50-65 (CDRH2), and 95-102 (CDRH3) according to Kabat *et al.*, *Id.* It is understood that the CDRs of different antibodies may contain insertions, thus the amino acid numbering may differ. The Kabat numbering system accounts for such insertions with a numbering scheme that utilizes letters attached to specific residues (*e.g.,* 27A, 27B, 27C, 27D, 27E, and 27F of CDRL1 in the light chain) to reflect any insertions in the numberings between different antibodies. Alternatively, in the light chain variable domain, the CDRs typically correspond to approximately residues 26-32 (CDRL1), 50-52 (CDRL2), and 91-96 (CDRL3), and in the heavy chain variable domain, the CDRs typically correspond to approximately residues 26-32 (CDRH1), 53-55 (CDRH2), and 96-101 (CDRH3) according to Chothia and Lesk (*J. Mol. Biol.*, 196: 901-917 (1987)).

[0108] As used herein, "framework region," "FW," or "FR" refers to framework amino acid residues that form a part of the antigen binding pocket or groove. In some embodiments, the framework residues form a loop that is a part of the antigen binding pocket or groove and the amino acids residues in the loop may or may not contact the antigen. Framework regions generally comprise the regions between the CDRs. In the light chain variable domain, the FRs typically correspond to approximately residues 0-23 (FRL1), 35-49 (FRL2), 57-88 (FRL3), and 98-109 and in the heavy chain variable domain the FRs typically correspond to approximately residues 0-30 (FRH1), 36-49 (FRH2), 66-94 (FRH3), and 103-133 according to Kabat *et al.*, *Id.* As discussed above with the Kabat numbering for the light chain, the heavy chain too accounts for insertions in a similar manner (*e.g.,* 35A, 35B of CDRH1 in the heavy chain). Alternatively, in the light chain

variable domain, the FRs typically correspond to approximately residues 0-25 (FRL1), 33-49 (FRL2) 53-90 (FRL3), and 97-109 (FRL4), and in the heavy chain variable domain, the FRs typically correspond to approximately residues 0-25 (FRH1), 33-52 (FRH2), 56-95 (FRH3), and 102-113 (FRH4) according to Chothia and Lesk, *Id.* The loop amino acids of a FR can be assessed and determined by inspection of the three-dimensional structure of an antibody heavy chain and/or antibody light chain. The three-dimensional structure can be analyzed for solvent accessible amino acid positions as such positions are likely to form a loop and/or provide antigen contact in an antibody variable domain. Some of the solvent accessible positions can tolerate amino acid sequence diversity and others (*e.g.,* structural positions) are, generally, less diversified. The three-dimensional structure of the antibody variable domain can be derived from a crystal structure or protein modeling.

[0109] In the present disclosure, the following abbreviations (in the parentheses) are used in accordance with the customs, as necessary: heavy chain (H chain), light chain (L chain), heavy chain variable region (VH), light chain variable region (VL), complementarity determining region (CDR), first complementarity determining region (CDR1), second complementarity determining region (CDR2), third complementarity determining region (CDR3), heavy chain first complementarity determining region (VH CDR1), heavy chain second complementarity determining region (VH CDR2), heavy chain third complementarity determining region (VH CDR3), light chain first complementarity determining region (VL CDR1), light chain second complementarity determining region (VL CDR2), and light chain third complementarity determining region (VL CDR3).

[0110] The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is generally defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. The Fc region of an immunoglobulin generally comprises two constant domains, C_H2 and C_H3.

[0111] "Antibodies" useful in the present disclosure encompass, but are not limited to, monoclonal antibodies, polyclonal antibodies, chimeric antibodies, bispecific antibodies, grafted antibodies, multispecific antibodies, heteroconjugate antibodies, humanized antibodies, human antibodies,

deimmunized antibodies, mutants thereof, fusions thereof, immunoconjugates thereof, antigen binding fragments thereof, and/or any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. In certain embodiments of the methods and conjugates provided herein, the antibody requires an Fc region to enable attachment of a linker between the antibody and the protein (e.g., attachment of the linker using an affinity peptide, such as in AJICAP™ technology).

[0112] In some instances, an antibody is a monoclonal antibody. As used herein, a "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen (epitope). The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method.

[0113] In some instances, an antibody is a humanized antibody. As used herein, "humanized" antibodies refer to forms of non-human (*e.g.,* murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or fragments thereof that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and biological activity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences but are included to further refine and optimize antibody performance. In general, a humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at

least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in, for example, WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

[0114] If needed, an antibody or an antigen binding fragment described herein can be assessed for immunogenicity and, as needed, be deimmunized (*i.e.*, the antibody is made less immunoreactive by altering one or more T cell epitopes). As used herein, a "deimmunized antibody" means that one or more T cell epitopes in an antibody sequence have been modified such that a T cell response after administration of the antibody to a subject is reduced compared to an antibody that has not been deimmunized. Analysis of immunogenicity and T-cell epitopes present in the antibodies and antigen binding fragments described herein can be carried out via the use of software and specific databases. Exemplary software and databases include iTope™ developed by Antitope of Cambridge, England. iTope™, is an *in silico* technology for analysis of peptide binding to human MHC class II alleles. The iTope™ software predicts peptide binding to human MHC class II alleles and thereby provides an initial screen for the location of such "potential T cell epitopes." iTope™ software predicts favorable interactions between amino acid side chains of a peptide and specific binding pockets within the binding grooves of 34 human MHC class II alleles. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by one amino acid spanning the test antibody variable region sequence. Each 9mer peptide can be tested against each of the 34 MHC class II allotypes and scored based on their potential "fit" and interactions with the MHC class II binding groove. Peptides that produce a high mean binding score (>0.55 in the iTope™ scoring function) against >50% of the MHC class II alleles are considered as potential T cell epitopes. In such regions, the core 9 amino acid sequence for peptide binding within the MHC class II groove is analyzed to determine the MHC class II pocket residues (P1, P4, P6, P7, and P9) and the possible T cell receptor (TCR) contact residues (P-l, P2, P3, P5, P8). After identification of any T-cell epitopes, amino acid residue changes, substitutions, additions, and/or deletions can be introduced to remove the identified T-cell epitope. Such changes can be made so as to preserve antibody structure and function while still removing the identified epitope. Exemplary changes can include, but are not limited to, conservative amino acid changes.

[0115] An antibody can be a human antibody. As used herein, a "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or that has been made using any suitable technique for making human antibodies. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies. Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, *e.g.,* mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized *in vitro*).

[0116] Any of the antibodies herein can be bispecific. Bispecific antibodies are antibodies that have binding specificities for at least two different antigens and can be prepared using the antibodies disclosed herein. Traditionally, the recombinant production of bispecific antibodies was based on the co-expression of two immunoglobulin heavy chain-light chain pairs, with the two heavy chains having different specificities. Bispecific antibodies can be composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure, with an immunoglobulin light chain in only one half of the bispecific molecule, facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations.

[0117] According to one approach to making bispecific antibodies, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion can be with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. The first heavy chain constant region (CH1), containing the site necessary for light chain binding, can be present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three

polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0118] In some instances, an antibody herein is a chimeric antibody. "Chimeric" forms of non-human (*e.g.,* murine) antibodies include chimeric antibodies which contain minimal sequence derived from a non-human Ig. For the most part, chimeric antibodies are murine antibodies in which at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin, is inserted in place of the murine Fc. Chimeric or hybrid antibodies also may be prepared *in vitro* using suitable methods of synthetic protein chemistry, including those involving cross-linking agents.

[0119] Provided herein are antibodies and antigen binding fragments thereof, modified antibodies and antigen binding fragments thereof, and binding agents that specifically bind to one or more epitopes on one or more target antigens. In one instance, a binding agent selectively binds to an epitope on a single antigen. In another instance, a binding agent is bivalent and either selectively binds to two distinct epitopes on a single antigen or binds to two distinct epitopes on two distinct antigens. In another instance, a binding agent is multivalent (*i.e.*, trivalent, quatravalent, *etc.*) and the binding agent binds to three or more distinct epitopes on a single antigen or binds to three or more distinct epitopes on two or more (multiple) antigens.

[0120] Antigen binding fragments of any of the antibodies herein are also contemplated. The terms "antigen binding portion of an antibody," "antigen binding domain," "antibody fragment," or a "functional fragment of an antibody" are used interchangeably herein to refer to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. Representative antigen binding fragments include, but are not limited to, a Fab, a Fab', a $F(ab')_2$, a bispecific $F(ab')_2$, a trispecific $F(ab')_2$, a variable fragment (Fv), a single chain variable fragment (scFv), a dsFv, a bispecific scFv, a variable heavy domain, a variable light domain, a variable NAR domain, bispecific scFv, an AVIMER®, a minibody, a diabody, a bispecific diabody, triabody, a tetrabody, a minibody, a maxibody, a camelid, a VHH, a minibody, an intrabody, fusion proteins comprising an antibody portion (*e.g.,* a domain antibody), a single chain binding polypeptide, a scFv-Fc, a Fab-Fc, a bispecific T cell engager (BiTE; two scFvs produced as a single polypeptide chain, where each scFv comprises an amino acid sequences a combination of CDRs or a combination of VL/VL

described herein), a tetravalent tandem diabody (TandAb; an antibody fragment that is produced as a non-covalent homodimer folder in a head-to-tail arrangement, *e.g.*, a TandAb comprising an scFv, where the scFv comprises an amino acid sequences a combination of CDRs or a combination of VL/VL described herein), a Dual-Affinity Re-targeting Antibody (DART; different scFvs joined by a stabilizing interchain disulphide bond), a bispecific antibody (bscAb; two single-chain Fv fragments joined via a glycine-serine linker), a single domain antibody (sdAb), a fusion protein, a bispecific disulfide-stabilized Fv antibody fragment (dsFv–dsFv'; two different disulfide-stabilized Fv antibody fragments connected by flexible linker peptides). In certain embodiments of the invention, a full length antibody (e.g., an antigen binding fragment and an Fc region) are preferred.

[0121] Heteroconjugate polypeptides comprising two covalently joined antibodies or antigen binding fragments of antibodies are also within the scope of the disclosure. Suitable linkers may be used to multimerize binding agents. Non-limiting examples of linking peptides include, but are not limited to, $(GS)_n$ (SEQ ID NO: 224), $(GGS)_n$ (SEQ ID NO: 225), $(GGGS)_n$ (SEQ ID NO: 226), $(GGSG)_n$ (SEQ ID NO: 227), or $(GGSGG)_n$ (SEQ ID NO: 228), $(GGGGS)_n$ (SEQ ID NO: 229), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. For example, a linking peptide can be $(GGGGS)_3$ (SEQ ID NO: 230) or $(GGGGS)_4$ (SEQ ID NO: 231). In some embodiments, a linking peptide bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used. Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports.

[0122] As used herein, the term "avidity" refers to the resistance of a complex of two or more agents to dissociation after dilution. Apparent affinities can be determined by methods such as an enzyme-linked immunosorbent assay (ELISA) or any other suitable technique. Avidities can be determined by methods such as a Scatchard analysis or any other suitable technique.

[0123] As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as K_D. The binding affinity (K_D) of an antibody or antigen binding fragment herein can be less than 500 nM, 475 nM, 450 nM, 425 nM, 400 nM, 375 nM, 350 nM, 325 nM, 300 nM, 275 nM, 250 nM, 225 nM, 200 nM, 175 nM, 150 nM, 125 nM, 100 nM, 90 nM, 80 nM, 70 nM, 50 nM, 50 nM, 49 nM, 48 nM, 47 nM, 46 nM, 45 nM, 44 nM, 43 nM, 42 nM, 41 nM, 40 nM, 39 nM, 38 nM, 37 nM, 36 nM, 35 nM, 34 nM, 33 nM, 32 nM, 31 nM, 30

nM, 29 nM, 28 nM, 27 nM, 26 nM, 25 nM, 24 nM, 23 nM, 22 nM, 21 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 990 pM, 980 pM, 970 pM, 960 pM, 950 pM, 940 pM, 930 pM, 920 pM, 910 pM, 900 pM, 890 pM, 880 pM, 870 pM, 860 pM, 850 pM, 840 pM, 830 pM, 820 pM, 810 pM, 800 pM, 790 pM, 780 pM, 770 pM, 760 pM, 750 pM, 740 pM, 730 pM, 720 pM, 710 pM, 700 pM, 690 pM, 680 pM, 670 pM, 660 pM, 650 pM, 640 pM, 630 pM, 620 pM, 610 pM, 600 pM, 590 pM, 580 pM, 570 pM, 560 pM, 550 pM, 540 pM, 530 pM, 520 pM, 510 pM, 500 pM, 490 pM, 480 pM, 470 pM, 460 pM, 450 pM, 440 pM, 430 pM, 420 pM, 410 pM, 400 pM, 390 pM, 380 pM, 370 pM, 360 pM, 350 pM, 340 pM, 330 pM, 320 pM, 310 pM, 300 pM, 290 pM, 280 pM, 270 pM, 260 pM, 250 pM, 240 pM, 230 pM, 220 pM, 210 pM, 200 pM, 190 pM, 180 pM, 170 pM, or any integer therebetween. Binding affinity may be determined using surface plasmon resonance (SPR), KINEXA® Biosensor, scintillation proximity assays, enzyme linked immunosorbent assay (ELISA), ORIGEN immunoassay (IGEN), fluorescence quenching, fluorescence transfer, yeast display, or any combination thereof. Binding affinity may also be screened using a suitable bioassay.

[0124] Also provided herein are affinity matured antibodies. The following methods may be used for adjusting the affinity of an antibody and for characterizing a CDR. One way of characterizing a CDR of an antibody and/or altering (such as improving) the binding affinity of a polypeptide, such as an antibody, is termed "library scanning mutagenesis." Generally, library scanning mutagenesis works as follows. One or more amino acid position in the CDR is replaced with two or more (such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids. This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of two or more members (if two or more amino acids are substituted at every position). Generally, the library also includes a clone comprising the native (unsubstituted) amino acid. A small number of clones, for example, about 20-80 clones (depending on the complexity of the library), from each library can be screened for binding specificity or affinity to the target polypeptide (or other binding target), and candidates with increased, the same, decreased, or no binding are identified. Binding affinity may be determined using Biacore surface plasmon resonance analysis, which detects differences in binding affinity of about 2-fold or greater.

[0125] In some instances, an antibody or antigen binding fragment is bispecific or multispecific and can specifically bind to more than one antigen. In some cases, such a bispecific or multispecific antibody or antigen binding fragment can specifically bind to 2 or more different antigens. In some cases, a bispecific antibody or antigen binding fragment can be a bivalent antibody or antigen binding fragment. In some cases, a multi specific antibody or antigen binding fragment can be a bivalent antibody or antigen binding fragment, a trivalent antibody or antigen binding fragment, or a quatravalent antibody or antigen binding fragment.

[0126] An antibody or antigen binding fragment described herein can be isolated, purified, recombinant, or synthetic.

[0127] It is contemplated that generic or biosimilar versions of the named antibodies herein which share the same amino acid sequence as the indicated antibodies are also encompassed when the name of the antibody is used.

[0128] The antibodies described herein may be made by any suitable method. Antibodies can often be produced in large quantities, particularly when utilizing high level expression vectors.

[0129] In one embodiment, an antibody or an antigen binding fragment of the disclosure comprises a fusion protein or a peptide immunotherapeutic agent.

Immune Cell Associated Antigen Specific Antibodies

[0130] In some embodiments, the antibody or antigen binding fragment thereof is specific for an immune cell associated antigen. In some embodiments, the immune cell associated antigen is associated with an immune cell subtype (e.g., lymphocyte, neutrophil, macrophage, etc.). In some embodiments, the immune cell associated antigen is associated with a T cell, a monocyte, and/or a natural killer (NK) cell. In embodiments, the immune cell antigen is associated with a T cell. In some embodiments, the immune cell antigen is associated with an effector T cell, a cytotoxic T cell, a helper T cell, a regulatory T cell, and/or a memory T cell.

[0131] In some embodiments, the immune cell associated antigen is an immune checkpoint molecule. In some embodiments, the immune cell associated antigen is a costimulatory antigen. In some embodiments, the immune cell associated antigen is an macrophage cell surface antigen. In some embodiments, the immune cell associated antigen is an NK cell surface antigen. In some embodiments, the immune cell associated antigen is a T cell surface antigen (*e.g.,* CD8A, CD8B).

[0132] In some embodiments, the immune cell associated antigen is 4-IBB, B7-H3, B7-H4, BTLA, CD3, CCR8, CD8A, CD8B, CD16A, CD27, CD28, CD33, CD38, CD39, CD40, CD47,

CD70, CD80, CD86, CD96, CD163, CLEC-1, CLEVER-1, CTLA-4, D40, GITR, ICOS, ILT2/3/4, LAG-3, MHCI, MHCII, NKG2A, NKG2D, NKp30, NKp44, NKp46, OX40, PD-1, PD-L1, PD-L2, PSGL-1, SIGLEC-9, SIGLEC-15, SIRP-α, TCR,TIGIT, TIM-3,VISTA, or VSIG4.. In some embodiments, the immune cell associated antigen is PD-1. In some embodiments, the immune cell associated antigen is CCR8, CD8A, CD8B, CD16A, CD96, CD226, CTLA-4, ICOS, LAG-3, NKG2A, NKG2D, NKp30, NKp44, NKp46, PD-1, PD-L1, TIGIT, or TIM-3.

[0133] In some embodiments, the immune cell associated antigen is 4-1BB. In some embodiments, the immune cell associated antigen is B7-H3. In some embodiments, the immune cell associated antigen is B7-H4. In some embodiments, the immune cell associated antigen is BTLA. In some embodiments, the immune cell associated antigen is CD3. In some embodiments, the immune cell associated antigen is CCR8. In some embodiments, the immune cell associated antigen is CD8A. In some embodiments, the immune cell associated antigen is CD8B. In some embodiments, the immune cell associated antigen is CD16A. In some embodiments, the immune cell associated antigen is CD27. In some embodiments, the immune cell associated antigen is CD33. In some embodiments, the immune cell associated antigen is CD38. In some embodiments, the immune cell associated antigen is CD39. In some embodiments, the immune cell associated antigen is CD40. In some embodiments, the immune cell associated antigen is CD47. In some embodiments, the immune cell associated antigen is CD80. In some embodiments, the immune cell associated antigen is CD86. In some embodiments, the immune cell associated antigen is CD96. In some embodiments, the immune cell associated antigen is CD163. In some embodiments, the immune cell associated antigen is CLEC-1. In some embodiments, the immune cell associated antigen is CLEVER-1. In some embodiments, the immune cell associated antigen is CTLA4. In some embodiments, the immune cell associated antigen is D40. In some embodiments, the immune cell associated antigen is GITR. In some embodiments, the immune cell associated antigen is ICOS. In some embodiments, the immune cell associated antigen is ILT2/3/4. In some embodiments, the immune cell associated antigen is LAG-3. In some embodiments, the immune cell associated antigen is MHCI. In some embodiments, the immune cell associated antigen is MHCII. In some embodiments, the immune cell associated antigen is NKG2A. In some embodiments, the immune cell associated antigen is NKp30. In some embodiments, the immune cell associated antigen is NKp44. In some embodiments, the immune cell associated antigen is NKp46. In some embodiments, the immune cell associated antigen is OX40. In some embodiments, the immune

cell associated antigen is PD-1. In some embodiments, the immune cell associated antigen is PD-L1. In some embodiments, the immune cell associated antigen is PD-L2. In some embodiments, the immune cell associated antigen is PSGL-1. In some embodiments, the immune cell associated antigen is SIGLEC-9. In some embodiments, the immune cell associated antigen is SIGLEC-15. In some embodiments, the immune cell associated antigen is SIRP-α. In some embodiments, the immune cell associated antigen is TCR. In some embodiments, the immune cell associated antigen is TIGIT. In some embodiments, the immune cell associated antigen is TIM-3. In some embodiments, the immune cell associated antigen is VISTA. In some embodiments, the immune cell associated antigen is VSIG4.

[0134] In some embodiments, the antibody or antigen binding fragment thereof is an anti-PD-1 antibody or antigen binding fragment. Programmed cell death protein 1 (also known as PD-1 and CD279), is a cell surface receptor that plays an role in down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. PD-1 is an immune cell inhibitory molecule that is expressed on activated B cells, T cells, and myeloid cells. PD-1 represents an immune checkpoint and guards against autoimmunity via a dual mechanism of promoting apoptosis (programmed cell death) in antigen-specific T-cells in lymph nodes while reducing apoptosis in regulatory T cells. PD-1 is a member of the CD28/CTLA-4/ICOS costimulatory receptor family that delivers negative signals that affect T and B cell immunity. PD-1 is monomeric both in solution as well as on cell surface, in contrast to CTLA-4 and other family members that are all disulfide-linked homodimers. Signaling through the PD-1 inhibitory receptor upon binding its ligand, PD-L1, suppresses immune responses against autoantigens and tumors and plays a role in the maintenance of peripheral immune tolerance. The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T cell receptor mediated proliferation, and immune evasion by the cancerous cells. A non-limiting, exemplary, human PD-1 amino acid sequence is MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSN TSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRN DSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGG LLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTP EPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL (SEQ ID NO: 331).

[0135] In one embodiment, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment of the disclosure comprises a combination of a heavy chain variable region (VH) and a light chain variable region (VL) described herein. In another embodiment, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment of the disclosure comprises a combination of complementarity determining regions (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) described herein. In one embodiment, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment of the disclosure comprises a modified Tislelizumab, Baizean, 0KVO411B3N, BGB-A317, hu317-1/IgG4mt2, Sintilimab, Tyvyt, IBI-308, Toripalimab, TeRuiPuLi, Terepril, Tuoyi, JS-001, TAB-001, Camrelizumab, HR-301210, INCSHR-01210, SHR-1210, Cemiplimab, Cemiplimab-rwlc, LIBTAYO®, 6QVL057INT, H4H7798N, REGN-2810, SAR-439684, Avelumab, BAVENCIO®, 451238, KXG2PJ551I, MSB-0010682, MSB-0010718C, PF-06834635, Durvalumab, IMFINZI®, 28X28X9OKV, MEDI-4736, Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11, Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538, Prolgolimab, Forteca, BCD-100, Penpulimab, AK-105, Zimberelimab, AB-122, GLS-010, WBP-3055, Balstilimab, 1Q2QT5M7EO, AGEN-2034, AGEN-2034w, Genolimzumab, Geptanolimab, APL-501, CBT-501, GB-226, Dostarlimab, ANB-011, GSK-4057190A, P0GVQ9A4S5, TSR-042, WBP-285, Serplulimab, HLX-10, CS-1003, Retifanlimab, 2Y3T5IF01Z, INCMGA-00012, INCMGA-0012, MGA-012, Sasanlimab, LZZ0IC2EWP, PF-06801591, RN-888, Spartalizumab, NVP-LZV-184, PDR-001, QOG25L6Z8Z, Relatlimab/nivolumab, BMS-986213, Cetrelimab, JNJ-3283, JNJ-63723283, LYK98WP91F, Tebotelimab, MGD-013, BCD-217, BAT-1306, HX-008, MEDI-5752, JTX-4014, Cadonilimab, AK-104, BI-754091, Pidilizumab, CT-011, MDV-9300, YBL-006, AMG-256, RG-6279, RO-7284755, BH-2950, IBI-315, RG-6139, RO-7247669, ONO-4685, AK-112, 609-A, LY-3434172, T-3011, MAX-10181, AMG-404, IBI-318, MGD-019, INCB-086550, ONCR-177, LY-3462817, RG-7769, RO-7121661, F-520, XmAb-23104, Pd-1-pik, SG-001, S-95016, Sym-021, LZM-009, Budigalimab, 6VDO4TY3OO, ABBV-181, PR-1648817, CC-90006, XmAb-20717, 2661380, AMP-224, B7-DCIg, EMB-02, ANB-030, PRS-332, [89Zr]Deferoxamide-pembrolizumab, 89Zr-Df-Pembrolizumab, [89Zr]Df-Pembrolizumab, STI-1110, STI-A1110, CX-188, mPD-1 Pb-Tx, MCLA-134, 244C8, ENUM 224C8, ENUM C8, 388D4, ENUM 388D4, ENUM D4, MEDI0680, or AMP-514.

[0136] In some embodiments, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment of the disclosure comprises a Tislelizumab, Sintilimab, Toripalimab, Terepril, Camrelizumab, Cemiplimab, Pembrolizumab Nivolumab, Prolgolimab, Penpulimab, Zimberelimab, Balstilimab, Genolimzumab, Geptanolimab, Dostarlimab, Serplulimab, Retifanlimab, Sasanlimab, Spartalizumab, Cetrelimab, Tebotelimab, Cadonilimab, A Pidilizumab, LZM-009, or Budigalimab. In one embodiment, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment of the disclosure comprises a modified Tislelizumab, Sintilimab, Toripalimab, Terepril, Camrelizumab, Cemiplimab, Pembrolizumab Nivolumab, Prolgolimab, Penpulimab, Zimberelimab, Balstilimab, Genolimzumab, Geptanolimab, Dostarlimab, Serplulimab, Retifanlimab, Sasanlimab, Spartalizumab, Cetrelimab, Tebotelimab, Cadonilimab, A Pidilizumab, LZM-009, or Budigalimab.

[0137] In some embodiments, the anti-PD-1 polypeptide is Nivolumab, Pembrolizumab, LZM-009, Dostarlimab, Sintilimab, Spartalizumab, Tislelizumab, or Cemiplimab. In some embodiment, the anti-PD-1 polypeptide is Dostarlimab, Sintilimab, Spartalizumab, or Tislelizumab. In some embodiments, the anti-PD-1 polypeptide is Nivolumab, Pembrolizumab, LZM-009, or Cemiplimab.

[0138] In some embodiments, the anti-PD-1 antibody is Pembrolizumab. In some embodiments, the anti-PD-1 antibody is modified Pembrolizumab.

[0139] In some embodiments, the anti-PD-1 antibody is a biosimilar of Tislelizumab, Sintilimab, Toripalimab, Terepril, Camrelizumab, Cemiplimab, Pembrolizumab Nivolumab, Prolgolimab, Penpulimab, Zimberelimab, Balstilimab, Genolimzumab, Geptanolimab, Dostarlimab, Serplulimab, Retifanlimab, Sasanlimab, Spartalizumab, Cetrelimab, Tebotelimab, Cadonilimab, A Pidilizumab, LZM-009, or Budigalimab. In some embodiments, the anti-PD-1 antibody is a biosimilar of any one of the antibodies provided herein.

[0140] TABLE 1 provides the sequences of exemplary anti-PD-1 antibodies and anti-PD-1 antigen binding fragments that can be modified to prepare anti-PD-1 immunoconjugates. TABLE 1 also shows provides combinations of CDRs that can be utilized in a modified anti-PD-1 immunoconjugate. Reference to an anti-PD-1 antibody herein may alternatively refer to an anti-PD-1 antigen binding fragment.

[0141] In some instances, the SEQ ID NOs listed in Table 1 contain full-length heavy or light chains of the indicated antibodies with the VH or VL respectively indicated in bold. Where there

is a reference herein to a VH or VL of a SEQ ID NO in Table 1 which contains a full-length heavy or light chain, it is intended to reference the bolded portion of the sequence. For example, reference to "a VH having an amino acid sequence shown in SEQ ID NO: 332" refers to the bolded portion of SEQ ID NO: 332 in Table 1.

[0142] An anti-PD-1 antibody or an anti-PD-1 antigen binding fragment can comprise a VH having an amino acid sequence of any one of SEQ ID NOS: 332, 334, 336, 338, 340, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, and 378. An anti-PD-1 antibody or an anti-PD-1 antigen binding fragment can comprise a VL having an amino acid sequence of any one of SEQ ID NOS: 333, 335, 337, 339, 341, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, and 379.

[0143] An anti-PD-1 antibody or an anti-PD-1 antigen binding fragment can comprise a heavy chain or VH having an amino acid sequence of any one of SEQ ID NOS: 332, 334, 336, 338, 340, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, and 378, or a portion corresponding to a VH thereof. An anti-PD-1 antibody or an anti-PD-1 antigen binding fragment can comprise a light chain or VL having an amino acid sequence of any one of SEQ ID NOS: 333, 335, 337, 339, 341, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, and 379, or a portion corresponding to a VL thereof.

[0144] In one instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 332, and a VL having an amino acid sequence shown in SEQ ID NO: 333. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 334, and a VL having an amino acid sequence shown in SEQ ID NO: 335. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 336, and a VL having an amino acid sequence shown in SEQ ID NO: 337. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 338, and a VL having an amino acid sequence shown in SEQ ID NO: 339. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 340, and a VL having an amino acid sequence shown in SEQ ID NO: 341. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 346, and a VL having an amino acid sequence

shown in SEQ ID NO: 347. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 348, and a VL having an amino acid sequence shown in SEQ ID NO: 349. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 350, and a VL having an amino acid sequence shown in SEQ ID NO: 351. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 352, and a VL having an amino acid sequence shown in SEQ ID NO: 353. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 354, and a VL having an amino acid sequence shown in SEQ ID NO: 355. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 356, and a VL having an amino acid sequence shown in SEQ ID NO: 357. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 358, and a VL having an amino acid sequence shown in SEQ ID NO: 359. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 360, and a VL having an amino acid sequence shown in SEQ ID NO: 361. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 362, and a VL having an amino acid sequence shown in SEQ ID NO: 363. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 364, and a VL having an amino acid sequence shown in SEQ ID NO: 365. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 366, and a VL having an amino acid sequence shown in SEQ ID NO: 367. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 368, and a VL having an amino acid sequence shown in SEQ ID NO: 369. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 370, and a VL having an amino acid sequence shown in SEQ ID NO: 371. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 372, and a VL having an

amino acid sequence shown in SEQ ID NO: 373. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 374, and a VL having an amino acid sequence shown in SEQ ID NO: 375. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 376, and a VL having an amino acid sequence shown in SEQ ID NO: 377. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 378, and a VL having an amino acid sequence shown in SEQ ID NO: 379.

[0145] In one instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 332, and a VL having an amino acid sequence of SEQ ID NO: 333. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 334, and a VL having an amino acid sequence of SEQ ID NO: 335. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 336, and a VL having an amino acid sequence of SEQ ID NO: 337. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 338, and a VL having an amino acid sequence of SEQ ID NO: 339. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 340, and a VL having an amino acid sequence of SEQ ID NO: 341. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 346, and a VL having an amino acid sequence of SEQ ID NO: 347. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 348, and a VL having an amino acid sequence of SEQ ID NO: 349. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 350, and a VL having an amino acid sequence of SEQ ID NO: 351. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 352, and a VL having an amino acid sequence of SEQ ID NO: 353. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 354, and a VL having an amino acid sequence of SEQ ID NO: 355. In another instance, an anti-PD-1 antibody or an anti-

PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 356, and a VL having an amino acid sequence of SEQ ID NO: 357. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 358, and a VL having an amino acid sequence of SEQ ID NO: 359. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 360, and a VL having an amino acid sequence of SEQ ID NO: 361. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 362, and a VL having an amino acid sequence of SEQ ID NO: 363. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 364, and a VL having an amino acid sequence of SEQ ID NO: 365. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 366, and a VL having an amino acid sequence of SEQ ID NO: 367. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 368, and a VL having an amino acid sequence of SEQ ID NO: 369. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 370, and a VL having an amino acid sequence of SEQ ID NO: 371. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 372, and a VL having an amino acid sequence of SEQ ID NO: 373. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 374, and a VL having an amino acid sequence of SEQ ID NO: 375. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 376, and a VL having an amino acid sequence of SEQ ID NO: 377. In another instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 378, and a VL having an amino acid sequence of SEQ ID NO: 379.

[0146] In one instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 380, a VH CDR2 having an amino acid sequence of SEQ ID NO: 381, a VH CDR3 having an amino acid sequence of SEQ ID NO: 382, VL CDR1 having an amino acid sequence of SEQ ID NO: 383, a VL CDR2 having an amino

acid sequence of SEQ ID NO: 384, and a VL CDR3 having an amino acid sequence of SEQ ID NO: 385. In one instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 386, a VH CDR2 having an amino acid sequence of SEQ ID NO: 387, a VH CDR3 having an amino acid sequence of SEQ ID NO: 388, VL CDR1 having an amino acid sequence of SEQ ID NO: 389, a VL CDR2 having an amino acid sequence of SEQ ID NO: 390, and a VL CDR3 having an amino acid sequence of SEQ ID NO: 391. In one instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 392, a VH CDR2 having an amino acid sequence of SEQ ID NO: 393, a VH CDR3 having an amino acid sequence of SEQ ID NO: 394, VL CDR1 having an amino acid sequence of SEQ ID NO: 395, a VL CDR2 having an amino acid sequence of SEQ ID NO: 396, and a VL CDR3 having an amino acid sequence of SEQ ID NO: 397. In one instance, an anti-PD-1 antibody or an anti-PD-1 antigen binding fragment comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 398, a VH CDR2 having an amino acid sequence of SEQ ID NO: 399, a VH CDR3 having an amino acid sequence of SEQ ID NO: 400, VL CDR1 having an amino acid sequence of SEQ ID NO: 401, a VL CDR2 having an amino acid sequence of SEQ ID NO: 402, and a VL CDR3 having an amino acid sequence of SEQ ID NO: 403.

[0147] In one instance, an anti-PD-1 antibody comprises a fusion protein. Such fusion protein can be, for example, a two-sided Fc fusion protein comprising the extracellular domain (ECD) of programmed cell death 1 (PD-1) and the ECD of tumor necrosis factor (ligand) superfamily member 4 (TNFSF4 or OX40L) fused via hinge-CH2-CH3 Fc domain of human IgG4, expressed in CHO-K1 cells, where the fusion protein has an exemplary amino acid sequence of SEQ ID NO: 104.

[0148] In some embodiments, the antibody or antigen binding fragment thereof is an anti-PD-L1 antibody or antigen binding fragment. Programmed death-ligand 1 (PD-L1) is a ligand for an immunosuppressive receptor "programmed death receptor 1 (PD-1)" that is predominantly expressed in activated T and B cells, which can negatively regulate antigen receptor signaling. The ligands (PD-L1 and PD-L2) for PD-1 may be constitutively expressed or may be derived into a number of cell types, including non-hematopoietic cell tissues and various tumor types. PD-L1 is expressed in B cells, T cells, bone marrow cells and dendritic cells (DCs), but also on non-lymphatic organs such as peripheral cells, pseudo-vascular endothelial cells and heart, lungs, *etc.*

A non-limiting, exemplary, human PD-L1 amino acid sequence is MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWE MEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMI SYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVL SGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNE RTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET (SEQ ID NO: 330)

[0149] In one embodiment, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment of the disclosure comprises a combination of a heavy chain variable region (VH) and a light chain variable region (VL) described herein. In another embodiment, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment of the disclosure comprises a combination of complementarity determining regions (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) described herein. In one embodiment, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment of the disclosure comprises a modified Avelumab (Bavencio, 451238, KXG2PJ551I, MSB-0010682, MSB-0010718C, PF-06834635, CAS 1537032-82-8: EMD Serono, Merck & Co., Merck KGaA, Merck Serono, National Cancer Institute (NCI), Pfizer), Durvalumab (Imfinzi, 28X28X9OKV (UNII code), MEDI-4736, CAS 1428935-60-7: AstraZeneca, Celgene, Children's Hospital Los Angeles (CHLA), City of Hope National Medical Center, MedImmune, Memorial Sloan-Kettering Cancer Center, Mirati Therapeutics, National Cancer Institute (NCI), Samsung Medical Center (SMC), Washington University), Atezolizumab (Tecentriq, 52CMI0WC3Y, MPDL-3280A, RG-7446, RO-5541267, CAS 1380723-44-3: Academisch Medisch Centrum (AMC), Chugai Pharmaceutical, EORTC, Genentech, Immune Design (Merck & Co.), Memorial Sloan-Kettering Cancer Center, National Cancer Institute (NCI), Roche, Roche Center for Medical Genomics), Sugemalimab (CS-1001, WBP-3155: CStone Pharmaceuticals, EQRx, Pfizer), KN-046 (CAS 2256084-03-2: Jiangsu Alphamab Biopharmaceuticals, Sinovent), APL-502 (CBT-502, TQB-2450: Apollomics, Jiangsu Chia Tai Tianqing Pharmaceutical), Envafolimab (3D-025, ASC-22, KN-035, hu56V1-Fc-m1, CAS 2102192-68-5: 3D Medicines, Ascletis, Jiangsu Alphamab Biopharmaceuticals, Suzhou Alphamab, Tracon Pharmaceuticals, Inc.), Bintrafusp alfa (M-7824, MSB-0011359C, NW9K8C1JN3, CAS 1918149-01-5: EMD Serono, GlaxoSmithKline, Merck KGaA, National Cancer Institute (NCI)), STI-1014 (STI-A1014, ZKAB-001: Lee's Pharmaceutical, Sorrento Therapeutics), PD-L1 t-haNK (ImmunityBio, NantKwest), A-167

(HBM-9167, KL-A167: Harbour BioMed, Sichuan Kelun-Biotech Biopharmaceutical), IMC-001 (STI-3031, STI-A-1015, STI-A1015, ImmuneOncia Therapeutics, Sorrento Therapeutics), HTI-1088 (SHR-1316: Atridia, Jiangsu Hengrui), IO-103 (IO Biotech), CX-072 (CytomX Therapeutics), AUPM-170 (CA-170: Aurigene, Curis), GS-4224 (Gilead), ND-021 (NM21-1480, PRO-1480: CStone Pharmaceuticals, Numab Therapeutics), BNT-311 (DuoBody-PD-L1x4-1BB, GEN-1046: BioNTech, Genmab), BGB-A333 (BeiGene), IBI-322 (Innovent Biologics), NM-01 (Nanomab Technology, Shanghai First People's Hospital), LY-3434172 (Eli Lilly), LDP (Dragonboat Biopharmaceutical), CDX-527 (Celldex Therapeutics), IBI-318 (Innovent Biologics, Lilly), 89Zr-DFO-REGN3504 (Regeneron), ALPN-202 (CD80 vIgD-Fc: Alpine Immune Sciences), INCB-086550 (Incyte), LY-3415244 (Eli Lilly), SHR-1701 (Jiangsu Hengrui), JS-003 (JS003-30, JS003-SD: Shanghai Junshi Biosciences), HLX-20 (PL2#3: Henlix Biotech, Shanghai Henlius Biotech), ES-101 (INBRX-105, INBRX-105-1: Elpiscience BioPharma, Inhibrx), MSB-2311 (MabSpace Biosciences), PD-1-Fc-OX40L (SL-279252, TAK-252: Heat Biologics, Shattuck Labs, Takeda), FS-118, FS118 mAb2, LAG-3/PD-L1 mAb2: F-star Therapeutics, Merck & Co., Merck KGaA), FAZ-053 (LAE-005: Laekna Therapeutics, Novartis), Lodapolimab (LY-3300054, NR4MAD6PPB, CAS 2118349-31-6: Eli Lilly), MCLA-145 (Incyte, Merus), BMS-189 (BMS-986189, PD-L1-Milla from Bristol-Myers Squibb), Cosibelimab (CK-301, TG-1501, CAS 2216751-26-5: Checkpoint Therapeutics, Dana-Farber Cancer Institute, Samsung Biologics, TG Therapeutics), IL-15Ralpha-SD/IL-15 (KD-033: Kadmon), WP-1066 (CAS 857064-38-1: M.D. Anderson Cancer Center, Moleculin Biotech), BMS-936559 (MDX-1105: Bristol-Myers Squibb, Medarex, National Institute Allergy Infect Dis.), BMS-986192 (Bristol-Myers Squibb), RC-98 (RemeGen), CD-200AR-L (CD200AR-L: OX2 Therapeutics, University of Minnesota), ATA-3271 (Atara Biotherapeutics), IBC-Ab002 (ImmunoBrain Checkpoint), BMX-101 (Biomunex Pharmaceuticals), AVA-04-VbP (Avacta), ACE-1708 (Acepodia Biotech), KY-1043 (Kymab, Provenance Biopharmaceuticals), ACE-05 (YBL-013: Y-Biologics), ONC-0055 (ONC0055, PRS-344 S-095012: Pieris Pharmaceuticals, Servier), TLJ-1-CK (I-Mab Biopharma), GR-1405 (Chinese Academy of Medical Sciences), PD-1ACR-T (Taipei Medical University), N-809 (N-IL15/PD-L1: ImmunityBio), CB-201 (Crescendo Biologics), MEDI-1109 (MedImmune), AVA-004 (AVA-04: Avacta), CA-327 (Aurigene, Curis), ALN-PDL (Alnylam Pharmaceuticals), KY-1003 (Kymab), CD22(aPD-L1)CAR-T cells (SL-22P: Hebei Senlang Biotechnology), ATA-2271

(M28z1XXPD-1DNR CAR T cells: Atara Biotherapeutics), and Zeushield cytotoxic T lymphocytes (Second Xiangya Hosp Central South Univ.).

[0150] In some embodiments, the anti-PD-L1 antibody is Avelumab, Durvalumab, Atezolizumab, Sugemalimab, Envafolimab, Lodapolimab, or Cosibelimab, or a modified version thereof. In some embodiments, the anti-PD-L1 antibody is Avelumab, Durvalumab, Atezolizumab, Sugemalimab, Envafolimab, Lodapolimab, or Cosibelimab. In some embodiments, the antibody is a biosimilar of Avelumab, Durvalumab, Atezolizumab, Sugemalimab, Envafolimab, Lodapolimab, or Cosibelimab.

[0151] TABLE 1 provides the sequences of exemplary anti-PD-L1 antibodies and anti-PD-L1 antigen binding fragments that can be modified to prepare anti-PD-L1 immunoconjugates. TABLE 1 also provides exemplary combinations of CDRs that can be utilized in a modified anti-PD-L1 immunoconjugate. Reference to an anti-PD-L1 antibody herein may alternatively refer to an anti-PD-L1 antigen binding fragment.

[0152] In some embodiments, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of any one of SEQ ID NOS: 232, 234, 236, 238, 242, 244, or 248. An anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of any one of SEQ ID NOS: 233, 235, 237, 239, 243, 245, or 249. In one instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of SEQ ID NO: 232, and a light chain or VL having an amino acid sequence of SEQ ID NO: 233. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of SEQ ID NO: 234, and a light chain or VL having an amino acid sequence of SEQ ID NO: 235. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of SEQ ID NO: 236, and a light chain or VL having an amino acid sequence of SEQ ID NO: 237. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of SEQ ID NO: 238, and a light chain or VL having an amino acid sequence of SEQ ID NO: 239. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a VH having an amino acid sequence of SEQ ID NO: 242, and a VL having an amino acid sequence of SEQ ID NO: 243. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a

VH having an amino acid sequence of SEQ ID NO: 244, and a VL having an amino acid sequence of SEQ ID NO: 245. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of SEQ ID NO: 248, and a light chain or VL having an amino acid sequence of SEQ ID NO: 249.

[0153] In some embodiments, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence of any one of SEQ ID NOS: 232, 234, 236, 238, 242, 244, or 248, or a portion corresponding to a VH thereof. An anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a light chain or VL having an amino acid sequence of any one of SEQ ID NOS: 233, 235, 237, 239, 243, 245, or 249, or a portion corresponding to a VL thereof. In one instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence shown in SEQ ID NO: 232, and a light chain or VL having an amino acid sequence shown in SEQ ID NO: 233. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence shown in SEQ ID NO: 234, and a light chain or VL having an amino acid sequence shown in SEQ ID NO: 235. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence shown in SEQ ID NO: 236, and a light chain or VL having an amino acid sequence shown in SEQ ID NO: 237. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence shown in SEQ ID NO: 238, and a light chain or VL having an amino acid sequence shown in SEQ ID NO: 239. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 242, and a VL having an amino acid sequence shown in SEQ ID NO: 243. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a VH having an amino acid sequence shown in SEQ ID NO: 244, and a VL having an amino acid sequence shown in SEQ ID NO: 245. In another instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a heavy chain or VH having an amino acid sequence shown in SEQ ID NO: 248, and a light chain or VL having an amino acid sequence shown in SEQ ID NO: 249.

[0154] In one instance, an anti-PD-L1 antibody or an anti-PD-L1 antigen binding fragment comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 250, a VH CDR2 having an amino acid sequence of SEQ ID NO: 251, a VH CDR3 having an amino acid sequence of SEQ

ID NO: 252, VL CDR1 having an amino acid sequence of SEQ ID NO: 253, a VL CDR2 having an amino acid sequence of SEQ ID NO: 254, and a VL CDR3 having an amino acid sequence of SEQ ID NO: 255.

[0155] In one instance, an anti-PD-L1 antibody comprises a single domain binding antibody having an amino acid sequence of SEQ ID NO: 256, a tri-specific fusion single chain antibody construct having an amino acid sequence of SEQ ID NO: 257, or a bispecific tetrameric antibody like engager having an amino acid sequence of SEQ ID NO: 258.

TABLE 1 – Exemplary Antibodies for Immune Cell Associated Antigens

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Tislelizumab, Baizean, 0KVO411B3N, BGB-A317, hu317-1/IgG4mt2 Heavy Chain (**VH in Bold**)	PD-1	**QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVHWIRQPPGKGLEWIGVIYADGSTNYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVYYCARAYGNYWYIDVWGQGTTVTV**SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPPVAGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	332
Tislelizumab, Baizean, 0KVO411B3N, BGB-A317, hu317-1/IgG4mt2 Light Chain (**VL in Bold**)	PD-1	**DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAWYQQKPGQPPKLLINYAFHRFTGVPDRFSGSGYGTDFTLTISSLQAEDVAVYYCHQAYSSPYTFGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	333

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Sintilimab, Tyvyt, IBI-308 Heavy Chain (**VH in Bold**)	PD-1	**QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGLIIPMFDTAGYAQKFQGRVAITVDESTSTAYMELS SLRSEDTAVYYCARAEHSSTGTFDYWGQGTLVTV**SSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	334
Sintilimab, Tyvyt, IBI-308 Light Chain (**VL in Bold**)	PD-1	**DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGK APKLLISAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQANHLPFTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	335
Toripalimab, TeRuiPuLi, Terepril, Tuoyi, JS-001, TAB-001 Heavy Chain (**VH in Bold**)	PD-1	**QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQA PIHGLEWIGVIESETGGTAYNQKFKGRVTITADKSTSTAYMEL SSLRSEDTAVYYCAREGITTVATTYYWYFDVWGQGTTVTV**SS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS NTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK	336
Toripalimab, TeRuiPuLi, Terepril, Tuoyi, JS-001, TAB-	PD-1	**DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLEWYLQ KPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCFQGSHVPLTFGQGTKLEIK**RTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	337

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
001 Light Chain (**VL in Bold**)			
Camrelizumab, HR-301210, INCSHR-01210, SHR-1210 Heavy Chain (**VH in Bold**)	PD-1	**EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYMMSWVRQAP GKGLEWVATISGGGANTYYPDSVKGRFTISRDNAKNSLYLQM NSLRAEDTAVYYCARQLYYFDYWGQGTTVTV**SSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL SLSLGK	338
Camrelizumab, HR-301210, INCSHR-01210, SHR-1210 Light Chain (**Light Chain in Bold**)	PD-1	**DIQMTQSPSSLSASVGDRVTITCLASQTIGTWLTWYQQKPGK APKLLIYTATSLADGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQVYSIPWTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	339
Cemiplimab, Cemiplimab-rwlc, LIBTAYO®, 6QVL057INT, H4H7798N,	PD-1	**EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMTWVRQAPG KGLEWVSGISGGGRDTYFADSVKGRFTISRDNSKNTLYLQMN SLKGEDTAVYYCVKWGNIYFDYWGQGTLVTV**SSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP	340

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
REGN-2810, SAR-439684 Heavy Chain (**VH in Bold**)		SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL SLSLGK	
Cemiplimab, Cemiplimab-rwlc, LIBTAYO®, 6QVL057INT, H4H7798N, REGN-2810, SAR-439684 Light Chain (**VL in Bold**)	PD-1	**DIQMTQSPSSLSASVGDSITITCRASLSINTFLNWYQQKPGKAP NLLIYAASSLHGGVPSRFSGSGSGTDFTLTIRTLQPEDFATYYC QQSSNTPFTFGPGT**VVDFRRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (Disulfide bridge) H22-H96, H131-L214, H144-H200, H223-H'223, H226-H'226, H258-H318, H364-H422, H'22-H'96, H'131-L'214, H'114-H'200, H'258-H'318, H'364-H'422, L23-L88, L134-L194, L'23-L'88, L'134-L'194)	341
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 Heavy Chain (**VH in Bold**)	PD-1	**QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAP GQGLEWMGGINPSNGGTNFNEKFKNRVTLTTDSSTTTAYME LKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTV**SSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK	346

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 **Light Chain (VL in Bold)**	PD-1	**EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQK PGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDF AVYYCQHSRDLPLTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	347
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 **VH**	PD-1	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPG QGLEWMGGFPSNGGTNFNEKFKNRVTLTTDSSTTTAYMELKSLQ FDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSS (Disulfide bridge) H22-H96, H134-L218, H147-H203, H226-H'226, H229-H'229, H261-H321, H367-H425, H'22-H'96, H'134-L'218, H'147-H'203, H'261-H'321, H'367-H'425, L23-L92, L138-L198, L'23-L'92, L'138-L'198)	348
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 **VL**	PD-1	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPG QAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFAVYYC QHSRDLPLTFGGGTKVEIK	349

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 **VH**	PD-1	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGK GLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLR AEDTAVYYCATNDDYWGQGTLVTVSS	350
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 **VL**	PD-1	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSS NWPRTFGQGTKVEIK (Disulfide bridge) H22-H96, H127-L214, H140-H196, H219-H'219, H222-H'222, H254-H314, H360-H418, H'22-H'96, H'127-L'214, H'140-H'196, H'254-H'314, H'360-H'418, L-23-L88, L134-L194, L'23-L'88, L'134-L'194)	351
Prolgolimab, Forteca, BCD-100 Heavy Chain (**VH in Bold**)	PD-1	**QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQVP GKGLEWVSAIDTGGGRTYYADSVKGRFAISRVNAKNTMYLQ MNSLRAEDTAVYYCARDEGGGTGWGVLKDWPYGLDAWGQ GTLVTV**SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK	352
Prolgolimab, Forteca, BCD-100	PD-1	**QPVLTQPLSVSVALGQTARITCGGNNIGSKNVHWYQQKPGQ APVLVIYRDSNRPSGIPERFSGSNSGNTATLTISRAQAGDEADY YCQVWDSSTAVFGTGTKLTV**LQRTVAAPSVFIFPPSDEQLKSGT	353

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Light Chain (**VL in Bold**)		ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	
Balstilimab, 1Q2QT5M7EO, AGEN-2034, AGEN-2034w Heavy Chain (**VH in Bold**)	PD-1	**QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCASNGDHWGQGTLVTV**SSASTKGPSVFPL APCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESK YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL SLG	354
Balstilimab, 1Q2QT5M7EO, AGEN-2034, AGEN-2034w Light Chain (**VL in Bold**)	PD-1	**EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQ APRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYY CQQYNNWPRTFGQGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	355
Dostarlimab, ANB-011, GSK-4057190A, P0GVQ9A4S5, TSR-042, WBP-285 Heavy	PD-1	**EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPG KGLEWVSTISGGGSYTYYQDSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCASPYYAMDYWGQGTTVTV**SSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVES KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL	356

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Chain (**VH in Bold**)		DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS LSLGK	
Dostarlimab, ANB-011, GSK-4057190A, P0GVQ9A4 S5, TSR-042, WBP-285 Light Chain (**VL in Bold**)	PD-1	**DIQLTQSPSFLSAYVGDRVTITCKASQDVGTAVAWYQQKPGK APKLLIYWASTLHTGVPSRFSGSGSGTEFTLTISSLQPEDFATY YCQHYSSYPWTFGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	357
Serplulimab, HLX-10 Heavy Chain (**VH in Bold**)	PD-1	**QVQLVESGGGLVKPGGSLRLSCAASGFTFSNYGMSWIRQAPG KGLEWSTISGGGSNIYYADSVKGRFTISRDNAKNSLYLQMNSL RAEDT**AVYYCVSYYYGIDFWGQGTSVTVSSASKYGPSVFPLAPC SRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGP PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL GK	358
Serplulimab, HLX-10 Light Chain (**VL in Bold**)	PD-1	**DIQMTQSPSSLSASVGDRVTITCKASQDVTTAVAWYQQKPGK APKLLIYWASTRHTGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHYTIPWTFGGGTKLEIKR**TVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	359
Retifanlimab,	PD-1	**QVQLVQSGAEVKKPGASVKVSCKASGYSFTSYWMNWVRQAP GQGLEWIGVIHPSDSETWLDQKFKDRVTITVDKSTSTAYMEL**	360

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
2Y3T5IF01Z, INCMGA-00012, INCMGA-0012, MGA-012 **Heavy Chain (VH in Bold)**		**SSLRSEDTAVYYCAREHYGTSPFAYWGQGTLVTV**SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG	
Retifanlimab, 2Y3T5IF01Z, INCMGA-00012, INCMGA-0012, MGA-012 Light Chain **(VL in Bold)**	PD-1	**EIVLTQSPATLSLSPGERATLSCRASESVDNYGMSFMNWFQQKPGQPPKLLIHAASNQGSGVPSRFSGSGSGTDFTLTISSLEPEDFAVYFCQQSKEVPYTFGGGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	361
Sasanlimab, LZZ0IC2EWP, PF-06801591, RN-888 Heavy Chain **(VH in Bold)**	PD-1	**QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWINWVRQAPGQGLEWMGNIYPGSSLTNYNEKFKNRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSTGTFAYWGQGTLVTV**SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	362

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Sasanlimab, LZZ0IC2EWP, PF-06801591, RN-888 **Light Chain (VL in Bold)**	PD-1	**DIVMTQSPDSLAVSLGERATINCKSSQSLWDSGNQKNFLTWY QQKPGQPPKLLIYWTSYRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQNDYFYPHTFGGGTKVEIK**RTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	363
Spartalizumab, NVP-LZV-184, PDR-001, QOG25L6Z8Z Heavy Chain (**VH in Bold**)	PD-1	**EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMHWVRQAT GQGLEWMGNIYPGTGGSNFDEKFKNRVTITADKSTSTAYME LSSLRSEDTAVYYCTRWTTGTGAYWGQGTTVTV**SSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLG	364
Spartalizumab, NVP-LZV-184, PDR-001, QOG25L6Z8Z Light Chain (**VL in Bold**)	PD-1	**EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQKNFLTWYQ QKPGQAPRLLIYWASTRESGVPSRFSGSGSGTDFTFTISSLEAE DAATYYCQNDYSYPYTFGQGTKVEIK**RTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C	365
Cetrelimab, JNJ-3283, JNJ-63723283, LYK98WP9	PD-1	**QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFDTANYAQKFQGRVTITADESTSTAYMELSS LRSEDTAVYYCARPGLAAAYDTGSLDYWGQGTLVTV**SSASTK GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV	366

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
1F Heavy Chain (**VH in Bold**)		DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSLGK	
Cetrelimab, JNJ-3283, JNJ-63723283, LYK98WP9 1F Light Chain (**VL in Bold**)	PD-1	**EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPGQA PRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQRNYWPLTFGQGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	367
Tebotelimab, MGD-013 **Heavy Chain (VL in Bold)**	PD-1	**DIQMTQSPSSLSASVGDRVTITCRASQDVSSVVAWYQQKPGK APKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHYSTPWTFGGGTKLEIK**GGGSGGGGQVQLVQSGAEVKK PGASVKVSCKASGYSFTSYWMNWVRQAPGQGLEWIGVIHPSDSE TWLDQKFKDRVTITVDKSTSTAYMELSSLRSEDTAVYYCAREHY GTSPFAYWGQGTLVTVSSGGCGGGEVAACEKEVAALEKEVAAL EKEVAALEKESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLYITR EPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLG	368
Tebotelimab, MGD-013 Light Chain (**VL in Bold**)	PD-1	**EIVLTQSPATLSLSPGERATLSCRASESVDNYGMSFMNWFQQ KPGQPPKLLIHAASNQGSGVPSRFSGSGSGTDFTLTISSLEPED FAVYFCQQSKEVPYTFGGGTKVEIK**GGGSGGGGQVQLVQSGA EVKKPGASVKVSCKASGYTFTDYNMDWVRQAPGQGLEWMGDI	369

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
		NPDNGVTIYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYY CAREADYFYFDYWGQGTTLTVSSGGCGGGKVAACKEKVAALKE KVAALKEKVAALKE	
Pidilizumab, CT-011, MDV-9300 Heavy Chain (**VH in Bold**)	PD-1	**QVQLVQSGSELKKPGASVKISCKASGYTFTNYGMNWVRQAP GQGLQWMGWINTDSGESTYAEEFKGRFVFSLDTSVNTAYLQI TSLTAEDTGMYFCVRVGYDALDYWGQGTLVTV**SSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	370
Pidilizumab, CT-011, MDV-9300 Light Chain (**VL in Bold**)	PD-1	**EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWFQQKPGKAP KLWIYRTSNLASGVPSRFSGSGSGTSYCLTINSLQPEDFATYYC QQRSSFPLTFGGGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	371
SG-001 **VH**	PD-1	QVQLVESGGGVVQPGRSLRLTCKASGLTFSSSGMHWVRQAPGK GLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLR AEDTAVYYCATNNDYWGQGTLVTVSS	372
SG-001 **VL**	PD-1	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPR LLIYTASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYS NWPRTFGQGTKVEIK	373
mpLZM-009 **VH** (Murine Precursor of LZM-009)	PD-1	EVQLQQSGPVLVKPGASVKMSCKASGYTFTSYYMYWVKQSHGK SLEWIGGVNPSNGGTNFNEKFKSKATLTVDKSSSTAYMELNSLTS EDSAVYYCARRDYRYDMGFDYWGQGTTLTVSS	374

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
mpLZM-009 **VL** (Murine Precursor of LZM-009)	PD-1	QIVLTQSPAIMSASPGEKVTMTCRASKGVSTSGYSYLHWYQQKPGSSPRLLIYLASYLESGVPVRFSGSGSGTSYSLTISRMEAEDAATYYCQHSRELPLTFGTGTRLEIK	375
LZM-009 **VH**	PD-1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWMGGVNPSNGGTNFNEKFKSRVTITADKSTSTAYMELSSLRSEDTAVYYCARRDYRYDMGFDYWGQGTTVTVSS	376
LZM-009 **VL**	PD-1	EIVLTQSPATLSLSPGERATISCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARFSGSGSGTDFTLTISSLEPEDFATYYCQHSRELPLTFGTGTKVEIK	377
Budigalimab, 6VDO4TY3OO, ABBV-181, PR-1648817 Heavy Chain (**VH in Bold**)	PD-1	**EIQLVQSGAEVKKPGSSVKVSCKASGYTFTHYGMNWVRQAPGQGLEWVGWVNTYTGEPTYADDFKGRLTFTLDTSTSTAYMELSSLRSEDTAVYYCTREGEGLGFGDWGQGTTVTV**SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	378
Budigalimab, 6VDO4TY3OO, ABBV-181, PR-1648817 Light Chain (**VL in Bold**)	PD-1	**DVVMTQSPLSLPVTPGEPASISCRSSQSIVHSHGDTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPVTFGQGTKLEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	379

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 VH CDR1	PD-1	NYYMY	380
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 VH CDR2	PD-1	GINPSNGGTNFNEKFKN	381
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 VH CDR3	PD-1	RDYRFDMGFDY	382
Lambrolizumab,	PD-1	RASKGVSTSGYSYLH	383

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 VL CDR1			
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 VL CDR2	PD-1	LASYLES	384
Lambrolizumab, Pembrolizumab, KEYTRUDA®, MK-3475, SCH-900475, h409A11 VL CDR3	PD-1	QHSRDLPLT	385
Nivolumab, Nivolumab BMS, OPDIVO®,	PD-1	NSGMH	386

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
BMS-936558, MDX-1106, ONO-4538 VH CDR1			
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 VH CDR2	PD-1	VIWYDGSKRYYADSVKG	387
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 VH CDR3	PD-1	NDDY	388
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 VL CDR1	PD-1	RASQSVSSYLA	389

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 VL CDR2	PD-1	DASNRAT	390
Nivolumab, Nivolumab BMS, OPDIVO®, BMS-936558, MDX-1106, ONO-4538 VL CDR3	PD-1	QQSSNWPRT	391
Serplulimab, HLX-10 VH CDR1	PD-1	FTFSNYGMS	392
Serplulimab, HLX-10 VH CDR2	PD-1	TISGGGSNIY	393
Serplulimab, HLX-10 VH CDR3	PD-1	VSYYYGIDF	394
Serplulimab, HLX-10 VL CDR1	PD-1	KASQDVTTAVA	395

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
Serplulimab, HLX-10 VL CDR2	PD-1	WASTRHT	396
Serplulimab, HLX-10 VL CDR3	PD-1	QQHYTIPWT	397
SG-001 VH CDR1	PD-1	GLTFSSSG	398
SG-001 VH CDR2	PD-1	IWYDGSKR	399
SG-001 VH CDR3	PD-1	ATNNDY	400
SG-001 VL CDR1	PD-1	RASQSVSSYLA	401
SG-001 VL CDR2	PD-1	TASNRAT	402
SG-001 VL CDR3	PD-1	QQYSNWPRT	403
PD-1-Fc-OX40L (Code), SL-279252 (Code), TAK-252 (Code)	PD-1	MQIPQAPWPWWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVV TEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQP GQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQ IKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQSKYGPPCPSCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCWVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLSGKEYK CKVSSKGLPSSIEKTISNATGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT VDKSSWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKIEGRMDQ VSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINC DGFYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSLMVAS LTYKDKVYLNVTTDNTSLDDFHVNGGELILIHQNPGEFCVLMQIP QAPWPWWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGD NATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDC	404

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
		RFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL RAELRVTERRAEVPTAHPSPSPRPAGQFQQVSHRYPRIQSIKVQFT EYKKEKGFILTSQKEDEIMKVQNNSVIINCDGFYLISLKGYFSQEV NISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVTTDN TSLDDFHVNGGELILIHQNPGEFCVL	
Avelumab (Generic) Bavencio (Brand) 451238 KXG2PJ551 I MSB-0010682 MSB-0010718C PF-06834635 Heavy Chain (**VH in Bold**)	PD-L1	**EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPG KGLEWVSSIYPSGGITFYADTVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARIKLGTVTTVDYWGQGTLVTV**SSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	232
Avelumab (Generic) Bavencio (Brand) 451238 KXG2PJ551 I MSB-0010682 MSB-0010718C	PD-L1	**QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPG KAPKLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEA DYYCSSYTSSSTRVFGTGTKVTV**LGQPKANPTVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	233

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
PF-06834635 Light Chain **(VL in Bold)**			
Durvalumab (Generic) Imfinzi (Brand) 28X28X9OKV (UNII code) MEDI-4736 Heavy Chain **(VH in Bold)**	PD-L1	**EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLVTV**SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	234
Durvalumab (Generic) Imfinzi (Brand) 28X28X9OKV (UNII code) MEDI-4736 Light Chain **(VL in Bold)**	PD-L1	**EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	235
Atezolizumab (Generic) Tecentriq (Brand) 52CMI0WC	PD-L1	**EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTV**SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK	236

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
3Y MPDL-3280A RG-7446 RO-5541267 Heavy Chain (**VH in Bold**)		VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	
Atezolizumab (Generic) Tecentriq (Brand) 52CMI0WC 3Y MPDL-3280A RG-7446 RO-5541267 Light Chain (**VL in Bold**)	PD-L1	**DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGK APKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQYLYHPATFGQGTKVEIK**RTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	237
Sugemalimab (Generic) CS-1001 WBP-3155 Heavy Chain (**VH in Bold**)	PD-L1	**EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSGISGSGGFTYYADSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCAKPPRGYNYGPFDYWGQGTLVTV**SSASTKG PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK	238

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
		TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSLGK	
Sugemalimab (Generic) CS-1001 WBP-3155 **Light Chain (VL in Bold)**	PD-L1	**SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQA PVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYY CQVWDSSSDHVVFGGGTKLTV**LGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	239
JS-003 JS003-30 JS003-SD **VH**	PD-L1	QGQLQESGPSLVKPSQTLSLTCTVSGDSITRGYWNWIRKHPGKGL EYIGYISYTGSTYSNLSLKSRVTISRDTSKNQYYLKLSSVTAADTA VYYCATSTGWLDPVDYWGQGTLVTVSS	242
JS-003 JS003-30 JS003-SD **VL**	PD-L1	DIVMTQSPDSLAVSLGERATINCKASQNVDTSVAWFQQKPGQPP KALIYSASFRYSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQ YYGYPFTFGQGTKLEIK	243
HLX-20 PL2#3 **VH**	PD-L1	EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYTMNWVRQAPGK GLEWVSSISSGSDYLYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARNELRWYPQAGAFDRWGQGTMVTVSS	244
HLX-20 PL2#3 **VL**	PD-L1	QSVVTQPPSMSAAPGQRVTISCSGSSSYIESSYVGWYQQLPGTAP RLLIYDDDMRPSGIPDRFSGSKSGTSATLAITGLQTGDEADYYCEI WRSGLGGVFGGGTKLTVL	245
Lodapolimab (Generic) LY-3300054 NR4MAD6 PPB Heavy Chain (**VH in Bold**)	PD-L1	**QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSS LRSEDTAVYYCARSPDYSPYYYYGMDVWGQGTTVTV**SSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN	248

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
		NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	
Lodapolimab (Generic) LY-3300054 NR4MAD6 PPB Light Chain (**VL in Bold**)	PD-L1	**QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGT APKLLIYGNSNRPSGVPDRFSGSKSGTSASLAISGLQSEDEADY YCQSYDSSLSGSVFGGGIKLTV**LGQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECS	249
HLX-20 PL2#3 VH CDR1	PD-L1	SYTMN	250
HLX-20 PL2#3 VH CDR2	PD-L1	SISSGSDYLYYADSVKG	251
HLX-20 PL2#3 VH CDR3	PD-L1	NELRWYPQAGAFDR	252
HLX-20 PL2#3 VL CDR1	PD-L1	SGSSSYIESSYVG	253
HLX-20 PL2#3 VL CDR2	PD-L1	DDDMRPS	254
HLX-20 PL2#3 VL CDR3	PD-L1	EIWRSGLGGV	255
Envafolimab (Generic) 3D-025 ASC-22 KN-035	PD-L1	**QVQLVESGGGLVQPGGSLRLSCAASGKMSSRRCMAWFRQAP GKERERVAKLLTTSGSTYLADSVKGRFTISRDNSKNTVYLQM NSLRAEDTAVYYCAADSFEDPTCTLVTSSGAFQYWGQGTLVT** VSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV	256

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
hu56V1-Fc-m1 single-domain antibody **(VH in Bold)**		VSVLTVLHQDWLNGKEYKCKVSNKALPAGIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	
ND-021 NM21-1480 PRO-1480 Tri-specific fusion single-chain antibody construct	PD-L1	DIQMTQSPASLSASVGDRVTITCQASQSIGTYLAWYQQKPGKPPK LLIYRAFILASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNF YSDSTTIGPNAFGTGTKVTVLGGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFSFSANYYPCWVRQAPGKGLEWIGCIYGGSSDITYD ANWTKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARSAWYS GWGGDLWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSIQMTQ SPSSLSASVGDRVTITCQASQSISNRLAWYQQKPGKAPKLLIYSAS TLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSTYYGNDGN AFGTGTKVTVLGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF SFNSDYWIYWVRQAPGKGLEWIASIYGGSSGNTQYASWAQGRFT ISRDNSKNTVYLQMNSLRAEDTAVYFCARGYVDYGGATDLWGQ GTLVTVSSGGGGSGGGGSIQMTQSPSSLSASVGDRVTITCQSSESV YSNNQLSWYQQKPGQPPKLLIYDASDLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCAGGFSSSSDTAFGGGTKLTVLGGGGGSGG GGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSLSS NAMGWVRQAPGKGLEYIGIISVGGFTYYASWAKGRFTISRDNSK NTVYLQMNSLRAEDTATYFCARDRHGGDSSGAFYLWGQGTLVT VSS	257
ACE-05 YBL-013 Bispecific tetrameric antibody-like cell engager (ALiCE)	PD-L1	QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQG LEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSED TAVYYCAKPRDGYNLVAFDIWGQGTMVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPGGGGSEVQLQQSGPELVKPGPSMKIS CKASGYSFTGYTMNWVKQSHGKNLEWMGLINPYKGVSTYNQK FKDKATLTVDKSSSTAYMELLSLTSEDSAVYYCARSGYYGDSDW	258

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
comprising two identical light chains (LC) consisting of antigen binding domains (ABDs) targeting programmed cell death-ligand 1 (PD-L1), and two different heavy chain (HC)-like chains (ACE-05-VH and ACE-05-VL) each consisting of an anti-PD-L1 ABD and an anti-CD3 ABD ; wherein each HC comprises a G4S linker		YFDVWGQGTTLTVFSQMQLVQSGAEVKKPGSSVKVSCKASGGT FSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRVTITAD KSTSTAYMELSSLRSEDTAVYYCAKPRDGYNLVAFDIWGQGTM VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPGGGGSDIQ MTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKPDGTVKLLI YYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PWTFAGGTKLEIKRQLVLTQPPSVSGAPGQRVTISCTGSSSNIGAG YDVHWYQQLPGAAPKLLIYGDINRPSGVPDRFSGSKSGISASLAIT GLQAEDEADYYCQSYDSSLSGGVFGGGTKLTVLRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC	

Antibody or Ag-binding fragment	Antigen Bound	Sequence	SEQ ID NO
(SEQ ID NO: 405) between the hinge region and the second ABD			

Modification to Fc region

[0156] Disclosed herein are antibodies or antigen binding fragments thereof that comprise an Fc region, wherein the Fc region comprises at least one covalently linked linker. In some embodiments, the linker is a chemical linker. In some embodiments, the chemical linker is covalently attached to a tyrosine, aspartic acid, glutamic acid, arginine, histidine, or lysine residue. In some embodiments, the chemical linker is covalently attached to a lysine, cysteine, or tyrosine residue. In some embodiments, the chemical linker is covalently attached to a cysteine residue. In some embodiments, the chemical linker is covalently attached to a lysine residue. In some embodiments, the chemical linker is covalently attached to a constant region of the antibody.

[0157] In some embodiments, the antibody comprises an Fc region. In some embodiments, the Fc region is an IgG Fc region, an IgA Fc region, an IgD Fc region, an IgM Fc region, or an IgE Fc region. In some embodiments, the Fc region is an IgG Fc region, an IgA Fc region, or an IgD Fc region. In some embodiments, the Fc region is a human Fc region. In some embodiments, the Fc region is a humanized. Fc region. In some embodiments, the Fc region is an IgG Fc region. In some instances, an IgG Fc region is an IgG1 Fc region, an IgG2a Fc region, or an IgG4 Fc region.

[0158] One or more mutations may be introduced in an Fc region to reduce Fc-mediated effector functions of an antibody or antigen-binding fragment such as, for example, antibody-dependent cellular cytotoxicity (ADCC) and/or complement function. In some instances, a modified Fc comprises a humanized IgG4 kappa isotype that contains a S229P Fc mutation. In some instances, a modified Fc comprises a human IgG1 kappa where the heavy chain CH2 domain is engineered

with a triple mutation such as, for example: (a) L238P, L239E, and P335S; or (2) K248; K288; and K317.

[0159] In some embodiments, the Fc region has an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence as set forth in SEQ ID NO: 260 (Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Xaa Xaa Gly Xaa Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asp Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Xaa Glu Xaa Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Xaa Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly, where Xaa can be any naturally occurring amino acid). In some embodiments, the Fc region comprises one or more mutations which make the Fc region susceptible to modification or conjugation at a particular residue, such as by incorporation of a cysteine residue at a position which does not contain a cysteine in SEQ ID NO: 260. Alternatively, the Fc region could be modified to incorporate a modified natural amino acid or an unnatural amino acid which comprises a conjugation handle, such as one connected to the modified natural amino acid or unnatural amino acid through a linker. In some embodiments, the Fc region does not comprise any mutations which facilitate the attachment of a linker to an additional cytokine (*e.g.,* an IL-18 polypeptide). In some embodiments, the chemical linker is attached to a native residue as set forth in SEQ ID NO: 260. In some embodiments, the chemical linker is attached to a native lysine residue of SEQ ID NO: 260.

[0160] In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at one of positions 10-90 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at one of positions 10-20, 10-30, 10-40, 10-50, 10-60, 10-70, 1-80, 10-90, 10-100, 10-110, 10-120, 10-130, 10-140, 10-150, 10-160, 10-170, 10-180, 10-190, or 10-200 of SEQ ID NO: 260. In some embodiments, the chemical linker is

attached to the Fc region at an amino acid residue at one of positions 10-30, 50-70, or 80-100 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at any one of positions 20-40, 65-85, or 90-110 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at one of positions 15-26, 55-65, or 85-90 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at any one of positions 25-35, 70-80, or 95-105 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at any one of positions 30, 32, 72, 74, or 101 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at an amino acid residue at any one of positions K30, K32, K72, K74, or K101 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at amino acid residue 30 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at amino acid residue 32 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at amino acid residue 72 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at amino acid residue 74 of SEQ ID NO: 260. In some embodiments, the chemical linker is attached to the Fc region at amino acid residue 101 of SEQ ID NO: 260.

[0161] The chemical linker can be covalently attached to one amino acid residue of an Fc region of the antibody. In some embodiments, the chemical linker is covalently attached to a non-terminal residue of the Fc region. In some embodiments, the non-terminal residue is in the CH1, CH2, or CH3 region of the antibody. In some embodiments, the non-terminal residue is in the CH2 region of the antibody.

[0162] In some embodiments, the chemical linker is covalently attached at an amino acid residue of the antibody or antigen binding fragment which selectively binds a immune associated antigen (*e.g.,* an anti-PD-1 antibody) such that the function of the antibody or antigen binding fragment is maintained (*e.g.,* without denaturing the polypeptide). For example, when the antibody or antigen binding fragment is a human IgG (*e.g.,* human IgG1), exposed lysine residues and exposed tyrosine residues are present at the following positions (refer to web site www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html by EU numbering). Exemplary exposed Lysine Residues: CH2 domain (position 246, position 248, position 274, position 288, position 290, position 317, position 320, position 322, and position 338) CH3 domain

(position 360, position 414, and position 439). Exemplary exposed Tyrosine Residues: CH2 domain (position 278, position 296, and position 300) CH3 domain (position 436).

[0163] The human IgG, such as human IgG1, may also be modified with a lysine or tyrosine residue at any one of the positions listed above in order provide a residue which is ideally surface exposed for subsequent modification.

[0164] In some embodiments, the chemical linker is covalently attached at an amino acid residue in the constant region of an antibody. In some embodiments, the chemical linker is covalently attached at an amino acid residue in the CH1, CH2, or CH3 region. In some embodiments, the chemical inker is covalently attached at an amino acid residue in the CH2 region. In some embodiments, the chemical linker may be covalently attached to one amino acid residue in the following groups of residues following EU numbering in human IgG Fc: amino acid residues 1-478, amino acid residues 2-478, amino acid residues 1-477, amino acid residues 2-477, amino acid residues 10-467, amino acid residues 30-447, amino acid residues 50-427, amino acid residues 100-377, amino acid residues 150-327, amino acid residues 200-327, amino acid residues 240-327, and amino acid residues 240-320.

[0165] In some embodiments, the chemical linker is covalently attached to one lysine residue of a human IgG Fc region. In some embodiments, the chemical linker is covalently attached at Lys 246, Lys 248, Lys 288, Lys 290, or Lys 317 of the Fc region (EU numbering). In some embodiments, the chemical linker is covalently attached at Lys 246 of an Fc region of the antibody, wherein amino acid residue position number is based on EU numbering. In some embodiments, the chemical linker is covalently attached at Lys 248 of an Fc region of the antibody, wherein amino acid residue position number is based on EU numbering. In some embodiments, the chemical linker is covalently attached at Lys 288 of an Fc region of the antibody, wherein amino acid residue position number is based on EU numbering. In some embodiments, the chemical linker is covalently attached at Lys 290 of an Fc region of the antibody, wherein amino acid residue position number is based on EU numbering. In some embodiments, the chemical linker is covalently attached at Lys 317 of the antibody, wherein amino acid residue position number is based on EU numbering.

[0166] The chemical linker can be covalently attached to an amino acid residue selected from a subset of amino acid residues. In some embodiments, the subset comprises two three, four, five,

six, seven, eight, nine, or ten amino acid residues of an Fc region of the antibody. The chemical linker can be covalently attached to one of two lysine residues of an Fc region of the antibody.

[0167] In some embodiments, the antibody will comprise two linkers covalently attached to the Fc region of the antibody. In some embodiments, each of the two linkers will be covalently attached to a different heavy chain of the antibody. In some embodiments, each of the two linkers will be covalently attached to a different heavy chain of the antibody at a residue position which is the same. In some embodiments, each of the two linkers will be covalently attached to a different heavy chain of antibody at a residue position which is different. When the two linkers are covalently attached to residue positions which differ, any combination of the residue positions provided herein may be used in combination.

Method of Modifying an Fc Region

[0168] Also provided herein are method of preparing a modified Fc region of an antibody or antigen binding fragment, such as for the attachment of a linker, a conjugation handle, the IL-18 polypeptide, or any combination thereof to the antibody or antigen binding fragment. A variety of methods for site-specific modification of Fc regions of antibodies are known in the art.

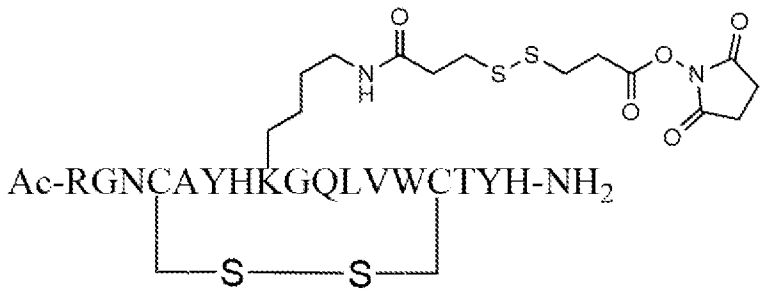
Modification with an affinity peptide configured to site-specifically attach linker to the antibody

[0169] In some embodiments, an Fc region is modified to incorporate a linker, a conjugation handle, or a combination thereof. In some embodiments, the modification is performed by contacting the Fc region with an affinity peptide bearing a payload configured to attach a linker or other group to the Fc region, such as at a specific residue of the Fc region. In some embodiments, the linker is attached using a reactive group which forms a bond with a residue of the Fc region. In some embodiments, the affinity peptide comprises a cleavable linker. The cleavable linker is configured on the affinity peptide such that after the linker or other group is attached to the Fc region, the affinity peptide can be removed, leaving behind only the desired linker or other group attached to the Fc region. The linker or other group can then be used further to add attach additional groups, such as a cytokine or a linker attached to a cytokine, to the Fc region.

[0170] Non-limiting examples of such affinity peptides can be found at least in PCT Publication No. WO2018199337A1, PCT Publication No. WO2019240288A1, PCT Publication No. WO2019240287A1, and PCT Publication No. WO2020090979A1, each of which is incorporated by reference as if set forth herein in its entirety. In some embodiments, the affinity peptide is a peptide which has been modified to deliver the linker/conjugation handle payload one or more

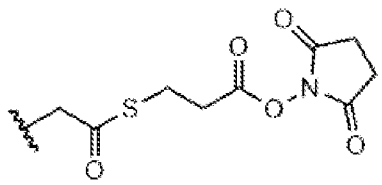
specific residues of the Fc region of the antibody. In some embodiments, the affinity peptide has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identify to a peptide selected from (1) QETNPTENLYFQQKNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDDC (SEQ ID NO: 261); (2) QTADNQKNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDCSQSANLLAEAQQLNDAQA PQA (SEQ ID NO: 262); (3) QETKNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDDC (SEQ ID NO:263); (4) QETFNKQCQRRFYEALHDPNLNEEQRNARIRSIRDDDC (SEQ ID NO: 264); (5) QETFNMQCQRRFYEALHDPNLNKEQRNARIRSIRDDDC (SEQ ID NO: 265); (6) QETFNMQCQRRFYEALHDPNLNEEQRNARIRSIKDDC (SEQ ID NO: 266); (7) QETMQCQRRFYEALHDPNLNEEQRNARIRSIKDDC (SEQ ID NO: 267); (8) QETQCQRRFYEALHDPNLNEEQRNARIRSIKDDC (SEQ ID NO: 268); (9) QETCQRRFYEALHDPNLNEEQRNARIRSIKDDC (SEQ ID NO: 269); (10) QETRGNCAYHKGQLVWCTYH (SEQ ID NO: 270); and (11) QETRGNCAYHKGQIIWCTYH (SEQ ID NO: 271), or a corresponding peptide which has been truncated at the N-terminus by one, two, three, four, or five residues.

[0171] An exemplary affinity peptide with cleavable linker and conjugation handle payload capable of attaching the payload to residue K248 of an antibody as provided herein is shown below (SEQ ID NO: 406) (as reported in Matsuda *et al.*, "Chemical Site-Specific Conjugation Platform to Improve the Pharmacokinetics and Therapeutic Index of Antibody-Drug Conjugates," *Mol. Pharmaceutics* 2021, 18, 11, 4058-4066.

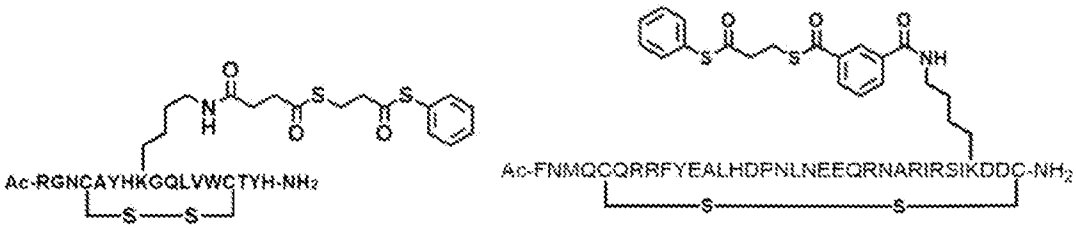


[0172] Alternative affinity peptides targeting alternative residues of the Fc region are described in the references cited above for AJICAP™ technology, and such affinity peptides can be used to attach the desired functionality to an alternative residue of the Fc region (e.g., K246, K288, etc.).

For example, the disulfide group of the above affinity peptide could instead be replaced with a thioester to provide a sulfhydryl protecting group as a cleavable portion of the linking group (e.g., the relevant portion of the affinity peptide would have a structure of



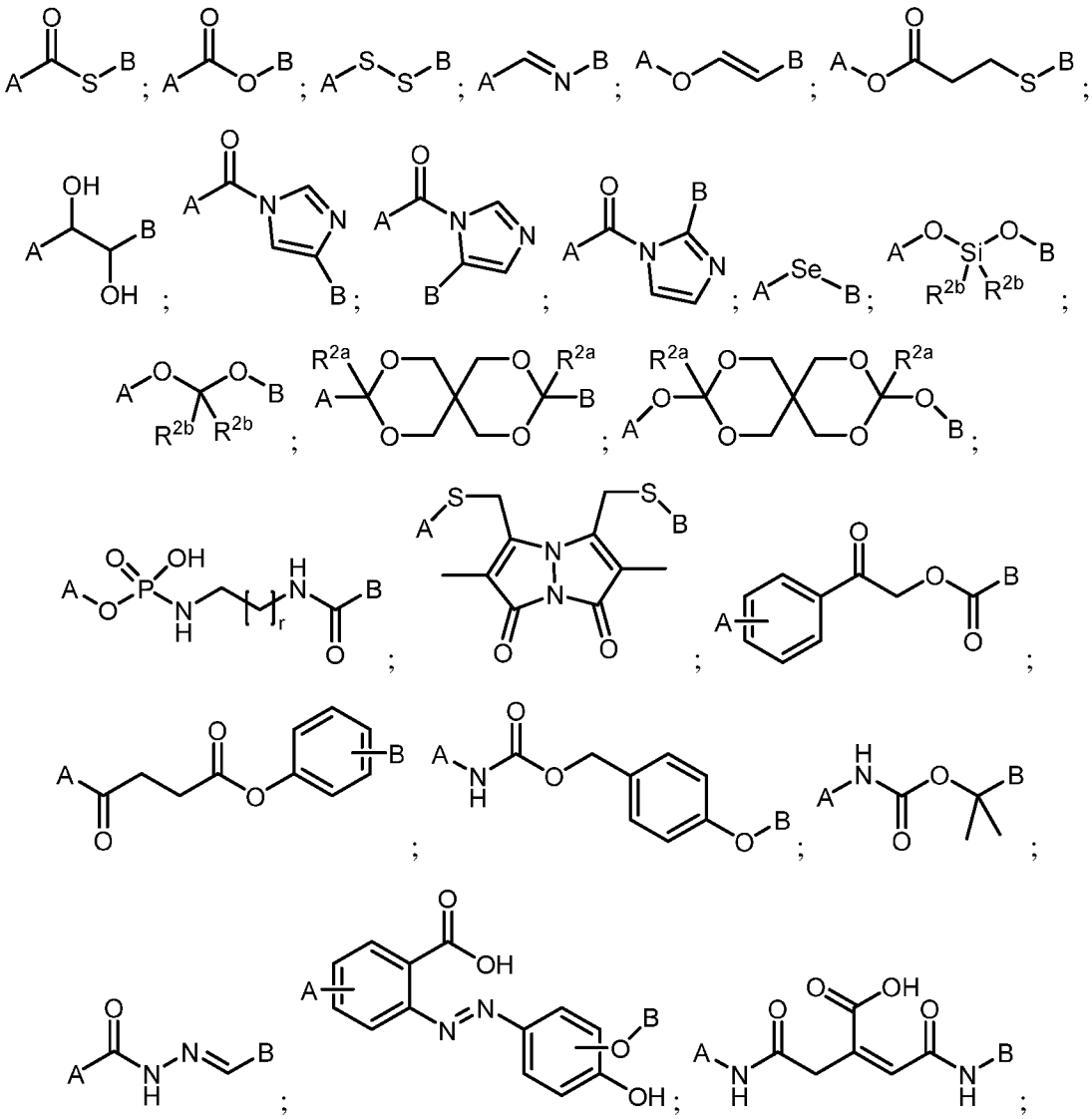
, or another of the cleavable linkers discussed below). Such alternative affinity peptides include those described in, for example "AJICAP Second Generation: Improved Chemical Site-Specific Conjugation Technology for Antibody-Drug Conjugation Technology for Antibody-Drug Conjugate Production" (Working Paper, Fujii et al., DOI: 10.26434/chemrxiv-2023-9p5p7, chemrxiv.org/engage/chemrxiv/article-details/63d5f7131125965a9e7df8a5 (Accessed 20 February 2023, Version 1 published 30 Jan 2023)). Exemplary affinity peptides provided therein include those shown below, wherein the left structure (SEQ ID NO: 407) targets K248 of the Fc region and the right structure (SEQ ID NO: 408) targets K288 of the Fc region (EU numbering).

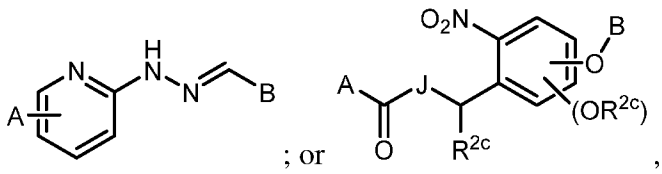


[0173] The affinity peptide of the disclosure can comprise a cleavable linker. In some embodiments, the cleavable linker of the affinity peptide connects the affinity peptide to the group which is to be attached to the Fc region and is configured such that the peptide can be cleaved after the group comprising the linker or conjugation handle has been attached. In some embodiments, the cleavable linker is a divalent group. In some embodiments, the cleavable linker can comprise a thioester group, an ester group, a sulfane group; a methanimine group; an oxyvinyl group; a thiopropanoate group; an ethane-1,2-diol group; an (imidazole-1-yl)methan-1-one group; a seleno ether group; a silylether group; a di-oxysilane group; an ether group; a di-oxymethane group; a tetraoxospiro[5.5]undecane group; an acetamidoethyl phosphoramidite group; a bis(methylthio)-pyrazolopyrazole-dione group; a 2-oxo-2-phenylethyl formate group; a 4-oxybenzylcarbamate

group; a 2-(4-hydroxy-oxyphenyl)diazinyl)benzoic acid group; a 4-amino-2-(2-amino-2-oxoethyl)-4-oxobut-2-enoic acid group; a 2-(2-methylenehydrazineyl)pyridine group; an N′-methyleneformohydrazide group; or an isopropylcarbamate group, any of which is unsubstituted or substituted. Composition and points of attachment of the cleavable linker to the affinity peptide, as well as related methods of use, are described in, at least, PCT Publication No. WO2018199337A1, PCT Publication No. WO2019240288A1, PCT Publication No. WO2019240287A1, and PCT Publication No. WO2020090979A1.

[0174] In some embodiments, the cleavable linker is:





wherein:

-one of A or B is a point of attachment the linker and the other of A or B is a point of attachment to the affinity peptide;

- each R^{2a} is independently H or optionally substituted alkyl;

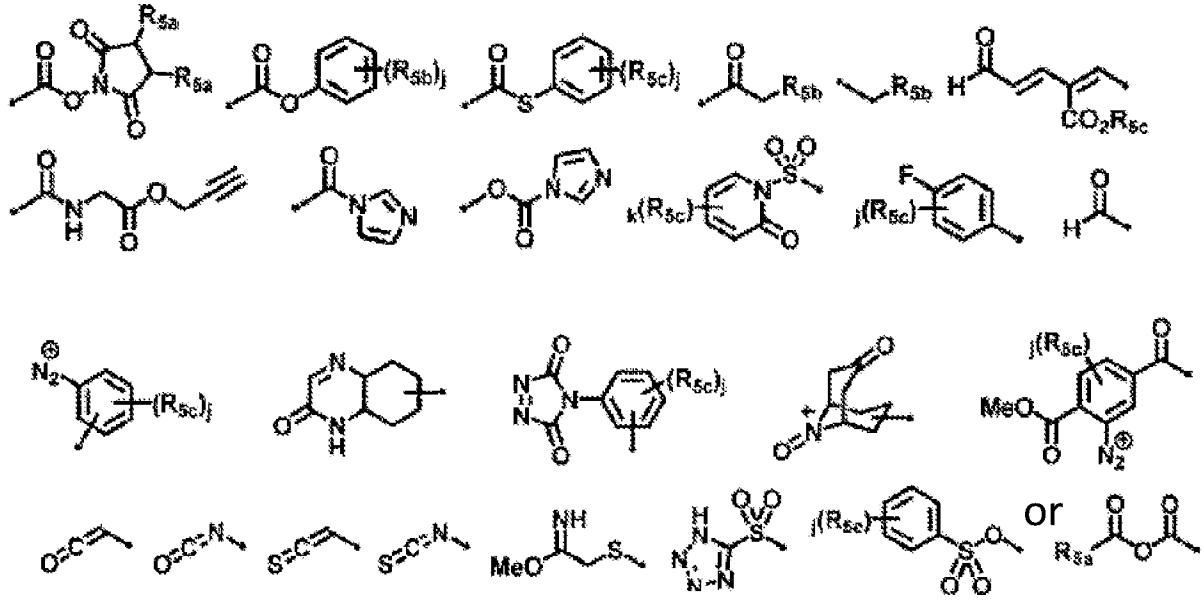
- each R^{2b} is independently H or optionally substituted alkyl;

- R^{2c} is a H or optionally substituted alkyl;

-J is a methyl, a N, a S, a Si, or an O atom; and

- r is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0175] The affinity peptide comprises a reactive group which is configured to enable the covalent attachment of the linker / conjugation handle to the Fc region. In some embodiments, the reactive group is selective for a functional group of a specific amino acid residue, such as a lysine residue, tyrosine residue, serine residue, cysteine residue, or an unnatural amino acid residue of the Fc region incorporated to facilitate the attachment of the linker. The reactive group may be any suitable functional group, such as an activated ester for reaction with a lysine (*e.g.,* N-hydroxysuccinimide ester or a derivate thereof, a pentafluorophenyl ester, *etc.*) or a sulfhydryl reactive group for reaction with a cysteine (*e.g.,* a Michael acceptor, such as an alpha-beta unsaturated carbonyl or a maleimide). In some embodiments, the reactive group is:



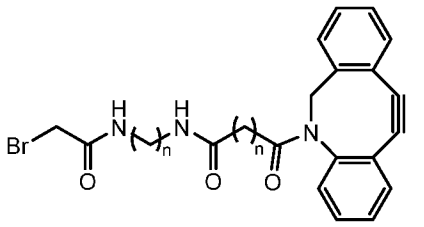
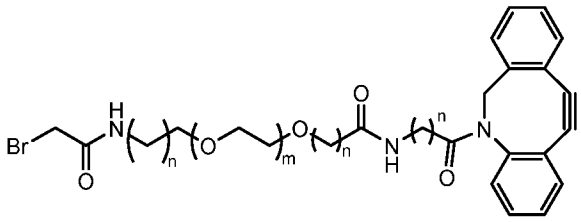
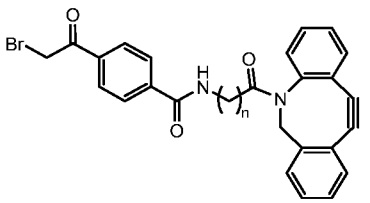
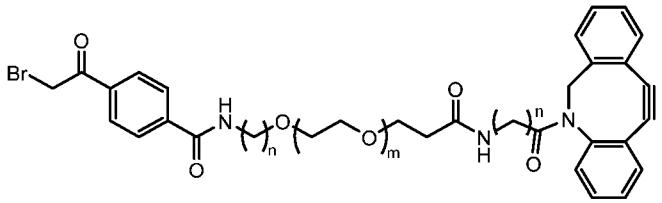
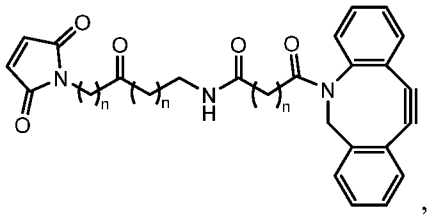
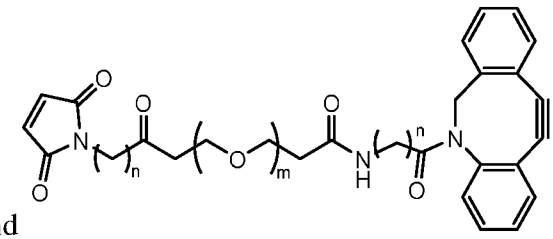
, wherein:

- each R_{5a}, R_{5b}, and R_{5c} is independently H, halogen, or optionally substituted alkyl;
- each j is 1, 2, 3, 4, or 5; and
- each k is 1, 2, 3, 4, or 5.

[0176] In some embodiments, the affinity peptide is used to deliver a reactive moiety to the desired amino acid residue such that the reactive moiety is exposed upon cleavage of the cleavable linker. By way of non-limiting example, the reactive group forms a covalent bond with a desired residue of the Fc region of the antibody or antigen binding fragment due to an interaction between the affinity peptide and the Fc region. Following this covalent bond formation, the cleavable linker is cleaved under appropriate conditions to reveal a reactive moiety (*e.g.,* if the cleavable linker comprises a thioester, a free sulfhydryl group is attached to the Fc region following cleavage of the cleavable linker). This new reactive moiety can then be used to subsequently add an additional moiety, such as a conjugation handle, by way of reagent comprising the conjugation handle tethered to a sulfhydryl reactive group (*e.g.,* alpha-halogenated carbonyl group, alpha-beta unsaturated carbonyl group, maleimide group, *etc.*).

[0177] In some embodiments, an affinity peptide is used to deliver a free sulfhydryl group to a lysine of the Fc region. In some embodiments, the free sulfhydryl group is then reacted with a bifunctional linking reagent to attach a new conjugation handle to the Fc region. In some embodiments, the new conjugation handle is then used to form the linker to the attached cytokine. In some embodiments, the new conjugation handle is an alkyne functional group. In some embodiments, the new conjugation handle is a DBCO functional group.

[0178] Exemplary bifunctional linking reagents useful for this purpose are of a formula A-B-C, wherein A is the sulfhydryl reactive conjugation handle (e.g., maleimide, α,β-unsaturated carbonyl, a-halogenated carbonyl), B is a linking group, and C is the new conjugation handle (e.g., an alkyne such as DBCO). Specific non-limiting examples of bifunctional linking reagents include


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, wherein each n is independently an integer from 1-6 and each m is independently an integer from 1-30, and related molecules (e.g., isomers).

[0179] Alternatively, the affinity peptide can be configured such that a conjugation handle is added to the Fc region (such as by a linker group) immediately after covalent bond formation between the reactive group and a residue of the Fc region. In such cases, the affinity peptide is cleaved and the conjugation handle is immediately ready for subsequent conjugation to the IL-18 polypeptide.

Alternative Methods of Modifying Fc Region

[0180] While the affinity peptide mediated modification of an Fc region of an antibody provide *supra* possesses many advantages over other methods which can be used to site-specifically modify the Fc region (*e.g.,* ease of use, ability to rapidly generate many different antibody conjugates, ability to use many "off-the-shelf" commercial antibodies without the need to do time consuming protein engineering, *etc.*), other methods of performing the modification are also contemplated as being within the scope of the present disclosure.

[0181] In some embodiments, an IL-18 polypeptide can be conjugated to a suitable antibody utilizing transglutaminase-mediated site-specific antibody-drug conjugate (ADC) strategies, such as those comprising: 1) glutamine-containing tags, endogenous glutamines (*e.g.*, native glutamines without engineering, such as glutamines in variable domains, CDRs, *etc.*), and/or endogenous glutamines made reactive by antibody engineering or an engineered transglutaminase; and 2)

amine donor agents comprising amine donor units, linkers, and agent moieties. Non-limiting examples of such transglutaminase mediated site-specific modifications can be found at least in publications PCT Publication No. WO2020188061, US Patent Publication No. US2019194641, US Patent Publication No. US2021128743, US Patent No. US9764038, and US Patent No. US10434180, which are incorporated by reference as if set forth herein in their entirety. Such strategies can be employed to either add a suitable conjugation handle to a desired site of the antibody or antigen binding fragment, or can alternatively be used to directly conjugate the IL-18 polypeptide to the antibody, though it is preferable that such a strategy is used to add a conjugation handle, then the IL-18 polypeptide is later reacted with the conjugation handle to form the immunocytokine.

[0182] In some embodiments, an IL-18 polypeptide can be conjugated to a suitable antibody utilizing a transpeptide-mediated strategy for either direct attachment of the IL-18 polypeptide to the antibody or via addition of a suitable conjugation handle to the antibody. For example, a sortase based system (such as those described in, for example, U.S. Patent No. 10,081,684, U.S. Patent No. 10,864,277, U.S. Patent No. 11,421,022, and/or U.S. Patent No. 9,862,779) can be used, either by addition of a sortase tag to the C-terminus of antibody heavy or light chain or though a suitable linker armed with a sortase tag, to add a linker with a conjugation handle to the antibody or antigen binding fragment thereof, followed by a subsequent conjugation of the IL-18 polypeptide to the antibody or antigen binding fragment thereof using the conjugation handle.

[0183] In another aspect, the disclosure provides methods of generating immunocytokines using an engineered Fc-containing polypeptide conjugate comprising the formula: (Fc-containing polypeptide-T-A), wherein T is an acyl donor glutamine-containing tag engineered at a specific site, wherein A is an amine donor agent, wherein the amine donor agent is site-specifically conjugated to the acyl donor glutamine-containing tag at a carboxyl terminus, an amino terminus, or at an another site in the Fc-containing polypeptide, wherein the acyl donor glutamine-containing tag comprises an amino acid sequence XXQX, wherein X is any amino acid (*e.g.*, X can be the same or different amino acid), and wherein the engineered Fc-containing polypeptide conjugate comprises an amino acid substitution from glutamine to asparagine at position 295 (Q295N; EU numbering scheme).

[0184] In some embodiments, the acyl donor glutamine-containing tag is not spatially adjacent to a reactive Lys (*e.g.*, the ability to form a covalent bond as an amine donor in the presence of an

acyl donor and a transglutaminase) in the polypeptide or the Fc-containing polypeptide. In some embodiments, the polypeptide or the Fc-containing polypeptide comprises an amino acid modification at the last amino acid position in the carboxyl terminus relative to a wild-type polypeptide at the same position. The amino acid modification can be an amino acid deletion, insertion, substitution, mutation, or any combination thereof.

[0185] In some embodiments, the immunocytokine composition comprises a full length antibody heavy chain and an antibody light chain, wherein the acyl donor glutamine-containing tag is located at the carboxyl terminus of a heavy chain, a light chain, or both the heavy chain and the light chain.

[0186] In some embodiments, the immunocytokine composition comprises an antibody, wherein the antibody is a monoclonal antibody, a polyclonal antibody, a human antibody, a humanized antibody, a chimeric antibody, a bispecific antibody, a minibody, a diabody, or an antibody fragment. In some embodiments, the antibody is an IgG.

[0187] In another aspect, provided herein is a method for preparing an engineered Fc-containing polypeptide conjugate comprising the formula: (Fc-containing polypeptide-T-A), wherein T is an acyl donor glutamine-containing tag engineered at a specific site, wherein A is an amine donor agent, wherein the amine donor agent is site-specifically conjugated to the acyl donor glutamine-containing tag at a carboxyl terminus, an amino terminus, or at an another site in the Fc-containing polypeptide, wherein the acyl donor glutamine-containing tag comprises an amino acid sequence XXQX, wherein X is any amino acid (*e.g.*, X can be the same or a different amino acid), and wherein the engineered Fc-containing polypeptide conjugate comprises an amino acid substitution from glutamine to asparagine at position 295 (Q295N; EU numbering scheme), comprising the steps of: a) providing an engineered (Fc-containing polypeptide)-T molecule comprising the Fc-containing polypeptide and the acyl donor glutamine-containing tag; b) contacting the amine donor agent with the engineered (Fc-containing polypeptide)-T molecule in the presence of a transglutaminase; and c) allowing the engineered (Fc-containing polypeptide)-T to covalently link to the amine donor agent to form the engineered Fc-containing polypeptide conjugate.

[0188] In another aspect, provided herein is a method for preparing an engineered polypeptide conjugate comprising the formula: polypeptide-T-A, wherein T is an acyl donor glutamine-containing tag engineered at a specific site, wherein A is an amine donor agent, wherein the amine donor agent is site-specifically conjugated to the acyl donor glutamine-containing tag at a carboxyl

terminus, an amino terminus, or at an another site in the polypeptide, and wherein the acyl donor glutamine-containing tag comprises an amino acid sequence LLQGPX, wherein X is A or P (SEQ ID NO: 272), or GGLLQGPP (SEQ ID NO: 273), comprising the steps of: a) providing an engineered polypeptide-T molecule comprising the polypeptide and the acyl donor glutamine-containing tag; b) contacting the amine donor agent with the engineered polypeptide-T molecule in the presence of a transglutaminase; and c) allowing the engineered polypeptide-T to covalently link to the amine donor agent to form the engineered Fc-containing polypeptide conjugate.

[0189] In some embodiments, the engineered polypeptide conjugate (*e.g.*, the engineered Fc-containing polypeptide conjugate, the engineered Fab-containing polypeptide conjugate, or the engineered antibody conjugate) as described herein has conjugation efficiency of at least about 51%. In another aspect, the invention provides a pharmaceutical composition comprising the engineered polypeptide conjugate as described herein (*e.g.*, the engineered Fc-containing polypeptide conjugate, the engineered Fab-containing polypeptide conjugate, or the engineered antibody conjugate) and a pharmaceutically acceptable excipient.

[0190] In some embodiments, provided herein is a method for conjugating a moiety of interest (Z) to an antibody, comprising the steps of: (a) providing an antibody having (*e.g.*, within the primary sequence of a constant region) at least one acceptor amino acid residue (*e.g.*, a naturally occurring amino acid) that is reactive with a linking reagent (linker) in the presence of a coupling enzyme, *e.g.*, a transamidase; and (b) reacting said antibody with a linking reagent (*e.g.*, a linker comprising a primary amine) comprising a reactive group (R), optionally a protected reactive group or optionally an unprotected reactive group, in the presence of an enzyme capable of causing the formation of a covalent bond between the acceptor amino acid residue and the linking reagent (other than at the R moiety), under conditions sufficient to obtain an antibody comprising an acceptor amino acid residue linked (covalently) to a reactive group (R) via the linking reagent. Optionally, said acceptor residue of the antibody or antibody fragment is flanked at the +2 position by a non-aspartic acid residue. Optionally, the residue at the +2 position is a non-aspartic acid residue. In one embodiment, the residue at the +2 position is a non-aspartic acid, non-glutamine residue. In one embodiment, the residue at the +2 position is a non-aspartic acid, non-asparagine residue. In one embodiment, the residue at the +2 position is a non-negatively charged amino acid (an amino acid other than an aspartic acid or a glutamic acid). Optionally, the acceptor glutamine is in an Fc domain of an antibody heavy chain, optionally further-within the CH2 domain

Optionally, the antibody is free of heavy chain N297-linked glycosylation. Optionally, the acceptor glutamine is at position 295 and the residue at the +2 position is the residue at position 297 (EU index numbering) of an antibody heavy chain.

[0191] In one aspect, provided herein is a method for conjugating a moiety of interest (Z) to an antibody, comprising the steps of: (a) providing an antibody having at least one acceptor glutamine residue; and (b) reacting said antibody with a linker comprising a primary amine (a lysine-based linker) comprising a reactive group (R), preferably a protected reactive group, in the presence of a transglutaminase (TGase), under conditions sufficient to obtain an antibody comprising an acceptor glutamine linked (covalently) to a reactive group (R) via said linker. Optionally, said acceptor glutamine residue of the antibody or antibody fragment is flanked at the +2 position by a non-aspartic acid residue. Optionally, the residue at the +2 position is a non-aspartic acid residue. In one embodiment, the residue at the +2 position is a non-aspartic acid, non-glutamine residue. In one embodiment, the residue at the +2 position is a non-aspartic acid, non-asparagine residue. In one embodiment, the residue at the +2 position is a non-negatively charged amino acid (an amino acid other than an aspartic acid or a glutamic acid). Optionally, the acceptor glutamine is in an Fc domain of an antibody heavy chain, optionally further-within the CH2 domain Optionally, the antibody is free of heavy chain N297-linked glycosylation. Optionally, the acceptor glutamine is at position 295 and the residue at the +2 position is the residue at position 297 (EU index numbering) of an antibody heavy chain. The antibody comprising an acceptor residue or acceptor glutamine residue linked to a reactive group (R) via a linker comprising a primary amine (a lysine-based linker) can thereafter be reacted with a reaction partner comprising a moiety of interest (Z) to generate an antibody comprising an acceptor residue or acceptor glutamine residue linked to a moiety of interest (Z) via the linker. Thus, in one embodiment, the method further comprises a step (c): reacting (i) an antibody of step b) comprising an acceptor glutamine linked to a reactive group (R) via a linker comprising a primary amine (a lysine-based linker), optionally immobilized on a solid support, with (ii) a compound comprising a moiety of interest (Z) and a reactive group (R') capable of reacting with reactive group R, under conditions sufficient to obtain an antibody comprising an acceptor glutamine linked to a moiety of interest (Z) via a linker comprising a primary amine (a lysine-based linker). Preferably, said compound comprising a moiety of interest (Z) and a reactive group (R') capable of reacting with reactive group R is provided at a less than 80 times, 40 times, 20 times, 10 times, 5 times or 4 molar equivalents to the antibody. In one

embodiment, the antibody comprises two acceptor glutamines and the compound comprising a moiety of interest (Z) and a reactive group (R') is provided at 10 or less molar equivalents to the antibody. In one embodiment, the antibody comprises two acceptor glutamines and the compound comprising a moiety of interest (Z) and a reactive group (R') is provided at 5 or less molar equivalents to the antibody. In one embodiment, the antibody comprises four acceptor glutamines and the compound comprising a moiety of interest (Z) and a reactive group (R') is provided at 20 or less molar equivalents to the antibody. In one embodiment, the antibody comprises four acceptor glutamines and the compound comprising a moiety of interest (Z) and a reactive group (R') is provided at 10 or less molar equivalents to the antibody. In one embodiment, steps (b) and/or (c) are carried out in aqueous conditions. Optionally, step (c) comprises: immobilizing a sample of an antibody comprising a functionalized acceptor glutamine residue of Formula II on a solid support to provide a sample comprising immobilized antibodies, reacting the sample comprising immobilized antibodies, optionally recovering any unreacted compound and re-introducing such recovered compound to the solid support for reaction with immobilized antibodies, and eluting the antibody conjugates to provide an antibody composition comprising a Z moiety.

[0192] In an alternative embodiment, an amino acid residue comprising a conjugation handle can be incorporated into the Fc region of the antibody (*e.g.,* during expression of the antibody) at a desired location (*e.g.,* any of the locations provided herein). In some embodiments, the amino acid residue comprising the conjugation handle is an unnatural amino acid.

Conjugation Handle Chemistry

[0193] In some embodiments, the appropriately modified Fc region of the antibody or antigen binding fragment will comprise a conjugation handle which is used to conjugate the antibody or antigen binding fragment to an IL-18 polypeptide to produce an immunocytokine composition provided herein.

[0194] Any suitable reactive group capable of reacting with a complementary reactive group attached to the IL-18 polypeptide can be used as the conjugation handle. In some embodiments, the conjugation handle comprises a reagent for a Cu(I)-catalyzed or "copper-free" alkyne-azide triazole-forming reaction (*e.g.,* strain promoted cycloadditions), the Staudinger ligation, inverse-electron-demand Diels-Alder (IEDDA) reaction, "photo-click" chemistry, tetrazine cycloadditions with trans-cyclooctenes, potassium acyl trifluoroborate (KAT) ligation or a metal-mediated process such as olefin metathesis and Suzuki- Miyaura or Sonogashira cross-coupling.

[0195] In some embodiments, the conjugation handle comprises a reagent for a "copper-free" alkyne azide triazole-forming reaction. Non-limiting examples of alkynes for said alkyne, azide triazole forming reaction include cyclooctyne reagents (*e.g.,* (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol containing reagents, dibenzocyclooctyne-amine reagents, difluorocyclooctynes, or derivatives thereof). In some embodiments, the alkyne functional group is attached to the Fc region. In some embodiments, the azide functional group is attached to the Fc region.

[0196] In some embodiments, the conjugation handle comprises a reactive group selected from azide, alkyne, tetrazine, halide, sulfhydryl, disulfide, maleimide, activated ester, alkene, aldehyde, ketone, imine, hydrazine, potassium acyl trifluoroborate, hydroxylamine (e.g., O-substituted hydroxylamine) and hydrazide. In some embodiments, the IL-18 polypeptide comprises a reactive group complementary to the conjugation handle of the Fc region. In some embodiments, the conjugation handle and the complementary conjugation handle comprise "CLICK" chemistry reagents. Exemplary groups of click chemistry residue are shown in Hein *et al.*, "Click Chemistry, A Powerful Tool for Pharmaceutical Sciences," Pharmaceutical Research, volume 25, pages 2216–2230 (2008); Thirumurugan *et al.*, "Click Chemistry for Drug Development and Diverse Chemical–Biology Applications," *Chem. Rev.* 2013, 113, 7, 4905–4979; US20160107999A1; US10266502B2; and US20190204330A1, each of which is incorporated by reference in its entirety.

Linker Structure

[0197] In some embodiments, the linker used to attach the antibody or antigen binding fragment and the IL-18 polypeptide comprises points of attachment at both moieties. The points of attachment can be any of the residues for facilitating the attachment as provided herein. The linker structure can be any suitable structure for creating the spatial attachment between the two moieties. In some embodiments, the linker provides covalent attachment of both moieties. In some embodiments, the linker is a chemical linker (*e.g.,* not an expressed polypeptide as in a fusion protein). In some embodiments, the linker is a peptide linker. In some embodiments, the linker is a non-peptide linker (*e.g.,* does not consist of amino acid residues).

Chemical Linkers

[0198] In some embodiments, the linker is a chemical linker. In some embodiments, the chemical linker comprises at least one portion which is not comprised of amino acid residues. In some embodiments, the linker comprises a polymer. In some embodiments, the linker comprises a water soluble polymer. In some embodiments, the linker comprises poly(alkylene oxide),

polysaccharide, poly(vinyl pyrrolidone), poly(vinyl alcohol), polyoxazoline, poly(acryloylmorpholine), or a combination thereof. In some embodiments, the linker comprises poly(alkylene oxide). In some embodiments, the poly(alkylene oxide) is polyethylene glycol or polypropylene glycol, or a combination thereof. In some embodiments, the poly(alkylene oxide) is polyethylene glycol.

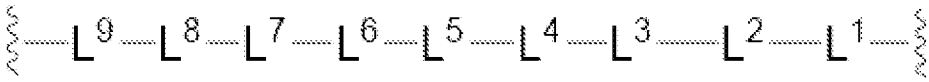
[0199] In some embodiments, the linker is a bifunctional linker. In some embodiments, the bifunctional linker comprises an amide group, an ester group, an ether group, a thioether group, or a carbonyl group. In some embodiments, the linker comprises a non-polymer linker. In some embodiments, the linker comprises a non-polymer, bifunctional linker. In some embodiments, the non-polymer, bifunctional linker comprises succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate; Maleimidocaproyl; Valine-citrulline; Allyl(4-methoxyphenyl)dimethylsilane; 6-(Allyloxycarbonylamino)-1-hexanol; 4-Aminobutyraldehyde diethyl acetal; or (E)-N-(2-Aminoethyl)-4-{2-[4-(3-azidopropoxy)phenyl]diazenyl}benzamide hydrochloride.

[0200] The linker can be branched or linear. In some embodiments, the linker is linear. In some embodiments, the linker is branched. In some embodiments, the linker comprises a linear portion (*e.g.,* between the first point of attachment and the second point of attachment) of a chain of at least 10, 20, 50, 100, 500, 1000, 2000, 3000, or 5000 atoms. In some embodiments, the linker comprises a linear portion of a chain of at least 10, 20, 30, 40, or 50 atoms. In some embodiments, the linker comprises a linear portion of at least 10 atoms. In some embodiments, the linker is branched and comprises a linear portion of a chain of at least 10, 20, 50, 100, 500, 1000, 2000, 3000, or 5000 atoms. In some embodiments, the linker comprises a linear portion of at from 1 to 1000 atoms, 1 to 900 atoms, 1 to 800 atoms, 1 to 500 atoms, 1 to 400 atoms, 1 to 300 atoms, 1 to 200 atoms, 1 to 100 atoms, 1 to 50 atoms, 10 to 1000 atoms, 10 to 900 atoms, 10 to 800 atoms, 10 to 500 atoms, 10 to 400 atoms, 10 to 300 atoms, 10 to 200 atoms, 10 to 100 atoms, 10 to 50 atoms, 25 to 1000 atoms, 25 to 900 atoms, 25 to 800 atoms, 25 to 500 atoms, 25 to 400 atoms, 25 to 300 atoms, 25 to 200 atoms, 25 to 100 atoms,25 to 50 atoms, 50 to 1000 atoms, 50 to 900 atoms, 50 to 800 atoms, 50 to 500 atoms, 50 to 400 atoms, 50 to 300 atoms, 50 to 200 atoms, or 50 to 100 atoms. In some embodiments, the linker has a linear length of from about 10 angstroms to about 200 angstroms. In some embodiments, the linker has a linear length of from about 10 to 500, 10 to 200, 10 to 150, 10 to 125, 10 to 100, 10 to 75, 10 to 50, 25 to 200, 25 to 150, 25 to 125, 25 to 100, 25 to 75, 25 to 50, 50 to 200, 50 to 150, 50 to 100, or 50 to 75 angstroms.

[0201] In some embodiments, the linker has a molecular weight of about 200 Daltons to about 2000 Daltons. In some embodiments, the linker has a molecular weight of about 200 Daltons to about 5000 Daltons. In some embodiments, the linker has a molecular weight of 200 Daltons to 100,000 Daltons. In some embodiments, the linker has a molecular weight of at least about 500 Daltons, at least about 1,000 Daltons, at least about 5,000 Daltons, at least about 10,000 Daltons, at least about 15,000 Daltons, at least about 20,000 Daltons, at least about 25,000 Daltons, or at least about 30,000 Daltons. In some embodiments, the linker as a molecular weight of at most about 100,000 Daltons, at most about 50,000 Daltons, at most about 40,000 Daltons, at most about 30,000 Daltons, at most about 25,000 Daltons, at most about 20,000 Daltons at most about 15,000 Daltons, at most about 10,000 Daltons, or at most about 5,000 Daltons.

[0202] In some embodiments, the linker comprises a reaction product of one or more pairs of conjugation handles and a complementary conjugation handle thereof. In some embodiments, the reaction product comprises a triazole, a hydrazone, pyridazine, a sulfide, a disulfide, an amide, an ester, an ether, an oxime, an alkene, or any combination thereof. In some embodiments, the reaction product comprises a triazole. The reaction product can be separated from the first point of attachment and the second point of attachment by any portion of the linker. In some embodiments, the reaction product is substantially in the center of the linker. In some embodiments, the reaction product is substantially closer to one point of attachment than the other is.

[0203] In some embodiments, the linker comprises a structure of Formula (X)



wherein each of L^1, L^2, L^3, L^4, L^5, L^6, L^7, L^8 , and L^9 is independently -O-, $-NR^L$-, $-(C_1$-C_6 alkylene)NR^L-, $-NR^L(C_1$-C_6 alkylene)-, $-N(R^L)_2^+$-, $-(C_1$-C_6 alkylene)$N(R^L)_2^+$-, $-N(R^L)_2^+-(C_1$-C_6 alkylene)-, -OP(=O)(OR^L)O-, -S-, -(C_1-C_6 alkylene)S-, -S(C_1-C_6 alkylene)-, -S(=O)-, -$S(=O)_2$-, -C(=O)-, -(C_1-C_6 alkylene)C(=O)-, -C(=O) (C_1-C_6 alkylene)-, -C(=O)O-, -OC(=O)-, -OC(=O)O-, -C(=O)NR^L-, -C(=O)NR^L(C_1-C_6 alkylene)-, -(C_1-C_6 alkylene)C(=O)NR^L-, -NR^LC(=O)-, -(C_1-C_6 alkylene)NR^LC(=O)-, -NR^LC(=O)(C_1-C_6 alkylene)-, -OC(=O)NR^L-, -NR^LC(=O)O-, -NR^LC(=O)NR^L-, -NR^LC(=S)NR^L-, -CR^L=N-, -N=CR^L, -NR^LS(=O)$_2$-, -$S(=O)_2NR^L$-, -C(=O)NR^LS(=O)$_2$-, -$S(=O)_2NR^L$C(=O)-, substituted or unsubstituted C_1-C_6 alkylene, substituted or unsubstituted C_1-C_6 heteroalkylene, substituted or unsubstituted C_2-C_6 alkenylene, substituted or unsubstituted C_2-C_6 alkynylene, substituted or unsubstituted C_6-C_{20}

arylene, substituted or unsubstituted C_2-C_{20} heteroarylene, -$(CH_2$-CH_2-$O)_{qa}$-, -$(O$-CH_2-$CH_2)_{qb}$-, -$(CH_2$-$CH(CH_3)$-$O)_{qc}$-, -$(O$- $CH(CH_3)$-$CH_2)_{qd}$-, a reaction product of a conjugation handle and a complementary conjugation handle, or absent;

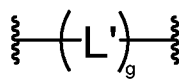
each R^L is independently hydrogen, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, substituted or unsubstituted C_2-C_6 alkenyl, substituted or unsubstituted C_2-C_5 alkynyl, substituted or unsubstituted C_3-C_8 cycloalkyl, substituted or unsubstituted C_2-C_7 heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and

each of qa, qb, qc and qd is independently an integer from 1-100,

wherein each  is a point of attachment to the antibody or antigen binding fragment or the IL-18 polypeptide.

[0204] In some embodiments, the linker consists of a plurality of structures of Formula (X) to form the linkage between the antibody or antigen binding fragment and the IL-18 polypeptide (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more structures of Formula (X) appended from end to end, where only the terminal  denote points of attachment to the antibody or antigen binding fragment or the IL-18 polypeptide).

[0205] In some embodiments, the polymer comprises a linker comprising a structure of Formula (X')



wherein each L' is independently -O-, $–NR^L$-, $–(C_1$-C_6 alkylene$)NR^L$-, $–NR^L(C_1$-C_6 alkylene)-, $–N(R^L)_2{}^+$-, $–(C_1$-C_6 alkylene$)N(R^L)_2{}^+$-, $–N(R^L)_2{}^+–(C_1$-C_6 alkylene)-, -$OP(=O)(OR^L)O$-, -S-, -$(C_1$-C_6 alkylene$)S$-, -$S(C_1$-C_6 alkylene)-, -S(=O)-, -$S(=O)_2$-, -C(=O)-, -$(C_1$-C_6 alkylene$)C(=O)$-, -C(=O) (C_1-C_6 alkylene)-, -C(=O)O-, -OC(=O)-, -OC(=O)O-, -$C(=O)NR^L$-, -$C(=O)NR^L(C_1$-C_6 alkylene)-, -$(C_1$-C_6 alkylene$)C(=O)NR^L$-, -$NR^LC(=O)$-, -$(C_1$-C_6 alkylene$)NR^LC(=O)$-, -$NR^LC(=O)(C_1$-C_6 alkylene)-, -$OC(=O)NR^L$-, -$NR^LC(=O)O$-, -$NR^LC(=O)NR^L$-, -$NR^LC(=S)NR^L$-, -CR^L=N-, -N=CR^L, -$NR^LS(=O)_2$-, -$S(=O)_2NR^L$-, -$C(=O)NR^LS(=O)_2$-, -$S(=O)_2NR^LC(=O)$-, substituted or unsubstituted C_1-C_6 alkylene, substituted or unsubstituted C_1-C_6 heteroalkylene, substituted or unsubstituted C_2-C_6 alkenylene, substituted or

unsubstituted C_2-C_6 alkynylene, substituted or unsubstituted C_6-C_{20} arylene, substituted or unsubstituted C_2-C_{20} heteroarylene, -$(CH_2$-CH_2-$O)_{qa}$-, -$(O$-CH_2-$CH_2)_{qb}$-, -$(CH_2$-$CH(CH_3)$-$O)_{qc}$-, -$(O$- $CH(CH_3)$-$CH_2)_{qd}$-, a reaction product of a conjugation handle and a complementary conjugation handle, or absent; (C_1-C_6 alkylene);

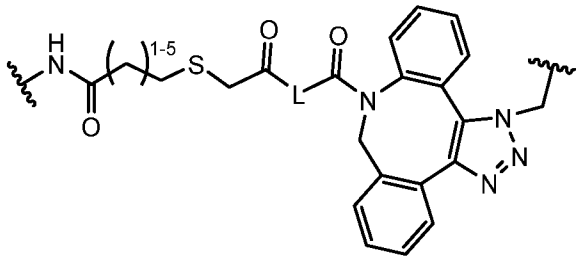
each R^L is independently hydrogen, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, substituted or unsubstituted C_2-C_6 alkenyl, substituted or unsubstituted C_2-C_5 alkynyl, substituted or unsubstituted C_3-C_8 cycloalkyl, substituted or unsubstituted C_2-C_7 heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and

each of qa, qb, qc and qd is independently an integer from 1-100,

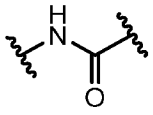
g is an integer from 1-100,

wherein each  is a point of attachment to the IL-18 polypeptide or the antibody or antigen binding fragment.

[0206] In some embodiments, the linker of Formula (X) or Formula (X') comprises the structure:

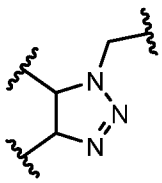


wherein



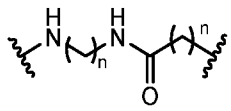
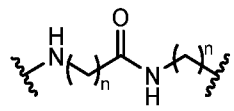
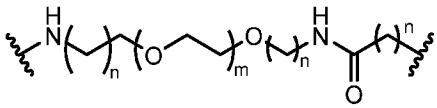
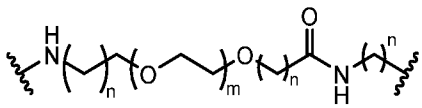
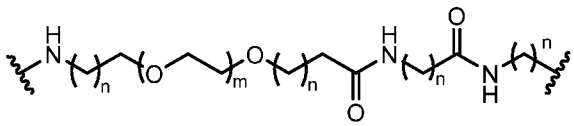
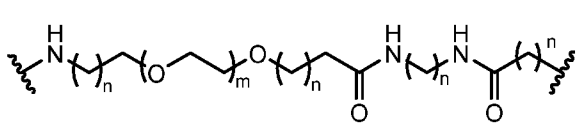
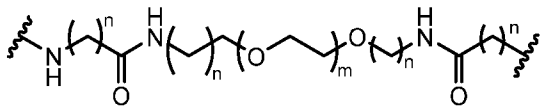
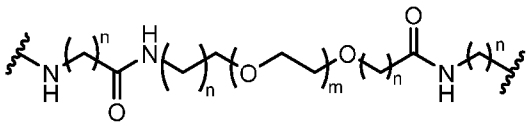
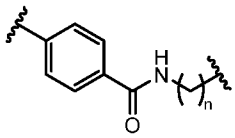
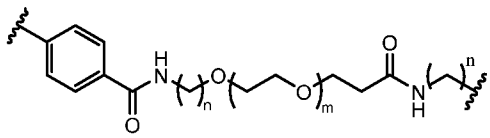
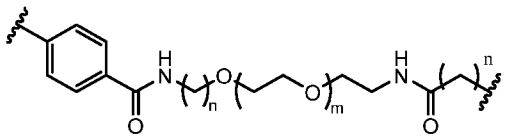
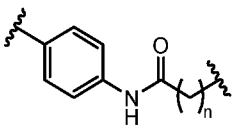
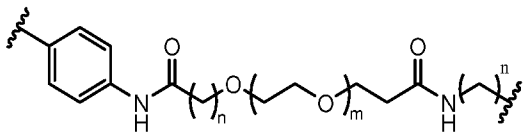
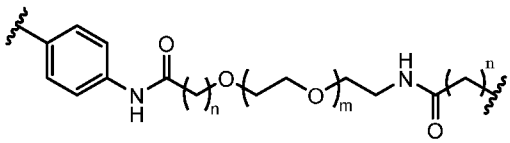
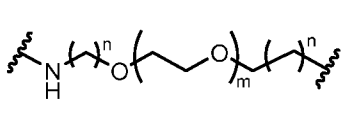
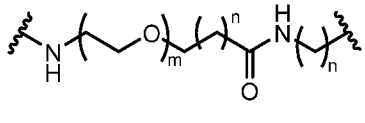
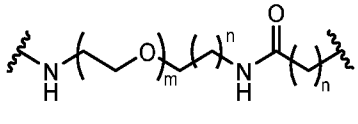
is the attachment to a lysine residue of the antibody or antigen binding fragment;

L is a linking group; and

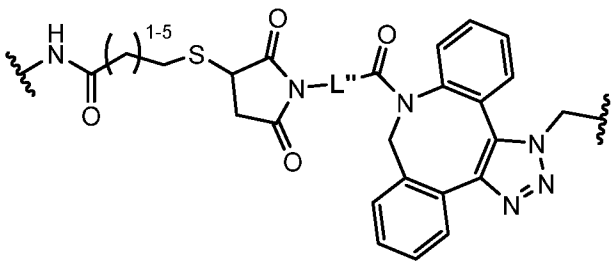


is a point of attachment to a linking group which connects to point of attachment on the IL-18 polypeptide,

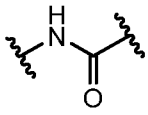
or a regioisomer thereof.

[0207] In some embodiments, L has a structure , , , , , , , , , , , , , , , , or , wherein each n is independently an integer from 1-6 and each m is an integer from 1-30. In some embodiments, each m is independently 2 or 3. In some embodiments, each m is an integer from 1-24, from 1-18, from 1-12, or from 1-6.

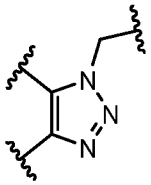
[0208] In some embodiments, the linker of Formula (X) or of Formula (X') comprises the structure:



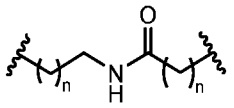
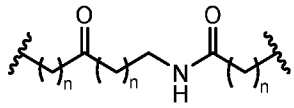
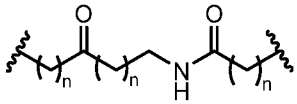
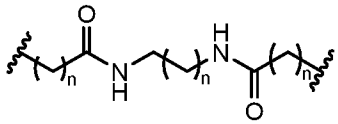
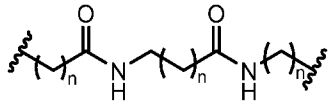
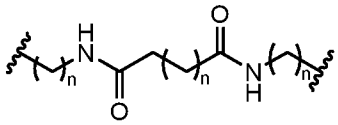
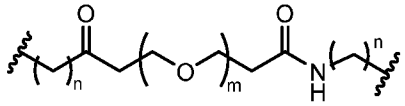
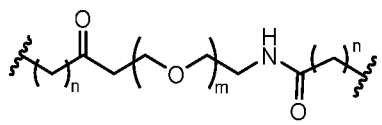
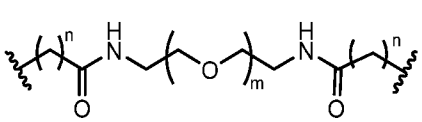
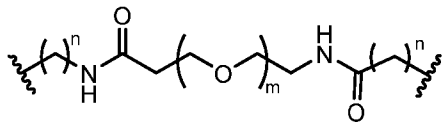
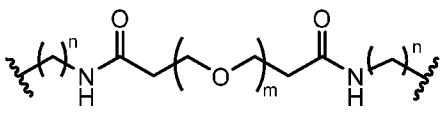
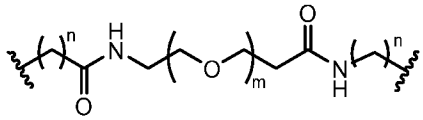
wherein

 is the first point of attachment to a lysine residue of the polypeptide which selectively binds to PD-1;

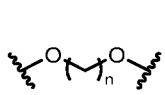
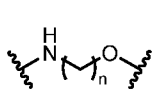
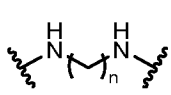
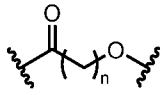
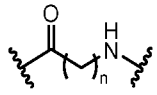
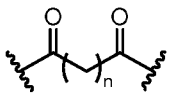
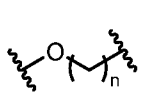
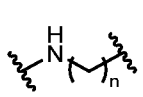
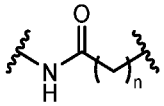
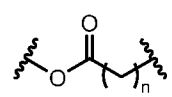
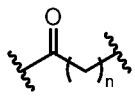
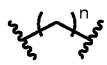
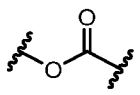
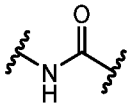
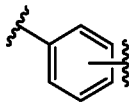
L'' is a linking group; and

 is a point of attachment to a linking group which connects to the first point of attachment,

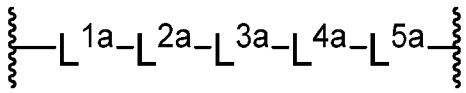
or a regioisomer thereof.

[0209] In some embodiments, L'' has a structure , , , , , , , , , , , or , wherein each n is independently an integer from 1-6 and each m is independently an integer from 1-30. In some

embodiments, each m is independently 2 or 3. In some embodiments, each m is an integer from 1-24, from 1-18, from 1-12, or from 1-6.

[0210] In some embodiments, L or L'' comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more subunits each independently selected from , , , , , , , , , , , , , , and , wherein each n is independently an integer from 1-30. In some embodiments, each n is independently an integer from 1-6. In some embodiments, L or L'' comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the subunits.

[0211] In some embodiments, L or L'' is a structure of Formula (X'')



wherein each of L^{1a}, L^{2a}, L^{3a}, L^{4a}, L^{5a}, is independently -O-, $-NR^{La}$-, $-(C_1\text{-}C_6$ alkylene)NR^{La}-, $-NR^{La}(C_1\text{-}C_6$ alkylene)-, $-N(R^L)_2^+$-, $-(C_1\text{-}C_6$ alkylene)$N(R^{La})_2^+(C_1\text{-}C_6$ alkylene)-,$-N(R^L)_2^+$-, -$OP(=O)(OR^{La})O$-, -S-, -$(C_1\text{-}C_6$ alkylene)S-, -$S(C_1\text{-}C_6$ alkylene)-, -S(=O)-, -$S(=O)_2$-, -C(=O)-, -$(C_1\text{-}C_6$ alkylene)C(=O)-, -C(=O)$(C_1\text{-}C_6$ alkylene)-, -C(=O)O-, -OC(=O)-, -OC(=O)O-, -$C(=O)NR^{La}$-, -$C(=O)NR^{La}(C_1\text{-}C_6$ alkylene)-, -$(C_1\text{-}C_6$ alkylene)$C(=O)NR^{La}$-, -$NR^{La}C(=O)$-, -$(C_1\text{-}C_6$ alkylene)$NR^{La}C(=O)$-, -$NR^{La}C(=O)(C_1\text{-}C_6$ alkylene)-, -OC(=O)NR^{La}-, -$NR^{La}C(=O)O$-, -$NR^{La}C(=O)NR^{La}$-, -$NR^{La}C(=S)NR^{La}$-, -CR^{La}=N-, -N=CR^{La}, -$NR^{La}S(=O)_2$-, -$S(=O)_2NR^{La}$-, -$C(=O)NR^{La}S(=O)_2$-, -$S(=O)_2NR^{La}C(=O)$-, substituted or unsubstituted C_1-C_6 alkylene, substituted or unsubstituted C_1-C_6 heteroalkylene, substituted or unsubstituted C_2-C_6 alkenylene, substituted or unsubstituted C_2-C_6 alkynylene, substituted or unsubstituted C_6-C_{20} arylene, substituted or unsubstituted C_2-C_{20} heteroarylene, -$(CH_2\text{-}CH_2\text{-}O)_{qe}$-, -$(O\text{-}CH_2\text{-}CH_2)_{qf}$-, -$(CH_2\text{-}CH(CH_3)\text{-}O)_{qg}$-, -$(O\text{-}CH(CH_3)\text{-}CH_2)_{qh}$-, a reaction product of a conjugation handle and a complementary conjugation handle, or absent; (C_1-C_6 alkylene)

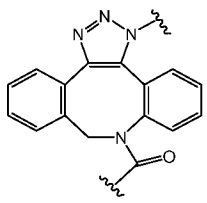
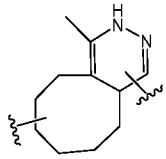
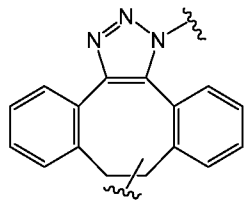
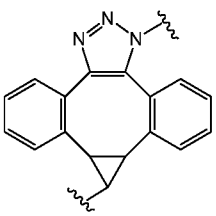
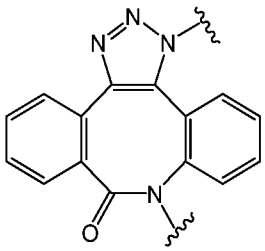
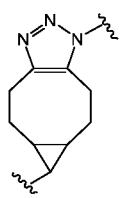
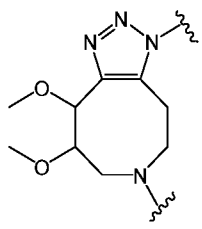
each R^{La} is independently hydrogen, substituted or unsubstituted C_1-C_4 alkyl, substituted or unsubstituted C_1-C_4 heteroalkyl, substituted or unsubstituted C_2-C_6 alkenyl, substituted or

unsubstituted C_2-C_5 alkynyl, substituted or unsubstituted C_3-C_8 cycloalkyl, substituted or unsubstituted C_2-C_7 heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and

each of qe, qf, qg and qh is independently an integer from 1-100.

[0212] In some embodiments, L or L'' comprises a linear chain of 2 to 10, 2 to 15, 2 to 20, 2 to 25, or 2 to 30 atoms. In some embodiments, the linear chain comprises one or more alkyl groups (e.g., lower alkyl (C_1-C_4)), one or more aromatic groups (e.g., phenyl), one or more amide groups, one or more ether groups, one or more ester groups, or any combination thereof.

[0213] In some embodiments, the linking group which connects to the first point of attachment (e.g., the point of attachment to the IL-18 polypeptide) comprises poly(ethylene glycol). In some embodiments, the linking group comprises about 2 to about 30 poly(ethylene glycol) units. In some embodiments, the linking group which connects to the first point of attachment (e.g., the point of attachment to the IL-18 polypeptide) is a functionality attached to a cytokine provided herein which comprises an azide (e.g., the triazole is the reaction product of the azide).

[0214] In some embodiments, each reaction product of a conjugation handle and a complementary conjugation handle independently comprises a triazole, a hydrazone, pyridazine, a sulfide, a disulfide, an amide, an ester, an ether, an oxime, or an alkene. In some embodiments, each reaction product of a conjugation handle and a complementary conjugation handle comprises a triazole. In some embodiments, each reaction product of a conjugation handle and a complementary conjugation handle comprise a structure of , , , , , , or , or a regioisomer or derivative thereof.

Peptide Linkers

[0215] In some embodiments, the antibody or antigen binding fragment is linked to the IL-18 polypeptide through a peptide linker. In some embodiments, the antibody or antigen binding fragment is linked to the IL-18 polypeptide as a fusion protein. In such instances, the linker comprises one or more peptide bonds between the antibody or antigen binding fragment and the IL-18 polypeptide. In some embodiments, the linker between the fusion protein of the antibody or antigen binding fragment and the IL-18 polypeptide is a bond. In some embodiments, the linker between the fusion protein of the antibody or antigen binding fragment and the IL-18 polypeptide is a linking peptide. Non-limiting examples of linking peptides include, but are not limited to $(GS)_n$ (SEQ ID NO: 224), $(GGS)_n$ (SEQ ID NO: 225), $(GGGS)_n$ (SEQ ID NO: 226), $(GGSG)_n$ (SEQ ID NO: 227), or $(GGSGG)_n$ (SEQ ID NO: 228), $(GGGGS)_n$ (SEQ ID NO: 229), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. For example, a linking peptide can be $(GGGGS)_3$ (SEQ ID NO: 230) or $(GGGGS)_4$ (SEQ ID NO: 231). In some embodiments, the IL-18 polypeptide is fused to the C-terminal end of the antibody or antigen binding fragment (optionally through a linking peptide). In some embodiments, the IL-18 polypeptide is fused to the N-terminal end of the antibody or antigen binding fragment (optionally through a linking peptide).

Cleavable linkers

[0216] In some embodiments, the linker (*e.g.,* a chemical or peptide linker as provided herein) is a cleavable linker. In some embodiments, the cleavable linker is cleaved at, near, or in a tumor microenvironment. In some embodiments, the tumor is mechanically or physically cleaved at, near, or in the tumor microenvironment. In some embodiments, the tumor is chemically cleaved at, near, or in a tumor microenvironment. In some embodiments, the cleavable linker is a reduction sensitive linker. In some embodiments, the cleavable linker is an oxidation sensitive linker. In some embodiments, the cleavable linker is cleaved as a result of pH at, near, or in the tumor microenvironment. In some embodiments, the cleavable linker is cleaved by a tumor metabolite at, near, or in the tumor microenvironment. In some embodiments, the cleavable linker is cleaved by a protease at, near, or in the tumor microenvironment.

IL-18 Polypeptides

[0217] The present disclosure describes antibodies or antigen binding fragments linked to interleukin-18 (IL-18) polypeptides as immunocytokine compositions and their use as therapeutic agents. IL-18 is a pro-inflammatory cytokine that elicits biological activities that initiate or promote host defense and inflammation following infection or injury. IL-18 has been

implicated in autoimmune diseases, myocardial function, emphysema, metabolic syndromes, psoriasis, inflammatory bowel disease, hemophagocytic syndromes, macrophage activation syndrome, sepsis, and acute kidney injury. In some models of disease, IL-18 plays a protective role.

[0218] IL-18 also plays a major role in the production of IFNγ from T-cells and natural killer cells. IFNγ is a T helper type 1 cytokine mainly produced by T cells, NK cells, and macrophages and is critical for innate and adaptive immunity against viral, some bacterial, and protozoal infections. IFNγ is also an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression.

[0219] IL-18 forms a signaling complex by binding to the IL-18 alpha chain (IL-18Rα), which is the ligand binding chain for mature IL-18. However, the binding affinity of IL-18 to IL-18Rα is low. In cells that express the co-receptor, IL-18 receptor beta chain (IL-18Rβ), a high affinity heterodimer complex is formed, which then activates cell signaling.

[0220] The activity of IL-18 is balanced by the presence of a high affinity, naturally occurring IL-18 binding protein (IL-18BP). IL-18BP binds IL-18 and neutralizes the biological activity of IL-18. Cell surface IL-18Rα competes with IL-18BP for IL-18 binding. Increased disease severity can be associated with an imbalance of IL-18 to IL-18BP such that levels of free IL-18 are elevated in the circulation. **FIGURE 4** illustrates the mechanism of action of IL-18, IFNγ production, IL-18BP production, and inhibition of IL-18 activity by IL-18BP. IL-18 induces IFNγ production, which in turn induces IL-18BP production. IL-18BP then competes with IL-18Rα to inhibit IL-18 activity.

[0221] In some embodiments, the IL-18 polypeptides of the immunocytokines provided herein display reduced binding to IL-18BP and retain binding to the IL-18 receptor. The IL-18 polypeptides with this property provided herein are able to retain IL-18 receptor signaling activity (including inducing production of IFNγ) even in the presence of IL-18BP. This allows the immunocytokines provided herein to retain IL-18 signaling activity well beyond a short period of time after administration, or upon repeat administrations. In some embodiments, the IL-18 polypeptides with this property comprise a modification (*e.g.*, substitution, polymer attachment, or deletion) at one or more amino acid residues which convey this property to the IL-18 polypeptide. Examples of IL-18 polypeptides with this property are provided herein, as well as those otherwise known, such as those described in Patent Cooperation Treaty Publication No.

WO2019051015A1, which is hereby incorporated by reference as if set forth herein in its entirety. In addition to IL-18 polypeptides provided herein, these otherwise known IL-18 polypeptides or their analogs may similarly be modified with points of attachment to the linker as provided herein. In some embodiments, an IL-18 can by prepared synthetically, such as according to the route shown in FIGURE 5.

Points of Attachment of Chemical Linkers to IL-18 Polypeptides

[0222] The immunocytokines provided herein comprise linkers which have a point of attachment to the IL-18 polypeptide. In some embodiments, the linker is a chemical linker. As discussed *supra*, the linker has another point of attachment to the antibody or antigen binding fragment at any residue as provided herein. The point of attachment to the IL-18 polypeptide is to a residue as provided herein.

[0223] In some embodiments, the linker is attached to an amino acid residue of the IL-18 polypeptide. In some embodiments, the linker is attached to any amino acid residue of the IL-18 polypeptide (e.g., at a position corresponding to any one of positions 1-157 of SEQ ID NO: 1). In some embodiments, the linker is attached at a non-terminal residue of the IL-18 polypeptide (e.g., a residue at position corresponding to any one of positions 2-156 of SEQ ID NO: 1). In some embodiments, the linker is attached at a non-terminal residue of the IL-18 polypeptide, wherein the IL-18 polypeptide has been extended or truncated by one or more amino acids relative to SEQ ID NO: 1.

[0224] In some embodiments, the linker is attached to the IL-18 polypeptide at a residue in a region comprising residues 2-156, wherein residue position numbering is based on SEQ ID NO: 1 as a reference sequence. In some embodiments, the linker is attached to the IL-18 polypeptide at a residue in a region comprising residues 30-150. In some embodiments, the linker is attached to the IL-18 polypeptide at a residue in a region comprising residues 33-43, residues 60-100, residues 65-75, residues 80-90, residues 85-100, residues 90-110, residues 115-130, residues 120-130, or residues 140-150. In some embodiments, the linker is attached to the IL-18 polypeptide at a residue selected from residue 38, 68, 69, 70, 76, 78, 85, 86, 95, 98, 121, 127, and 144. In some embodiments, the linker is attached to the IL-18 polypeptide at a residue selected from 68, 69, 70, 85, 86, and 98. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 68, 69, or 70. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 85, 86, 95, or 98. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 68. In

some embodiments, the linker is attached to the IL-18 polypeptide at residue 69. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 70. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 85. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 86. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 95. In some embodiments, the linker is attached to the IL-18 polypeptide at residue 98.

[0225] In some embodiments, the linker is attached to the IL-18 polypeptide at a residue which is known in the art to be compatible with attachment of a polymer to the IL-18 polypeptide without having a profound impact on the bioactivity of the IL-18 polypeptide. Examples of these residues include residues 38, 76, 78, 121, 127, and 144, as described in PCT Pub. No. WO2004091517A2, which is hereby incorporated by reference as if set forth in its entirety.

[0226] In some embodiments, the residue to which the linker is attached is a natural amino acid residue. In some embodiments, the residue to which the linker is covalently attached is selected from cysteine, aspartate, asparagine, glutamate, glutamine, serine, threonine, lysine, and tyrosine. In some embodiments, the residue to which the linker is covalently attached is selected from asparagine, aspartic acid, cysteine, glutamic acid, glutamine, lysine, and tyrosine. In some embodiments, the linker is covalently attached to a cysteine. In some embodiments, the linker is covalently attached to a lysine. In some embodiments, the linker is covalently attached to a glutamine. In some embodiments, the linker is covalently attached to an asparagine. In some embodiments, the residue to which the linker is attached is a tyrosine. In some embodiments, the residue to which the linker is attached is the natural amino acid in that position in SEQ ID NO: 1.

[0227] In some embodiments, the linker is attached to a different natural amino acid which is substituted at the relevant position. The substitution can be for a naturally occurring amino acid which is more amenable to attachment of additional functional groups (*e.g.*, aspartic acid, cysteine, glutamic acid, lysine, serine, threonine, or tyrosine), a derivative of modified version of any naturally occurring amino acid, or any unnatural amino acid (*e.g.*, an amino acid containing a desired conjugation handle, such as a CLICK chemistry reagent such as an azide, alkyne, *etc.*). In some embodiments, the linker is covalently attached to site-specifically to a natural amino acid.

[0228] In some embodiments, the linker is attached at an unnatural amino acid residue. In some embodiments, the unnatural amino acid residue comprises a conjugation handle. In some embodiments, the conjugation handle facilitates the addition of the linker to the IL-18 polypeptide.

The conjugation handle can be any of the conjugation handles provided herein. In some embodiments, the linker is covalently attached site-specifically to the unnatural amino acid. Non-limiting examples of amino acid residues comprising conjugation handles can be found, for example, in PCT Pub. Nos. WO2015054658A1, WO2014036492A1, WO2021133839A1 WO2006069246A2, and WO2007079130A2, each of which is incorporated by reference as if set forth in its entirety.

[0229] In some embodiments, the linker is covalently attached at residue 68. In some embodiments, the linker is covalently attached at residue C68, C68E, C68D, C68Q, C68K, C68N, or C68Y. In some embodiments, the linker is covalently attached at residue C68. In some embodiments, the linker is covalently attached to an unnatural amino acid at residue 68.

[0230] In some embodiments, the linker is covalently attached at residue 69. In some embodiments, the linker is covalently attached at residue E69, E69C, E69D, E69Q, E69K, E69N, or E69Y. In some embodiments, the linker is covalently attached at residue E69. In some embodiments, the linker is covalently attached residue E69C. In some embodiments, the linker is covalently attached to an unnatural amino acid at residue 69.

[0231] In some embodiments, the linker is covalently attached at residue 70. In some embodiments, the linker is covalently attached at residue K70, K70C, K70D, K70Q, K70E, K70N, or K70Y. In some embodiments, the linker is covalently attached at residue K70. In some embodiments, the linker is covalently attached residue K70C. In some embodiments, the linker is covalently attached to an unnatural amino acid at residue 70.

[0232] In some embodiments, the linker is covalently attached at residue 85. In some embodiments, the linker is covalently attached at residue E85, E85C, E85D, E85Q, E85K, E85N, or E85Y. In some embodiments, the linker is covalently attached at residue E85. In some embodiments, the linker is covalently attached residue E85C. In some embodiments, the linker is covalently attached to an unnatural amino acid at residue 85.

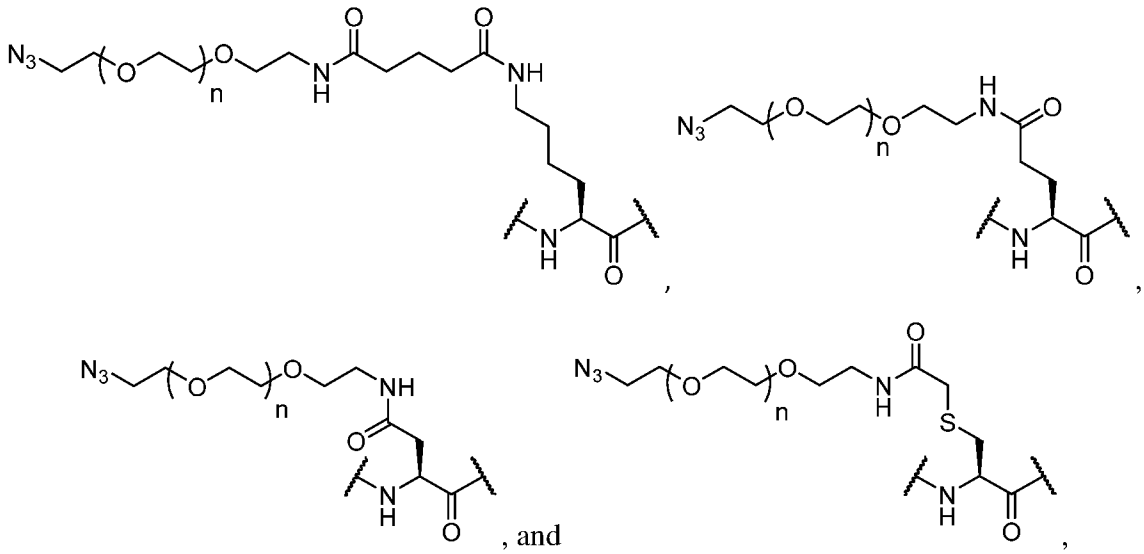
[0233] In some embodiments, the linker is covalently attached at residue 86. In some embodiments, the linker is covalently attached at residue M86C, M86D, M86Q, M86K, M86N, M86E, or M86Y. In some embodiments, the linker is covalently attached M86C. In some embodiments, the linker is covalently attached to an unnatural amino acid at residue 86.

[0234] In some embodiments, the polymer is covalently attached at residue 95. In some embodiments, the polymer is covalently attached at residue T95, T95C, T95D, T95Q, T95K,

T95N, T95E, or T95Y. In some embodiments, the polymer is covalently attached at residue T95C, T95D, T95Q, T95K, T95N, T95E, or T95Y. In some embodiments, the polymer is covalently attached at residue T95C. In some embodiments, the polymer is covalently attached to an unnatural amino acid at residue 95.

[0235] In some embodiments, the linker is covalently attached at residue 98. In some embodiments, the linker is covalently attached at residue D98, D98C, D98Q, D98K, D98N, D98E, or D98Y. In some embodiments, the linker is covalently attached at residue D98C. In some embodiments, the linker is covalently attached to an unnatural amino acid at residue 98.

[0236] In some embodiments, the linker is covalently attached through a modified natural amino acid. In some embodiments, the modified natural amino acid comprises a conjugation handle. In some embodiments, the linker is covalently attached through a modified amino acid α. In some embodiments, the modified amino acid α is an amino-acid-PEG-azide group. In some embodiments, the modified amino acid α is a glutamate, aspartate, lysine, cysteine, or tyrosine modified to incorporate an azide group linked to the amino acid through a PEG spacer. In some embodiments, the modified amino acid α has a structure selected from:



, and

wherein each n is independently an integer from 1-30. In some embodiments, n is an integer from 1-20, 1-10, 2-30, 2-20, 2-10, 5-30, 5-20, or 5-10. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30. In some embodiments, n is 10. In some embodiments, n is 8. In some embodiments, n is 6. In some embodiments, n is 12. The modified amino acid α can be incorporated at any point of attachment

of the IL-18 polypeptide as provided herein. In some embodiments, the modified amino acid α is located at a position on the IL-18 polypeptide selected from residue 68, residue 69, residue 70, residue 85, residue 86, residue 95, or residue 98.

[0237] Where IL-18 polypeptides contain unnatural amino acids or modified natural amino acids (*e.g.,* those provided herein for purposes of conjugation), these amino acids may be incorporated into the IL-18 polypeptides using many techniques known in the art for introduction such modifications. For example, recombinant proteins with unnatural amino acids can be made using methods as described in Patent Cooperation Treaty Publication Nos. WO2016115168, WO2002085923, WO2005019415, and WO2005003294. Alternatively or in combination, unnatural or modified natural amino acids can be incorporated into chemically synthesized proteins during synthesis.

Modifications to IL-18 polypeptides

[0238] In some embodiments, the IL-18 polypeptide of the immunocytokine comprises one or more modifications to that of SEQ ID NO: 1. The modifications provided herein are in addition to any modification at the point of attachment as discussed *supra*. In some embodiments, the residue position numbering of the IL-18 polypeptide is based on SEQ ID NO: 1 as a reference sequence.

[0239] Modifications to the IL-18 polypeptide described herein encompass mutations, addition of various functionalities, deletion of amino acids, addition of amino acids, or any other alteration of the wild-type version of the protein or protein fragment. Functionalities which may be added to polypeptides include polymers, linkers, alkyl groups, detectable molecules such as chromophores or fluorophores, reactive functional groups, or any combination thereof. In some embodiments, functionalities are added to individual amino acids of the polypeptides. In some embodiments, functionalities are added site-specifically to the polypeptides.

[0240] In some embodiments, the IL-18 polypeptide of the immunocytokine comprise one or more modifications in addition to a modification needed to attach the linker to the relevant residue of the IL-18 polypeptide (*e.g.*, an amino acid substitution at a residue to which the linker is not attached). In some embodiments, the modification is in the range of amino acid residues 1-127, based on the sequence of human $IL\text{-}18^{37\text{-}193}$ (SEQ ID NO: 1). SEQ ID NO: 1 reflects the bioactive form of IL-18. Endogenously, IL-18 is initially expressed with an additional 36 amino acid segment at the N-terminus which is cleaved by caspases to mediate biologic activity.

[0241] In some embodiments, the IL-18 polypeptide of the immunocytokine described herein contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more modified amino acid residues.

[0242] In some embodiments, the IL-18 polypeptide of the immunocytokine comprises an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1.

[0243] In some embodiments, the IL-18 polypeptide of the immunocytokine provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 2-72 provided herein. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence at least 85% identical to the sequence of any one of SEQ ID NOs: 2-272. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence of SEQ ID NO: 30. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence at least 85% identical to the sequence of SEQ ID NO: 30. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence of SEQ ID NO: 59. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence at least 85% identical to the sequence of SEQ ID NO: 59.

[0244] In some embodiments, the IL-18 polypeptide of the immunocytokine described herein comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, or at least 9 amino acid substitutions, wherein the amino acid substitutions are relative to SEQ ID NO: 1. In some embodiments, the IL-18 polypeptide comprises 1 to 9 amino acid substitutions. In some embodiments, the IL-18 polypeptide comprises 1 or 2 amino acid substitutions, 1 to 3 amino acid substitutions, 1 to 4 amino acid substitutions, 1 to 5 amino acid substitutions, 1 to 6 amino acid substitutions, 1 to 7 amino acid substitutions, 1 to 8 amino acid substitutions, 2 to 3 amino acid substitutions, 2 to 4 amino acid substitutions, 2 to 5 amino acid substitutions, 2 to 6 amino acid substitutions, 2 to 7 amino acid substitutions, 2 to 8 amino acid substitutions, 2 to 9 amino acid substitutions 3 or 4 amino acid substitutions, 3 to 5 amino acid substitutions, 3 to 6 amino acid substitutions, 3 to 7 amino acid substitutions, 3 to 9 amino acid substitutions, 4 or 5 amino acid substitutions, 4 to 6 amino acid substitutions, 4 to 7 amino acid substitutions, 4 to 9 amino acid substitutions, 5 or 6 amino acid substitutions, 5 to 7 amino acid substitutions, 5 to 9 amino acid substitutions, 6 or 7 amino acid substitutions, 6 to 9 amino acid substitutions, or 7 to 9 amino acid substitutions. In some embodiments, the IL-18 polypeptide comprises 3 amino acid substitutions, 4 amino acid substitutions, 5 amino acid substitutions, 6 amino acid substitutions, 7 amino acid

substitutions, or 9 amino acid substitutions. In some embodiments, the IL-18 polypeptide comprises at most 4 amino acid substitutions, 5 amino acid substitutions, 6 amino acid substitutions, 7 amino acid substitutions, or 9 amino acid substitutions.

[0245] In some embodiments, one modification is at amino acid residue 6. In some embodiments, one modification is in the range of amino acid residues 53-63. In some embodiments, one modification is at amino acid residue 53. In some embodiments, one modification is at amino acid residue 63.

[0246] In some embodiments, the IL-18 polypeptide comprises at least one modification to the amino acid sequence of SEQ ID NO: 1 selected from: Y01X, F02X, E06X, S10X, V11X, D17X, C38X, M51X, K53X, D54X, S55X, T63X, C68X, C76X, AND C127X, wherein each X is independently a natural or non-natural amino acid. In some embodiments, the IL-18 polypeptide further comprises an amino acid substitution at the point of attachment of the linker, such as residue 69, residue 70, residue 85, residue 86, residue 95, or residue 98. In some embodiments, the IL-18 polypeptide comprises at least one modification to the amino acid sequence of SEQ ID NO: 1 selected from: Y01G, F02A, E06K, S10T, V11I, D17N, C38S, C38A, C38Q, M51G, K53A, D54A, S55A, T63A, C68S, C68A, C76S, C76A, C127A, and C127S. In some embodiments, the IL-18 polypeptide further comprises an amino acid substitution at the point of attachment of the linker, such as E69C, K70C, E85C, M86C, T95C, or D98C.

[0247] In some embodiments, the interleukin-18 (IL-18) polypeptide comprising E06K and K53A, wherein residue position numbering of the IL-18 polypeptide is based on SEQ ID NO: 1 as a reference sequence. In some embodiments, the IL-18 polypeptide further comprises V11I. In some embodiments, the IL-18 polypeptide further comprises T63A. In some embodiments, the IL-18 polypeptide further comprises at least one of Y01X, S55X, F02X, D54X, C38X, C68X, E69X, K70X, C76X, or C127X, wherein each X is independently an amino acid or an amino acid derivative. In some embodiments, the IL-18 polypeptide further comprises at least one of Y01G, S55A, F02A, D54A, C38S, C38A, C38Q, C68S, C68A, E69C, K70C, C76S, C76A, C127S, or C127A. In some embodiments, the IL-18 polypeptide further comprises an amino acid substitution at the point of attachment of the linker, such as residue 69, residue 70, residue 85, residue 86, residue 95, or residue 98.

[0248] In some embodiments, the IL-18 peptide comprises at least one modification to the amino acid sequence of SEQ ID NO: 1, wherein the modification is E06X, V11X, K53X, S55X, or T63X,

wherein X is a natural or non-natural amino acid. In some embodiments, the IL-18 peptide comprises at least two modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06X and K53X; E06X and S55X; K53X and S55X; E06X and T63X; or K53X and T63X, wherein X is a natural or non-natural amino acid. In some embodiments, the IL-18 peptide comprises at least three modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06X, K53X, and S55X; or E06X, K53X, and T63X, wherein X is a natural or non-natural amino acid. In some embodiments, the IL-18 peptide comprises at least four modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06X, K53X, S55X, and T63X; E06X, K53X, S55X, and Y01X; E06X, K53X, S55X, and F02X; E06X, K53X, S55X, and D54X; E06X, K53X, S55X, and M51X; or C38X, C68X, C76X, and C127X, wherein X is a natural or non-natural amino acid. In each embodiment wherein a plurality of amino acids residues are replaced with a natural or non-natural amino acid X, each X is independently the same or a different amino acid.

[0001] In certain embodiments, the IL-18 polypeptide comprises a substitution at residue Y1. In certain embodiments, the IL-18 polypeptide can comprises Y1M substitution. Unless specifically mentioned otherwise, the residue position numbering is provided in this paragraph, and elsewhere in this disclosure is based on SEQ ID NO: 1, as a reference sequence. Unless specifically mentioned otherwise, the amino acid substitutions provided in this paragraph, and elsewhere in this disclosure is with respect to SEQ ID NO: 1, as a reference sequence. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue F2. In certain embodiments, the IL-18 polypeptide can comprises F2A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue E6. In certain embodiments, the IL-18 polypeptide comprises E6K substitution. In certain embodiments, the IL-18 polypeptide comprises E6R substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue K8. In certain embodiments, the IL-18 polypeptide comprises K8L substitution. In certain embodiments, the IL-18 polypeptide comprises K8E substitution. In certain embodiments, the IL-18 polypeptide comprises K8R substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue V11. In certain embodiments, the IL-18 polypeptide can comprises V11I substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue E31. In certain embodiments, the IL-18 polypeptide comprises E31A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue T34. In certain

embodiments, the IL-18 polypeptide comprises T34A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue D35. In certain embodiments, the IL-18 polypeptide comprises D35A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue S36. In certain embodiments, the IL-18 polypeptide comprises S36A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue D37. In certain embodiments, the IL-18 polypeptide comprises D37A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue D40. In certain embodiments, the IL-18 polypeptide comprises D40A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue N41. In certain embodiments, the IL-18 polypeptide comprises N41A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue I49. In certain embodiments, the IL-18 polypeptide comprises I49E substitution. In certain embodiments, the IL-18 polypeptide comprises I49M substitution. In certain embodiments, the IL-18 polypeptide comprises I49R substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue K53. In certain embodiments, the IL-18 polypeptide comprises K53A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue D54. In certain embodiments, the IL-18 polypeptide comprises D54A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue S55. In certain embodiments, the IL-18 polypeptide comprises S55A substitution. In certain embodiments, the IL-18 polypeptide comprises S55T substitution. In certain embodiments, the IL-18 polypeptide comprises S55H substitution. In certain embodiments, the IL-18 polypeptide comprises S55R substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue T63. In certain embodiments, the IL-18 polypeptide comprises T63A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue Q103. In certain embodiments, the IL-18 polypeptide comprises Q103R substitution. In certain embodiments, the IL-18 polypeptide comprises Q103E substitution. In certain embodiments, the IL-18 polypeptide comprises Q103K substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue G108. In certain embodiments, the IL-18 polypeptide comprises G108A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue H109. In certain embodiments, the IL-18 polypeptide comprises H109A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue D110. In certain embodiments, the IL-18 polypeptide comprises D110A substitution. In certain embodiments, the

IL-18 polypeptide comprises a substitution at residue D132. In certain embodiments, the IL-18 polypeptide comprises D132A substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue V153. In certain embodiments, the IL-18 polypeptide comprises V153R substitution. In certain embodiments, the IL-18 polypeptide comprises V153E substitution. In certain embodiments, the IL-18 polypeptide comprises V153Y substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue C38. In certain embodiments, the IL-18 polypeptide comprises C38A substitution. In certain embodiments, the IL-18 polypeptide comprises C38S substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue C68. In certain embodiments, the IL-18 polypeptide comprises C68A substitution. In certain embodiments, the IL-18 polypeptide comprises C68S substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue C76. In certain embodiments, the IL-18 polypeptide comprises C76A substitution. In certain embodiments, the IL-18 polypeptide comprises C76S substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue C127. In certain embodiments, the IL-18 polypeptide comprises C127A substitution. In certain embodiments, the IL-18 polypeptide comprises C127S substitution. In certain embodiments, the IL-18 polypeptide comprises a substitution at residue C38, C68, C76, and/or C127. In certain embodiments, the IL-18 polypeptide comprises a C38A, C38S, C68A, C68S, C76A, C76S, C127A, and/or C127S substitution. In certain embodiments, the IL-18 polypeptide comprises C38A, C76A, and C127A substitutions. In certain embodiments, the IL-18 polypeptide comprises C38S, C76S and C127S substitutions.

[0249] In some embodiments, the IL-18 peptide comprises at least one modification to the amino acid sequence of SEQ ID NO: 1, wherein the modification is E06K, V11I, K53A, S55A, or T63A. In some embodiments, the IL-18 peptide comprises at least two modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06K and K53A; E06K and S55A; K53A and S55A; E06K and T63A; or K53A and T63A. In some embodiments, the IL-18 peptide comprises at least three modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06K, K53A, and S55A; E06K, V11I, and K53A; E06K, C38A, and K53A; or E06K, K53A, and T63A. In some embodiments, the IL-18 peptide comprises at least four modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06K, K53A, S55A, and T63A; E06K, K53A, S55A, and Y01G; E06K, K53A, S55A, and F02A; E06K, K53A, S55A, and D54A; E06K, K53A, S55A, and M51G; or C38S, C68S,

C76S, and C127S. In some embodiments, the IL-18 peptide comprises at least six modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise E06K, K53A, C38S, C68S, C76S, and C127S; or K53A, T63A, C38S, C68S, C76S, and C127S. In some embodiments, the IL-18 polypeptide comprises at least seven modifications to the sequence of SEQ ID NO: 1, wherein the seven modifications comprise E6K, V11I, C38A, K53A, T63A, C76A, C127A. In some embodiments, the IL-18 peptide comprises at least eight modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise Y01G, F02A, E06K, M51G, K53A, D54A, S55A, and T63A. In some embodiments, the IL-18 peptide comprises at least eight modifications to the amino acid sequence of SEQ ID NO: 1, wherein the modifications comprise Y01G, F02A, E06K, M51G, K53A, D54A, S55A, and T63A.

[0250] In some embodiments, IL-18 polypeptide as provided herein comprises E06K and K53A, wherein residue position numbering of the IL-18 polypeptide is based on SEQ ID NO: 1 as a reference sequence. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to the amino acid sequence of SEQ ID NO: 30. In some embodiments, the IL-18 polypeptide comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to the amino acid sequence of SEQ ID NO: 59. In some embodiments, the IL-18 polypeptide further comprises an amino acid substitution at one or more cysteine residues. In some embodiments, the IL-18 polypeptide comprises one or more cysteines substituted with either serine or alanine. In some embodiments, the IL-18 polypeptide comprise amino acid substitutions at each cysteine residue of SEQ ID NO: 1.

[0251] In some embodiments, the IL-18 polypeptide comprises a polypeptide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or about 100 % sequence identity to SEQ ID NO: 2-72. In some embodiments, the IL-18 polypeptide comprises a polypeptide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or about 100 % sequence identity to SEQ ID NO: 13-23. In some embodiments, the IL-18 polypeptide comprises a polypeptide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99 % sequence identity to SEQ ID NO: 24-33 In some embodiments, the polypeptide sequence is at least about 80% identical to SEQ ID NO: 30 or SEQ ID NO: 59. In some embodiments, the polypeptide sequence is at least about

80% identical to SEQ ID NO: 30. In some embodiments, the polypeptide sequence is at least about 90% identical to SEQ ID NO: 30. In some embodiments, the polypeptide sequence is at least about 95% identical to SEQ ID NO: 30. In some embodiments, the polypeptide sequence is at least about 98% identical to SEQ ID NO: 30 .In some embodiments, the polypeptide sequence is identical to SEQ ID NO: 30. In some embodiments, the polypeptide sequence is at least about 80% identical to SEQ ID NO: 59. In some embodiments, the polypeptide sequence is at least about 90% identical to SEQ ID NO: 59. In some embodiments, the polypeptide sequence is at least about 95% identical to SEQ ID NO: 59. In some embodiments, the polypeptide sequence is at least about 98% identical to SEQ ID NO: 59. In some embodiments, the polypeptide sequence is identical to SEQ ID NO: 59. In some embodiments, the IL-18 polypeptide is recombinant. In some embodiments, the IL-18 polypeptide is one provided in Table 2. In some embodiments, the IL-18 polypeptide is one described in Table 3.

Biological Activity

[0252] In some embodiments, the immunocytokine composition exhibits one or more activities associated with the antibody or antigen binding fragment and/or an IL-18 polypeptide.

[0253] In some embodiments, the immunocytokine composition exhibits an ability to bind to the IL-18 receptor. In some embodiments, the immunocytokine composition exhibits an ability to bind to the IL-18 receptor which is comparable to WT IL-18. In some embodiments, immunocytokine composition exhibits an ability to bind to the IL-18 receptor (IL-18Rαβ) which is reduced by at most 2-fold, at most 5-fold, at most 10-fold, at most 20-fold, at most 50-fold, at most 100-fold, at most 200-fold, at most 300-fold, at most 400-fold, or at most 1000-fold compared to WT IL-18. In some embodiments, the immunocytokine composition exhibits an enhanced ability to bind the IL-18Rαβ. In some embodiments, the immunocytokine composition exhibits an ability to bind to the IL-18Rαβ which is increased by at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold compared to WT IL-18.

[0254] In some embodiments, immunocytokine composition exhibits an ability to stimulate production of IFNγ upon contact with a cell (*e.g.,* an immune cell, such as an NK cell). In some embodiments, the ability of the immunocytokine composition to stimulate IFNγ production is somewhat reduced compared to WT IL-18. In some embodiments, a half-maximal effective concentration (EC_{50}) of the ability of the immunocytokine composition to stimulate production of IFNγ is at most 100-fold higher than, at most 50-fold higher than, at most 20-fold higher than, at

most 10-fold higher than, at most 5-fold higher than, or at most 2-fold higher than that of a WT IL-18. In some embodiments, the ability of the immunocytokine composition to stimulate IFNγ production is enhanced compared to WT IL-18. In some embodiments, a half-maximal effective concentration (EC_{50}) of the ability of the immunocytokine composition to stimulate production of IFNγ is at least 5-fold lower than, at least 10-fold lower than, at least 20-fold lower than, at least 50-fold lower than, at least 75-fold lower than, or at least 100-fold higher than that of a WT IL-18.

[0255] In some embodiments, the immunocytokine composition exhibits an ability to stimulate production of IFNγ upon contact with a cell (*e.g.,* an immune cell, such as an NK cell) which is only somewhat reduced as compared to the IL-18 polypeptide not comprised in the immunocytokine composition (e.g., unconjugated IL-18 polypeptide). In some embodiments, the EC_{50} of IFNγ stimulation is at most 5-fold greater than, at most 10-fold greater than, at most 50-fold greater than, or at most 100-fold greater than that that of the IL-18 polypeptide not comprised in the immunocytokine composition. In some embodiments, the immunocytokine composition exhibits an ability to induce IFNγ production in a cell as measured by half-maximal effective concentration (EC_{50}) which is within about 100-fold of the corresponding IL-18 polypeptide not comprised in the immunocytokine composition. In some embodiments, the immunocytokine composition exhibits a lower EC_{50} than WT IL-18. In some embodiments, the immunocytokine composition exhibits a lower EC_{50} than WT IL-18 by at least 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 50-fold, or 100-fold.

[0256] In some embodiments, the immunocytokine composition exhibits a reduced ability to bind IL-18 binding protein (IL-18BP). In some embodiments, the ability of immunocytokine composition to bind IL-18BP is reduced by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 30-fold, at least 50-fold, or at least 100-fold compared to WT IL-18. In some embodiments, the immunocytokine composition does not display any substantial ability to bind IL-18 BP.

[0257] In some embodiments, the immunocytokine composition exhibits a reduced ability to have its IFNγ production stimulatory activity inhibited by IL-18BP. In some embodiments, the ability of the immunocytokine composition to be inhibited by IL-18BP is measured as a half maximal inhibitory concentration (IC_{50}). In some embodiments, the immunocytokine composition exhibits an IC_{50} by IL-18BP that is at least 2-fold higher than, at least 5-fold higher than, at least 10-fold higher than, at least 15-fold higher than, at least 20-fold higher than, at least 25-fold higher than,

at least 30-fold higher than, at least 40-fold higher than, or at least 50-fold higher than an IC_{50} of WT IL-18's inhibition by IL-18BP. In some embodiments, the immunocytokine composition exhibits an IC_{50} by IL-18BP that is at least 100-fold higher than an IC_{50} of WT IL-18's inhibition by IL-18BP. In some embodiments, the immunocytokine composition exhibits an IC_{50} by IL-18BP that is at least 200-fold higher than an IC_{50} of WT IL-18's inhibition by IL-18BP. In some embodiments, the immunocytokine composition exhibits an IC_{50} by IL-18BP that is at least 500-fold higher than an IC_{50} of WT IL-18's inhibition by IL-18BP. In some embodiments, the immunocytokine composition exhibits an IC_{50} by IL-18BP that is at least 1000-fold higher than an IC_{50} of WT IL-18's inhibition by IL-18BP.

[0258] In some embodiments, the immunocytokine composition retains binding associated with the antibody or antigen binding fragment. In some embodiments, the immunocytokine composition retains binding to the antigen of the antibody or antigen binding fragment. In some embodiments, the immunocytokine composition exhibits binding affinity (K_D) to the antigen of the antibody which is within 5-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine composition exhibits binding affinity (K_D) to the antigen of the antibody which is within 2.5-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the binding is determined by ELISA. In some embodiments, the binding is determined by BLI.

[0259] In some embodiments, the immunocytokine composition retains binding to one or more Fc receptors associated with the antibody or antigen binding fragment. In some embodiments, the Fc receptor is selected from FcRn, CD64, CD32a, CD16, and CD32b, or any combination thereof. In some embodiments, the immunocytokine composition exhibits a binding affinity (K_D) to at least one Fc receptor which is within 10-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine composition exhibits a binding affinity (K_D) to at least one Fc receptor which is less than 10-fold higher, less than 5-fold higher, less than 4-fold higher, less than 3-fold higher, less than 2-fold higher, or less than the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine composition exhibits a binding affinity (K_D) to each of FcRn, CD64, CD32a, CD16, and CD32B which is less than 10-fold higher, less than 5-fold higher, less than 4-fold higher, less than 3-fold higher, less than 2-fold higher, or less than the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine

composition exhibits a binding affinity (K_D) to each of FcRn, CD64, CD32a, CD16, and CD32B which is within 10-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine composition exhibits a binding affinity (K_D) to each of FcRn, CD64, CD32a, CD16, and CD32B which is within 20-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine composition exhibits a binding affinity (K_D) to each of FcRn, CD64, CD32a, CD16, and CD32B which is within 50-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide. In some embodiments, the immunocytokine composition exhibits a binding affinity (K_D) to each of FcRn, CD64, CD32a, CD16, and CD32B which is within 100-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide.

[0260] In some embodiments, the immunocytokine composition exhibits synergistic efficacy owing to the presence of both molecules in one molecule. In some embodiments, the immunocytokine composition exhibits enhanced activity compared to either molecule alone. In some embodiments, the immunocytokine composition exhibits enhanced anti-tumor growth inhibition compared to the antibody alone. In some embodiments, the immunocytokine composition exhibits enhanced anti-tumor growth inhibition compared to the antibody and the IL-18 polypeptide administered in combination. In some embodiments, the IL-18 polypeptide is administered as a half-life extended version (e.g., PEGylated, attached to an Fc region (e.g., an Fc fusion), or attached to a negative control antibody). In some embodiments, the immunocytokine composition exhibits similar or enhanced antitumor activity at the same concentration as the antibody administered alone. In some embodiments, the immunocytokine composition exhibits similar or enhanced antitumor activity when administered at a dose which is less that 0.5-fold, 0.25-fold, or 0.1-fold the dose of the antibody alone.

[0261] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) stimulates IFNg secretion by a PD-1^+ cell more potently than PD-1^- cell. In some embodiments, the EC_{50} value is at least 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the EC_{50} value is at least 10-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the EC_{50} value is at least 50-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the EC_{50} value is at least 100-fold lower for the PD-1^+ cell than the PD-1^- cell. In some embodiments, the immunocytokine composition exhibits a reduced ability to

be inhibited by IL-18BP when stimulating IFNg secretion of a PD-1$^+$ cell as compared to a PD-1$^-$ cell. In some embodiments, the reduced ability to be inhibited by IL-18 BP is assessed as an IC_{50} value. In some embodiments, the IC_{50} value is at least 10-fold, 20-fold, 50-fold, 100-fold, 150-fold, 200-fold, or 250-fold higher for the PD-1$^+$ cell as compared to the PD-1$^-$ cell. In some embodiments, the IC_{50} value is at least 10-fold higher for the PD-1$^+$ cell as compared to the PD-1$^-$ cell. In some embodiments, the IC_{50} value is at least 100-fold higher for the PD-1$^+$ cell as compared to the PD-1$^-$ cell. In some embodiments, the IC_{50} value is at least 200-fold higher for the PD-1$^+$ cell as compared to the PD-1$^-$ cell.

[0262] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) exhibits a reduced ability to be inhibited by IL-18 BP when stimulating IFNg secretion of a PD-1$^+$ cell compared to a corresponding IL-18 polypeptide not comprised in an immunocytokine composition. In some embodiments, the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold higher. In some embodiments, the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 5-fold higher. In some embodiments, the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 10-fold higher.

[0263] In some embodiments, an immunocytokine composition described herien (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) is used in a method of expanding a population of CD8$^+$ T cells in a subject in need thereof. In some embodiments, the CD8$^+$ T cells are PD-1$^+$/IL-18Rα^+ CD8$^+$ T cells. In some embodiments, the immunocytokine composition selectively expands CD8$^+$ T cells in a tumor microenvironment relative to CD8$^+$ cells in blood of a subject. In some embodiments, the CD8$^+$ T cells are effector memory T cells. In some embodiments, the expansion of CD8$^+$ T cells is assessed as the fraction of CD8$^+$ T cells within CD45$^+$ leukocytes. In some embodiments, the fraction of CD8$^+$ T cells within CD45$^+$ leukocytes increases by at least 2-fold, 3-fold, or 4-fold within a tumor after administration of the immunocytokine composition. In some embodiments, the fraction of CD8$^+$ T cells within CD45$^+$ leukocytes reaches a fraction of at least 50%, 60%, 70%, 75%, or 80% within a tumor after administration of the immunocytokine composition. In some embodiments, the fraction of CD8$^+$ T cells within CD45$^+$ leukocytes increases by at most 4-fold, 3-fold, or 2-fold in the blood of the subject after administration of the immunocytokine composition. In some embodiments, the fraction of CD8$^+$ T cells within CD45$^+$

leukocytes reaches a fraction of at most 50%, 45%, 40%, 35%, 30%, or 20% within the blood of the subject after administration of the immunocytokine composition.

[0264] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) selectively suppresses macrophage cells in a tumor microenvironment relative to macrophage cells in blood of a subject. In some embodiments, suppression of macrophage cells is assessed as the fraction of macrophages within $CD45^+$ leukocytes. In some embodiments, fraction of macrophage cells within $CD45^+$ leukocytes decreases by at least 2-fold, 3-fold, 4-fold, 5-fold, 7.5-fold, or 10-fold within a tumor after administration of the immunocytokine composition. In some embodiments, fraction of macrophage cells within $CD45^+$ leukocytes reaches a fraction of below 40%, 30%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% within a tumor after administration of the immunocytokine composition. In some embodiments, fraction of macrophage cells within $CD45^+$ leukocytes does not have any significant effect on macrophage levels in the blood of the subject (e.g., macrophage levels remain with ~5% of the total fraction of $CD45^+$ leukocytes in the blood).

[0265] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) has minimal effect on $CD4^+$ T cells or NK cells (e.g., $CD45^+$ leukocytes) in a tumor. In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) has minimal effect on the population of $CD4^+$ T cells or NK cells (e.g., $CD45^+$ leukocytes) in a tumor (e.g., does not significantly expand the population, either in absolute terms or relative to $CD8^+$ T cells). In some embodiments, the immunocytokine composition expands expression of IL-18Ra on $CD4^+$ T cells within a tumor, optionally wherein the expression of IL-18Ra is increased by at least 2-fold, 3-fold, or 4-fold.

[0266] In some embodiments, the immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) expands 1, 2, 3, or 4 cytokines selected from IFNg, GM-CSF, TNFa, and IL-6 within a tumor after administration of the immunocytokine composition. In some embodiments, the immunocytokine composition expands an amount of IFNg within a tumor after administration of the immunocytokine composition. In some embodiments, the immunocytokine composition expands an amount of GM-CSF within a tumor after administration of the immunocytokine composition. In some embodiments, the immunocytokine composition expands an amount of TNFa within a tumor after administration of the immunocytokine composition. In some embodiments, the immunocytokine composition expands an amount of IL-6 within a tumor

after administration of the immunocytokine composition. In some embodiments, the immunocytokine composition does not substantially expand the cytokine in blood of a subject (e.g., each of the 1, 2, 3, or 4 cytokines). In some embodiments, the immunocytokine composition expands the cytokine (e.g., 1, 2, 3, or 4 of the cytokines) substantially more (e.g., at least 10-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, or 1000-fold more) in the tumor of a subject than in the blood of a subject. In some embodiments, the level of the cytokine is measured at a specific amount of time after the administration (e.g., after 6h, 12h, 24h, 48h, 72h, etc.).

[0267] In some embodiments, in the immunocytokine composition preferentially expands a population of IL-18 receptor alpha / PD-1 double-positive T cells after administration in vivo (e.g., to a subject such as a human subject or a non-human primate subject). In some embodiments, the IL-18 receptor alpha / PD-1 double-positive T cells are memory T cells. In some embodiments, the IL-18 receptor alpha / PD-1 double-positive T cells are increased by a factor of at least 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 1000-fold, or more compared to those in the subject prior to the administration. In some embodiments, the IL-18 receptor alpha / PD-1 double-positive T cells reach a peak concentration after about 4-14 days (e.g., at about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, o1r 14 days). In some embodiments, the immunocytokine composition does not substantially expand non T cells which are not double positive for PD-1 and IL-18 receptor alpha. (e.g., $CD3^+$ T cells which are not double-positive for PD-1 and IL-18 receptor alpha are not preferentially expanded, only $CD3^+$ T cells which are double-positive for PD-1 and IL-18 receptor alpha are expanded).

Compositions

[0268] In one aspect, provided herein is a pharmaceutical composition comprising an antibody linked to an IL-18 polypeptide described herein; and a pharmaceutically acceptable carrier or excipient. In some embodiments, the pharmaceutical composition further comprises one or more excipients, wherein the one or more excipients include, but are not limited to, a carbohydrate, an inorganic salt, an antioxidant, a surfactant, a buffer, or any combination thereof. In some embodiments the pharmaceutical composition further comprises one, two, three, four, five, six, seven, eight, nine, ten, or more excipients, wherein the one or more excipients include, but are not limited to, a carbohydrate, an inorganic salt, an antioxidant, a surfactant, a buffer, or any combination thereof.

[0269] In some embodiments, the pharmaceutical composition further comprises a carbohydrate. In certain embodiments, the carbohydrate is selected from the group consisting of fructose, maltose, galactose, glucose, D-mannose, sorbose, lactose, sucrose, trehalose, cellobiose raffinose, melezitose, maltodextrins, dextrans, starches, mannitol, xylitol, maltitol, lactitol, xylitol, sorbitol (glucitol), pyranosyl sorbitol, myoinositol, cyclodextrins, and combinations thereof.

[0270] Alternately, or in addition, the pharmaceutical composition further comprises an inorganic salt. In certain embodiments, the inorganic salt is selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium phosphate, potassium phosphate, sodium sulfate, or combinations thereof.

[0271] Alternately, or in addition, the pharmaceutical composition further comprises an antioxidant. In certain embodiments, the antioxidant is selected from the group consisting of ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, potassium metabisulfite, propyl gallate, sodium metabisulfite, sodium thiosulfate, vitamin E, 3,4-dihydroxybenzoic acid, and combinations thereof.

[0272] Alternately, or in addition, the pharmaceutical composition further comprises a surfactant. In certain embodiments, the surfactant is selected from the group consisting of polysorbates, sorbitan esters, lipids, phospholipids, phosphatidylethanolamines, fatty acids, fatty acid esters, steroids, EDTA, zinc, and combinations thereof.

[0273] Alternately, or in addition, the pharmaceutical composition further comprises a buffer. In certain embodiments, the buffer is selected from the group consisting of citric acid, sodium phosphate, potassium phosphate, acetic acid, ethanolamine, histidine, amino acids, tartaric acid, succinic acid, fumaric acid, lactic acid, tris, HEPES, or combinations thereof.

[0274] In some embodiments, the pharmaceutical composition is formulated for parenteral or enteral administration. In some embodiments, the pharmaceutical composition is formulated for intravenous (IV) or subcutaneous (SQ) administration. In some embodiments, the pharmaceutical composition is in a lyophilized form.

[0275] In one aspect, described herein is a liquid or lyophilized composition that comprises a described antibody or antigen binding fragment linked to an IL-18 polypeptide. In some embodiments, the antibody or antigen binding fragment linked to the IL-18 polypeptide is a lyophilized powder. In some embodiments, the lyophilized powder is resuspended in a buffer solution. In some embodiments, the buffer solution comprises a buffer, a sugar, a salt, a surfactant,

or any combination thereof. In some embodiments, the buffer solution comprises a phosphate salt. In some embodiments, the phosphate salt is sodium Na_2HPO_4. In some embodiments, the salt is sodium chloride. In some embodiments, the buffer solution comprises phosphate buffered saline. In some embodiments, the buffer solution comprises mannitol. In some embodiments, the lyophilized powder is suspended in a solution comprising about 10 mM Na_2HPO_4 buffer, about 0.022% SDS, and about 50 mg/mL mannitol, and having a pH of about 7.5.

Dosage Forms

[0276] The immunocytokine compositions described herein can be in a variety of dosage forms. In some embodiments, the immunocytokine composition is dosed as a lyophilized powder. In some embodiments, the immunocytokine composition is dosed as a suspension. In some embodiments, the immunocytokine composition is dosed as a solution. In some embodiments, the immunocytokine composition is dosed as an injectable solution. In some embodiments, the immunocytokine composition is dosed as an IV solution.

Methods of Treatment

[0277] In one aspect, described herein, is a method of treating cancer in a subject in need thereof, comprising: administering to the subject an effective amount of an immunocytokine composition (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) or a pharmaceutical composition as described herein. In another aspect provided herien is a method of method of expanding a population of $CD8^+$ T cells in a subject in need thereof, the method comprising administering to the subject an immunocytokine composition described herien (*e.g.*, an anti-PD-1 / IL-18 immunocytokine composition) or a pharmaceutical composition provided herein. In some embodiments, the $CD8^+$ T cells are $PD\text{-}1^+/IL\text{-}18R\alpha^+$ $CD8^+$ T cells. In some embodiments, the $CD8^+$ T cells are preferentially expanded in a tumor microenvironment. In some embodiments, expanding the population of $CD8^+$ T cells in the subject is useful in the treatment of cancer.

[0278] In some embodiments, the cancer is a solid cancer. A cancer or tumor can be, for example, a primary cancer or tumor or a metastatic cancer or tumor. In some embodiments, the cancer is a solid cancer. In some embodiments, the solid cancer is adrenal cancer, anal cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, carcinoid cancer, cervical cancer, colorectal cancer, esophageal cancer, eye cancer, gallbladder cancer, gastrointestinal stromal tumor, germ cell cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, neuroendocrine cancer,

oral cancer, oropharyngeal cancer, ovarian cancer, pancreatic cancer, pediatric cancer, penile cancer, pituitary cancer, prostate cancer, skin cancer, soft tissue cancer, spinal cord cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, ureteral cancer, uterine cancer, vaginal cancer, or vulvar cancer.

[0279] In some embodiments, the cancer is a blood cancer. In some embodiments, the blood cancer is leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, an AIDS-related lymphoma, multiple myeloma, plasmacytoma, post-transplantation lymphoproliferative disorder, or Waldenstrom macroglobulinemia.

[0280] In some embodiments, the cancer is resistant to treatment with an immune checkpoint inhibitor alone (e.g., resistant to treatment with an anti-PD-1 antibody not comprised in an immunocytokine composition as described herein). In some embodiments, a cancer is resistant to treatment with an immune checkpoint inhibitor alone when treatment with the immune checkpoint inhibitor alone is deemed clinically ineffective or sub-optimal (e.g., does not improve patient outcomes or extend patient life or quality of life beyond statistically significant measures, or otherwise fails to meet clinically relevant endpoints in patients). In some embodiments, the cancer is resistant to treatment with an anti-PD-1 antibody alone. In some embodiments, the cancer is resistant to treatment with the checkpoint inhibitor (e.g., anti-PD-1 antibody) in combination with another active agent (e.g., an additional standard of care therapeutic). In some embodiments, treatment with the checkpoint inhibitor in combination with the other active agent offers no clinically significant benefit (i.e., administration of the checkpoint inhibitor does not improve patient outcome to a clinically significant amount as compered to the additional therapeutic alone). In some embodiments, administration of an immunocytokine composition as provided herein results in an improved patient outcome compared to a corresponding checkpoint inhibitor either alone or in combination with an additional active agent (e.g., an immunocytokine composition comprising an anti-PD-1 antibody or antigen binding fragment thereof is more efficacious than an anti-PD-1 antibody not comprised in an immunocytokine composition, either alone or in a corresponding therapy regimen with an additional active agent).

[0281] Combination therapies with one or more additional active agents are contemplated herein.

[0282] An effective response is achieved when the subject experiences partial or total alleviation or reduction of signs or symptoms of illness, and specifically includes, without

limitation, prolongation of survival. The expected progression-free survival times may be measured in months to years, depending on prognostic factors including the number of relapses, stage of disease, and other factors. Prolonging survival includes without limitation times of at least 1 month (mo), about at least 2 mos., about at least 3 mos., about at least 4 mos., about at least 6 mos., about at least 1 year, about at least 2 years, about at least 3 years, about at least 4 years, about at least 5 years, *etc.* Overall or progression-free survival can be also measured in months to years. Alternatively, an effective response may be that a subject's symptoms or cancer burden remain static and do not worsen. Further treatment of indications are described in more detail below. In some instances, a cancer or tumor is reduced by at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0283] In some embodiments, the immunocytokine composition is administered in a single dose of the effective amount of immunocytokine composition, including further embodiments in which (i) the immunocytokine composition is administered once a day; or (ii) the immunocytokine composition is administered once a day; or (ii) the immunocytokine composition is administered to the subject multiple times over the span of one day. In some embodiments, the conjugate is administered daily, every other day, twice a week, 3 times a week, once a week, every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 12 weeks, every 3 days, every 4 days, every 5 days, every 6 days, 2 times a week, 3 times a week, 4 times a week, 5 times a week, 6 times a week, once a month, twice a month, 3 times a month, 4 times a month, once every 2 months, once every 3 months, once every 4 months, once every 5 months, or once every 6 months. Administration includes, but is not limited to, injection by any suitable route (*e.g.*, parenteral, enteral, intravenous, subcutaneous, *etc.*).

Methods of Manufacturing

[0284] In one aspect, described herein, is a method of making an immunocytokine composition, comprising providing an antibody or antigen binding fragment thereof (*e.g.,* an antibody or antigen binding fragment provided herein), wherein the antibody comprises a reactive group (*e.g.*, a conjugation handle), contacting the reactive group with a complementary reactive group attached to an IL-18 polypeptide, and forming the immunocytokine composition. The resulting composition is any of the compositions provided herein.

[0285] In some embodiments, providing the antibody comprising the reactive group comprises attaching the reactive group to the antibody. In some embodiments, the reactive group is added

site-specifically. In some embodiments, attaching the reactive group to the antibody comprises contacting the antibody with an affinity group comprising a reactive functionality which forms a bond with a specific residue of the antibody. In some embodiments, attaching the reactive group to the antibody comprises contacting the antibody with an enzyme. In some embodiments, the enzyme is configured to site-specifically attach the reactive group to a specific residue of the antibody. In some embodiments, the enzyme is glycosylation enzyme or a transglutaminase enzyme.

[0286] In some embodiments, the method further comprises attaching the complementary reactive group to the cytokine.

Definitions

[0287] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0288] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.,* to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0289] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. In this application, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0290] In this application, the use of "or" means "and/or" unless stated otherwise. The terms "and/or" and "any combination thereof" and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any combination is specifically contemplated. Solely for illustrative purposes, the following phrases "A, B, and/or C" or "A, B, C, or any combination thereof" can mean "A individually; B individually; C individually; A and B; B and C; A and C; and A, B, and C." The term "or" can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[0291] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 15%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, within 5-fold, or within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0292] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0293] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures. To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

[0294] Referred to herein are groups which are "attached" or "covalently attached" to residues of IL-18 polypeptides or other polypeptides. As used herein, "attached" or "covalently attached" means that the group is tethered to the indicated reside, and such tethering can include a linking group (*i.e.,* a linker). Thus, for a group "attached" or "covalently attached" to a residue, it is expressly contemplated that such linking groups are also encompassed.

[0295] Binding affinity refers to the strength of a binding interaction between a single molecule and its ligand/binding partner. A higher binding affinity refers to a higher strength bond than a lower binding affinity. In some instances, binding affinity is measured by the dissociation constant (K_D) between the two relevant molecules. When comparing K_D values, a binding interaction with

a lower value will have a higher binding affinity than a binding interaction with a higher value. For a protein-ligand interaction, K_D is calculated according to the following formula:

$$K_D = \frac{[L][P]}{[LP]}$$

where [L] is the concentration of the ligand, [P] is the concentration of the protein, and [LP] is the concentration of the ligand/protein complex.

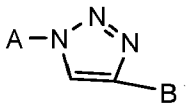
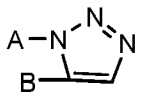
[0296] Referred to herein are certain amino acid sequences (*e.g.*, polypeptide sequences) which have a certain percent sequence identity to a reference sequence or refer to a residue at a position corresponding to a position of a reference sequence. Sequence identity is measured by protein-protein BLAST algorithm using parameters of Matrix BLOSUM62, Gap Costs Existence:11, Extension:1, and Compositional Adjustments Conditional Compositional Score Matrix Adjustment. This alignment algorithm is also used to assess if a residue is at a "corresponding" position through an analysis of the alignment of the two sequences being compared.

[0297] The term "pharmaceutically acceptable" refers to approved or approvable by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia (U.S.P.) or other generally recognized pharmacopeia for use in animals, including humans.

[0298] A "pharmaceutically acceptable excipient, carrier, or diluent" refers to an excipient, carrier, or diluent that can be administered to a subject, together with an agent, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

[0299] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9. With respect to sub-ranges, "nested sub-ranges" that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

[0300] Throughout the instant description, certain numerical or other similar values may be described as, for example, "at least" or "at most" a set of values indicated in a list form (e.g., "at least 2, 3, 4, 5, or 6"). In such cases, unless context clearly indicates otherwise, it is intended that the phrase "at least," "at most," or other similar term is applied individually to each value in the list. For example, the phrase "at least 2, 3, 4, 5, or 6" is to be interpreted as "at least 2, at least 3, at least 4, at least 5, or at least 6."

[0301] Certain formulas and other illustrations provided herein depict triazole reaction products resulting from azide-alkyne cycloaddition reactions. While such formulas generally depict only a single regioisomer of the resulting triazole formed in the reaction, it is intended that the formulas encompass both resulting regioisomers. Thus, while the formulas depict only a single regioisomer (*e.g.* ), it is intended that the other regioisomer (*e.g.* ) is also encompassed.

[0302] The term "subject" refers to an animal which is the object of treatment, observation, or experiment. By way of example only, a subject includes, but is not limited to, a mammal, including, but not limited to, a human or a non-human mammal, such as a non-human primate, bovine, equine, canine, ovine, or feline.

[0303] The term "optional" or "optionally" denotes that a subsequently described event or circumstance can but need not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not.

[0304] The term "moiety" refers to a specific segment or functional group of a molecule. Chemical moieties are often recognized chemical entities embedded in or appended to a molecule.

[0305] As used herein, the term "number average molecular weight" (Mn) means the statistical average molecular weight of all the individual units in a sample, and is defined by Formula (1):

$$Mn = \frac{\sum N_i \, M_i}{\sum N_i}$$

Formula (1)

where M_i is the molecular weight of a unit and N_i is the number of units of that molecular weight.

[0306] As used herein, the term "weight average molecular weight" (Mw) means the number defined by Formula (2):

$$Mw = \frac{\sum N_i \, M_i^2}{\sum N_i \, M_i}$$

Formula (2)

where M_i is the molecular weight of a unit and N_i is the number of units of that molecular weight.

[0307] As used herein, "peak molecular weight" (Mp) means the molecular weight of the highest peak in a given analytical method (*e.g.,* mass spectrometry, size exclusion chromatography, dynamic light scattering, analytical centrifugation, *etc.*).

[0308] As used herein, "conjugation handle" refers to a reactive group capable of forming a bond upon contacting a complementary reactive group. In some instances, a conjugation handle preferably does not have a substantial reactivity with other molecules which do not comprise the intended complementary reactive group. Non-limiting examples of conjugation handles, their respective complementary conjugation handles, and corresponding reaction products can be found in the table below. While table headings place certain reactive groups under the title "conjugation handle" or "complementary conjugation handle," it is intended that any reference to a conjugation handle can instead encompass the complementary conjugation handles listed in the table (*e.g.,* a trans-cyclooctene can be a conjugation handle, in which case tetrazine would be the complementary conjugation handle). In some instances, amine conjugation handles and conjugation handles complementary to amines are less preferable for use in biological systems owing to the ubiquitous presence of amines in biological systems and the increased likelihood for off-target conjugation.

Table of Conjugation Handles

Conjugation Handle	**Complementary Conjugation Handle**	**Reaction Product**
Sulfhydryl	alpha-halo-carbonyl (*e.g.,* bromoacetamide), alpha-beta unsaturated carbonyl (e.g., maleimide, acrylamide)	thioether
Azide	alkyne (*e.g.,* terminal alkyne, substituted cyclooctyne (*e.g.,* dibenzocycloocytne (DBCO), difluorocyclooctyne, bicyclo[6.1.0]nonyne, etc.))	triazole
Phosphine	Azide/ester pair	amide
Tetrazine	*trans*-cyoclooctene	dihydropyridazine
Amine	Activated ester (*e.g.,* N-hydroxysuccinimide ester, pentaflurophenyl ester)	amide
isocyanate	amine	urea

epoxide	amine	alkyl-amine
hydroxyl amine	aldehyde, ketone	oxime
hydrazide	aldehyde, ketone	hydrazone
potassium acyl trifluoroborate	O-substituted hydroxylamine (e.g., O-carbamoylhydroxylamine)	amide

[0309] Throughout the instant application, prefixes are used before the term "conjugation handle" to denote the functionality to which the conjugation handle is linked. For example, a "protein conjugation handle" is a conjugation handle attached to a protein (either directly or through a linker), an "antibody conjugation handle" is a conjugation handle attached to an antibody (either directly or through a linker), and a "linker conjugation handle" is a conjugation handle attached to a linker group (*e.g.*, a bifunctional linker used to link a synthetic protein and an antibody).

[0310] The term "alkyl" refers to a straight or branched hydrocarbon chain radical, having from one to twenty carbon atoms, and which is attached to the rest of the molecule by a single bond. An alkyl comprising up to 10 carbon atoms is referred to as a C_1-C_{10} alkyl, likewise, for example, an alkyl comprising up to 6 carbon atoms is a C_1-C_6 alkyl. Alkyls (and other moieties defined herein) comprising other numbers of carbon atoms are represented similarly. Alkyl groups include, but are not limited to, C_1-C_{10} alkyl, C_1-C_9 alkyl, Ci-C_8 alkyl, C_1-C_7 alkyl, C_1-C_6 alkyl, C_1-C_5 alkyl, C_1-C_4 alkyl, C_1-C_3 alkyl, C_1-C_2 alkyl, C_2-C_8 alkyl, C_3-C_8 alkyl and C_4-C_8 alkyl. Representative alkyl groups include, but are not limited to, methyl, ethyl, *n*-propyl, 1 -methyl ethyl (*i*-propyl), *n*-butyl, *i*-butyl, 5-butyl, *n*-pentyl, 1,1 -dimethyl ethyl (*i*-butyl), 3-methylhexyl, 2- methylhexyl, 1 -ethyl-propyl, and the like. In some embodiments, the alkyl is methyl or ethyl. In some embodiments, the alkyl is -$CH(CH_3)_2$ or -$C(CH_3)_3$. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted. "Alkylene" or "alkylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group. In some embodiments, the alkylene is -CFF-, -CH_2CH_2-, or -$CH_2CH_2CH_2$-. In some embodiments, the alkylene is -CH_2-. In some embodiments, the alkylene is -CH_2CH_2-. In some embodiments, the alkylene is -$CH_2CH_2CH_2$-. Unless stated otherwise specifically in the specification, an alkylene group may be optionally substituted.

[0311] The term "alkenylene" or "alkenylene chain" refers to a straight or branched divalent hydrocarbon chain in which at least one carbon-carbon double bond is present linking the rest of the molecule to a radical group. In some embodiments, the alkenylene is -CH=CH-, -CH_2CH=CH-

, or -CH=CHCH$_2$-. In some embodiments, the alkenylene is -CH=CH-. In some embodiments, the alkenylene is -CH$_2$CH=CH-. In some embodiments, the alkenylene is -CH=CHCH$_2$-.

[0312] The term "alkynyl" refers to a type of alkyl group in which at least one carbon-carbon triple bond is present. In one embodiment, an alkenyl group has the formula -C≡C-R^X, wherein R^x refers to the remaining portions of the alkynyl group. In some embodiments, R^x is H or an alkyl. In some embodiments, an alkynyl is selected from ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the like. Non-limiting examples of an alkynyl group include -C≡CH, -C≡CCH3, - C≡CCH$_2$CH , and -CH$_2$C≡CH.

[0313] The term "aryl" refers to a radical comprising at least one aromatic ring wherein each of the atoms forming the ring is a carbon atom. Aryl groups can be optionally substituted. Examples of aryl groups include, but are not limited to phenyl, and naphthyl. In some embodiments, the aryl is phenyl. Depending on the structure, an aryl group can be a monoradical or a diradical (i.e., an arylene group). Unless stated otherwise specifically in the specification, the term "aryl" or the prefix "ar-"(such as in "aralkyl") is meant to include aryl radicals that are optionally substituted. In some embodiments, an aryl group comprises a partially reduced cycloalkyl group defined herein (e.g., 1,2-dihydronaphthalene). In some embodiments, an aryl group comprises a fully reduced cycloalkyl group defined herein (e.g., 1,2,3,4-tetrahydronaphthalene). When aryl comprises a cycloalkyl group, the aryl is bonded to the rest of the molecule through an aromatic ring carbon atom. An aryl radical can be a monocyclic or polycyclic (e.g., bicyclic, tricyclic, or tetracyclic) ring system, which may include fused, spiro or bridged ring systems.

[0314] The term "cycloalkyl" refers to a monocyclic or polycyclic non-aromatic radical, wherein each of the atoms forming the ring (i.e. skeletal atoms) is a carbon atom. In some embodiments, cycloalkyls are saturated or partially unsaturated. In some embodiments, cycloalkyls are spirocyclic or bridged compounds. In some embodiments, cycloalkyls are fused with an aromatic ring (in which case the cycloalkyl is bonded through a non-aromatic ring carbon atom). Cycloalkyl groups include groups having from 3 to 10 ring atoms. Representative cycloalkyls include, but are not limited to, cycloalkyls having from three to ten carbon atoms, from three to eight carbon atoms, from three to six carbon atoms, or from three to five carbon atoms. Monocyclic cycloalkyl radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. In some embodiments, the monocyclic cycloalkyl is cyclopentyl. In some embodiments, the monocyclic cycloalkyl is cyclopentenyl or cyclohexenyl. In some embodiments,

the monocyclic cycloalkyl is cyclopentenyl. Polycyclic radicals include, for example, adamantyl, 1,2-dihydronaphthalenyl, 1,4-dihydronaphthalenyl, tetrainyl, decalinyl, 3,4- dihydronaphthalenyl-l(2H)-one, spiro[2.2]pentyl, norbornyl and bicycle[l.l.l]pentyl. Unless otherwise stated specifically in the specification, a cycloalkyl group may be optionally substituted.

[0315] The term "heteroalkylene" or "heteroalkylene chain" refers to a straight or branched divalent heteroalkyl chain linking the rest of the molecule to a radical group. Unless stated otherwise specifically in the specification, the heteroalkyl or heteroalkylene group may be optionally substituted as described below. Representative heteroalkylene groups include, but are not limited to $-CH_2-O-CH_2-$, $-CH_2-N(alkyl)-CH_2-$, $-CH_2-N(aryl)-CH_2-$, $-OCH_2CH_2O-$, $-OCH_2CH_20CH_2CH_2O-$, or - O CH_2CH_2 O CH_2CH_2 O CH_2CH_2O -.

[0316] The term "heteocycloalkyl" refers to a cycloalkyl group that includes at least one heteroatom selected from nitrogen, oxygen, and sulfur. Unless stated otherwise specifically in the specification, the heterocycloalkyl radical may be a monocyclic, or bicyclic ring system, which may include fused (when fused with an aryl or a heteroaryl ring, the heterocycloalkyl is bonded through a non-aromatic ring atom) or bridged ring systems. The nitrogen, carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidized. The nitrogen atom may be optionally quatemized. The heterocycloalkyl radical is partially or fully saturated. Examples of heterocycloalkyl radicals include, but are not limited to, dioxolanyl, thienyl[l,3]dithianyl, tetrahydroquinolyl, tetrahydroisoquinolyl, decahydroquinolyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl, 1,1-dioxo-thiomorpholinyl. The term heterocycloalkyl also includes all ring forms of carbohydrates, including but not limited to monosaccharides, disaccharides and oligosaccharides. Unless otherwise noted, heterocycloalkyls have from 2 to 12 carbons in the ring. In some embodiments, heterocycloalkyls have from 2 to 10 carbons in the ring. In some embodiments, heterocycloalkyls have from 2 to 10 carbons in the ring and 1 or 2 N atoms. In some embodiments, heterocycloalkyls have from 2 to 10 carbons in the ring and 3 or 4 N atoms. In some embodiments, heterocycloalkyls have from 2 to 12 carbons, 0-2 N atoms, 0-2 O atoms, 0-2 P atoms, and 0-1 S atoms in the ring. In some embodiments, heterocycloalkyls have from 2 to 12

carbons, 1-3 N atoms, 0-1 O atoms, and 0-1 S atoms in the ring. It is understood that when referring to the number of carbon atoms in a heterocycloalkyl, the number of carbon atoms in the heterocycloalkyl is not the same as the total number of atoms (including the heteroatoms) that make up the heterocycloalkyl (i.e. skeletal atoms of the heterocycloalkyl ring). Unless stated otherwise specifically in the specification, a heterocycloalkyl group may be optionally substituted.

[0317] The term "heteroaryl" refers to an aryl group that includes one or more ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, heteroaryl is monocyclic or bicyclic. Illustrative examples of monocyclic heteroaryls include pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, pyridazinyl, triazinyl, oxadiazolyl, thiadiazolyl, furazanyl, indolizine, indole, benzofuran, benzothiophene, indazole, benzimidazole, purine, quinolizine, quinoline, isoquinoline, cinnoline, phthalazine, quinazoline, quinoxaline, 1,8-naphthyridine, and pteridine. Illustrative examples of monocyclic heteroaryls include pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, pyridazinyl, triazinyl, oxadiazolyl, thiadiazolyl, and furazanyl. Illustrative examples of bicyclic heteroaryls include indolizine, indole, benzofuran, benzothiophene, indazole, benzimidazole, purine, quinolizine, quinoline, isoquinoline, cinnoline, phthalazine, quinazoline, quinoxaline, 1,8-naphthyridine, and pteridine. In some embodiments, heteroaryl is pyridinyl, pyrazinyl, pyrimidinyl, thiazolyl, thienyl, thiadiazolyl or furyl. In some embodiments, a heteroaryl contains 0-6 N atoms in the ring. In some embodiments, a heteroaryl contains 1-4 N atoms in the ring. In some embodiments, a heteroaryl contains 4-6 N atoms in the ring. In some embodiments, a heteroaryl contains 0-4 N atoms, 0-1 0 atoms, 0-1 P atoms, and 0- 1 S atoms in the ring. In some embodiments, a heteroaryl contains 1-4 N atoms, 0-1 0 atoms, and 0-1 S atoms in the ring. In some embodiments, heteroaryl is a C_1-C_9 heteroaryl. In some embodiments, monocyclic heteroaryl is a C_1-C_5 heteroaryl. In some embodiments, monocyclic heteroaryl is a 5-membered or 6-membered heteroaryl. In some embodiments, a bicyclic heteroaryl is a C6-C9 heteroaryl. In some embodiments, a heteroaryl group comprises a partially reduced cycloalkyl or heterocycloalkyl group defined herein (e.g., 7,8-dihydroquinoline). In some embodiments, a heteroaryl group comprises a fully reduced cycloalkyl or heterocycloalkyl group defined herein (e.g., 5,6,7, 8-tetrahydroquinoline). When heteroaryl comprises a cycloalkyl or heterocycloalkyl group, the heteroaryl is bonded to the rest of the molecule through a

heteroaromatic ring carbon or hetero atom. A heteroaryl radical can be a monocyclic or polycyclic (e.g., bicyclic, tricyclic, or tetracyclic) ring system, which may include fused, spiro or bridged ring systems.

[0318] The term "optionally substituted" or "substituted" means that the referenced group is optionally substituted with one or more additional group(s) individually and independently selected from D, halogen, -CN, -NH_2, -NH(alkyl), -$N(alkyl)_2$, -OH, -CO_2H, -CO_2alkyl, -$C(=0)NH_2$, -C(=0)NH(alkyl), -$C(=0)N(alkyl)_2$, -$S(=0)_2NH_2$, -$S(=0)_2$NH(alkyl), -$S(=0)_2N(alkyl)_2$, alkyl, cycloalkyl, fluoroalkyl, heteroalkyl, alkoxy, fluoroalkoxy, heterocycloalkyl, aryl, heteroaryl, aryloxy, alkylthio, arylthio, alkylsulfoxide, arylsulfoxide, alkylsulfone, and arylsulfone. In some other embodiments, optional substituents are independently selected from D, halogen, -CN, -NH_2, -$NH(CH_3)$, -$N(CH_3)_2$, -OH, -$C0_2H$, -$C0_2$(Ci-C_4alkyl), - $C(=0)NH_2$, - C(=0)NH(C 1 -C_4alkyl), -$C(=0)N$(Ci-C_4alkyl)$_2$, -$S(=0)_2NH_2$, -$S(=0)_2$NH(Ci- C_4alkyl), - $S(=0)_2N(C_1$-C_4alkyl)$_2$, C_1-C_4alkyl, C_3-C_6cycloalkyl, C_1-C_4fluoroalkyl, C_1-C_4heteroalkyl, C_1-C_4alkoxy, C_1-C_4fluoroalkoxy, -SC_1-C_4alkyl, -$S(=0)C_1$-C_4alkyl, and -$S(=0)_2C_1$- C_4alkyl. In some embodiments, optional substituents are independently selected from D, halogen, -CN, -NH_2, -OH, -$NH(CH_3)$, -$N(CH_3)_2$, -NH(cyclopropyl), -CH_3, -CH_2CH_3, -CF_3, -OCH_3, and - OCF_3. In some embodiments, substituted groups are substituted with one or two of the preceding groups. In some embodiments, an optional substituent on an aliphatic carbon atom (acyclic or cyclic) includes oxo (=0).

[0319] As used herein, "AJICAP^{TM} technology," "AJICAP^{TM} methods," and similar terms refer to systems and methods (currently produced by Ajinomoto Bio-Pharma Services ("Ajinomoto")) for the site specific functionalization of antibodies and related molecules using affinity peptides to deliver the desired functionalization to the desired site. General protocols for the AJICAP^{TM} methodology are found at least in PCT Publication No. WO2018199337A1, PCT Publication No. WO2019240288A1, PCT Publication No. WO2019240287A1, PCT Publication No. WO2020090979A1, Matsuda et al., *Mol. Pharmaceutics* 2021, 18, 4058-4066, and Yamada et al., AJICAP: Affinity Peptide Mediated Regiodivergent Functionalization of Native Antibodies. *Angew. Chem., Int. Ed.* 2019, 58, 5592-5597, and in particular Examples 2-4 of US Patent Publication No. US20200190165A1. In some embodiments, such methodologies site specifically incorporate the desired functionalization at lysine residues at a position selected from position 246, position 248, position 288, position 290, and position 317 of an antibody Fc region (e.g., an IgG1

Fc region) (EU numbering). In some embodiments, the desired functionalization is incorporated at residue position 248 of an antibody Fc region (EU numbering). In some embodiments, position 248 corresponds to the 18th residue in a human IgG CH2 region (EU numbering).

Sequences (SEQ ID NOS) of IL-18 Polypeptides

TABLE 2

SEQ ID NO:	Modification	Sequence
1	Native sequence	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYKDSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
2	E6K, C38A, K53A, C68A, E85C	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKAEK ISTLSCENKI ISFKCMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
3	E6K, V11I, C38A, K53A, T63A, C68A, C76A, E85C, C127A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKAEK ISTLSAENKI ISFKCMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
4	E6K, C38A, K53A, C68A, M86C	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKAEK ISTLSCENKI ISFKECNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
5	E6K, V11I, C38A, K53A, T63A, C68A, C76A, M86C, C127A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKAEK ISTLSAENKI ISFKECNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
6	E6K, V11I, C38A, K53A, T63A, C68A, C76A, D98C, C127A	YFGKL**K**SKLS **I**IRNLNDQVL FIDQGNRPLF EDMTDSD**A**RD NAPRTIFIIS MY**A**DSQPRGM AV**A**ISVK**A**EK ISTLS**A**ENKI ISFKEMNPPD NIKDTKS**C**II FFQRSVPGHD NKMQFESSSY EGYFLA**A**EKE RDLFKLILKK EDELGDRSIM FTVQNED
7	E6K, C38A, K53A, C68A, C76A, M86C, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKAEK ISTLSAENKI ISFKECNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
8	E6K, V11I, C38A, K53A, T63A, C68A, C76A, T95C, C127A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKAEK ISTLSAENKI ISFKEMNPPD NIKDCKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
9	E6K, C38A, K53A, C68A, D98C	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKAEK ISTLSCENKI ISFKEMNPPD NIKDTKSCII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED

10	E6K, C38A, K53A, C68A, C76A, D98C, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKAEK ISTLSAENKI ISFKEMNPPD NIKDTKSCII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
11	E6K, V11I, C38A, K53A, C76A, C127A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
12	E6K, C38A, K53A, T63A, C76A, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
13	E6K, K53A, T63N	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVNISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
14	E6K, K53A, S50A, T63N	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIA MYADSQPRGM AVNISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
15	E6K, K53A, S50H, T63N	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIH MYADSQPRGM AVNISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
16	E6K, K53A, T63N, S65A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVNIAVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
17	E6K, K53A, S50H	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIH MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
18	E6K, C38A, K53A, C68A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKAEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
19	E6K, K53A, K79A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENAI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED

20	E6K, K53A, R104A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENAI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
21	E6K, K53A, G108A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPAHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
22	E6K, K53A, H109A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGAD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
23	E6K, K53A, K112A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NAMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
24	E6K, C38A, K53A, T63A, C76A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
25	E6K, C38Q, K53A, T63A, C76A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDQRD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
26	E6K, C38A, K53A, T63A, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
27	E6K, C38Q, K53A, T63A, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDQRD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
28	E6K, C38A, K53A, T63A, C76A, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
29	E6K, V11I, C38A, K53A, T63A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED

30	E6K, V11I, C38A, K53A, T63A, C76A, C127A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
31	C38A, C76A, C127A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYKDSQPRGM AVTISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
32	C38A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYKDSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
33	E6K, C38A, K53A, T63A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
34	E06K, K53A, S55A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
35	Y01G, F02A, E06K, M51G, K53A, D54A, S55A, T63A	GAGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS GYAAAQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
36	K53A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
37	S55A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYKDAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
38	E06K	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYKDSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
39	E06K, K53A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED

40	E06K, S55A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYKDAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
41	K53A, S55A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
42	E06K, K53A, S55A, T63A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADAQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
43	E06K, K53A, S55A, Y01G	GFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
44	E06K, K53A, S55A, F02A	YAGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
45	E06K, K53A, S55A, D54A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYAAAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
46	E06K, K53A, S55A, M51G	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS GYADAQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
47	C38S, C68S, C76S, C127S	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYKDSQPRGM AVTISVKSEK ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED
48	C38S, C68S, C76S, C127S, K70C	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYKDSQPRGM AVTISVKSEC ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED
49	E06K, K53A, S55A, C38S, C68S, C76S, C127S, K70C	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYADAQPRGM AVTISVKSEC ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED

50	E06K, K53A, T63A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
51	T63A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYKDSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
52	E06K, T63A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYKDSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
53	K53A, T63A	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
54	E06K, K53A, C38S, C68S, C76S, C127S, K70C	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYADSQPRGM AVTISVKSEC ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED
55	K53A, T63A, C38S, C68S, C76S, C127S, K70C	YFGKLESKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYADSQPRGM AVAISVKSEC ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED
56	E6K, K53A, C38S, C76S, C127S	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED
57	E6K, C38S, K53A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
58	E6K, K53A, C38S, C68S, C76S, C127S, K70C	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDSRD NAPRTIFIIS MYADSQPRGM AVTISVKSEC ISTLSSENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLASEKE RDLFKLILKK EDELGDRSIM FTVQNED
59	E6K, C38A, K53A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED

60	E6K, C38Q, K53A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDQRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
61	E6K, C38A, K53A, C76A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
62	E6K, C38A, K53A, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
63	E6K, C38A, K53A, C76A, C127A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
64	E6K, K53A, C38A, S55A, T63A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS MYADAQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
65	E6K, C38Q, K53A, S55A, T63A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDQRD NAPRTIFIIS MYADAQPRGM AVAISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
66	E6K, K53A, K84A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFAEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
67	E6K, K53A, D98A	YFGKLKSKLS VIRNLNDQVL FIDQGNRPLF EDMTDSDCRD NAPRTIFIIS MYADSQPRGM AVTISVKCEK ISTLSCENKI ISFKEMNPPD NIKDTKSAII FFQRSVPGHD NKMQFESSSY EGYFLACEKE RDLFKLILKK EDELGDRSIM FTVQNED
68 (a.k.a. C146)	V11I, C38A, M51G, K53A, C76A, C127A	YFGKLESKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS GYADSQPRGM AVTISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED
69 (a.k.a. C183)	E6K, V11I, C38A, M51G, K53A, T63A, C76A, C127A	YFGKLKSKLS IIRNLNDQVL FIDQGNRPLF EDMTDSDARD NAPRTIFIIS GYADSQPRGM AVAISVKCEK ISTLSAENKI ISFKEMNPPD NIKDTKSDII FFQRSVPGHD NKMQFESSSY EGYFLAAEKE RDLFKLILKK EDELGDRSIM FTVQNED

70 (a.k.a. C192)	N-terminal G, E6K, V11I, C38A, M51G, K53A, T63A, C76A, C127A	GYFGKLKSKL SIIRNLNDQV LFIDQGNRPL FEDMTDSDAR DNAPRTIFII SGYADSQPRG MAVAISVKCE KISTLSAENK IISFKEMNPP DNIKDTKSDI IFFQRSVPGH DNKMQFESSS YEGYFLAAEK ERDLFKLILK KEDELGDRSI MFTVQNED
71 (a.k.a. C141)	N-terminal G, E6K, V11I, C38A, K53A, T63A, C76A, C127A	GYFGKLKSKL SIIRNLNDQV LFIDQGNRPL FEDMTDSDAR DNAPRTIFII SMYADSQPRG MAVAISVKCE KISTLSAENK IISFKEMNPP DNIKDTKSDI IFFQRSVPGH DNKMQFESSS YEGYFLAAEK ERDLFKLILK KEDELGDRSI MFTVQNED
72 (a.k.a. C140)	N-terminal 4xG, E6K, V11I, C38A, K53A, T63A, C76A, C127A	GGGGYFGKLK SKLSIIRNLN DQVLFIDQGN RPLFEDMTDS DARDNAPRTI FIISMYADSQ PRGMAVAISV KCEKISTLSA ENKIISFKEM NPPDNIKDTK SDIIFFQRSV PGHDNKMQFE SSSYEGYFLA AEKERDLFKL ILKKEDELGD RSIMFTVQNE D

ADDITIONAL EXEMPLARY IL-18 CONSTRUCTS

[0320] Also provided herein are IL-18 polypeptides which comprise the modifications to SEQ ID NO: 1 listed in the table below, each of which is assigned a Composition ID, which can be incorporated into an immunocytokine composition as provided herein. In some embodiments, the IL-18 polypeptide of an immunocytokine composition comprises the set of amino acid substitutions shown for any one of the constructs depicted below. In the constructs depicted below, each of the substitutions is listed using SEQ ID NO: 1 as a reference sequence. In some embodiments, the IL-18 polypeptide an immunocytokine composition comprises only the substitutions shown for a construct below relative to SEQ ID NO: 1 (i.e., the IL-18 polypeptide has only the indicated set of substitutions and the remaining residues are those set forth in SEQ ID NO: 1).

Table 3 – Additional IL-18 Polypeptide

Composition ID / Substitutions to SEQ ID NO: 1		Composition ID / Substitutions to SEQ ID NO: 1		Composition ID / Substitutions to SEQ ID NO: 1	
C143	V11I, C38A, K53A, C76A, C127A	C156	V11I, C38A, N41A, K53A, C76A, C127A	C168	V11I, C38A, C76A, S105K, C127A
C144	V11I, C38A, K53A, T63A, C76A, C127A	C157	V11I, C38A, K53A, C76A, C127A, D132A	C174	K8L, E6K, V11I, C38A, K53A, T63A, C76A, C127A

C145	V11I, C38A, K53A, S55A, C76A, C127A	C158	V11I, C38A, K53A, C76A, G108A, C127A	C175	E6K, V11I, C38A, I49E, K53A, T63A, C76A, C127A
C147	V11I, C38A, K53A, D54A, C76A, C127A	C159	V11I, C38A, K53A, C76A, H109A, C127A	C176	E6K, V11I, C38A, I49M, K53A, T63A, C76A, C127A
C148	F2A, V11I, C38A, K53A, C76A, C127A	C160	V11I, C38A, K53A, C76A, D110A, C127A	C177	E6K, V11I, C38A, I49R, K53A, T63A, C76A, C127A
C149	V11I, E31A, C38A, K53A, C76A, C127A	C161	K8R, V11I, C38A, C76A, Q103E, C127A	C178	E6K, V11I, C38A, K53A, T63A, C76A, Q103R, C127A
C150	V11I, T34A, C38A, K53A, C76A, C127A	C162	K8E, V11I, C38A, C76A, Q103R, C127A	C179	E6K, K8E, V11I, C38A, K53A, T63A, C76A, Q103R, C127A
C151	V11I, D35A, C38A, K53A, C76A, C127A	C163	V11I, C38A, C76A, Q103K, C127A	C180	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153R
C152	V11I, S36A, C38A, K53A, C76A, C127A	C164	V11I, C38A, S55H, C76A, C127A	C181	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153E
C153	V11I, D37A, C38A, K53A, C76A, C127A	C165	V11I, C38A, S55R, C76A, C127A	C182	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153Y
C154	V11I, E31A, D37A, C38A, K53A, C76A, C127A	C166	V11I, C38A, S55T, C76A, C127A	C184	E6R, V11I, C38A, K53A, T63A, C76A, C127A
C155	V11I, C38A, D40A, K53A, C76A, C127A	C167	V11I, C38A, C76A, S105I, C127A	C142	Y1M, E6K, V11I, C38A, K53A, T63A, C76A, C127A
CX90	M51K, K53S, Q56L, P57A, M60L, S105D, D110S, N111R	CX91	C38S, M51K, K53S, Q56L, P57A, M60L, C68D, S105D, D110S, N111R	CX92	M3L, C38S, C68S, C76S, C127S, E6A, K53A
CX93	C38M, C68S, C76S, C127S, E6A, K53A	CX94	C38S, C68S, C76S, C127S, E6Q, S10C, K53D, N111T, N155C	CX95	M51A, K53G, Q56R, P57A, M60K

CX96	C38M, C76S, C127S, E6A, K53A	CX97	C38S, C76S, C127S, E6Q, S10C, K53D, N111T, N155C	CX98	M3L, C38S, C76S, C127S, E6A, K53A

[0321] Although the present disclosure and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the disclosure as defined in the appended claims.

[0322] The present disclosure is further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the disclosure in any way.

EXAMPLES

Example 1A – Recombinant IL18 Expression and Purification

[0323] Recombinant IL-18 variants suitable for linking to an antibody or antigen binding fragment as provided herein can be prepared according to the protocols provided below. In some instances, the recombinant IL-18 will contain a cysteine residue at the desired point of attachment of the linker, or may include an unnatural amino acid (*e.g.,* azidolysine) suitable for attachment of the linker at the desired point of attachment.

Soluble His-SUMO-IL18 variants

[0324] *E. coli* BL21 (DE3) harboring a plasmid encoding a N-His-SUMO tagged IL-18 variant fusion is inoculated into 3 L LB culture medium and induced with 0.4 mM IPTG at 30 °C for 6h. Cells are pelleted and cell lysis is done by sonication in lysis buffer: PBS, pH 7.4. Soluble protein is purified via Ni-NTA beads 6FF (wash 1 with: PBS, 20 mM imidazole, pH7.4; wash 2 with PBS, 50 mM Imidazole, pH7.4; elution with PBS, 500 mM imidazole, pH7.4).

[0325] Fractions containing the protein are pooled, dialyzed into PBS pH 7.4 and followed by SUMO digestion. Then the protein is two-step purified with Ni-NTA beads (continue with flow through sample) and gel filtration. Fractions containing the protein are pooled and QC is performed using analytical techniques, such as SDS-PAGE and analytical SEC.

Insoluble His-SUMO-IL18 variants

[0326] *E. coli* BL21 (DE3) harboring a plasmid encoding a N-His-SUMO tagged IL-18 variant fusion are inoculated into 10 L LB culture medium and induced with 0.4 mM IPTG at 30 °C for 6h. Cells are pelleted and cell lysis is done by sonication in lysis buffer: PBS, 8 M urea, pH 7.4. Protein is purified via Ni-NTA beads 6FF (wash 1 with: PBS, 8 M urea, 20 mM imidazole, pH7.4;

wash 2 with PBS, 8 M urea, 50 mM Imidazole, pH7.4; elution with PBS, 8 M urea, 500 mM imidazole, pH7.4).

[0327] Fractions containing the protein are pooled, dialyzed into PBS pH 7.4 and followed by SUMO digestion. Then the protein is purified with Ni-NTA beads (equilibrate column with PBS, 8 M urea, pH 7.4, wash with PBS, 8 M urea, pH 7.4, elution with PBS, 8 M urea, pH 7.4). Fractions containing the protein are pooled, dialyzed into PBS pH 7.4 and QC is performed using analytical techniques, such as SDS-PAGE and analytical SEC.

Insoluble tagless IL18 variants

[0328] *E. coli* BL21 (DE3) harboring a plasmid encoding mIL-18 is inoculated into 2 L LB culture medium and induced with 0.4 mM IPTG at 30 °C for 6h. Cells are pelleted and cell lysis was done by sonication in lysis buffer: 110 mM Tris, 1.1 M guanidine HCl, 5 mM DTT, pH 8.9. Protein as purified via Q Sepharose FF (balance buffer 20 mM MES, pH 7.0, elution with an increasing gradient from 0 to 1 M NaCl).

Example 1B –Additional Methods for Recombinant IL18 Expression and Purification

[0329] The following protocols were also used to prepare certain IL-18 polypeptides provided herein which were subsequently used either in assays for conversion into immunocytokine compositions as provided herien.

[0330] Expression of IL-18 Polypeptides

[0331] IL-18 polypeptide were produced as an N-terminal fusion to N-His-SUMO-IL18. The gene was synthesized and cloned by a commercial vendor. Plasmids were transformed into *E. coli* BL21 (DE3). Expression was performed in shake flasks with TB medium. The cells were grown at 37 °C until an OD600 of approximately 1.2 was reached, after which they were induced by 0.1 mM IPTG and cultured for another 20 hours at 18 °C. Cells were harvested by centrifugation.

[0332] Purification of IL18 Polypeptides

[0333] *Cell lysis* - Cells were resuspended in lysis buffer (20 mM Tris/HCl, pH 8.0, 0.15 M NaCl, 10 mM Imidazole, 1 tablet of EDTA-free complete protease inhibitor (Roche, COEDTAF-RO) per liter production) at 100 mL buffer/L culture and disrupted twice with a homogenizer at 1000 bar. The lysate was cleared of debris by centrifugation at 40'000 g for 2x 45 minutes, changing flask in between, and subsequent filtration through a 0.22 µm filter.

[0334] *Affinity Purification and Endotoxin Removal* - The lysate was loaded on Ni NTA resin (Cytiva, 17524802) pre-equilibrated with 20 mM Tris/HCl, pH 8.0, 0.15 M NaCl, 10 mM

Imidazole, at 5 mL/min and washed with the same buffer for 5 CV. To remove endotoxins, the column was washed with 20 mM Tris/HCl, pH 8.0, 0.15 M NaCl, 10 mM Imidazole, 0.1% Tryton X-114 at 10 mL/min for 30 CV. The column was washed with 20 mM Tris/HCl, pH 8.0, 0.15 M NaCl, 10 mM Imidazole, for 5 CV at 5 mL/min and the protein of interest eluted by linear increase of imidazole concentration. The column was then regenerated by 0.5M NaOH.

[0335] *SUMO digestion and dialysis* - To cleave the SUMO tag, SUMO protease was added to the elution pool at a w/w ratio of 1:250 (protein:SUMO enzyme) and incubated for 18 hours at 4°C. At the same time, the protein was dialysed (20 mM Tris, pH 8.0, 150mM NaCl), to reduce the imidazole concentration.

[0336] *Purification by reverse IMAC* - In order to remove the cleaved tag and the SUMO protease, the digested protein was flown through a Ni NTA resin column pre-equilibrated with 20 mM Tris/HCl, pH 8.0, 0.15 M NaCl, 10 mM Imidazole, at 5 mL/min. The flow-through was collected.

[0337] *Buffer Exchange* - The flow-through was concentrated to 2.6 mg/mL and buffer exchanged into either 20mM HEPES, 150mM NaCl, 0.5mM TCEP, 10% glycerol, pH7.5 or PBS, 10% glycerol, pH7.4. Proteins were stored at -70°C until further quality controls.

Example 2 – Conjugation of IL-18 Polypeptide With Bifunctional Linking Group

[0338] An IL-18 polypeptide as provided herein can be conjugated to a bifunctional linking group prior to forming the full linker of the immunocytokine composition. In some cases, the bifunctional linking group first attaches to a desired residue of the IL-18 polypeptide at the point of attachment of the linker. Once attached to the IL-18 polypeptide, the second functionality of the bifunctional linking group is used to attach to a second portion. An exemplary schematic of such a process is shown in **FIGURE 6**. The process shown in **FIGURE 6** is not needed for all IL-18 polypeptides, as some IL-18 polypeptides provided herein (*e.g.,* an IL-18 with the desired conjugation handle for final linkage with the antibody already attached). An exemplary protocol on an IL-18 polypeptide with a cysteine residue point of attachment provided herein is described below.

[0339] *Conjugation* – The IL-18 polypeptide is stored at a concentration of 2.4 mg/mL at -80 °C in potassium phosphate buffer (pH 7.0) containing 50 mM KCl and 1 mM DTT. The sample is thawed on ice yielding a clear solution. The protein solution is diluted in PBS, pH 7.4. A clear solution is obtained at a concentration of ~ 0.4 mg/mL.

[0340] The protein solution is dialyzed against PBS, pH 7.4 (twice against 600 mL for 2 h and once against 800 mL for 18 h). After dialysis, a clear solution is obtained with no sign of

precipitation. Protein concentration is obtained using UV absorbance at 280 nm and by BCA protein assay.

[0341] A stock solution of bi-functional linking group (*e.g.,* bromoacetamido-PEG5-azide, CAS: 1415800-37-1) in water is prepared at a concentration of 20 mM. 500 µL of the protein solution are mixed with 25 µL of linking group solution. pH was adjusted to 7.5 and it was let to react for 3 h at 20 °C.

[0342] The progress of the synthesis is monitored by reverse-phase HPLC using a gradient of 5 to 30% (2.5 min) and 30 to 75% (7.5 min) CH_3CN with 0.1% TFA (*v/v*) on a Aeris WIDEPORE C18 200 Å column (3.6 µm, 150 x 4.6 mm) at a flow rate of 1 mL/min at 40 °C and by MALDI-TOF MS.

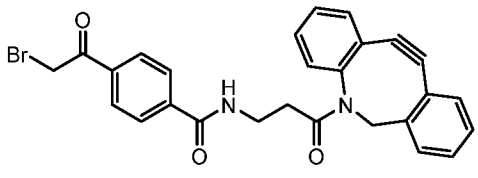
[0343] *Purification* - In some cases, ion-exchange chromatography is used to purify the conjugated protein. To remove the excess of probe, the reaction mixture (volume is around 500 µL) is flowed through a Hi-Trap-G-FF-1mL column using 25 mM Tris (pH 7.4) as the buffer. The column is eluted with a linear gradient of 0-0.35 M NaCl in the same buffer. The fractions containing the target protein are gathered, buffer exchanged (25 mM Tris, pH 7.4, 75 mM NaCl, 5% glycerol) and concentrated at 0.4 mg/mL. The concentration of purified protein is determined by UV absorbance at 280 nm and by BCA protein assay. The protein solution is kept at -80 °C

[0344] *Characterization* - The purity and identity of the recombinant protein from commercial source and the conjugated protein is confirmed by aSEC, HPLC and MALDI-TOF MS.

Example 3 – Conjugation of IL-18 Polypeptide to Antibody

[0345] *Preparation of a conjugatable antibody*

[0346] A modified antibody (*e.g.,* an anti-PD-1 antibody such as nivolumab or LZM-009) comprising a DBCO conjugation handle is prepared using a protocol modified from Examples 2-4 of US Patent Publication No. US2020019165A1. An exemplary illustration of this process resulting in the attachment of one DBCO conjugation handle is shown in **FIGURE 3.** Briefly, the CD20 antibody with a free sulfhydryl group attached to a lysine residue side chain in the Fc region is prepared by reacting the antibody with an affinity peptide configured to deliver a protected version of the sulfhydryl group (*e.g.,* a thioester) to the lysine residue. The protecting group is then removed to reveal the free sulfhydryl. The free sulfhydryl is then reacted with a bifunctional reagent comprising a bromoacetamide group connected to the DBCO conjugation handle through

a linking group (*e.g.,* ). The method can be used to produce an antibody with one DBCO group present (DAR1) and/or two DBCO groups attached to the antibody (DAR2, one DBCO group linked to each Fc of the antibody). Unless otherwise specified, all immunocytokines described herein are DAR1.

[0347] *Conjugation of antibody to IL-18 polypeptide*

[0348] The DBCO modified antibody is then conjugated to an IL-18 polypeptide comprising an azide moiety at a desired point of attachment (*e.g.,* an IL-18 polypeptide which contains an amino acid with an azide side chain or an IL-18 linked to an azide using a bifunctional linking group as in Example 4). DBCO modified antibody with one (DAR1) or two (DAR2) reactive handles are reacted with 2-10 equivalents of azide containing IL-18 (pH 5.2 buffer, 5% trehalose, rt, 24 h). In an alternative embodiment, antibody comprising two DBCO conjugation handles is reacted either as an excess reagent (e.g., 5-10 equivalents) with 1 equivalent of IL-18 comprising an azide functionality to produce a DAR1 antibody or the antibody comprising two DBCO conjugation handles is reacted with 1 equivalent of antibody with excess reagent of IL-18 comprising an azide (e.g., 5-10 equivalents) to produce a DAR2 antibody. An illustration of this protocol is shown in **FIGURE 7**.

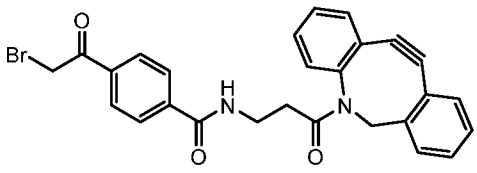
[0349] *Purification and Characterization of antibody-IL-18 immunocytokine*

[0350] The resulting immunocytokine is purified by cation-exchange chromatography and/or size exclusion chromatography to obtain purified immunocytokine. Antibody-IL-18 polypeptide immunocytokine is purified from unreacted IL-18 and aggregates using a desalting column, CIEX and SEC (GE Healthcare Life Sciences AKTA pure, mobile phase: Histidine 5.2/150 mM NaCl/5% Trehalose, column: GE Healthcare Life Sciences SUPERDEX™ 200 increase 3.2/300, flow rate: 0.5 mL/min).

[0351] The purity and identity of the antibody-IL-18 polypeptide immunocytokine is confirmed by RP-HPLC (HPLC: ThermoFisher Scientific UHPLC Ultimate 3000, column: Waters BEH C-4 300A, 3.0 µm, 4.6 mm, 250 mm, mobile phase A: 0.05% TFA in Water, mobile phase B: 0.05% TFA in mixture of ACN:IPA:ETOH:H2O (5:1.5:2:1.5), flow rate: 0.5 mL/min, injection amount: 10 µg (10 µL Injection of 1 mg/ mL), gradient: 0% to 20% mobile phase B in 50 min) and SDS-PAGE.

[0352] Exemplary chromatograms and analytical characterization of DAR1 immunocytokines of an IL-18 of SEQ ID NO: 60 conjugated via residue C68 and an anti-PD-1 antibody (LZM-009) prepared according to the described methods are shown in **FIGURES 8-10**.

[0353] Additional exemplary chromatograms and analytical characterization of a DAR1 immunocytokine of an IL-18 of SEQ ID NO: 30 conjugated via residue C68 to Fc residue K248 of the anti-PD-1 antibody LZM-009 are shown in **FIGURE 11** (RP HPLC), **FIGURE 12** (SEC-HPLC), and **FIGURE 13** (Q-TOF Mass Spectrometry). The immunocytokine shown is Composition A. The linker is formed from bifunctional linking reagent



attached to residue K248 of the antibody using AJICAP™ technology, then subsequently conjugated to the bromoacetamide-PEG_5-azide linker attached to residue C68 of the IL-18 polypeptide.

Example 4 – Characterization of Immunocytokine IL-18 Activity

[0354] The ability of the immunocytokine to perform various IL-18 activities is measured as provided below, as well as relevant comparisons to non-conjugated IL-18 polypeptides.

Surface Plasmon Resonance

[0355] The interaction of immunocytokines, wild type IL-18, and of modified IL-18 polypeptides with human IL-18 receptor subunits are measured with Surface Plasmon Resonance (SPR) technology. Anti-human IgG antibodies are bound by amine coupling onto a CM5 chip to capture 6 μg/mL of Fc fused human IL-18Rα, 6 μg/mL of Fc fused human IL-18Rβ, or 2 μg/mL of Fc fused human IL-18BP isoform a (IL-18BPa) for 30 min before capture. In other settings, 6 μg/mL of alpha and beta IL-18 receptors are mixed and pre-incubated for 30 min before capture of the alpha /beta heterodimer IL-18 receptor.

[0356] The kinetic binding of the IL-18 analytes and immunocytokines are measured with a Biacore 8K instrument in two-fold serial dilutions starting at 1 μM down to 0.98 nM. Regeneration of the surface back to amine coupled anti IgG antibody is done after every concentration of analyte. To measure the protein association to the receptors, the samples are injected with a flow rate of 50 μL/min for 60 s, followed by 300 s buffer only to detect the dissociation. The used running buffer is 1xPBS with 0.05% Tween20. The relative response units (RU, Y-axis) are plotted against time (s, X-axis) and analyzed in a kinetic 1:1 binding model for the monomer receptor binding and for

the binding to the IL-18BP. A kinetic heterogenous ligand fit model is applied for the alpha/beta heterodimer binding.

IL-18BP Binding alphaLISA Assay

[0357] A human IL-18BP AlphaLISA Assay Kit is used to determine the binding affinity of each immunocytokine and IL-18 variant for IL-18BP, which detected the presence of free form IL-18BP.

[0358] Sixteen three-fold serial dilutions of IL-18 analytes are prepared in aMEM medium supplemented with 20% FCS, Glutamax, and 25 μM β-mercaptoethanol in the presence of 5 ng/mL of His-tagged human IL-18BP. Final IL-18 analytes concentration range from 2778 nM to 0.2 pM.

[0359] After 1 hr incubation at room temperature, free IL-18BP levels are measured using a Human IFNγ AlphaLISA Assay Kit. In a 384 well OPTIplate, 5 μL of 5X Anti-IL-18BP acceptor beads are added to 7.5 μL of an IL-18/IL-18BP mix. After 30 min incubation at room temperature with shaking, 5 μL of biotinylated Anti-IL-18BP antibodies are added to each well. The plate is incubated further for 1 hr at room temperature. Under subdued light, 12.5 μL of 2X streptavidin (SA) donor beads are pipetted into each well, and the wells are incubated with shaking for an additional 30 min at room temperature. The AlphaLisa signal is then measured on an Enspire plate reader with 680 and 615 nm as excitation and emission wavelengths, respectively. The dissociation constant (K_D) is calculated based on a variable slope, four parameter analysis using GraphPad PRISM software.

IFNγ Induction Cellular Assay

[0360] The ability of immunocytokines and IL-18 polypeptides provided herein are assessed for ability to induce IFNγ in a cellular assay according to the protocol below.

[0361] The NK cell line NK-92 derived from a patient with lymphoma (ATCC® CRL-2407™) is cultured in aMEM medium supplemented with 20% FCS, Glutamax, 25 μM B-mercaptoethanol, and 100 IU/mL of recombinant human IL-2.

[0362] On the day of experiment, cells are harvested and washed with aMEM medium without IL-2 and containing 1 ng/mL of recombinant human IL-12. After counting, cells are seeded at 100,000 cells/well in a 384 well titer plate and incubated at 37 °C/5% CO_2. Sixteen 4-fold serial dilutions of IL-18 analytes are prepared in aMEM medium, and 1 ng/mL of IL-12 were added to the NK-92 cells. Final IL-18 analyte concentrations range from 56 nM to $5x10^{-5}$ pM.

[0363] After incubating the cells for 16-20 hr at 37 °C/5% CO_2, 5 µL of supernatant is carefully transferred to a 384 microwell OptiPlate. IFNγ levels are measured using a human IFNγ AlphaLISA Assay Kit. Briefly, 10 µL of 2.5X AlphaLISA Anti-IFNγ acceptor beads and biotinylated antibody anti-IFNγ mix are added to the 5µL of NK-92 supernatants. The mixtures are incubated for 1 hr at room temperature with shaking. Under subdued light, 2.5 µL of 2X streptavidin (SA) donor beads are pipetted into each well, and the wells are incubated for 30 min at room temperature with shaking. AlphaLISA signals are then measured on an EnSpire™ plate reader using 680 nm and 615 nm as excitation and emission wavelengths, respectively. Half maximal effective concentrations (EC_{50}) are calculated based on a variable slope and four parameter analysis using GraphPad PRISM software.

IL-18 Binding Protein Inhibition Cellular Assay

[0364] The NK cell line NK-92 derived from a patient with lymphoma (ATCC® CRL-2407™) is cultured in aMEM medium supplemented with 20% FCS-Glutamax, 25 µM B-mercaptoethanol, and 100 IU/mL of recombinant human IL-2.

[0365] On the day of experiment, cells are harvested and washed with aMEM medium without IL-2 and containing 1 ng/mL of recombinant human IL-12. After counting, the cells are seeded at 100,000 cells/well in a 384 well titer plate and incubated at 37 °C/5% CO_2. Sixteen 2-fold serial dilutions of Fc-fused human IL-18 binding protein isoform a (IL-18BPa) are prepared in aMEM medium. 1ng/mL of IL-12 containing 2 nM of each IL-18 polypeptide variant is added to the NK-92 cells. The final IL-18 analyte concentration is 1 nM, and the final IL-18BPa concentration ranged from 566 nM to 17 pM.

After incubating the cells for 16-20 hr at 37 °C/5% CO_2, 5 µL of the supernatant is carefully transferred to a 384 microwell OptiPlate. IFNγ levels are measured using a human IFNγ AlphaLISA Assay Kit. Briefly, 10 µL of 2.5X AlphaLISA anti-IFNγ acceptor beads and biotinylated antibody anti-IFNγ mix are added to 5 µL of NK-92 supernatants. The mixtures are incubated for 1 hr at room temperature with shaking. Under subdued light, 2.5 µL of 2X SA donor beads are pipetted in each well and incubated for 30 min at room temperature with shaking. AlphaLISA signals are then measured on an EnSpire™ plate reader using 680 nm and 615 nm as excitation and emission wavelengths, respectively. Half maximal inhibitory concentrations (IC_{50}) are calculated based on a variable slope and four parameter analysis using GraphPad PRISM software.

IFNγInduction on primary human cells

[0366] Ability of IL-18 variants to stimulate Human peripheral blood mononuclear cells (PBMCs) was assessed according to the following protocol.

[0367] Isolation of lymphocytes: Blood from Buffy Coats of healthy volunteers was diluted with equal volume of PBS and slowly poured on top of SepMate tube prefilled with 15mL Histopaque-1077. Tubes were centrifuged for 10 minutes at 1200g, the top layer was collected and washed 3 times with PBS containing 2% of Fetal Bovine Serum. PBMCs were counted and cryopreserved as aliquots of 20×10^6 cells.

[0368] Cryopreserved PBMCs were thawed and seeded at 150 000 cells/well in a 96w round bottom 96 well plate. PBMCs were stimulated with a gradient of human IL-18 variants ranging from 0.2 pg/mL to 3600 ng/mL. All stimulations were performed in the presence of hIL-12 (1ng/ml, Sino Biological, #CT011-H08H) for 24hrs in RPMI containing 10% Fetal Bovine Serum.

[0369] Cytokine production after 24hr stimulation were measured using Legendplex bead-based cytokine assay (Biolegend #740930) according to manufacturer protocol. Half maximal effective concentrations (EC_{50}) of IFNg released in culture supernatant were calculated based on a variable slope and four parameter analysis using GraphPad PRISM software.

IFNγInduction on primary mouse cells

[0370] Ability of IL-18 variants to stimulate murine splenocytes was assessed according to the following protocol.

[0371] Cryopreserved splenocytes isolated from BALB/c and C57BL6 mice were purchased from IQ Biosciences (Berkeley, CA, USA).

[0372] Cryopreserved splenocytes were thawed, treated with DNAseI, and seeded at 200 000 cells/well in a 96w round bottom 96 well plate. Splenocytes were stimulated with a gradient of human IL-18 variants ranging from 0.2 pg/mL to 3600 ng/mL. All stimulations were performed in the presence of mIL-12 (1ng/ml, Peprotech, cat # 210-12) for 24hrs in RPMI containing 10% Fetal Bovine Serum.

[0373] Cytokine production after 24hr stimulation were measured using Legendplex bead-based cytokine assay (Biolegend #740622) according to manufacturer protocol. Half maximal effective concentrations (EC_{50}) of IFNg released in culture supernatant were calculated based on a variable slope and four parameter analysis using GraphPad PRISM software.

Example 5 – Immune Cell Associated Antigen Binding ELISA Assay (Figure 14A, Figure 14B, Figure 15A, and Figure 15B)

[0374] The interaction of the unmodified antibodies and corresponding IL-18 immunocytokines with relevant immune cell associated antigen are measured by ELISA assay. For these studies, Corning high-binding half-area plates (Fisher Scientific, Reinach, Switzerland) are coated overnight at 4°C with 25 µl of unmodified antibodies corresponding IL-18 immunocytokines at 5 µg/ml in PBS. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Plate surfaces are blocked with 25 µl of PBS-0.02% Tween20-1% BSA at 37°C during 1h. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Twenty-five microliters (25 µl) of recombinant biotinylated human PD-1 (Biotinylated Recombinant Human PD-1/CD279-Fc Chimera,carrier-free, Biolegend #789406) or PD-L1 (Biotinylated Human PD-L1 / B7-H1, ACROBiosystems, PD1-H82E5-25UG) protein are added in seven-fold serial dilutions starting at 12 nM down to 0.15 pM into PBS-0.02% Tween20-0.1% BSA and incubated at 37°C during 2h. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Twenty-five microliters of Streptavidin- Horseradish peroxidase (#RABHRP3, Merck, Buchs, Switzerland) diluted at 1:500 into PBS-0.02% Tween20-0.1% BSA are added to each well and incubated at Room Temperature during 30 min. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Fifty microliters of TMB substrate reagent (#CL07, Merck, Buchs, Switzerland) are added to each well and incubated at 37°C during 5min. After 5 min at 37°C, Horseradish peroxidase reaction is stopped by adding 50 µl/well of 0.5M H_2SO_4 stop solution. ELISA signal is then measured at 450 nm on an ENSPIRE® plate reader from Perkin Elmer (Schwerzenbach, Switzerland). Results from this experiment are shown in the table below.

KD values of the interaction of immunocytokines with PD-1 and PD-L1 as measured by ELISA

Composition	Antibody	IL-18 polypeptide	PD-1 KD (pM)	PD-L1 KD (pM)
-	Pembrolizumab/Keytruda	-		>100000
	LZM-009	-	40.7	>100000
-	Nivolumab/Opdivo	-	91.9	>100000

-	Durvalumab/Imfinzi	-	>100000	146
-	Atezolizumab/Tecentriq	-	>100000	443
-	Avelumab/Bavencio	-	>100000	36
A	LZM-009	SEQ ID NO: 30	46.7	>100000
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	>100000	473

[0375] FIGURE 14A and FIGURE 14B show plots measuring ability of the unmodified and of conjugated anti-PD1 antibodies to bind with human PD1/CD279 ligand, with the figure showing ELISA signal on the y-axis and dosage of the biotinylated PD-1 protein on the x-axis. The unconjugated reference antibodies are Pembrolizumab, LZM-009, Nivolumab, Atezolizumab, Durvalumab, and Avelumab. The conjugated antibodies tested in this figure are compositions A and composition B.

[0376] FIGURE 15A and FIGURE 15B show plots measuring ability of the unmodified and of conjugated antibodies to bind with human PD-L1/B7-H1 ligand, with the figure showing ELISA signal on the y-axis and dosage of the biotinylated PD-L1 protein on the x-axis. The unconjugated reference antibodies are Pembrolizumab, LZM-009, Nivolumab, Atezolizumab, Durvalumab, and Avelumab. The conjugated antibodies tested in this figure are compositions A and composition B.

Example 6 – Kinetic Analysis of Binding of reference antibodies and Immunocytokines to Immune Cell Associated Antigens (Figure 16)

[0377] Based on Bio-Layer Interferometry (BLI), Octet® BLI systems enable real-time, label-free analysis for the determination of kinetics and affinity of a ligands to its receptor. Here anti-human IgG FC Capture (AHC) sensors are loaded with the test items (ICs). Sensors are first dipped into a kinetic buffer for baseline measurement, then into an analyte solution, here human PD1, to allow association and again into a buffer solution where the analyte is allowed to come off the ligand (dissociation). Several concentrations of analyte are run in parallel and enable the calculation of affinity parameters: Ka, Kd, KD.

[0378] Typically, first, the sensors are regenerated by 3 cycles of dipping into 10 mM glycine solution at pH=2 for 20 seconds, followed by 20 second kinetics buffer and a final 60 seconds in kinetics buffer to establish the initial signal (baseline). Second, the loading column will contain the ligand, here the unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1

antibody, at a fixed concentration determined in the loading scout experiment (20 ug/mL). Then another wash/baseline step allows non immobilized proteins to be washed away. The association column will contain the 2-fold dilution series of the analyte (His-tagged human PD1, R&D #8986-PD) including a no analyte control. The highest concentration should be ~10-fold the KD. The dissociation designates the sensors to return to previous baseline column with kinetics buffer. After acquisition, the data is analyzed with Data Analysis Studio software (Sartorius). Data sets are first preprocessed by subtracting references samples and aligning curves on the baseline step. Group fitting is then applied to the data series and kinetics parameters are calculated. Results from this experiment are shown in the table below.

Binding kinetics of the interaction of reference antibodies and immunocytokines with PD-1 as measured by Bio-Layer Interferometry (BLI)

Composition	Antibody	IL-18 polypeptide	ka (1/Ms)	kd (1/s)	KD (nM)
-	Pembrolizumab/Keytruda	-	4.00E+05	2.72E-03	6.77
	LZM-009	-	3.42E+05	7.70E-03	23.50
A	LZM-009	SEQ ID NO: 30	4.54E+05	4.79E-03	11.50

[0379] FIGURE 16 shows plots measuring ability of the unmodified and of conjugated antibodies to bind to human PD-L1/B7-H1 ligand, with the figure showing net BioLayer interferometry shift in nanometer on the y-axis and time of incubation dosage of the biotinylated PD-L1 protein on the x-axis. The figure shows mean ELISA signal on the y-axis and dosage of the human Fc gamma receptors on the x-axis. The unconjugated reference antibodies are Pembrolizumab and LZM-009. The conjugated antibodies tested is Compositions A.

Example 7 – PD-1/PD-L1 Blockade Assay (Figure 17A and 17B)

[0380] For immunocytokine compositions which comprise PD-1 or PD-L1 antibodies or antigen binding fragments, the experiment outlined below is performed to assess the ability of the immunocytokines to interfere with the PD-1/PD-L1 pathway. The assay is the PD-1/PD-L1 Blockade Bioassay from Promega (Cat# J1250, Madison, WI, USA). PD-1/PD-L1 Blockade Bioassay is a bioluminescent cell-based assay based on the co-culture of effector cells with target cells mimicking an immunological synapse. Jurkat T cells expressing human PD-1 and a luciferase reporter driven by a NFAT response element (NFAT-RE) are activated by CHO-K1 cells expressing human PD-L1 and an engineered cell surface protein designed to activate Jurkat cells cognate TCRs. Concurrent interaction PD-1/PD-L1 inhibits TCR signaling and represses NFAT-RE-mediated luminescence. Addition of either an anti-PD-1 or anti-PD-L1 antibody that blocks the PD-1/PD-L1 interaction releases the inhibitory signal, restoring TCR activation and resulting in a gain of signal of NFAT-RE luminescent reporter.

[0381] Briefly, PD-L1 aAPC/CHO-K1 Target cells were plated in white tissue culture 96- wells plates and cultured overnight at 37°C/5% CO_2. Test molecules were measured in four-fold serial dilutions starting at 1 µM down to 0.002 nM and pre-incubated on target cells for 10 min before the addition of freshly thawed PD-1 Jurkat effector cells. After 6h at 37°C/5% CO_2, activity NFAT-RE luminescent reporter was evaluated by the addition of Bio-Glo reagent and measured on an ENSPIRE® plate reader (1sec /well) from Perkin Elmer (Schwerzenbach, Switzerland). Results from this experiment are shown in the table below.

Activity of unconjugated IL-18 variants and corresponding IL-18 immunocytokines in the PD-1/PD-L1 blockade cellular assay

Composition	Antibody	IL-18 polypeptide	KD FcRn (nM)
-	Pembrolizumab/Keytruda	-	0.757
-	LZM-009	-	5.799
-	Durvalumab/Imfinzi	-	0.330
-	Atezolizumab/Tecentriq	-	0.664
-	Avelumab/Bavencio	-	0.676

-	Trastuzumab/Herceptin		>10000
A	LZM-009	SEQ ID NO: 30	4.075
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	0.573
C	Trastuzumab/Herceptin	SEQ ID NO: 30	NT

[0382] FIGURE 17A and **17B** shows plots measuring ability of the unmodified and of conjugated anti-PD1 antibodies to interfere with PD1/PDL1 pathway, with the figure showing normalized luminescence intensity of effector cells NFAT-Lucia reporter on the y-axis and dosage of the unmodified and of conjugated anti-PD1 antibodies on the x-axis. The unconjugated reference antibodies are Pembrolizumab, LZM-009, Nivolumab, Atezolizumab, Durvalumab, and Avelumab. The conjugated antibodies tested in this figure are compositions A and composition B.

Example 8 – Human FcγR Binding Assay (Figure 18A and Figure 18B)

[0383] The interaction of the unmodified and of conjugated antibodies with human Fc gamma receptors I (FcγRI/CD64), with human Fc gamma receptors IIa (FcγRIIa/CD32a), with inhibitory human Fc gamma receptors IIb (FcγRIIb/CD32b), and with human Fc gamma receptors III FcγRIIIa/CD16 were measured by ELISA.

[0384] Briefly, Corning high-binding half-area plates (Fisher Scientific, Reinach, Switzerland) were coated overnight at 4°C with 25 µl of unmodified and of conjugated anti-PD1 antibodies at 2.5 µg/ml in PBS. Plates were then washed four times with 100 µl of PBS-0.02% Tween20. Plates surfaces were blocked with 25 µl of PBS-0.02% Tween20-1% BSA at 37°C during 1h. Plates were then washed four times with 100 µl of PBS-0.02% Tween20. Then twenty-five microliters of either recombinant Human Fc gamma RI/CD64 Protein (R&D systems, 1257-FC-050, CF), recombinant Human Fc gamma RIIA/CD32a (H167) Protein (R&D systems, 9595-CD-050, CF), recombinant Human Fc gamma RIIB/CD32b Avi-tag Protein (R&D systems, AVI1875-050, CF), or recombinant Human Fc gamma RIIIA/CD16a Protein (R&D systems, 4325-FC-050; CF) were added in five-fold serial dilutions ranging from 1000 nM to 0.001 nM into PBS-0.02% Tween20-0.1% BSA and incubated at 37°C during 2h. Plates were then washed four times with 100 µl of PBS-0.02% Tween20. Twenty-five microliters of a 1/500 HRP-anti-His antibody in PBS - 0.02% Tween20- 0.1%BSA (R&D systems, anti-HIS-HRP Ab, #MAB050H) were added to each well and plates were incubated at Room Temperature during 1h. Plates were then washed four times with 100 µl of PBS-0.02% Tween20. Fifty microliters of TMB substrate reagent (#CL07, Merck,

Buchs, Switzerland) were added to each well and incubated at 37°C during 5min. After 5min at 37°C, Horseradish peroxidase reaction was stopped by adding 50 µl/well of 0.5M H2SO4 stop solution. ELISA signal was then measured at 450 nm on an EnSpire plate reader from Perkin Elmer (Schwerzenbach, Switzerland). Results from this experiment are shown in the table below.

Binding affinity of reference antibodies and immunocytokines with human Fc gamma Receptors as measured by ELISA

Composition	Antibody	IL-18 polypeptide	FcγRI/ (CD64) nM	FcγRIIa (CD32a) nM	FcγRIIIa (CD16) nM	FcγRIIb (CD32b) nM
-	Pembrolizumab/Keytruda	-	0.5932	3358	1660	>10000
	LZM-009	-	0.3150	1348	1707	2627
-	Durvalumab/Imfinzi	-	2.34	160	234	233
-	Atezolizumab/Tecentriq	-	9.17	1370	356	>10000
-	Trastuzumab/Herceptin	-	0.0785	350	807	360
A	LZM-009	SEQ ID NO: 30	0.25	1892	598	436
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	10.85	1740	492	>10000
C	Trastuzumab/Herceptin	SEQ ID NO: 30			3299	

NT: Not Tested

[0385] FIGURE 18A and FIGURE 18B show plots measuring ability of the unmodified and of conjugated antibodies to bind to human Fc gamma receptor I (CD64) on top panels, and to human Fc gamma receptor IIIa (CD16) on lower panels. The figure shows mean ELISA signal on the y-axis and dosage of the human Fc gamma receptors on the x-axis. The unconjugated reference antibodies are LZM-009 and Atezolizumab. The conjugated antibodies tested are Compositions A and B.

Example 9 – Human FcRn Binding Assay (Figure 19)

[0386] The interaction of the unmodified and of conjugated anti-PD1 antibodies with the human neonatal Fc receptor (FcRn) at pH 6 was measured using the AlphaLISA® Human FcRn Binding Kit (AL3095C) from Perkin Elmer (Schwerzenbach, Switzerland). The AlphaLISA® detection of FcRn and IgG binding uses IgG coated AlphaLISA® acceptor beads to interact with biotinylated human FcRn captured on Streptavidin-coated donor beads. When reference IgG binds to FcRn, donor and acceptor beads come into proximity enabling the transfer of singlet oxygen that trigger a cascade of energy transfer reactions in the acceptor beads, resulting in a sharp peak of light emission at 615 nm. Addition of a free IgG antibodies into the AlphaLISA® mixture creates a competition for the binding of FcRn to the reference antibody resulting in a loss of signal.

[0387] Briefly, test molecules were measured in serial dilutions starting at 5uM down to 64 pM and incubated with AlphaLISA® reaction mixture consisting of 800 nM of recombinant biotinylated human FcRn, 40 µg/ml of human IgG conjugated Acceptor beads, and 40 µg/ml of Streptavidin coated Donor beads in pH 6 MES buffer. After 90min at 23°C in the dark, AlphaLISA® signal was measured on an EnSpire plate reader (Excitation at 680 nm, Emission at 615 nm) from Perkin Elmer (Schwerzenbach, Switzerland). Results from this experiment are shown in the table below.

Binding affinity of reference antibodies and immunocytokines with the human neonatal Fc receptor as measured by AlphaLISA

Composition	Antibody	IL-18 polypeptide	KD FcRn (nM)
-	Pembrolizumab/Keytruda	-	7.45
-	LZM-009	-	6.00
-	Durvalumab/Imfinzi	-	8.31
-	Atezolizumab/Tecentriq	-	6.40
-	Avelumab/Bavencio	-	5.36
-	Trastuzumab/Herceptin		6.55
A	LZM-009	SEQ ID NO: 30	33.45

B	Atezolizumab/Tecentriq	SEQ ID NO: 30	36.68
C	Trastuzumab/Herceptin	SEQ ID NO: 30	25.12

[0388] FIGURE 19 shows plots measuring ability of the unmodified and of conjugated antibodies to bind to human Fc neonatal receptor. The figure shows mean AlphaLISA signal on the y-axis and dosage of the human Fc neonatal receptor (FcRn) on the x-axis. The unconjugated reference antibodies are LZM-009 and Atezolizumab. The conjugated antibodies tested are Compositions A and B.

Example 10 – IFN gamma secretion assay in NK92 cells (Figure 20 & 21)

[0389] The IFNγ-secretion stimulating activity of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines was evaluated on NK92 cell line. The NK cell line NK-92 derived from a patient with lymphoma (ATCC, Cat# CRL-2407) was cultured in aMEM medium supplemented with 12.5% FCS, 12.5% horse serum (HS), 50 uM B-mercaptoEthanol, and 2 ng/ml of recombinant Human Interleukin-2 (IL-2).

[0390] On the day of experiment, cells were harvested and washed with aMEM medium without IL-2 and resuspended in medium (w/o IL-2) containing 1 ng/ml of recombinant human Interleukin-12 (SinoBiologicals, Cat# CT011-H08H). After counting, cells were seeded at 100 000 cells/well in a 384 well titer plate and incubated at 37°C/5%CO2. Sixteen 4-fold serial dilutions of IL-18 analytes were prepared in aMEM medium- 1ng/ml IL-12 and were added to the NK-92 cells. Final IL-18 analytes concentration ranged from to 200 nM down to 0.01 pM.

[0391] After 16-20h incubation at 37°C/5%CO2, 5 µl of supernatant were carefully transferred to a 384 microwells OPTIPlate (Perkin Elmer; Cat# 6007270) and Interferon-gamma (IFNγ) levels measured using the Human IFNγ AlphaLISA Assay Kit (Perkin Elmer, Cat# AL217C). Briefly, 10µl of 2.5X AlphaLISA Anti-IFNγ acceptor beads and biotinylated Antibody Anti-IFNγ mix were added to the 5µl of NK-92 supernatants and incubated for 1h at room temperature under shaking. Under subdued light, 2.5µl of 2X streptavidin (SA) donor beads were pipetted in each well and incubated for 30min at room temperature under shaking. AlphaLisa signal was then measured on an Enspire plate reader (Perkin Elmer) using 680 and 615 nm as excitation and emission wavelengths respectively. Half maximal effective concentration (EC50) was calculated based on a variable slope, four parameter analysis using GraphPad PRISM software. Results from this experiment are shown in the table below.

Activity of unconjugated IL-18 variants and corresponding IL-18 immunocytokines in the IFNγ secretion NK92 assay

Composition	Antibody	IL-18 polypeptide	EC_{50} (nM)
-	-	SEQ ID NO: 1	0.374
-	-	SEQ ID NO: 30	0.002
-	-	SEQ ID NO: 31	0.103
		SEQ ID NO: 60	0.008
A	LZM-009	SEQ ID NO: 30	0.073
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	0.001
C	Trastuzumab/Herceptin	SEQ ID NO: 30	0.027
D	LZM-009	SEQ ID NO: 31	0.471
E	LZM-009	SEQ ID NO: 60	0.056
F	Trastuzumab/Herceptin	SEQ ID NO: 60	0.064

[0392] FIGURE 20 shows plots measuring the levels PD-1 and PD-L1 surface expression on NK92 cells

[0393] FIGURE 21 shows plots measuring ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by NK92 cells. The figure shows mean IFNg alphaLISA signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID N°:1), SEQ ID N°: 30, and SEQ ID N°: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

Example 11 – IFN gamma secretion assay in KG-1 cells (Figure 22-24)

[0394] The IL-18 responsive AML cell line KG-1 shows high expression of IL-18Rα and moderate levels of IL18Rβ, respectively. The KG-1 cell line was used to generate a PD-1 expressing cell line and furthermore, to measure IFNγ release upon incubation with IL-18 variants and corresponding IL-18 immunocytokines.

[0395] PD-1 expressing cell line generation: Briefly, KG-1 cells were transduced using lentiviral particles carrying the human PD-1 gene (PDCD1 NM_005018; Origene, CAT#: RC210364L3V) at a MOI (Multiplicity of Infection) of 30. Spinfection was performed at 1260g during 90 min at 37°C in the presence of 5 µg/ml of Polybrene and 10 mM of HEPES in complete culture media (RPMI, 10% FBS, 1% L-Glutamine). Five days after transduction, puromycin at a final concentration of 1 ug/ml was added to select for PD-1 positive cells. For culture maintenance, puromycin concentration was decreased to 0.5 µg/ml. Stable and homogenous expression of PD-1 was verified by surface staining (BD Pharmingen, #557860).

[0396] IFNγ release was assessed in PD-1 positive (transduced) KG-1 cells, as well as in the parental PD-1 negative cells. $0.5x10^5$ cells were seeded into a 96-well U-bottom plate in culture media (RPMI, 10% FBS, 1% L-Glutamine) and stimulated with IL-18 variants/ICs for 20-24h. The test items were diluted to 100nM in culture medium, followed by 7 10-fold serial dilutions. The lowest concentration assessed was 0.05 fM. After incubation, IFNγ release was measured using the LEGENDplex custom human mix and match KIT (Biolegend LEGENDplex™ Human IFN-γ Capture Bead B5, 13X #740942, LEGENDplex™ HU Essential Immune Response Panel Detection Abs, # 740931, LEGENDplex™ Buffer Set A # 740368). To this end, cell culture supernatant was collected and diluted 1:1 with Assay Buffer. Fluorescence measurements were done with a Quanteon Flow Cytometer from Acea Biosciences. For analysis, MFI values (median fluorescence intensity) were exported and plotted against concentrations used. The EC_{50} values (half maximal effective concentration) were calculated based on a variable slope and four parameter analysis using GraphPad PRISM software version 9. Results from this experiment are shown in the table below.

Stimulation of IFNγ secretion by unconjugated IL-18 variants and corresponding IL-18 immunocytokines in parental and PD-1 transduced KG-1 cells

Composition	Antibody	IL-18 polypeptide	Parental KG-1 EC_{50} (nM)	PD-1 positive KG-1 EC_{50} (nM)	PD-1^{neg} /PD-1^{pos} EC_{50} ratio
-	-	SEQ ID NO: 1	0.387	0.297	1.298

-	-	SEQ ID NO: 30	0.012	0.008	1.336
-	-	SEQ ID NO: 31	0.1485	0.1354	1.2
A	LZM-009	SEQ ID NO: 30	0.0110	0.0012	10.9
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	0.0016	0.0036	0.6
C	Trastuzumab/Herceptin	SEQ ID NO: 30	0.0166	0.0096	1.7
D	LZM-009	SEQ ID NO: 31	0.2033	0.0225	9.3

[0397] FIGURE 22 shows plots measuring the levels PD-1 and PD-L1 surface expression on KG-1 cells

[0398] FIGURE 23 shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by parental PD-$1^{negative}$ and by engineered PD-$1^{positive}$ KG-1 cells. The figure shows mean IFNg legendplex signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID N°:1), SEQ ID N°: 30, and SEQ ID N°: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

Example 12 – IL-18 Binding protein alphaLISA assay (Figure 24)

[0399] Wild type or modified IL-18 polypeptides samples were diluted at 5.6µM in a solution of 1x alphaLISA Immunoassay Buffer provided in the alphaLISA IFNγ Detection kit and were diluted applying 3-fold serial dilutions down to 1.7pM in 384 deep well plates. A solution of 10ng/ml of human IL-18BP-His was prepared with 1x alphaLISA Immunoassay Buffer. IL-18/IL-18BP complex formation was performed incubating 30µl of IL-18BP solution to IL-18 sample titrations for 1h at 20°C. IL-18BP standard was prediluted from stock solution supplied in alphaLISA IFNγ Detection kit at 100ng/ml with 1x alphaLISA Buffer and titration prepared from applying 2-fold serial dilutions. The following solutions were prepared: a 50µg/ml solution of anti-IL-18BP alphaLISA Acceptor beads, a 5nM solution of biotinylated anti-IL18BP antibody and a

80µg/ml light-protected solution of Streptavidin Donor beads in 1x alphaLISA Immunoassay Buffer. To detect unbound IL-18BP in IL-18/IL-18P complex samples, 5µl of pre-mixed Acceptor beads solution were transferred on top on 7.5µL of samples in 384-well Optiplates, followed by a short centrifugation step at 150g, and incubated for 30 minutes at 20°C under shaking at 750rpm.

[0400] 5µl of Biotinylated anti-IL-18BP antibody were added, followed by a short centrifugation step at 150g, and incubated for 60 minutes at 20°C under shaking at 750rpm. Under subdued light, 12.5µl of pre-mixed Donor beads were added, followed by a short centrifugation step at 150g, and incubated for 30 minutes at 20°C under shaking at 750rpm with no light. AlphaLisa signal was then measured on an Enspire plate reader (Perkin Elmer) using 680 and 615 nm as excitation and emission wavelengths respectively. Unbound IL-18BP concentration interpolated from the standard signal-concentration curve using GraphPad Prism. Results from this experiment are shown in the table below.

Binding affinity of reference antibodies and immunocytokines with the human IL-18 Binding Protein as measured by AlphaLISA

Composition	Antibody	IL-18 polypeptide	IL-18BP KD (nM)
-	-	SEQ ID NO: 1	0.411
-	-	SEQ ID NO: 30	24.600
-	-	SEQ ID NO: 31	0.103
A	LZM-009	SEQ ID NO: 30	10.930
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	6.382
C	Trastuzumab/Herceptin	SEQ ID NO: 30	10.496
D	LZM-009	SEQ ID NO: 31	15.263

[0401] FIGURE 24 shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to bind to the human IL-18 Binding Protein (IL-18BP). The figure shows mean free IL-18BP AlphaLISA signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The

unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID N°:1), SEQ ID N°: 30, and SEQ ID N°: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

Example 13 – Cellular IL-18 Binding protein resistance assay (Figure 25)

[0402] The NK cell line NK-92 derived from a patient with lymphoma (ATCC, Cat# CRL-2407) was cultured in αMEM medium supplemented with 20%FCS-Glutamax, 25µM B-mercaptoethanol, and 100 IU/ml of recombinant Human Interleukin-2 (IL-2). On the day of experiment, cells were harvested and washed with αMEM medium without IL-2. After counting, cells were seeded at 100 000 cells/well in a 384 well titer plate and incubated at 37°C/5%CO2. Sixteen 2-fold serial dilutions of Fc fused human IL-18 binding protein isoform a (IL-18BPa; R&D systems, Cat# 119-BP) were prepared in αMEM medium- 1ng/ml IL-12 containing 2nM of each IL-18 variants and were added to the NK-92 cells. Final IL-18 analytes concentration was 1nM and final IL-18BPa concentrations ranged from to 566nM down to 17pM.

[0403] After 16-20h incubation at 37°C/5%CO2, 5 µl of supernatant were carefully transferred to a 384 microwells OPTIplate (Perkin Elmer; Cat# 6007270) and Interferon-gamma (IFNγ) levels measured using the Human IFNγ AlphaLISA Assay Kit (Perkin Elmer, Cat# AL217C). Briefly, 10µl of 2.5X AlphaLISA Anti-IFNγ acceptor beads and biotinylated Antibody Anti-IFNγ mix were added to the 5µl of NK-92 supernatants and incubated for 1h at room temperature under shaking. Under subdued light, 2.5µl of 2X streptavidin (SA) donor beads were pipetted in each well and incubated for 30min at room temperature under shaking. AlphaLisa signal was then measured on an Enspire plate reader (Perkin Elmer) using 680 and 615 nm as excitation and emission wavelengths respectively. Half maximal inhibitory concentration (IC50) was calculated based on a variable slope, four parameter analysis using GraphPad PRISM software. Results from this experiment are shown in the table below.

IL-18BP-mediated inhibition of IFNγ secretion by NK92 cells stimulated with unconjugated IL-18 variants and corresponding IL-18 immunocytokines

Composition	Antibody	IL-18 polypeptide	IC_{50} (nM)
-	-	SEQ ID NO: 1	0.781
-	-	SEQ ID NO: 30	487.8
-	-	SEQ ID NO: 31	0.282

A	LZM-009	SEQ ID NO: 30	6.87
B	Atezolizumab/Tecentriq	SEQ ID NO: 30	>1000
C	Trastuzumab/Herceptin	SEQ ID NO: 30	21.44
D	LZM-009	SEQ ID NO: 31	0.098

[0404] FIGURE 25 shows plots measuring the ability of the human IL-18 Binding Protein to inhibit the secretion of IFNgamma by NK92 cells stimulated with 2nM of unconjugated IL-18 variants and corresponding IL-18 immunocytokines. The figure shows mean IFNg alphaLISA signal on the y-axis and dosage of the human IL-18 Binding Protein on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID N°: 1), SEQ ID N°: 30, and SEQ ID N°: 31. Corresponding IL-18 immunocytokines tested are Compositions A, B, C, and D.

Example 14 – *In Vivo* Antitumor Activity in MC38 colon carcinoma model (Figure 26-27)

[0405] An *in vivo* efficacy study was performed in mice. Naïve, 6-8 weeks old, C57BL/6-hPD1 female mice (GemPharmatech Co, Ltd, Nanjing, China) were inoculated subcutaneously at the right upper flank with MC38 tumor cells (3×10^5) in 0.1 mL of PBS for tumor development. The animals were randomized (using an Excel-based randomization software performing stratified randomization based upon tumor volumes), and treatment started when the average tumor volume reached approximately 120 mm^3. Animals treated with unmodified antibodies received two weekly 10 mL/kg bolus intraperitoneal (i.p.) injections. Animals treated with IL-18 polypeptide conjugated antibodies received two weekly 10 mL/kg bolus intravenous (i.v.) injections. After inoculation, the animals were checked daily for morbidity and mortality. At the time, animals were checked for effects on tumor growth and normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect. The major endpoints were delayed tumor growth or complete tumor regression. Tumor sizes were measured three times a week in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

[0406] FIGURE 26A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition A tested as a single agent at 0.3 and 1 mg/kg as two weekly i.v. injections. (n=9; mean ± SEM).

[0407] FIGURE 26B shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-L1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition B tested as a single agent at 1 and 3 mg/kg as two weekly i.v. injections. (n=9; mean ± SEM).

[0408] FIGURE 27A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the body weight of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition A tested as a single agent at 0.3 and 1 mg/kg as two weekly i.v. injections. (n=9; mean ± SEM).

[0409] FIGURE 27B shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-L1 antibody on the body weight of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure is Composition B tested as a single agent at 1 and 3 mg/kg as two weekly i.v. injections. (n=9; mean ± SEM).

Example 15 – *In Vivo* Antitumor Activity in MC38 colon carcinoma model (Figure 28-30)

[0410] An *in vivo* efficacy study was performed in mice. Naïve, 6-8 weeks old, C57BL/6-hPD1 female mice (GemPharmatech Co, Ltd, Nanjing, China) were inoculated subcutaneously at the right upper flank with MC38 tumor cells (3×10^5) in 0.1 mL of PBS for tumor development. The animals were randomized (using an Excel-based randomization software performing stratified randomization based upon tumor volumes), and treatment started when the average tumor volume reached approximately 110 mm^3. Animals treated with unmodified antibodies received two weekly 10 mL/kg bolus intraperitoneal (i.p.) injections. Animals treated with IL-18 polypeptide conjugated antibodies received two weekly 10 mL/kg bolus intravenous (i.v.) injections. After inoculation, the animals were checked daily for morbidity and mortality. At the time, animals were

checked for effects on tumor growth and normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect. The major endpoints were delayed tumor growth or complete tumor regression. Tumor sizes were measured three times a week in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a\ x\ b^2$ where a and b are the long and short diameters of the tumor, respectively. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

[0411] FIGURE 28A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9; mean ± SEM).

[0412] FIGURE 28B shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows the mean tumor volume on day 17 post treatment initiation on the y-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; One-way Anova test *** P-value<0.001, ** P-value<0.01, * P-value<0.1, ns not significant, TGI: Tumor Growth Inhibition).

[0413] FIGURE 28C shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of MC38 syngeneic colon carcinoma tumors in hPD1 C57BL/6 mice. The figure shows the tumor volume of each individual animal on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9; CR: Complete Response).

[0414] FIGURE 29 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the on the body weight of MC38 syngeneic colon

carcinoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9; mean ± SEM).

[0415] FIGURE 30 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the survival of MC38 syngeneic colon carcinoma tumor-bearing hPD1 C57BL/6 mice. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.1, 0.25, and 0.5 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 0.5 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 1 mg/kg (n=9; mean ± SEM ; CR: Complete response).

Example 16 – *In Vivo* Antitumor Activity in B16F10 melanoma model (Figure 31-33)

[0416] An *in vivo* efficacy study was performed in mice. Naïve, 6-8 weeks old, C57BL/6-hPD1 female mice (GemPharmatech Co, Ltd, Nanjing, China) were inoculated subcutaneously at the right upper flank with B16F10 tumor cells (5 x 10^4; 1:1 with Matrigel®) in 0.1 mL of PBS for tumor development. The animals were randomized (using an Excel-based randomization software performing stratified randomization based upon tumor volumes), and treatment started when the average tumor volume reached approximately 70 mm^3. Animals treated with unmodified antibodies received two weekly 10 mL/kg bolus intraperitoneal (*i.p.*) injections. Animals treated with IL-18 polypeptide conjugated antibodies received two weekly 10 mL/kg bolus intravenous (*i.v.*) injections. After inoculation, the animals were checked daily for morbidity and mortality. At the time, animals were checked for effects on tumor growth and normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect. The major endpoints were delayed tumor growth or complete tumor regression. Tumor sizes were measured three times a week in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

[0417] FIGURE 31A shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of B16F10 syngeneic melanoma tumors

in hPD1 C57BL/6 mice. The figure shows mean tumor volume on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; mean ± SEM).

[0418] FIGURE 31B shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of B16F10 syngeneic melanoma tumors in hPD1 C57BL/6 mice. The figure shows the mean tumor volume on day 10 post treatment initiation on the y-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; TGI: Tumor Growth Inhibition).

[0419] FIGURE 31C shows a plot describing the effect of unmodified PD-1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the growth of B16F10 syngeneic melanoma tumors in hPD1 C57BL/6 mice. The figure shows the tumor volume of each individual animal on the y-axis and time on the x-axis. The immunocytokine tested in this figure are composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; CR: Complete Response).

[0420] FIGURE 32 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the body weight of B16F10 syngeneic melanoma tumor-bearing hPD1 C57BL/6 mice. The figure shows mean body weight change on the y-axis and time on the x-axis. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; mean ± SEM).

[0421] FIGURE 33 shows a plot describing the effect of unmodified PD-L1 antibodies and of IL-18 polypeptide conjugated PD-1 antibody on the survival of B16F10 syngeneic melanoma tumor-bearing hPD1 C57BL/6 mice. The immunocytokine tested in this figure are Composition A tested as a single agent at 0.3, 1, and 3 mg/kg as two weekly *i.v.* injections. As a control, Her2-targeted

immunocytokine composition C was applied at 3 mg/kg as a single agent and in combination with LZM-009 anti-PD-1 antibody at 10 mg/kg (n=9; mean ± SEM ; CR: Complete response).

Example 17: IFN gamma secretion assay in PD-1^{negative} and PD-1^{positive} NK-92 cells (Figure 34, 35, 36)

[0422] To show selectivity of the anti-PD1-IL18 immunocytokine (IC) for PD-1 positive cells, the NK-92 cell line was used to generate a PD-1 expressing cell line and furthermore, to measure IFNγ release upon incubation with IL-18 variants and ICs.

[0423] On the day of experiment, parental PD-1negative and PD-1positive transduced cells were harvested and washed with PBS 1X and resuspended in medium (w/o IL-2) containing 1 ng/ml of recombinant human Interleukin-12 (SinoBiologicals, Cat# CT011-H08H). After counting, cells were seeded at 100 000 cells/well in a 384 well culture plate and incubated at 37°C/5%CO2. When indicated, cells were preincubated with anti-PD-1 antibody LZM-009 at 17 uM during 30min at 37°C. The test items were diluted to 200nM in culture medium, followed by twelve 5-fold serial dilutions. The lowest concentration assessed was 0.8 fM.

[0424] After 16-20h incubation at 37°C/5%CO2, 5 µl of supernatant were carefully transferred to a 384 microwell OptiPlate (Perkin Elmer; Cat# 6007270) and Interferon-gamma (IFNγ) levels were measured using the Human IFNγ AlphaLISA Assay Kit (Perkin Elmer, Cat# AL217C). Briefly, 10µl of 2.5X AlphaLISA Anti-IFNγ acceptor beads and biotinylated Antibody Anti-IFNγ mix were added to the 5µl of NK-92 supernatants and incubated for 1h at room temperature while shaking. Under subdued light, 2.5µl of 2X streptavidin (SA) donor beads were pipetted in each well and incubated for 30min at room temperature under shaking. AlphaLISA signal was then measured on an Enspire plate reader (Perkin Elmer) using 680 and 615 nm as excitation and emission wavelengths respectively. Half maximal effective concentration (EC50) was calculated based on a variable slope, four parameter analysis using GraphPad PRISM software.

Stimulation of IFNγ secretion by unconjugated IL-18 variants and corresponding IL-18 immunocytokines in parental PD-1^{negative} and PD-1^{positive} transduced NK-92 cells

Composition	Antibody	IL-18 polypeptide	PD-1^{negative} Parental NK-92 EC_{50} (nM)	PD-1^{positive} NK-92 EC_{50} (nM)	PD-1^{neg}/PD-1^{pos} EC_{50} ratio

-	-	SEQ ID NO: 1	0.1579	0.1300	1.4
-	-	SEQ ID NO: 30	0.0024	0.0028	0.9
A	LZM-009	SEQ ID NO: 30	0.1187	0.0013	120.3
C	Trastuzumab/Herceptin	SEQ ID NO: 30	0.1521	0.1176	1.0

Example 18: IL-18BP mediated inhibition of IFN gamma secretion assay in PD-1^{negative} and PD-1^{positive} NK-92 cells (Figure 35)

[0425] To characterize the resistance of anti-PD1-IL18 immunocytokines (IC) on PD-1 positive cells, the NK-92 cell line was used to generate a PD-1 expressing cell line and furthermore, to measure the inhibition of IFNγ release upon incubation with IL-18 variants and ICs in the presence of increasing quantities of human IL-18BP.

[0426] On the day of experiment, parental PD-1^{negative} and PD-1^{positive} transduced cells were harvested and washed with PBS 1X and resuspended in medium (w/o IL-2) containing 1 ng/ml of recombinant human Interleukin-12 (SinoBiologicals, Cat# CT011-H08H). After counting, cells were seeded at 100 000 cells/well in a 384 well culture plate and incubated at 37°C/5%CO2. When indicated, cells were preincubated with anti-PD-1 antibody LZM-009 at 17 uM during 30min at 37°C. Sixteen 2-fold serial dilutions of His-tag human IL-18 binding protein isoform a (IL-18BPa; SinoBiologicals # 10357-H08H) were prepared in αMEM medium, supplemented with 1ng/ml IL-12 and 2 nM of each IL-18 variants, and added to the NK-92 cells. Final IL-18 analytes concentration was 1nM and final IL-18BPa concentrations ranged from 4500 nM down to 5 pM.

[0427] After 16-20h incubation at 37°C/5%CO_2, 5 µl of supernatant were carefully transferred to a 384 microwell OptiIPlate (Perkin Elmer; Cat# 6007270) and Interferon-gamma (IFNγ) levels measured using the Human IFNγ AlphaLISA Assay Kit (Perkin Elmer, Cat# AL217C). Briefly, 10µl of 2.5X AlphaLISA Anti-IFNγ acceptor beads and biotinylated Antibody Anti-IFNγ mix were added to the 5µl of NK-92 supernatants and incubated for 1h at room temperature while shaking. Under subdued light, 2.5µl of 2X streptavidin (SA) donor beads were pipetted in each well and incubated for 30min at room temperature while shaking. AlphaLISA signal was then measured on an Enspire plate reader (Perkin Elmer) using 680 and 615 nm as excitation and

emission wavelengths respectively. Half maximal inhibitory concentration (IC_{50}) was calculated based on a variable slope, four parameter analysis using GraphPad PRISM software.

IL-18BP-mediated inhibition of IFNγ secretion by unconjugated IL-18 variants and corresponding IL-18 immunocytokines in parental PD-1^{negative} and PD-1^{positive} transduced NK-92 cells

Composition	Antibody	IL-18 polypeptide	PD-1^{negative} Parental NK-92 IC_{50} (nM)	PD-1^{positive} NK-92 IC_{50} (nM)	PD-1^{pos} /PD-1^{neg} IC_{50} ratio
-	-	SEQ ID NO: 1	2.97	2.67	1.2
-	-	SEQ ID NO: 30	109.54	80.32	1.0
A	LZM-009	SEQ ID NO: 30	4.3310	1359.50	294.8
C	Trastuzumab/Herceptin	SEQ ID NO: 30	3.8410	3.42	1.2

[0428] FIGURE 34 shows plots measuring the levels PD-1 surface expression on wild type NK-92 cells and on NK-92 cells transduced with human PD-1.

[0429] FIGURE 35 (left panel) shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by parental PD-1^{negative} (round symbols, dotted lines) and by engineered PD-1^{positive} NK-92 cells (squared symbols, solid lines). The figure shows mean IFNg AlphaLISA signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO:1) and SEQ ID NO: 30. Corresponding IL-18 immunocytokines tested are Compositions A and C.

[0430] FIGURE 35 (right panel) shows plots measuring the ability of the human IL-18 Binding Protein to inhibit the secretion of IFNgamma by NK92 cells stimulated with 2nM of unconjugated IL-18 variants and corresponding IL-18 immunocytokines by parental PD-1^{negative} (round symbols, dotted lines) and by engineered PD-1^{positive} NK-92 cells (squared symbols, solid lines). The figure shows mean IFNg alphaLISA signal on the y-axis and dosage of the human IL-18 Binding Protein

on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO:1) and SEQ ID NO: 30. Corresponding IL-18 immunocytokines tested are Compositions A and C.

[0431] FIGURE 36 shows plots measuring the ability of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines to stimulate the secretion of IFNgamma by parental PD-$1^{negative}$ (round symbols, dotted lines) and by engineered PD-$1^{positive}$ NK-92 cells (squared symbols, solid lines) in the absence (black plain symbols) or presence (grey open symbols) of an excess of anti-PD-1 antibody. The figure shows mean IFNg legendplex signal on the y-axis and dosage of the unconjugated IL-18 variants and corresponding IL-18 immunocytokines on the x-axis. The unconjugated IL-18 variants are native IL-18 wild-type (SEQ ID NO:1) and SEQ ID NO: 30. Corresponding IL-18 immunocytokines tested are Compositions A and C.

Example 19: *in vivo* mouse PK/PD study (Figures 37-43)

[0432] An *in vivo* PK/PD study was performed in mice. Naïve, 6-8 weeks old, C57BL/6-hPD1 female mice (GemPharmatech Co, Ltd, Nanjing, China) were inoculated subcutaneously at the upper right flank with wild type MC38 tumor cells (3×10^5) in 0.1 mL of PBS for tumor development. After inoculation, the animals were checked daily for morbidity and mortality. At the time, animals were checked for effects on tumor growth and normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect. Tumor sizes were measured three times a week in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset. The animals were randomized (using an Excel-based randomization software performing stratified randomization based upon tumor volumes), and treatment started when the average tumor volume reached approximately 230 (Group 01-1 to 01-5) and 400 mm^3 (Group 01-6 to 01-9, 02-1 to 02-4, and 03-1 to 03-2). Animals treated with Composition A received a single 10 mL/kg bolus intravenous (i.v.) injection of 0.25 mg/kg of PD-1 antibody conjugated with IL-18 polypeptide.

[0433] Pharmacokinetic study included 12 time points (5 min, 1h, 2h, 4h, 6h, 12h, 24h, 72h, 96h, 120h, 168h, 240h) with 3 mice sampled per time points. At indicated time points, blood samples were collected in the presence of EDTA either via tail vein sampling or via cardiac puncture (end-point). In addition, 6h, 24h, 48h, 72h, 96h, 120h, 168h, and 240h after injection 3 mice per group were sacrificed and tumor samples were collected.

[0434] Bioanalysis is conducted by detecting the total amount of IL-18 variants (free and IL-18BP-complexed). Corning high-binding half-area plates are coated overnight at 4°C with 50 µl of anti-IL18 monoclonal antibody (R&D, cat # D043-3, Clone 25-2G) at 2 µg/ml in PBS. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Plates surfaces are blocked with 100 µl of PBS-0.02% Tween20-3% BSA at 37°C during 1h. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Fifty microliters of IL-18 variants (or of mouse plasma) are added in eight-fold serial dilutions starting at 50 nM down to 0.02 nM into PBS-0.02% Tween20-0.1% BSA and incubated at 37°C during 2h. Plates are then washed four times with 100 µl of PBS-0.02% Tween20 and 50 µl of biotinylated Mouse anti-Human IgG Fc Secondary Antibody (3D3cc) [Biotin] at 0.1 µg/ml in PBS-0.02% Tween20-0.1% BSA. Plates are incubated during 2h at 37°C and ae then washed four times with 100 µl of PBS-0.02% Tween20. Fifty microliters of Streptavidin- Horseradish peroxidase (#RABHRP3, Sigma) diluted at 1:500 into PBS-0.02% Tween20-0.1% BSA are added to each well and incubated at Room Temperature during 30min. Plates are then washed four times with 100 µl of PBS-0.02% Tween20. Fifty microliters of TMB substrate reagent (SeraCareTM, 5120-0077 for plasma and 5120-0037 for tumor) are added to each well and incubated at 37°C during 5min. After 5min at 37°C, Horseradish peroxidase reaction is stopped by adding 50µl/well of 0.5M H_2SO_4 stop solution. ELISA signal is then measured at 450 nm on a SpectraMax M5e with SoftMax Pro GxP 7.0.3 (Molecular Devices).

[0435] Bioanalysis is conducted by detecting the total amount of IL-18 variants (free and IL-18BP-complexed). Nunc MaxiSorp™ Flat Bottom Plate are coated overnight at 4°C with 50 µl of human IL18BPa Fc (R&D, 119-BP-100) at 1 µg/ml in PBS. Plates are then washed four times with 100 µl of PBS-0.1% Tween20. Plates surfaces are blocked with 100 µl of PBS-0.1% Tween20-5% Milk at 37°C during 1h. Plates are then washed four times with 100 µl of PBS-0.1% Tween20. Fifty microliters of IL-18 variants (or of mouse plasma) are added in eight-fold serial dilutions starting at 50 nM down to 0.02 nM into PBS-0.1% Tween20-5% Milk and incubated at 37°C during 2h. Plates are then washed four times with 100 µl of PBS-0.02% Tween20 and 50 µl of biotinylated Goat (Fab')2 anti-human IgG (Fab')2 HRP (Abcam, ab98535) at 0.1 µg/ml in PBS-0.1% Tween20-5% Milk. Plates are incubated during 2h at 37°C and are then washed four times with 100 µl of PBS-0.1% Tween20. Fifty microliters of Streptavidin- Horseradish peroxidase (#RABHRP3, Sigma) diluted at 1:500 into PBS-0.1% Tween20-5% Milk are added to each well and incubated at Room Temperature during 30min. Plates are then washed four times with 100 µl

of PBS-0.1% Tween20. Fifty microliters of TMB substrate reagent (SeraCareTM, 5120-0077 for plasma and 5120-0037 for tumor) are added to each well and incubated at 37°C during 5min. After 5min at 37°C, Horseradish peroxidase reaction is stopped by adding 50µl/well of 0.5M H_2SO_4 stop solution. ELISA signal is then measured at 450 nm on a SpectraMax M5e with SoftMax Pro GxP 7.0.3 (Molecular Devices).

Plasma pharmacokinetic of composition A injected as a single bolus intravenous injections at 0.25 mg/kg in in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors

BLOOD	Cl_obs ($mL \cdot min^{-1} \cdot kg^{-1}$)	Vss_obs (L/kg)	AUC_{INF}_obs ($h \cdot ng \cdot mL^{-1}$)	$T_{1/2}$ (h)	$MRT_{0\text{-}inf}$ (h)
TOTAL	0.0382	0.061	109131	19	26.9
FREE	0.0566	0.0957	735652	18.8	28.2

Tumor pharmacokinetic of composition A injected as a single bolus intravenous injections at 0.25 mg/kg in in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors

Tumor	Cl_obs ($mL \cdot min^{-1} \cdot kg^{-1}$)	Vss_obs (L/kg)	AUC_{INF}_obs ($h \cdot ng \cdot mL^{-1}$)	$T_{1/2}$ (h)	$MRT_{0\text{-}inf}$ (h)
TOTAL	-	-	16045	30	52.6
FREE	Not determined				

[0436] FIGURE 37A shows a plot describing plasma exposure of Total (bound and unbound to IL-18BP) and free (unbound to IL-18BP) composition A in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows plasma concentrations on the y-axis, and time on the x-axis. The IL-18 immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0437] FIGURE 37B shows a plot describing tumor exposure of Total (bound and unbound to IL-18BP) and free (unbound to IL-18BP) composition A in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows tumor concentrations

on the y-axis, and time on the x-axis. The IL-18 immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0438] Pharmacodynamic study included 8 time points (6h, 24h, 48h, 72h, 96h, 120h, 168h, and 240h) with 3 mice sampled per time points. At indicated time points, blood samples were collected in the presence of EDTA either via tail vein sampling or via cardiac puncture (end-point). In addition, 6h, 24h, 48h, 72h, 96h, 120h, 168h, and 240h after injection 3 mice per group were sacrificed and tumor samples were collected.

[0439] Fresh tumor sample from each mouse was minced individually and digested with mixed enzymes in C tubes. C tubes were attached onto the sleeves of the Gentle MACS Dissociator before running the program "m_imptumor_01_01" one time. C tubes were then incubated for 30 minutes at 37 °C, followed by another round of program "m_imptumor_01_01". Digested tissues were filtered through 70 μm cell strainers. Cells were washed twice with DPBS before staining.

[0440] Collected mouse blood in the presence of EDTA was immediately centrifuged sample tube at 4,000 rpm for 5 min at 4 °C. Collected plasma (supernatant) and stored at -80 °C until Bioanalysis. Then mixed one volume of cell pellet with 20 volumes of 1 x Red Blood Cell Lysis Solution. Then incubated for 3 min and centrifuged. If the blood wasn't lysed well, it would be suspended with 2 mL 1 x Red Blood Cell Lysis Solution again and incubated for 3 min.

[0441] 100 μL of resuspended cells (in concentration of 10 million/mL) per test were seeded on 96 V-hole plate. After centrifugation, the cells were suspended with 100 μL DPBS. Zombie NIR™ live/dead was added and incubated for 30 min at 4 °C in the dark. The cells were washed twice with DPBS. The cells were suspended with 50 μL staining buffer, purified rat Anti-Mouse CD16/CD32 was added and incubated at 4°C in the dark for 5 min. Appropriate fluorescent-conjugated antibodies were prepared in 50 μL Brilliant Stain Buffer, which were subsequently added to the cell suspension and incubated at 4°C in the dark for 30 min. After extracellular antibodies incubation, cells were washed twice with staining buffer, fixed and permeabilized for 30 min. Purified rat anti-mouse CD16/CD32 were added for 5 min incubation and cells were stained according to the procedures indicated in specific intracellular antibodies specification. Then, the cells were washed twice and suspended with 200 μL staining buffer. Stained cells were analyzed by Cytek Aurora Flow Cytometer.

Table 15: Staining was performed with the following antibody panel.

#	Fluorochrome	Antibody	Supplier	Cat#	Clone
1	BV421	CD178 (FasL)	BD	740054	MFL3
2	BV421	Isotype	BD	562601	A19-3
3	BV480	CD366 (TIM-3)	BD	747618	5D12/TIM-3
4	BV480	Isotype	BD	565652	X40
5	BV570	CD44	BioLegend	103037	IM7
6	Pacific Orange	CD8	ThermoFisher	MCD0830	5H10
7	BV605	MHCII	BD	563413	M5/114.15.2
8	BV650	Isotype	BD	563906	A110-1
9	BV650	CD25	BD	564021	PC61
10	BV711	CD127	BioLegend	135035	A7R34
11	BV711	Isotype	BioLegend	400551	RTK2758
12	BV750	CD45	BioLegend	103157	30-F11
13	BV785	m PD-L1	BioLegend	124331	10F.9G2
14	BV785	Isotype	BioLegend	400647	RTK4530
15	FITC	CD62L	BioLegend	104406	MEL-14
16	AF532	CD11b	ThermoFisher	58-0112-82	M1/70
17	PE	CD39	BioLegend	143804	Duha59
18	PE	Isotype	BioLegend	400508	RTK2758
19	PE/Dazzle 594	CD49b	BioLegend	108924	DX5
20	PE-Cy5	CD69	BioLegend	104510	H1.2F3
21	PE-Cy5	Isotype	BioLegend	400910	HTK888
22	BB700	F4/80	BD	746070	T45-2342
23	PerCP-eFluor710	CD122 (IL-2R beta)	eBioscience	46-1222-82	TM-b1
24	PerCP-eFluor710	Isotype	eBioscience	46-4031-82	eB149/10H5
25	PE-Cy7	CD218a	eBioscience	25-5183-82	P3TUNYA
26	PE-Cy7	Isotype	eBioscience	25-4321-82	eBR2a

#	Fluorochrome	Antibody	Supplier	Cat#	Clone
27	APC	h PD-1	BD	558694	MIH4
28	APC	Isotype	BD	555751	MOPC-21
29	APC-Cy5.5	CD4	ThermoFisher	MCD0419	RM4-5
30	AF700	CD206	BioLegend	141734	C068C2
31	Zombie NIR™	L/D	BioLegend	423106	-
32	APC-Cy7	CD3	BioLegend	100222	17A2

[0442] Mouse tumor protein extract and plasma samples were subjected to Luminex analysis for cytokine array using Mouse ProcartaPlex Mix&Match 5-plex (GM-CSF, IFN gamma, IL-2, IL-6 and TNF alpha) following provider instructions.

[0443] FIGURE 38 shows a plot describing the levels of surface expression of PD-1 and IL-18 receptor alpha in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice treated with vehicle. The figure shows the median fluorescence intensity of PD-1 (upper panel) and of IL-18 receptor alpha (bottom panel) surface staining the y-axis, and the subset of immune cell on the x-axis. (48h and 7 days after i.v. Injection of formulation buffer, n=5 per time point).

[0444] FIGURE 39 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expansion of CD4+ T-cells, CD8+ T-cells, macrophages, and Natural Killer (NK) cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the frequency of indicated immune cell subsets within CD45+ leukocytes on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0445] FIGURE 40 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expansion of naïve ($CD62L^{high}$ $CD44^{low}$), central memory ($CD62L^{high}$ $CD44^{high}$) effector memory ($CD62L^{negative}$ $CD44^{high}$) CD8+ T-cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the frequency of indicated immune cell subsets within CD45+ leukocytes on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0446] FIGURE 41 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expression of CD69 surface marker on CD4+ T-cells, CD8+ T-cells, and Natural Killer (NK) cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the median fluorescence intensity of CD69 surface staining the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0447] FIGURE 42 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on the expression of IL-18 receptor alpha on the surface of CD4+ T-cells, CD8+ T-cells, and Natural Killer (NK) cells in the blood and tumors of MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the median fluorescence intensity of IL-18 receptor alpha surface staining the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0448] FIGURE 43 shows a plot describing the effect of PD-1 targeted IL-18 immunocytokine on levels of cytokines in blood and tumors of in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows plasma and tumor concentrations of IFNg, GM-CSF, TNFa, and IL-6 on the y-axis, and time on the x-axis. The IL-18 immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point; mean ± SEM)

Example 20: In vivo depletion efficacy study (Figure 44-48)

[0449] An *in vivo* efficacy study was performed in mice. Naïve, 6-8 weeks old, C57BL/6-hPD1 female mice (GemPharmatech Co, Ltd, Nanjing, China) were inoculated subcutaneously at the right upper flank with MC38 tumor cells (3×10^5) in 0.1 mL of PBS for tumor development. The animals were randomized (using an Excel-based randomization software performing stratified randomization based upon tumor volumes) when the average tumor volume reached approximately 80 mm^3. To trigger depletion of target immune cells, animals were treated intraperitoneally with 10 mg/kg of the following depleting antibodies two days before start of treatment with PD-1 IL-18 immunocytokine and twice a week for 4 weeks (10 injections in total). Two days after first depleting antibody injection, three mice per group were sacrificed to enumerate blood and tumoral macrophages, CD4+ T-cells, CD8+ T-cells, B-cells, NK cells using the following antibody panel.

	Panel
1	Live/dead
2	CD45
3	CD3
4	CD4
5	CD8
6	NKp46
7	CD19
8	CD11b
9	F4/80

[0450] Two days after treatment with depleting antibodies,. Animals received a single 10 mL/kg bolus intravenous (i.v.) of 0.5 mg/kg of PD-1 antibody conjugated with IL-18 polypeptide. After inoculation, the animals were checked daily for morbidity and mortality. At the time, animals were checked for effects on tumor growth and normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect. The major endpoints were delayed tumor growth or complete tumor regression. Tumor sizes were measured three times a week in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b are the long and short diameters of the tumor, respectively. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

[0451] FIGURE 44 shows a plot describing the effect of depleting antibody treatment on leukocytes populations of blood and tumors of in hPD-1 transgenic C57BL/6 mice implanted with MC38 syngeneic colon carcinoma tumors. The figure shows the frequency of indicated immune cell subsets within CD45+ leukocytes on the y-axis, and depleting antibody treatment on the x-axis. The IL-18 immunocytokine tested is Compositions A tested as a single agent at 0.25 mg/kg as a single i.v. injection. (n=3 per time point ; mean ± SEM).

[0452] FIGURE 45 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, Macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the volume of MC38 tumors on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group ; mean ± SEM).

[0453] FIGURE 46 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, Macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice 12 days after treatment. The figure shows the volume of MC38 tumors on the y-axis, and treatment received by mice on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group ; mean ± SEM ; TGI: Tumor Growth Inhibition).

[0454] FIGURE 47 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, Macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the volume of MC38 tumors on the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group ; CR: Complete Response).

[0455] FIGURE 48 shows a plot describing the effect CD4+ T-cells, CD8+ T-cells, macrophages, and Natural Killer (NK) cells depletion on the anti-tumoral activity of PD-1 targeted IL-18 immunocytokine in MC38 tumor bearing hPD1 humanized C57BL/6 mice after treatment. The figure shows the percentage of surviving mice within each treatment group the y-axis, and time on the x-axis. The immunocytokine tested is Compositions A tested as a single agent at 0.5 mg/kg as a single i.v. injection. (n=7 per group).

Example 21: PK/PD Study in Non-Human Primates (NHPs) (Figures 49-42)

[0456] A pharmacokinetic (PK) and pharmacodynamic (PD) study of Composition A was performed in non-human primates. The objective of this study was to evaluate the pharmacokinetics and pharmacodynamics of Composition A after a single 15-minute intravenous infusion to cynomolgus monkeys followed by a 3-week observation period.

[0457] Briefly, six male cynomolgus monkeys were assigned to the study and randomized into 3 groups as shown in the table below. Composition A was administered as a single 15-minute intravenous infusion at dose levels of 0.03 mg/kg (G1), 0.1 mg/kg (G2) and 0.3 mg/kg (G3) (see table below). Administrations of G2 and G3 were staggered by 1 week to ensure tolerability of the test item.

Group	Group size	Test article	Dosage mg/kg	Dose volume (mL/kg)	Route	Frequency

1	2	Composition A	0.03	2.5	IV infusion (15 min)	Single
2	2		0.1	2.5		
3	2		0.3	2.5		

[0458] Primary objectives of the study were to characterize PK/PD and associated tolerability profile of Composition A upon single dose. It was also desired to define a pharmacological dose range, identify potential tolerability, clinical signs, or clinical pathology perturbations. Acclimation biomarker baseline determination was made at 7 days before initial dose. Endpoints were body weight and clinical signs, PK (free and total assays), immunophenotyping, cytokine level measurements, hematology, clinical chemistry, anti-drug antibody assessment, and measurement of exploratory biomarkers.

[0459] Resulting PK parameters of individual animals are shown in the table below. The results of Composition A concentration in plasma indicated that all animals were exposed to Composition A with quantifiable plasma concentrations for at least 72 hours and up to 120 hours. AUC(0-last)/dose, half-life, and MRTlast increased with dise while clearance decrased with dose, suggesting a nonlinear PK. PK was slightly over-dose proportional, with volume of distribution consistent with physiological plasma volume. Half-life and mean residence time (MRT) was dose-dependent. **FIGURE 49** shows plots of both individual PK for individual animals and dose-normalized PK for individual animals at each dose.

Subject		**401**	**402**	**404**	**405**	**406**	**407**
Dose	mg/kg	0.03		0.1		0.3	
CL	mL/min/kg	0.0574	0.0975	0.133	0.0601	0.0425	0.0396
Vss	L/kg	0.0457	0.0548	0.0921	0.053	0.0416	0.0541
t1/2	h	10.2	9.94	9.81	10	12.3	13.1
MRTlast	h	12.7	9.1	11.2	14.2	15.2	22.7
Cmax	ng/mL	743	681	1210	2260	8460	7810
AUC(0-last)	h*ng/mL	8654	5112	12477	27524	115882	126271
AUC(0-inf)	h*ng/mL	8718	5130	12535	27724	117577	126390

AUC(0-last)/Dose	h*ng/mL/(mg/kg)	288473	170409	124770	275244	386273	420904

[0460] Additional PK analysis was conducted by analyzing the amount of Composition A which was free of IL-18BP versus total amount of Composition A observed in samples. Results of this analysis are shown in **FIGURE 50**. In **FIGURE 50**, "Free" represents the amount of Composition A not bound to IL-18BP and "Total" measures Composition A both free and in complex with IL-18 BP. The results showed that the PK profiles of both free and bound Composition A are superimposable, indicating that Composition A is not neutralized by circulated IL-18BP at the doses tested. Additionally, no faster decrease of free vs total levels of Composition A was observed in any of the animals, suggesting IL-18BP binding is not associated with faster clearance.

[0461] Linker stability of Composition A was also assessed. Briefly, quantification of the anti-PD-1 related circulating material in plasma (i.e., bearing or not bearing the IL-18 moiety) was assessed by performing an anti-PD-1 assay (i.e., quantifying all circulating anti-PD-1 mAb material (i.e., capture PD-1, detection of anti-human IgG) and an anti-immunocytokine assay to quantify all immunocytokine material (i.e., capture with anti-IL-18, then detect by anti-human IgG to ensure the material is intact).The resulting PK profiles in each sample are shown in **FIGURE 51**. These results show clear comparability across the two methods of detection, indicating that all or substantially all of the Composition A material remained intact (i.e., no separation of IL-18 and antibody portions of the immunocytokine), suggesting no cleavage of the linker occurring *in vivo*.

[0462] Finally, the ability of Composition A to expand IL-18Ra/PD-1 double positive T cells *in vivo*was assessed. Significant increases of these double positive T cells were observed, particulary at the highest dose (**FIGURE 52**). Additionally, this subset of double-positive T cells were preferentially expanded compared to other T cell subtypes. Investigation of immune cell subsets showed that IL-18R and PD-1 are both preferentially expressed in memory T cells at baseline. Upon Composition A administration, circulating lymphocytes transiently decreased (2-4 days post-dose) and returned to baseline about a week after administration (data not shown). Notably, T cells expressing both IL-18R1 and PD-1 showed a dose-dependent increase in total numbers.

Example 22: Characteristics of Additional IL-18 Polypeptides

[0463] Described below are characteristics of additional IL-18 polypeptides according to the instant disclosure which can also be incorporated into immunocytokines as described herein. It is

predicted that such IL-18 polypeptide would display similarly translatable characteristics compared to those immunocytokines explicity made and tested herein.

[0464] Example 22A – HEK-Blue Reporter Assay – An IL-18R positive HEK-Blue reporter cell line is used to determine binding of IL-18 variants to IL-18R and subsequent downstream signaling. The general protocol is outlined below.

[0465] 5× 104 cells HEK-Blue IL18R reporter cells (InvivoGen, #hkb-hmil18) are seeded into each well of a 96 well plate and stimulated with 0-100 nM of IL-18 polypeptide variants at 37 °C and 5 % CO2. After 20h incubation, 20 µL of cell culture supernatant is then taken from each well and mixed with 180 µL QUANTI-Blue media in a 96 well plate, incubated for 1 hour at 37 °C and 5 % CO2. The absorbance signal at 620nm is then measured on an Enspire plate reader with 680 and 615 nm as excitation and emission wavelengths, respectively. Half Maximal Effective dose (EC50) is calculated based on a variable slope, four parameter analysis using GraphPad PRISM software.

[0466] The HEK-Blue IL-18R reporter assay described above was performed on additional IL-18 polypeptides which can be incorporated into immunocytokine compositions provided herein. It is expected that the IL-18 polypeptides provided below would behave analogously to C086 (SEQ ID NO: 30) when incorporated into an immunocytokine composition as those otherwise provided herein.

SEQ ID NO: or Composition ID	Sequence modifications	EC_{50} (pM)
1	Native sequence	3.33
34	E6K, K53A, S55A	272.5
39	E6K, K53A	0.72
42	E6K, K53A, S55A, T63A	0.79
50	E6K, K53A, T63A	1.77
54	E6K, C38S, K53A, C68S, K70C, C76S, C127S	9.12
56	E6K, K53A, C38S, C76S, C127S	3.73
57	E6K, C38S, K53A	0.86
30	E6K, V11I, C38A, K53A, T63A, C76A, C127A	0.034
62	E6K, C38A, K53A, C127A	0.17
60	E6K, C38Q, K53A	0.203

59	E6K, C38A, K53A	0.268
57	E6K, C38S, K53A	0.53
C143	V11I, C38A, K53A, C76A, C127A	0.98
C144	V11I, C38A, K53A, T63A, C76A, C127A	0.17
C145	V11I, C38A, K53A, S55A, C76A, C127A	3.63
C146	V11I, C38A, M51G, K53A, C76A, C127A	0.8
C147	V11I, C38A, K53A, D54A, C76A, C127A	1
C148	F2A, V11I, C38A, K53A, C76A, C127A	7.28
C149	V11I, E31A, C38A, K53A, C76A, C127A	6.6
C150	V11I, T34A, C38A, K53A, C76A, C127A	0.7
C151	V11I, D35A, C38A, K53A, C76A, C127A	13.12
C152	V11I, S36A, C38A, K53A, C76A, C127A	0.25
C153	V11I, D37A, C38A, K53A, C76A, C127A	14.12
C154	V11I, E31A, D37A, C38A, K53A, C76A, C127A	11.95
C155	V11I, C38A, D40A, K53A, C76A, C127A	0.52
C156	V11I, C38A, N41A, K53A, C76A, C127A	11.7
C157	V11I, C38A, K53A, C76A, C127A, D132A	1.95
C158	V11I, C38A, K53A, C76A, G108A, C127A	15.56
C159	V11I, C38A, K53A, C76A, H109A, C127A	19.5
C160	V11I, C38A, K53A, C76A, D110A, C127A	2.02
C161	K8R, V11I, C38A, C76A, Q103E, C127A	2.01
C162	K8E, V11I, C38A, C76A, Q103R, C127A	2.3
C163	V11I, C38A, C76A, Q103K, C127A	1.5
C164	V11I, C38A, S55H, C76A, C127A	3.14
C165	V11I, C38A, S55R, C76A, C127A	1.91
C166	V11I, C38A, S55T, C76A, C127A	4.73
C167	V11I, C38A, C76A, S105I, C127A	5.37
C168	V11I, C38A, C76A, S105K, C127A	7.73
C174	K8L, E6K, V11I, C38A, K53A, T63A, C76A, C127A	0.29
C176	E6K, V11I, C38A, I49M, K53A, T63A, C76A, C127A	0.07
C177	E6K, V11I, C38A, I49R, K53A, T63A, C76A, C127A	0.04
C178	E6K, V11I, C38A, K53A, T63A, C76A, Q103R, C127A	0.26

C179	E6K, K8E, V11I, C38A, K53A, T63A, C76A, Q103R, C127A	0.4
C181	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153E	0.1
C182	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153Y	0.08
C183	E6K, V11I, C38A, M51G, K53A, T63A, C76A, C127A	0.1
C184	E6R, V11I, C38A, K53A, T63A, C76A, C127A	0.04
C140	E6K, V11I, C38A, K53A, T63A, C76A, C127A	2.5
C141	E6K, V11I, C38A, K53A, T63A, C76A, C127A	1.68
C142	Y1M, E6K, V11I, C38A, K53A, T63A, C76A, C127A	0.02
C192	E6K, V11I, C38A, M51G, K53A, T63A, C76A, C127A	13.99

[0467] Example 22B – IL-18 BP AlphaLISA assay – An IL-18 binding protein AlphaLISA experiment substantially as described in Example 4 was performed on IL-18 polypeptide which can be incorporated into immunocytokine compositions as provided herein to assess ability to bind to IL-18BP. Results are shown in the Table below. It is expected that the IL-18 polypeptides provided below would behave analogously to C086 (SEQ ID NO: 30) when incorporated into an immunocytokine composition as those otherwise provided herein.

SEQ ID NO: or Composition ID	**Sequence modifications**	**KD (nM)**
1	Native Sequence	0.67
34	E06K, K53A, S55A	>1500
35	Y01G, F02A, E06K, M51G, K53A, D54A, S55A, T63A	969.0
36	K53A	513.8
37	S55A	10.7
38	E06K	0.13
39	E06K, K53A	130.3
40	E06K, S55A	12.3
41	K53A, S55A	500.0

SEQ ID NO: or Composition ID	Sequence modifications	KD (nM)
42	E06K, K53A, S55A, T63A	822.0
43	E06K, K53A, S55A, Y01G	
44	E06K, K53A, S55A, F02A	>1000
45	E06K, K53A, S55A, D54A	>1000
46	E06K, K53A, S55A, M51G	>1000
47	C38S, C68S, C76S, C127S	0.03
48	C38S, C68S, C76S, C127S, K70C	0.21
49	E06K, K53A, S55A, C38S, C68S, C76S, C127S, K70C	>1000
50	E06K, K53A, T63A	339.8
51	T63A	2.59
52	E06K, T63A	0.83
53	K53A, T63A	198
54	E06K, K53A, C38S, C68S, C76S, C127S, K70C	446.0
55	K53A, T63A, C38S, C68S, C76S, C127S, K70C	913
56	E6K, K53A, C38S, C76S, C127S	435.5
57	E6K, K53A, C38S	50.2
C143	V11I, C38A, K53A, C76A, C127A	8.86
C144	V11I, C38A, K53A, T63A, C76A, C127A	0.66
C145	V11I, C38A, K53A, S55A, C76A, C127A	9.74
C146	V11I, C38A, M51G, K53A, C76A, C127A	373.30
C147	V11I, C38A, K53A, D54A, C76A, C127A	25.77
C148	F2A, V11I, C38A, K53A, C76A, C127A	57.21

SEQ ID NO: or Composition ID	Sequence modifications	KD (nM)
C149	V11I, E31A, C38A, K53A, C76A, C127A	0.64
C150	V11I, T34A, C38A, K53A, C76A, C127A	1.24
C151	V11I, D35A, C38A, K53A, C76A, C127A	2.88
C152	V11I, S36A, C38A, K53A, C76A, C127A	1.12
C153	V11I, D37A, C38A, K53A, C76A, C127A	4.55
C154	V11I, E31A, D37A, C38A, K53A, C76A, C127A	2.12
C155	V11I, C38A, D40A, K53A, C76A, C127A	0.74
C156	V11I, C38A, N41A, K53A, C76A, C127A	18.47
C157	V11I, C38A, K53A, C76A, C127A, D132A	13.70
C158	V11I, C38A, K53A, C76A, G108A, C127A	1.24
C159	V11I, C38A, K53A, C76A, H109A, C127A	0.55
C160	V11I, C38A, K53A, C76A, D110A, C127A	0.71
C161	K8R, V11I, C38A, C76A, Q103E, C127A	0.06
C162	K8E, V11I, C38A, C76A, Q103R, C127A	0.85
C163	V11I, C38A, C76A, Q103K, C127A	0.05
C164	V11I, C38A, S55H, C76A, C127A	0.08
C165	V11I, C38A, S55R, C76A, C127A	0.15
C166	V11I, C38A, S55T, C76A, C127A	0.02
C167	V11I, C38A, C76A, S105I, C127A	0.04
C168	V11I, C38A, C76A, S105K, C127A	0.05
C174	K8L, E6K, V11I, C38A, K53A, T63A, C76A, C127A	0.14
C176	E6K, V11I, C38A, I49M, K53A, T63A, C76A, C127A	25.84
C177	E6K, V11I, C38A, I49R, K53A, T63A, C76A, C127A	>2800
C178	E6K, V11I, C38A, K53A, T63A, C76A, Q103R, C127A	>2800

SEQ ID NO: or Composition ID	Sequence modifications	KD (nM)
C179	E6K, K8E, V11I, C38A, K53A, T63A, C76A, Q103R, C127A	>2800
C180	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153R	
C181	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153E	>2800
C182	E6K, V11I, C38A, K53A, T63A, C76A, C127A, V153Y	>2800
C183	E6K, V11I, C38A, M51G, K53A, T63A, C76A, C127A	>2800
C184	E6R, V11I, C38A, K53A, T63A, C76A, C127A	5.46
C140	E6K, V11I, C38A, K53A, T63A, C76A, C127A	>2800
C141	E6K, V11I, C38A, K53A, T63A, C76A, C127A	>2800
C142	Y1M, E6K, V11I, C38A, K53A, T63A, C76A, C127A	2.25
C192	E6K, V11I, C38A, M51G, K53A, T63A, C76A, C127A	>2800
62	E6K, C38A, K53A, C127A	69.62
60	E6K, C38Q, K53A	24.8
59	E6K, C38A, K53A	35.95

[0468] Example 22C – IFNγ Stimulation and IL-18BP Inhibition Assay– The experiments described in Example4 for determination of IFNg stimulation in NK92 cells (and inhibition by IL-18 BP) were performed substantially as described on IL-18 polypeptides in order to assess their activities and their suitability for incorporation into immunocytokine compositions. Results are shown in the table below. It is expected that the IL-18 polypeptides provided below would behave analagously to C086 (SEQ ID NO: 30) when incorporated into an immunocytokine composition as those otherwise provided herein.

SEQ ID NO: or Composition ID	Sequence modifications	IC_{50} (nM)	EC_{50} (nM)
1	Native sequence	1.47	0.276
34	E06K, K53A, S55A	229	0.824

SEQ ID NO: or Composition ID	Sequence modifications	IC_{50} (nM)	EC_{50} (nM)
35	Y01G, F02A, E06K, M51G, K53A, D54A, S55A, T63A	> 55.0	> 55.0
36	K53A	27.3	0.444
37	S55A	4.46	0.108
38	E06K	7.79	0.0567
39	E06K, K53A	> 703	0.0192
40	E06K, S55A	15	0.067
41	K53A, S55A	37.3	1.58
42	E06K, K53A, S55A, T63A	1060	0.144
43	E06K, K53A, S55A, Y01G	27.8	6.12
44	E06K, K53A, S55A, F02A	NT	> 1000
45	E06K, K53A, S55A, D54A	NT	30
46	E06K, K53A, S55A, M51G	0.189	7.4
47	C38S, C68S, C76S, C127S	0.444	0.115
48	C38S, C68S, C76S, C127S, K70C	0.114	0.488
49	E06K, K53A, S55A, C38S, C68S, C76S, C127S, K70C	NT	58.5
50	E06K, K53A, T63A	> 1000	0.0268
51	T63A	0.239	0.449
52	E06K, T63A	47.1	0.011
53	K53A, T63A	18.2	0.155
54	E06K, K53A, C38S, C68S, C76S, C127S, K70C	23.5	0.962
55	K53A, T63A, C38S, C68S, C76S, C127S, K70C	> 1000	17.2
6	E6K, V11I, C38A, K53A, T63A, C68A, C76A, C127A, D98C	5.847	1.366
5	E6K, V11I, C38A, K53A, T63A, C68A, C76A, C127A, M86C	62.37	0.075
9	E6K, C38A, K53A, C68A, D98C	960.8	0.069
4	E6K, C38A, K53A, C68A, M86C	396.3	0.022
62	E6K, C38A, K53A, C127A	283.6	0.026
60	E6K, C38Q, K53A	780.5	0.006
59	E6K, C38A, K53A	653.5	0.015

SEQ ID NO: or Composition ID	Sequence modifications	IC_{50} (nM)	EC_{50} (nM)
57	E6K, C38S, K53A	146.2	0.045
C143	V11I, C38A, K53A, C76A, C127A	1.625	0.138
C144	V11I, C38A, K53A, T63A, C76A, C127A	7.522	0.012
C145	V11I, C38A, K53A, S55A, C76A, C127A	10.24	0.087
C146	V11I, C38A, M51G, K53A, C76A, C127A	732.9	0.037
C147	V11I, C38A, K53A, D54A, C76A, C127A	47.63	0.079
C148	F2A, V11I, C38A, K53A, C76A, C127A	5.055	0.256
C149	V11I, E31A, C38A, K53A, C76A, C127A	1.167	0.187
C150	V11I, T34A, C38A, K53A, C76A, C127A	21.27	0.015
C151	V11I, D35A, C38A, K53A, C76A, C127A	3.622	0.061
C152	V11I, S36A, C38A, K53A, C76A, C127A	7.85	0.033
C153	V11I, D37A, C38A, K53A, C76A, C127A	2.222	0.175
C154	V11I, E31A, D37A, C38A, K53A, C76A, C127A	3.709	0.062
C155	V11I, C38A, D40A, K53A, C76A, C127A	3.233	0.067
C156	V11I, C38A, N41A, K53A, C76A, C127A	0.681	0.558
C157	V11I, C38A, K53A, C76A, C127A, D132A	6.082	0.056
C158	V11I, C38A, K53A, C76A, G108A, C127A	3.981	0.073
C159	V11I, C38A, K53A, C76A, H109A, C127A	1.807	0.123
C160	V11I, C38A, K53A, C76A, D110A, C127A	3.181	0.028
C161	K8R, V11I, C38A, C76A, Q103E, C127A	1.073	0.057
C162	K8E, V11I, C38A, C76A, Q103R, C127A	7.292	0.061
C163	V11I, C38A, C76A, Q103K, C127A	0.823	0.093
C164	V11I, C38A, S55H, C76A, C127A	0.456	0.414
C165	V11I, C38A, S55R, C76A, C127A	0.885	0.176
C166	V11I, C38A, S55T, C76A, C127A	0.44	0.098
C167	V11I, C38A, C76A, S105I, C127A	0.809	0.103

SEQ ID NO: or Composition ID	Sequence modifications	IC_{50} (nM)	EC_{50} (nM)
C168	V11I, C38A, C76A, S105K, C127A	0.176	0.098

[0469] Although the present disclosure and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the disclosure as defined in the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. An immunocytokine composition, comprising:
   an IL-18 polypeptide; and
   an antibody or an antigen binding fragment thereof specific for programmed cell death protein 1 (PD-1); and
   a linker, wherein the linker comprises:
      a first point of attachment to the IL-18 polypeptide; and
      a second point of attachment to the antibody or antigen binding fragment thereof.
2. The immunocytokine composition of claim 1, wherein the immunocytokine composition stimulates IFNg secretion by a PD-1^+ cell more potently than PD-1^- cell.
3. The immunocytokine composition of claim 2, wherein the potency is assessed as an EC_{50} value, and wherein the EC_{50} value is at least 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold lower for the PD-1^+ cell than the PD-1^- cell.
4. The immunocytokine composition of any one of claims 1-3, wherein the immunocytokine composition exhibits a reduced ability to be inhibited by IL-18BP when stimulating IFNg secretion of a PD-1^+ cell as compared to a PD-1^- cell.
5. The immunocytokine composition of claim 4, wherein the reduced ability to be inhibited by IL-18 BP is assessed as an IC_{50} value, and wherein the IC_{50} value is at least 10-fold, 20-fold, 50-fold, 100-fold, 150-fold, 200-fold, or 250-fold higher for the PD-1^+ cell as compared to the PD-1^- cell.
6. The immunocytokine composition of any one of claims 1-5, wherein the immunocytokine composition exhibits a reduced ability to be inhibited by IL-18 BP when stimulating IFNg secretion of a PD-1^+ cell compared to a corresponding IL-18 polypeptide not comprised in an immunocytokine composition, wherein the reduced ability to be inhibited is assessed as an IC_{50} value which is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold higher.
7. The immunocytokine composition of any one of claims 1-6, wherein the immunocytokine composition selectively expands $CD8^+$ T cells in a tumor microenvironment relative to

$CD8^+$ cells in blood of a subject, optionally wherein the $CD8^+$ T cells are effector memory T cells.

8. The immunocytokine composition of claim 7, wherein the expansion of $CD8^+$ T cells is assessed as the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes.
9. The immunocytokine composition of claim 8, wherein the fraction of $CD8^+$ T cells within $CD45^+$ leukocytes:
    a. Increases by at least 2-fold, 3-fold, or 4-fold within a tumor after administration of the immunocytokine composition;
    b. Reaches a fraction of at least 50%, 60%, 70%, 75%, or 80% within a tumor after administration of the immunocytokine composition;
    c. Increases by at most 4-fold, 3-fold, or 2-fold in the blood of the subject after administration of the immunocytokine composition; and/or
    d. Reaches a fraction of at most 50%, 45%, 40%, 35%, 30%, or 20% within the blood of the subject after administration of the immunocytokine composition.
10. The immunocytokine composition of any one of claims 1-9, wherein the immunocytokine composition selectively suppresses macrophage cells in a tumor microenvironment relative to macrophage cells in blood of a subject.
11. The immunocytokine composition of claim 10, wherein suppression of macrophage cells is assessed as the fraction of macrophages within $CD45^+$ leukocytes.
12. The immunocytokine composition of claim 11, wherein the fraction of macrophage cells within $CD45^+$ leukocytes:
    a. decreases by at least 2-fold, 3-fold, 4-fold, 5-fold, 7.5-fold, or 10-fold within a tumor after administration of the immunocytokine composition;
    b. reaches a fraction of below 40%, 30%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% within a tumor after administration of the immunocytokine composition; and/or
    c. does not have any significant effect on macrophage levels in the blood of the subject (e.g., macrophage levels remain with ~5% of the total fraction of $CD45^+$ leukocytes in the blood).
13. The immunocytokine composition of any one of claims 1-12, wherein the immunocytokine composition has minimal effect on $CD4^+$ T cells or NK cells (e.g., $CD45^+$ leukocytes) in a tumor.

14. The immunocytokine composition of any one of claims 1-13, wherein the immunocytokine composition expands expression of IL-18Ra on $CD4^+$ T cells within a tumor, optionally wherein the expression of IL-18Ra is increased by at least 2-fold, 3-fold, or 4-fold.
15. The immunocytokine composition of any one of claims 1-14, wherein the immunocytokine composition expands 1, 2, 3, or 4 cytokines selected from IFNg, GM-CSF, TNFa, and IL-6 within a tumor after administration of the immunocytokine composition.
16. The immunocytokine composition of claim 15, wherein the immunocytokine composition does not substantially expand the cytokine in blood of a subject.
17. The immunocytokine composition of any one of claims 1-16, wherein the immunocytokine composition preferentially expands a population of IL-18 receptor alpha / PD-1 double-positive T cells after administration *in vivo*.
18. The immunocytokine composition of claim 17, wherein the L-18 receptor alpha / PD-1 double-positive T cells are memory T cells.
19. The immunocytokine composition of any one of claims 1-18, wherein the linker comprises a polymer.
20. The immunocytokine composition of claim 19, wherein the polymer comprises a water-soluble polymer.
21. The immunocytokine composition of claim 20, wherein the water-soluble polymer comprises poly(alkylene oxide), polysaccharide, poly(vinyl pyrrolidone), poly(vinyl alcohol), polyoxazoline, poly(acryloylmorpholine), or a combination thereof.
22. The immunocytokine composition of any one of claims 19-21, wherein the polymer has a weight average molecular weight of from about 0.1 kDa to about 2 kDa.
23. The immunocytokine composition of any one of claims 1-22, wherein the first point of attachment is at a residue which is not the N-terminus or the C-terminus of the IL-18 polypeptide.
24. The immunocytokine composition of any one of claims 1-23, wherein the first point of attachment is to a residue in the region of residues 2-156 of the IL-18 polypeptide, wherein residue position numbering is based on SEQ ID NO: 1.

25. The immunocytokine composition of claim 23 wherein the first point of attachment is to a residue in the region of residues 38-144 of the IL-18 polypeptide.
26. The immunocytokine composition of claim 24 or 25, wherein the first point of attachment is at residue 38, 68, 69, 70, 76, 78, 85, 86, 95, 98, 121, 127, or 144 of the IL-18 polypeptide.
27. The immunocytokine composition of any one of claims 24-25, wherein the first point of attachment is at residue 68, 69, 70, 85, 86, 95, or 98 of the IL-18 polypeptide.
28. The immunocytokine composition of any one of claims 24-27, wherein the first point of attachment is at residue 68 of the IL-18 polypeptide.
29. The immunocytokine composition of any one of claims 1-28, wherein the IL-18 polypeptide comprises an amino acid substitution at the first point of attachment.
30. The immunocytokine composition of claim 29, wherein the amino acid substitution at the first point of attachment is for an unnatural amino acid.
31. The immunocytokine composition of claim 29, wherein the amino acid substitution at the first point of attachment is for a natural amino acid.
32. The immunocytokine composition of claim 29, wherein the amino acid substitution at the first point of attachment is E69C, K70C, E85C, M86C, T95C, or D98C.
33. The immunocytokine composition of any one of claims 1-32, wherein the second point of attachment is at an amino acid residue in an Fc region of the antibody or antigen binding fragment.
34. The immunocytokine composition of claims 33, wherein the second point of attachment is at a residue selected from Lys 246, Lys 248, Lys 288, Lys 290, or Lys 317 of the Fc region (EU numbering).
35. The immunocytokine composition of claim 34, wherein the second point of attachment is at Lys 248 (EU numbering).
36. The immunocytokine composition of any one of claims 1-35, wherein the second point of attachment is to a lysine residue on the antibody or antigen binding fragment.
37. The immunocytokine composition of any one of claims 1-36, wherein the IL-18 polypeptide displays reduced binding to IL-18 binding protein (IL-18BP) compared to a wild type IL-18 polypeptide of SEQ ID NO: 1.

38. The immunocytokine composition of any one of claims 1-37, wherein the IL-18 polypeptide contains one or more amino acid substitutions that are located at residue positions selected from Y01, F02, E06, V11, C38, K53, D54, S55, T63, E69, K70, E85, C76, M86, T95, D98, and C127, wherein residue position numbering of the IL-18 polypeptides are based on SEQ ID NO: 1 as a reference sequence.
39. The immunocytokine composition of claim 38, wherein the one or more amino acid substitutions in the IL-18 polypeptide are selected from Y01G, F02A, E06K, V11I, C38S, C38A, K53A, D54A, S55A, T63A, C68S, C68A, E69C, K70C, C76S, C76A, E85C, T95C, D98C, C127S, and C127A.
40. The immunocytokine composition of any one of claims 1-39, wherein the IL-18 polypeptide contains E06K and K53A amino acid substitutions.
41. The immunocytokine composition of any one of claims 1-40, wherein the IL-18 polypeptides contains a T63A amino acid substitution.
42. The immunocytokine composition of any one of claims 1-41, wherein the IL-18 polypeptide comprises a V11I amino acid substitution.
43. The immunocytokine composition of any one of claims 1-42, wherein the IL-18 polypeptide comprises substitutions at 1, 2, 3, or 4 cysteines of the sequence of SEQ ID NO: 1.
44. The immunocytokine composition of any one of claims 1-43, wherein the IL-18 polypeptide comprises substitutions residues C38, C76, and C127 of the sequence set forth in SEQ ID NO: 1.
45. The immunocytokine composition of claim 44, wherein the IL-18 polypeptide comprises C38A, C76A, and C127A substitutions or C38S, C76S, and C127S substitutions.
46. The immunocytokine composition of any one of claims 1-45, wherein the antibody or antigen binding fragment thereof is a monoclonal antibody, a humanized antibody, a grafted antibody, a chimeric antibody, a human antibody, a de-immunized antibody, or a bispecific antibody.
47. The immunocytokine composition of any one of claims 1-46, wherein the antibody or antigen binding fragment thereof an antigen binding fragment, wherein the antigen binding fragment comprises a Fab, a Fab', a F(ab')2, a bispecific F(ab')2, a trispecific F(ab')2, a variable fragment (Fv), a single chain variable fragment (scFv), a dsFv, a

bispecific scFv, a variable heavy domain, a variable light domain, a variable NAR domain, bispecific scFv, an AVIMER®, a minibody, a diabody, a bispecific diabody, triabody, a tetrabody, a minibody, a maxibody, a camelid, a VHH, a minibody, an intrabody, fusion proteins comprising an antibody portion (a domain antibody), a single chain binding polypeptide, a scFv-Fc, a Fab-Fc, a bispecific T cell engager (BiTE), a tetravalent tandem diabody (TandAb), a Dual-Affinity Re-targeting Antibody (DART), a bispecific antibody (bscAb), a single domain antibody (sdAb), a fusion protein, or a bispecific disulfide-stabilized Fv antibody fragment (dsFv–dsFv′).

48. The immunocytokine composition of any one of claims 1-47, wherein the antibody or antigen binding fragment thereof comprises an IgG, an IgM, an IgE, an IgA, an IgD, or is derived therefrom.

49. The immunocytokine composition of claim 48, wherein the antibody or antigen binding fragment thereof comprises the IgG, and wherein the IgG is an IgG1, an IgG4, or is derived therefrom.

50. The immunocytokine composition of claim any one of claims 1-49, wherein the antibody or antigen binding fragment thereof comprises tislelizumab, baizean, 0KVO411B3N, BGB-A317, hu317-1/IgG4mt2, sintilimab, tyvyt, IBI-308, toripalimab, TeRuiPuLi , terepril, tuoyi, JS-001, TAB-001, tamrelizumab , HR-301210, INCSHR-01210, SHR-1210, temiplimab, cemiplimab-rwlc, 6QVL057INT , H4H7798N, REGN-2810 , SAR-439684, lambrolizumab, pembrolizumab, MK-3475, SCH-900475, h409A11, nivolumab, nivolumab BMS, BMS-936558, MDX-1106, ONO-4538, prolgolimab, forteca, BCD-100, penpulimab, AK-105, zimberelimab, AB-122, GLS-010, WBP-3055, balstilimab, 1Q2QT5M7EO, AGEN-2034, AGEN-2034w, genolimzumab, geptanolimab, APL-501, CBT-501, GB-226, dostarlimab, ANB-011, GSK-4057190A, P0GVQ9A4S5, TSR-042, WBP-285, serplulimab, HLX-10, CS-1003, retifanlimab, 2Y3T5IF01Z, INCMGA-00012, INCMGA-0012, MGA-012, sasanlimab, LZZ0IC2EWP, PF-06801591, RN-888, spartalizumab, PDR-001, QOG25L6Z8Z, relatlimab/nivolumab, BMS-986213, cetrelimab, JNJ-3283, JNJ-63723283, LYK98WP91F, tebotelimab, MGD-013, BCD-217, BAT-1306, HX-008, MEDI-5752, JTX-4014, cadonilimab, AK-104, BI-754091, pidilizumab, CT-011, MDV-9300, YBL-006, AMG-256, RG-6279, RO-7284755, BH-2950, IBI-315, RG-6139, RO-7247669, ONO-4685, AK-112, 609-A, LY-3434172, T-

3011, AMG-404, IBI-318, MGD-019, ONCR-177, LY-3462817, RG-7769, RO-7121661, F-520, XmAb-23104, Pd-1-pik, SG-001, S-95016, Sym-021, LZM-009, budigalimab, 6VDO4TY3OO, ABBV-181, PR-1648817, CC-90006, XmAb-20717, 2661380, AMP-224, B7-DCIg, EMB-02, ANB-030, PRS-332, [89Zr]Deferoxamide-pembrolizumab, 89Zr-Df-Pembrolizumab, [89Zr]Df-Pembrolizumab, STI-1110, STI-A1110, CX-188, mPD-1, MCLA-134, 244C8, ENUM 224C8, ENUM C8, 388D4, ENUM 388D4, ENUM D4, MEDI0680, NVP-LZV-184, or AMP-514, or a modified version thereof..

51. The immunocytokine composition of any one of claims 1-50, wherein the antibody or antigen binding fragment thereof comprises nivolumab, pembrolizumab, LZM-009, or cemiplimab, or a modified version thereof.
52. The immunocytokine composition of any one of claims 1-51, wherein the antibody or antigen binding fragment thereof comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence of Table 1.
53. The immunocytokine composition of any one of claims 1-52, wherein the IL-18 polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1.
54. The immunocytokine composition of any one of claims 1-53, wherein the IL-18 polypeptide of the immunocytokine provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 2-67.
55. The immunocytokine composition of any one of claims 1-54, wherein the IL-18 polypeptide comprises an amino acid sequence of SEQ ID NO: 30.
56. The immunocytokine composition of any one of claims 1-55, wherein the IL-18 polypeptide comprises an amino acid sequence of SEQ ID NO: 68-72.
57. The immunocytokine composition of any one of claims 1-56, wherein the immunocytokine composition exhibits a binding affinity (K_D) to at least one Fc receptor which is within 10-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide.

58. The immunocytokine composition of any one of claims 1-57, wherein the immunocytokine composition exhibits binding affinity (K_D) to the antigen of the antibody which is within 5-fold of the binding affinity of the antibody not attached to the IL-18 polypeptide.
59. The immunocytokine composition of any one of claim 1-58, wherein the immunocytokine composition exhibits an ability to induce IFNg production in a cell as measured by half-maximal effective concentration (EC_{50}) which is within about 100-fold of the corresponding IL-18 polypeptide not comprised in the immunocytokine composition, and wherein the immunocytokine composition exhibits a lower EC_{50} than WT IL-18.
60. The immunocytokine composition of any one of claims 1-59, wherein the immunocytokine composition exhibits enhanced anti-tumor growth inhibition compared to the antibody alone.
61. The immunocytokine composition of any one of claims 1-60, wherein the immunocytokine composition exhibits enhanced anti-tumor growth inhibition compared to the antibody and the IL-18 polypeptide administered in combination.
62. The immunocytokine composition of any one of claims 1-61, further comprising a second linker, wherein the second linker comprises:
    a third point of attachment to the antibody or antigen binding fragment thereof, and
    a fourth point of attachment to an additional IL-18 polypeptide.
63. The immunocytokine composition of claim 62, wherein the third point of attachment is to a different heavy chain of the antibody or antigen binding fragment thereof from the second point of attachment.
64. A pharmaceutical composition comprising:
    an immunocytokine composition according to any one of the preceding claims; and
    one or more pharmaceutically acceptable carriers or excipients.
65. The pharmaceutical composition of claim 64, wherein the pharmaceutical composition is formulated for parenteral or enteral administration.
66. The pharmaceutical composition of claim 64 or 65, wherein the pharmaceutical composition is formulated for intravenous or subcutaneous administration.
67. The pharmaceutical composition of any one of claims 64-66, wherein the pharmaceutical composition is in a lyophilized form.

68. The pharmaceutical composition of any one of claims 64-67, wherein the one or more pharmaceutically acceptable carriers or excipients comprises one or more of each of: a carbohydrate, an inorganic salt, an antioxidant, a surfactant, a buffer, or any combination thereof.
69. The pharmaceutical composition of any one of claims 64-68, comprising one, two, three, four, five, six, seven, eight, nine, ten, or more excipients.
70. A method of expanding a population of $CD8^+$ T cells in a subject in need thereof, the method comprising administering to the subject the immunocytokine composition of any one of claims 1-63 or the pharmaceutical composition of any one of claims 64-69.
71. A method of expanding a population of IL-18 receptor alpha / PD-1 double-positive T cells in a subject in need thereof, the method comprising adminsiterting to the subject the immunocytokine composition of any one of claims 1-63 or the pharmaceutical composition of any one of claims 64-69.
72. The method of claim 70 or 71, wherein the subject has a tumor (i.e., a solid cancer).
73. A method of treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the immunocytokine composition of any one of claims 1-63 or a pharmaceutical composition according to any one of claims 62-69.
74. The method of claim 72 or 73 wherein the cancer is a melanoma, a lung cancer, a bladder cancer (BC), a microsatellite instability high (MSI-H)/ mismatch repair-deficient (dMMR) solid tumor, a tumor mutation burden high (TMB-H) solid tumor, a triple-negative breast cancer (TNBC), a gastric cancer (GC), a cervical cancer (CC), a pleural mesothelioma (PM), classical Hodgkin's lymphoma (cHL), a primary mediastinal large B cell lymphoma (PMBCL), or a combination thereof.
75. The method of claim 72 or 73, wherein the cancer is adrenal cancer, anal cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, carcinoid cancer, cervical cancer, colorectal cancer, esophageal cancer, eye cancer, gallbladder cancer, gastrointestinal stromal tumor, germ cell cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, neuroendocrine cancer, oral cancer, oropharyngeal cancer, ovarian cancer, pancreatic cancer, pediatric cancer, penile cancer, pituitary cancer, prostate cancer, skin

cancer, soft tissue cancer, spinal cord cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, ureteral cancer, uterine cancer, vaginal cancer, or vulvar cancer.

76. The method of claim 72 or 73, wherein the cancer is.a carcinoma, and wherein the carcinoma comprises a cutaneous squamous cell carcinoma (CSCC), a urothelial carcinoma (UC), a renal cell carcinoma (RCC), a hepatocellular carcinoma (HCC), a head and neck squamous cell carcinoma (HNSCC), an esophageal squamous cell carcinoma (ESCC), a gastroesophageal junction (GEJ) carcinoma, an endometrial carcinoma (EC), a Merkel cell carcinoma (MCC), or a combination thereof.

77. The method of claim 73, wherein the cancer is leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, an AIDS-related lymphoma, multiple myeloma, plasmacytoma, post-transplantation lymphoproliferative disorder, or Waldenstrom macroglobulinemia..

78. The method of claim 77, wherein the cancer is leukemia and the leukemia comprises, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, small lymphocytic lymphoma, or chronic myeloid leukemia

79. The method of claim 72 or 73, wherein the cancer is lymphoma and the lymphoma comprises Burkitt lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma, cutaneous lymphoma, diffuse large B cell lymphoma, follicular lymphoma, Hodgkin lymphoma, peripheral T cell lymphoma, Waldenstrom macroglobulinemia, lymphoplamacytic lymphoma, marginal zone lymphoma, B cell cutaneous lymphoma, extranodal natural killer T cell lymphoma, T cell lymphoblastic lymphoma, peripheral T cell lymphoma, or T cell cutaneous lymphoma.

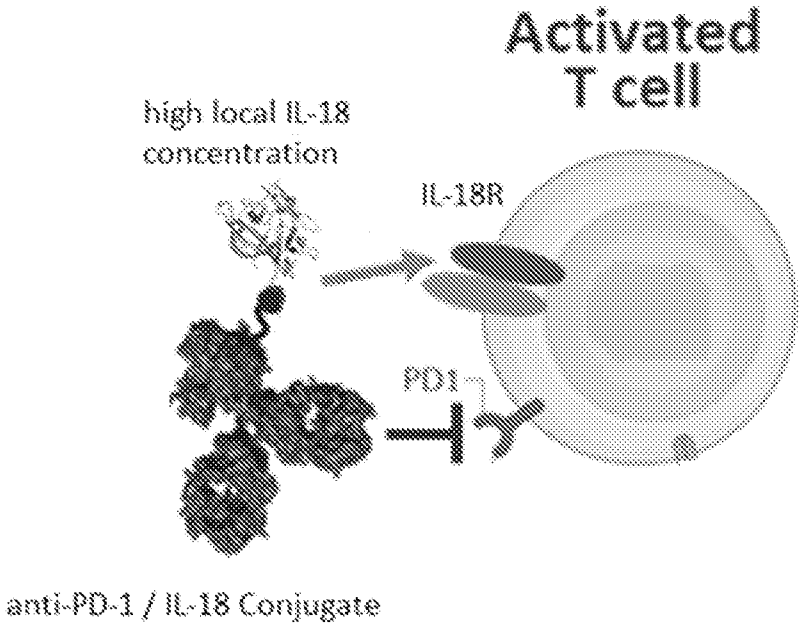


FIGURE 1A

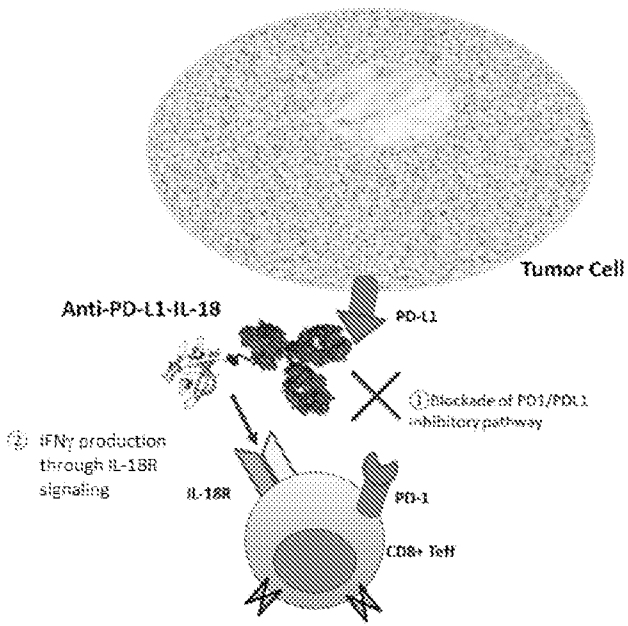


FIGURE 1B

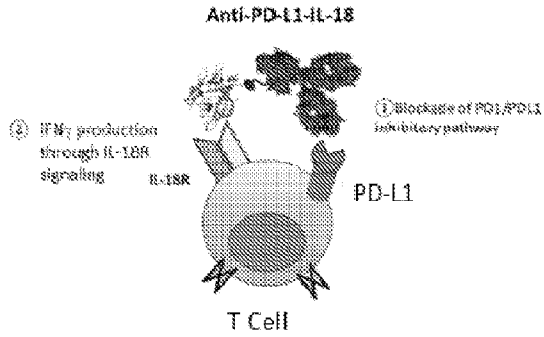


FIGURE 1C

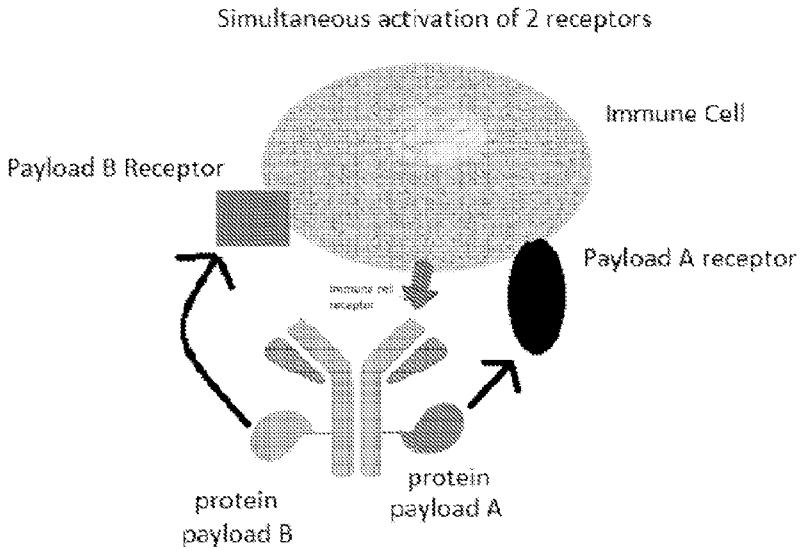


FIGURE 1D

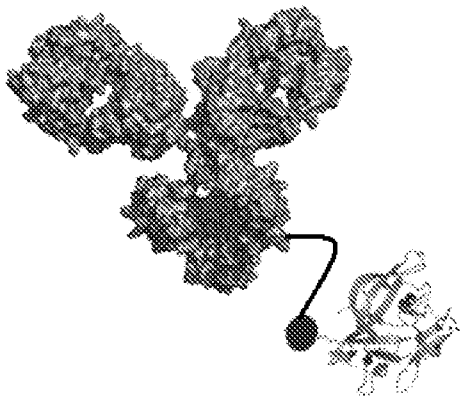


FIGURE 2

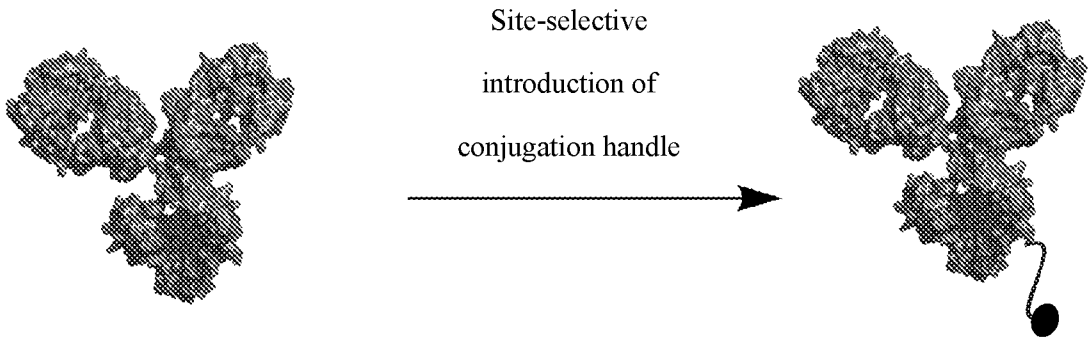


FIGURE 3

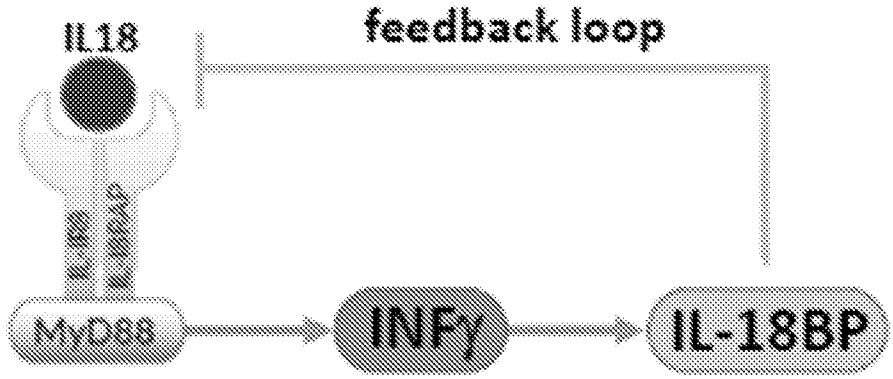


FIGURE 4

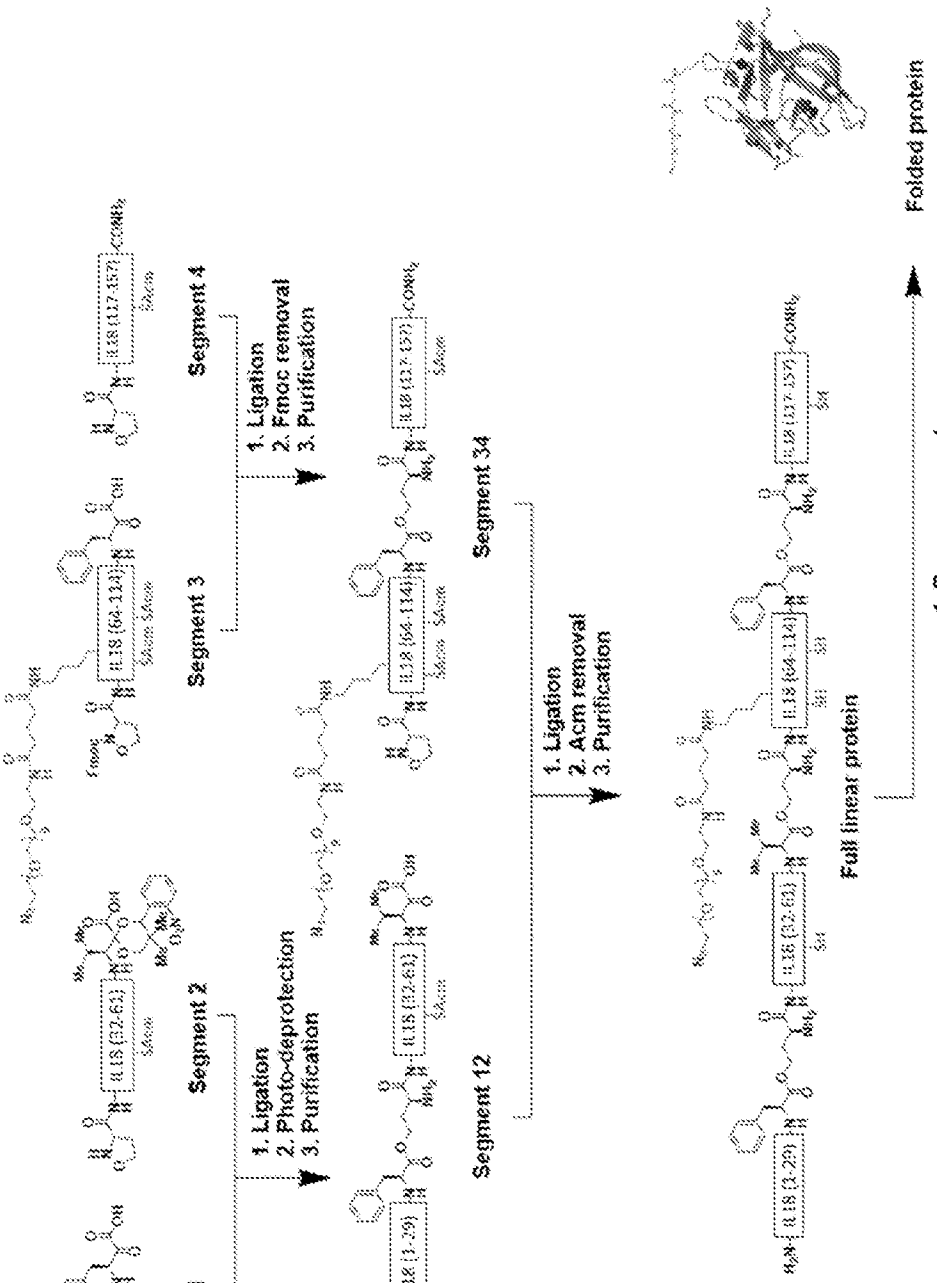

Segment 1
Segment 2
Segment 3
Segment 4
1. Ligation
2. Photo-deprotection
3. Purification
1. Ligation
2. Fmoc removal
3. Purification
Segment 12
Segment 34
1. Ligation
2. Acm removal
3. Purification
Full linear protein
1. Rearrangement
2. Folding
Folded protein

FIGURE 5

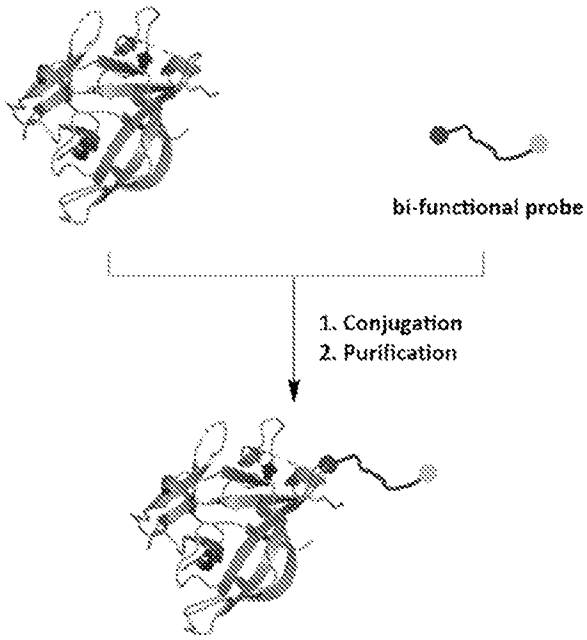


FIGURE 6

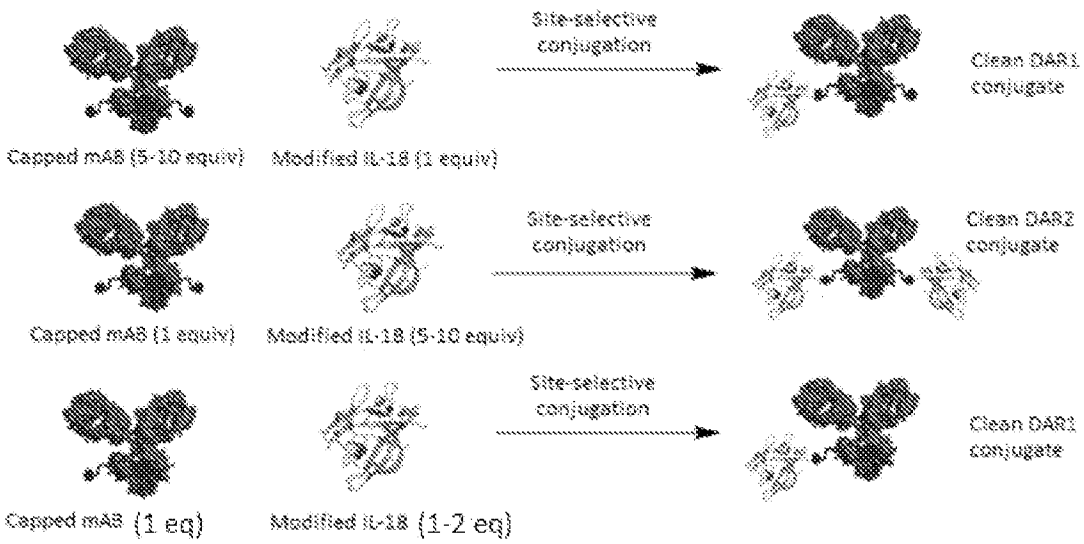


FIGURE 7

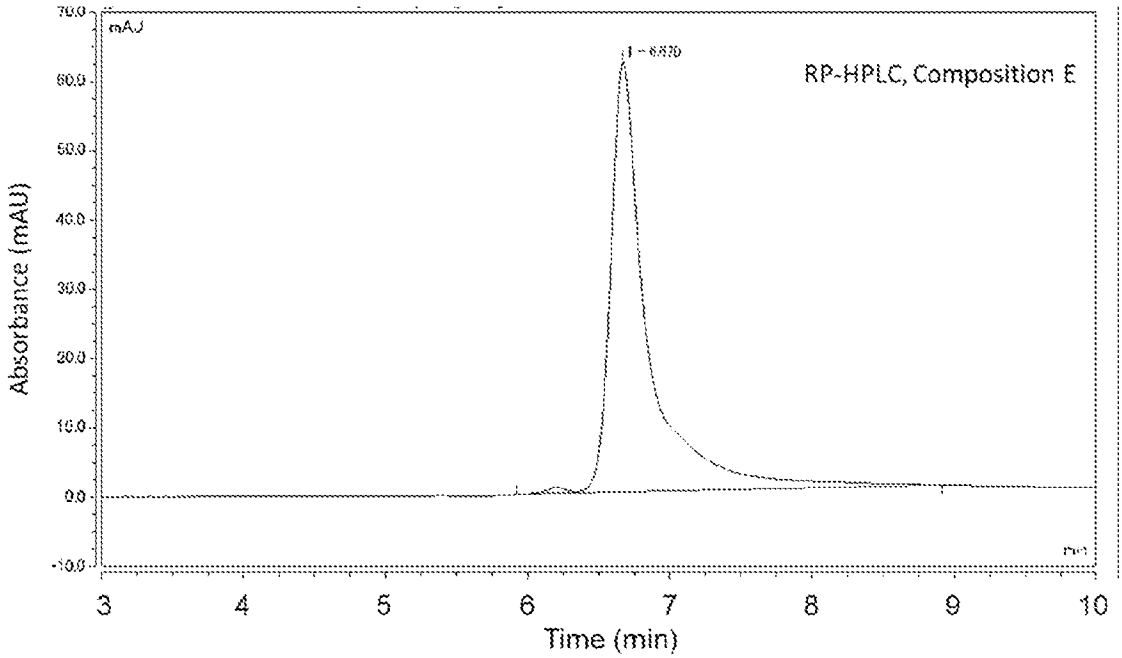


FIGURE 8

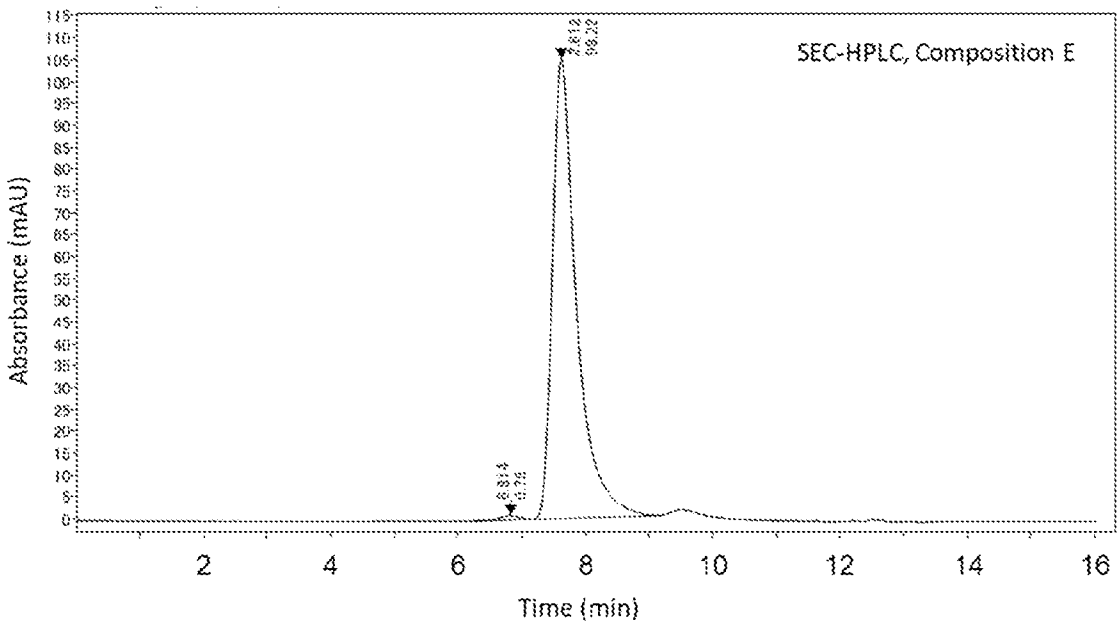


FIGURE 9

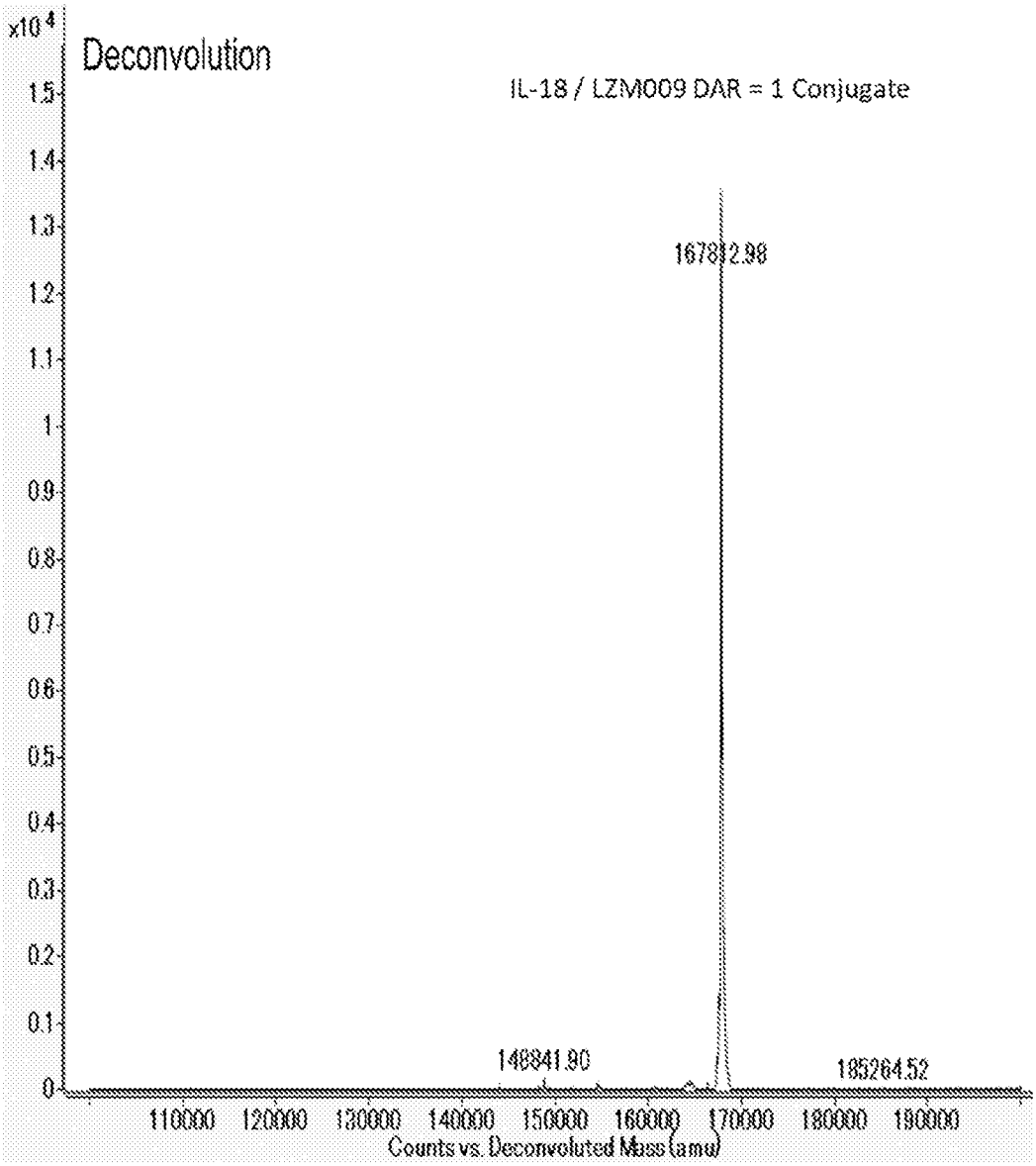

x10^4
Deconvolution
IL-18 / LZM009 DAR = 1 Conjugate
167812.98
148841.90
185264.52
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
110000 120000 130000 140000 150000 160000 170000 180000 190000
Counts vs. Deconvoluted Mass (amu)

FIGURE 10

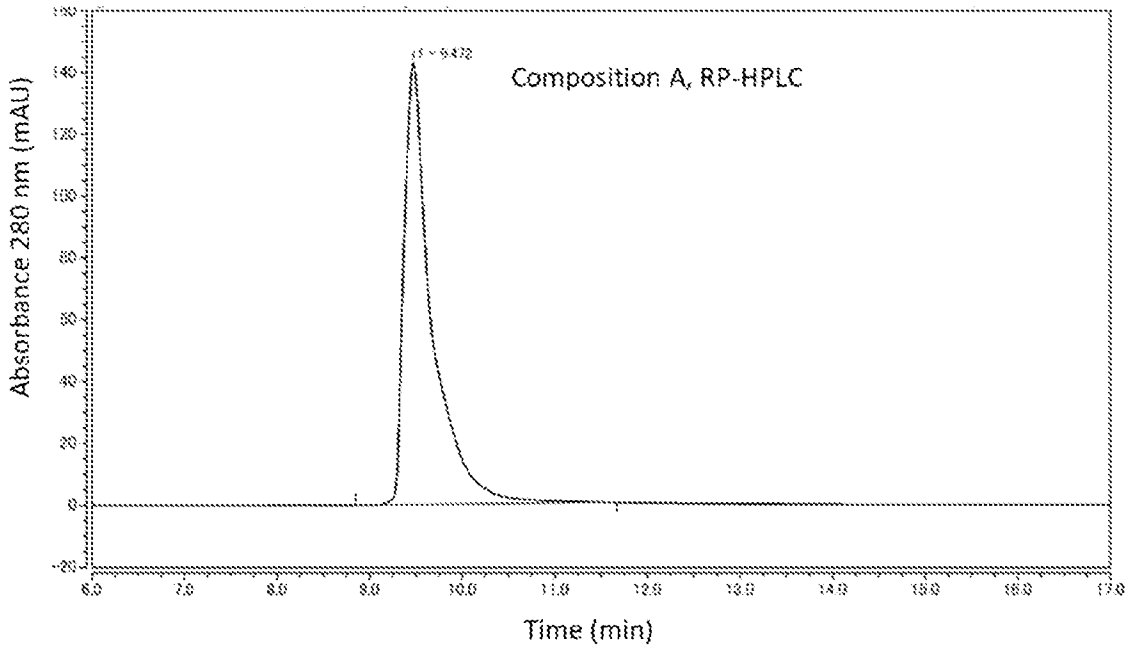


FIGURE 11

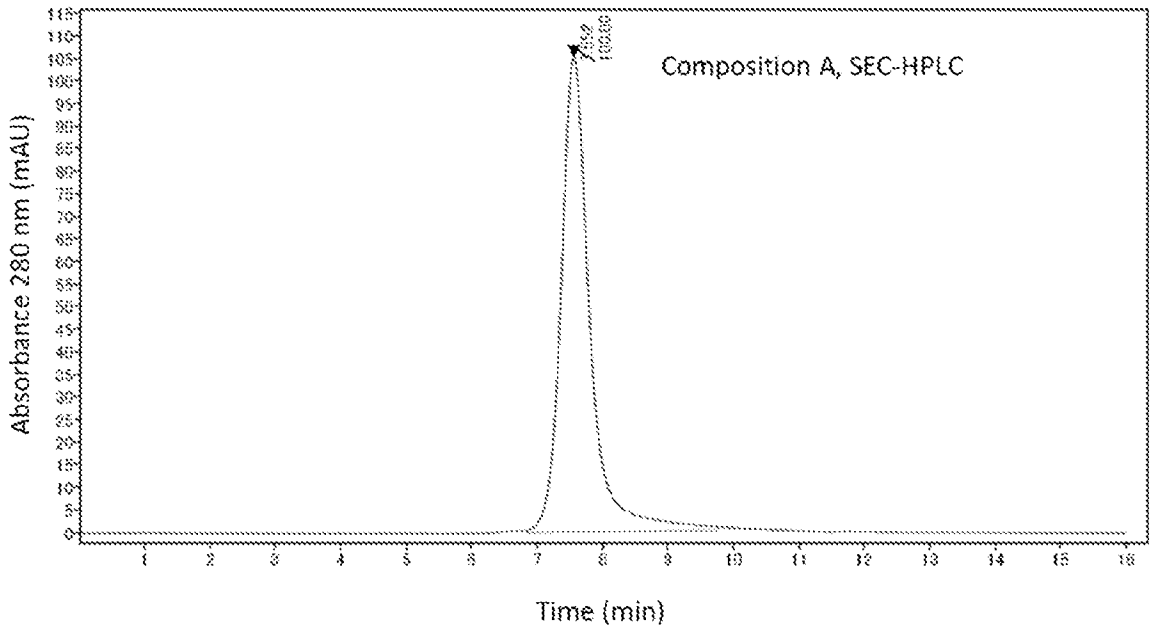


FIGURE 12

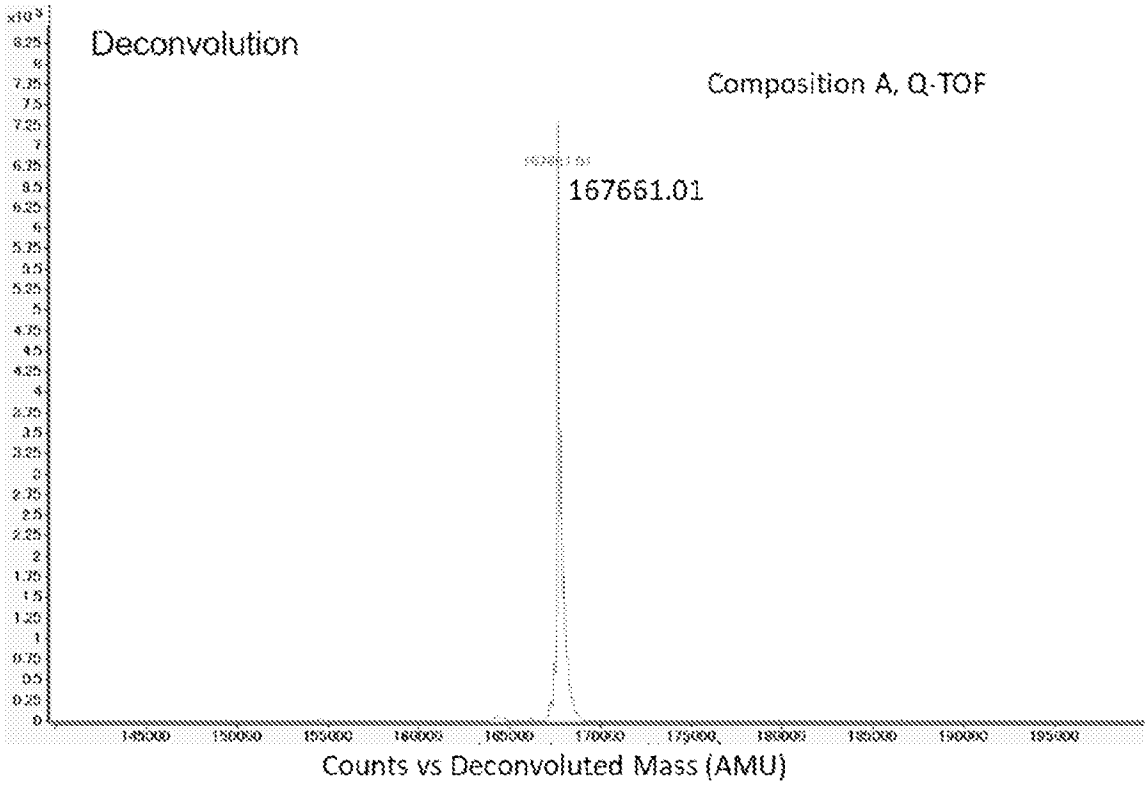

Deconvolution
Composition A, Q-TOF
167661.01
Counts vs Deconvoluted Mass (AMU)

FIGURE 13

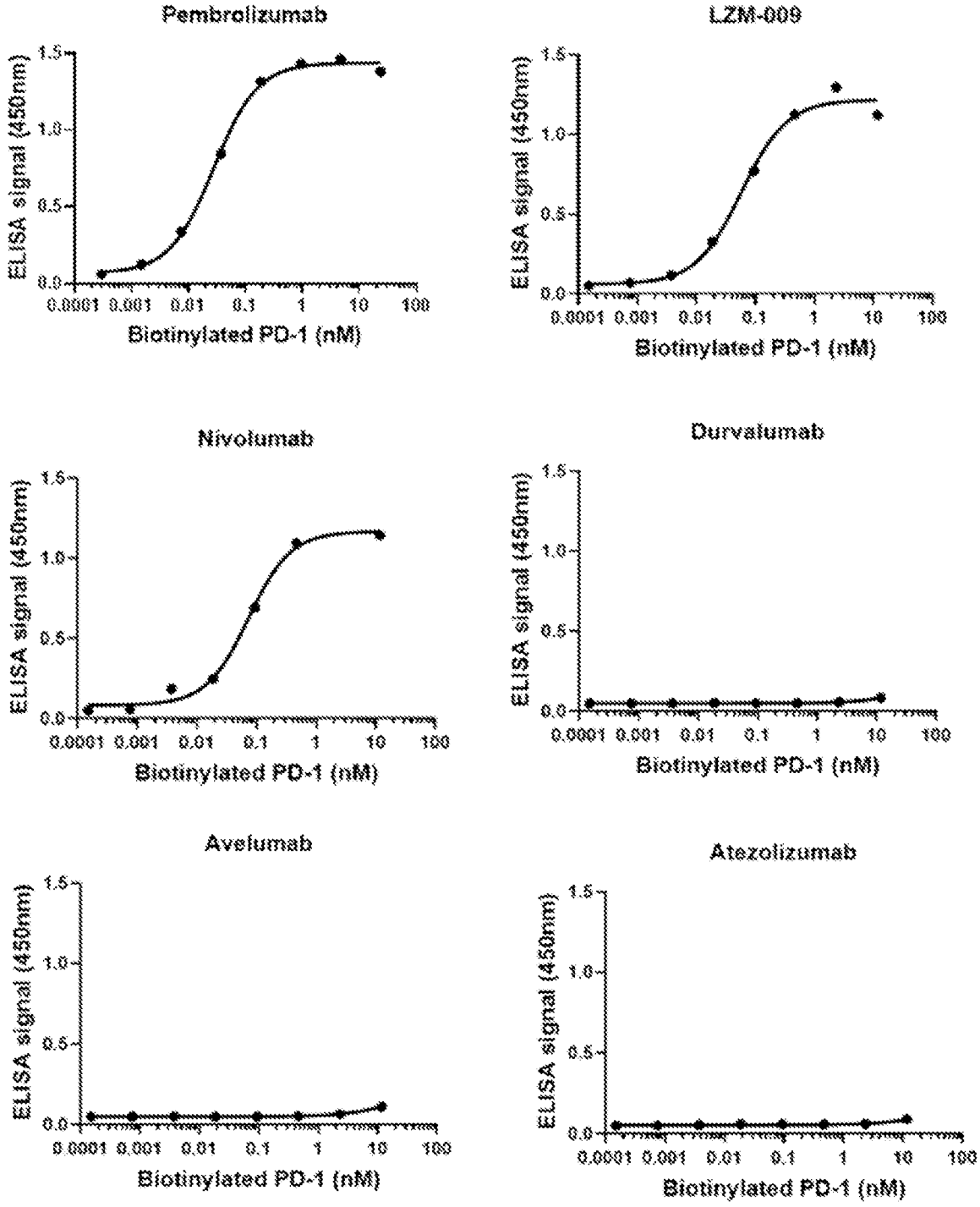
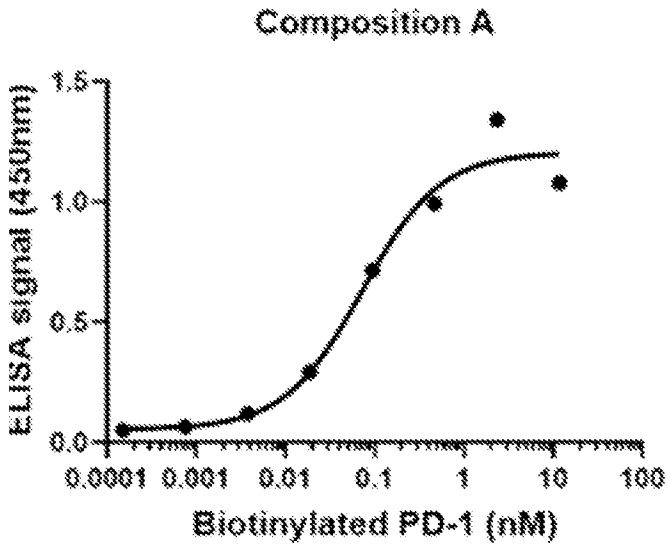

Pembrolizumab
LZM-009
Nivolumab
Durvalumab
Avelumab
Atezolizumab
ELISA signal (450nm)
Biotinylated PD-1 (nM)
0.0
0.5
1.0
1.5
0.0001
0.001
0.01
0.1
1
10
100

FIGURE 14A


Composition A
ELISA signal (450nm)
1.5
1.0
0.5
0.0
0.0001 0.001 0.01 0.1 1 10 100
Biotinylated PD-1 (nM)

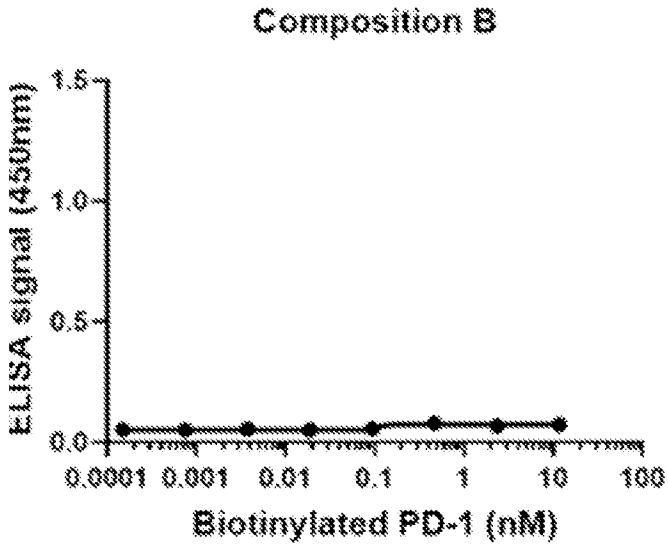

Composition B
ELISA signal (450nm)
1.5
1.0
0.5
0.0
0.0001 0.001 0.01 0.1 1 10 100
Biotinylated PD-1 (nM)

FIGURE 14B

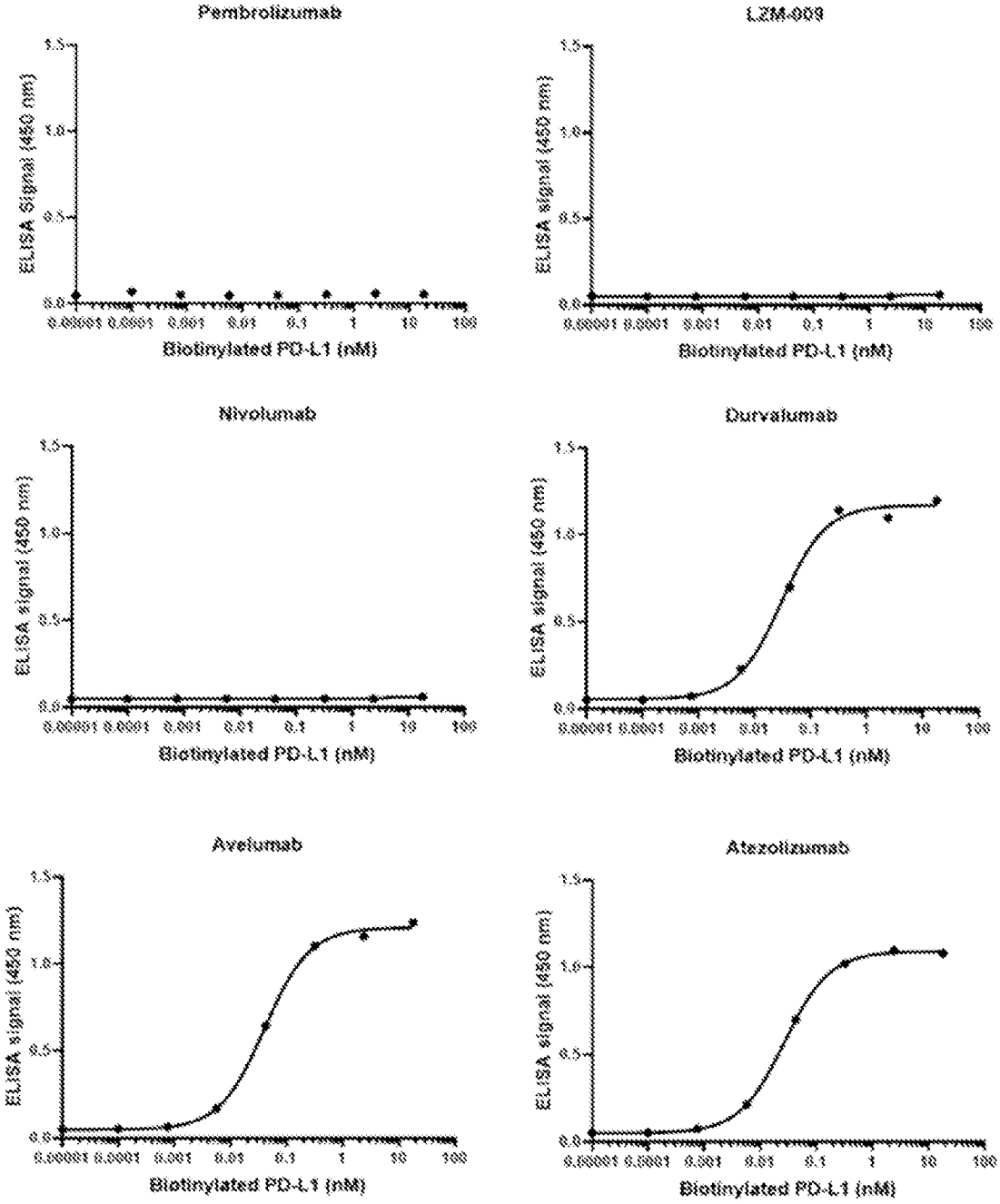
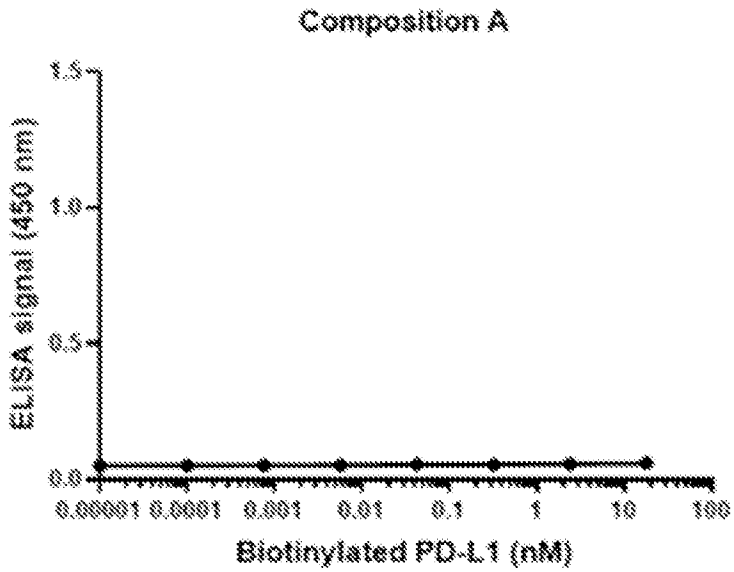

Pembrolizumab
LZM-009
Nivolumab
Durvalumab
Avelumab
Atezolizumab
ELISA signal (450 nm)
Biotinylated PD-L1 (nM)
0.0
0.5
1.0
1.5
0.00001
0.0001
0.001
0.01
0.1
1
10
100

FIGURE 15A


Composition A
ELISA signal (450 nm)
1.5
1.0
0.5
0.0
0.00001 0.0001 0.001 0.01 0.1 1 10 100
Biotinylated PD-L1 (nM)

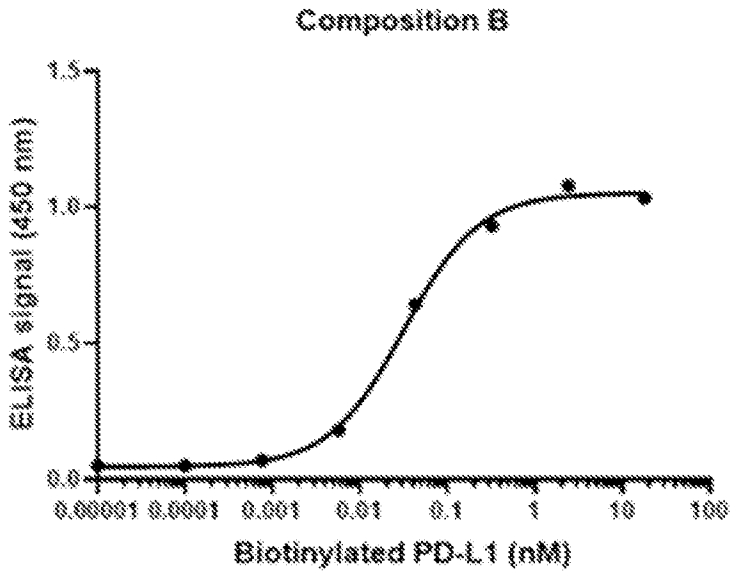
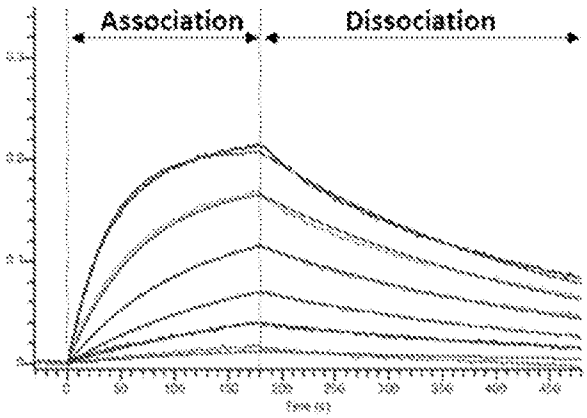
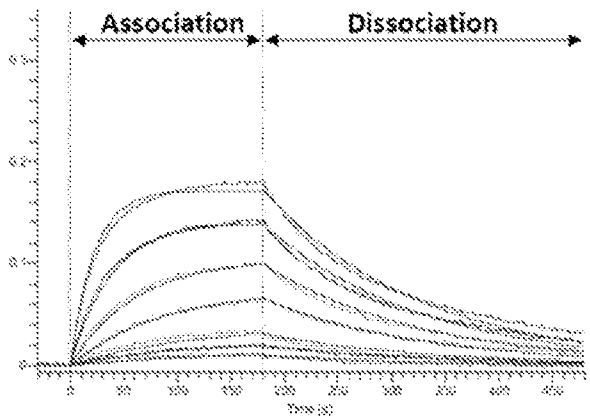

Composition B
ELISA signal (450 nm)
1.5
1.0
0.5
0.0
0.00001 0.0001 0.001 0.01 0.1 1 10 100
Biotinylated PD-L1 (nM)

FIGURE 15B

Pembrolizumab/Keytruda



LZM-009



Composition A

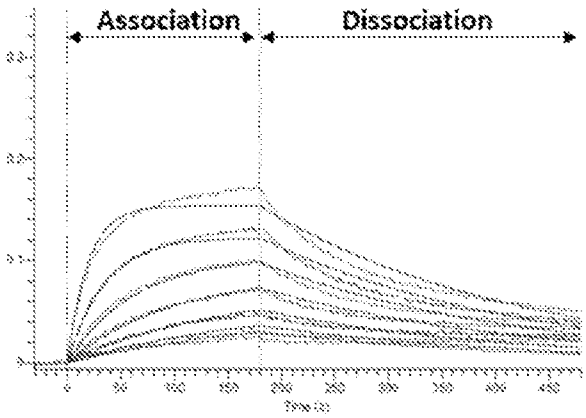


FIGURE 16

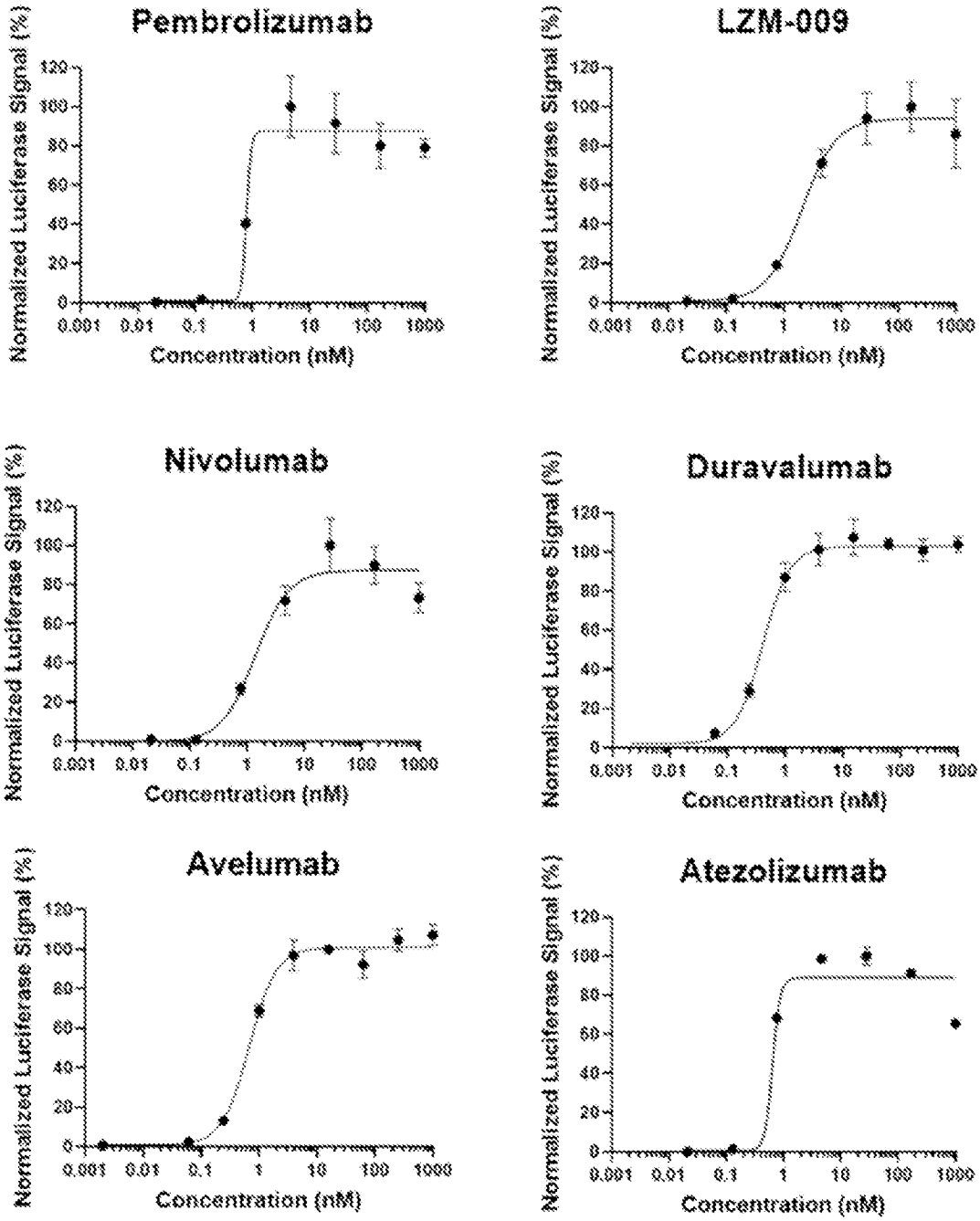
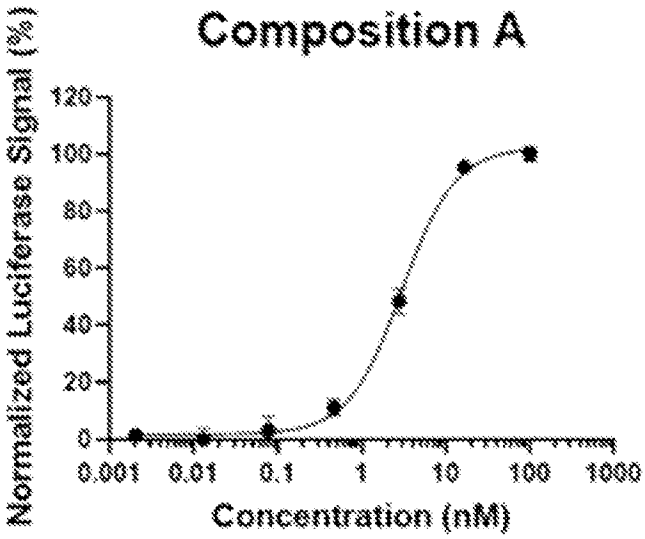

Pembrolizumab
LZM-009
Nivolumab
Duravalumab
Avelumab
Atezolizumab
Normalized Luciferase Signal (%)
Concentration (nM)
0.001
0.01
0.1
1
10
100
1000
0
20
40
60
80
100
120

FIGURE 17A


Composition A
Normalized Luciferase Signal (%)
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

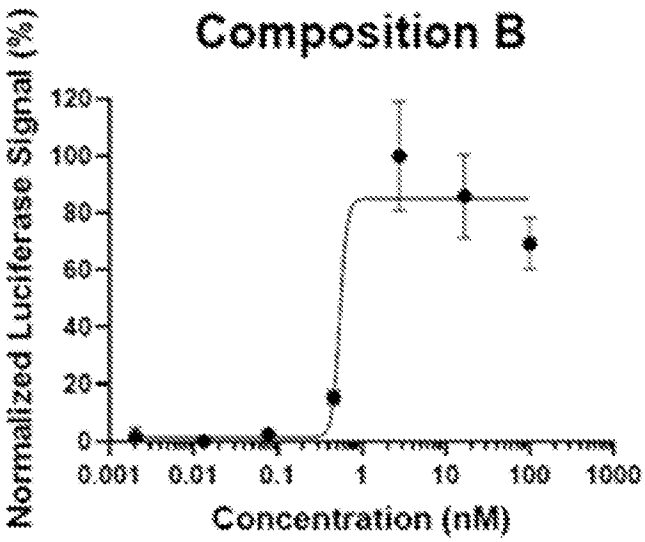

Composition B
Normalized Luciferase Signal (%)
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIGURE 17B

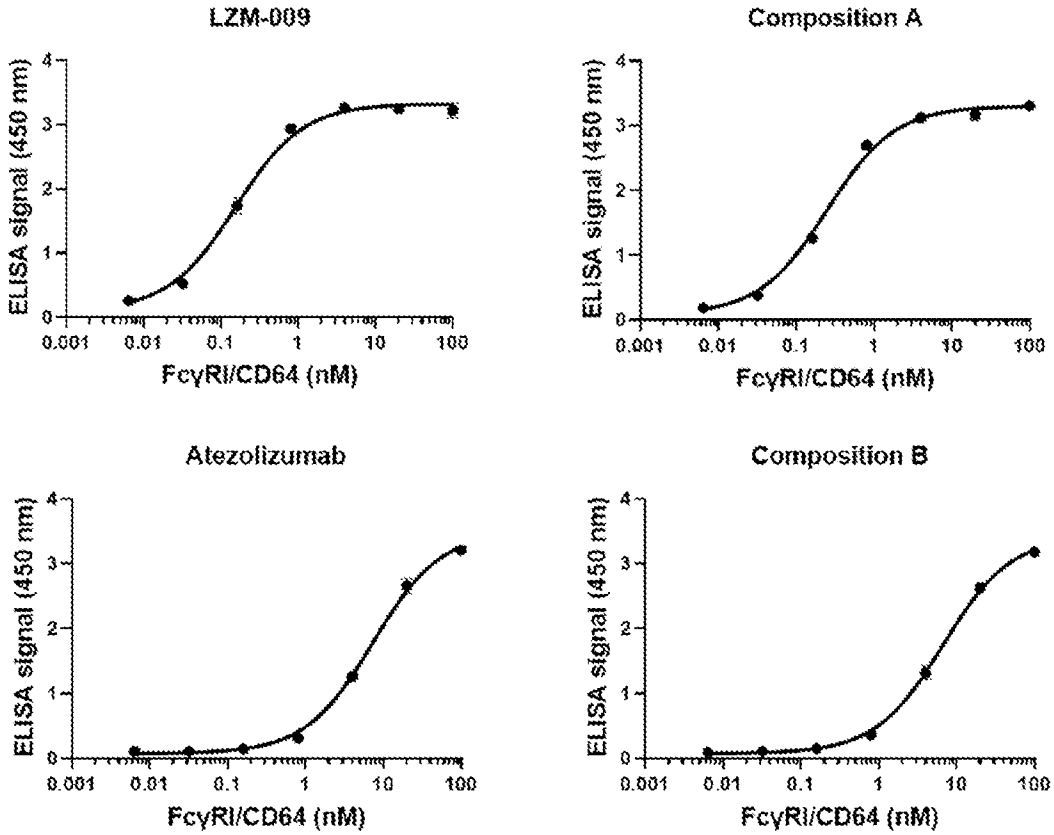

LZM-009
Composition A
Atezolizumab
Composition B
ELISA signal (450 nm)
FcγRI/CD64 (nM)
0
1
2
3
4
0.001
0.01
0.1
1
10
100

FIGURE 18A

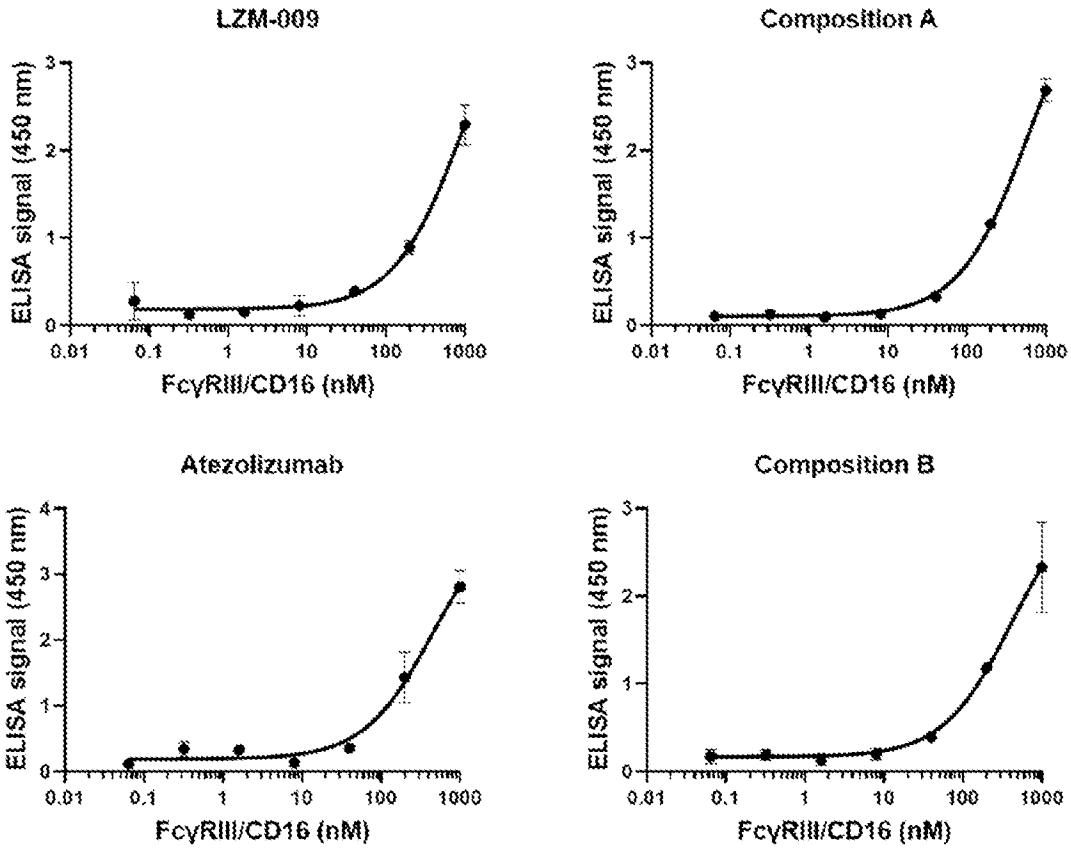

LZM-009
Composition A
Atezolizumab
Composition B
ELISA signal (450 nm)
FcγRIII/CD16 (nM)
0.01
0.1
1
10
100
1000

FIGURE 18B

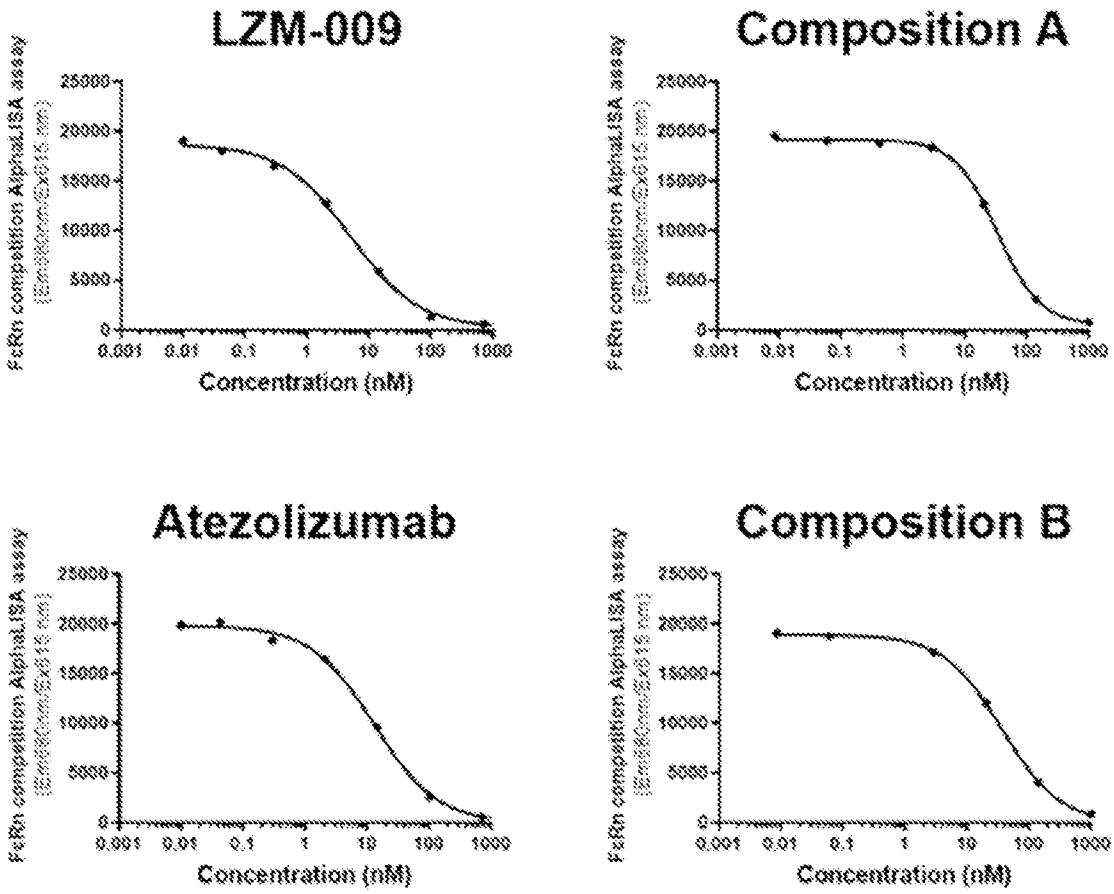

LZM-009
Composition A
Atezolizumab
Composition B
FcRn competition AlphaLISA assay
Concentration (nM)
0.001
0.01
0.1
1
10
100
1000
0
5000
10000
15000
20000
25000

FIGURE 19

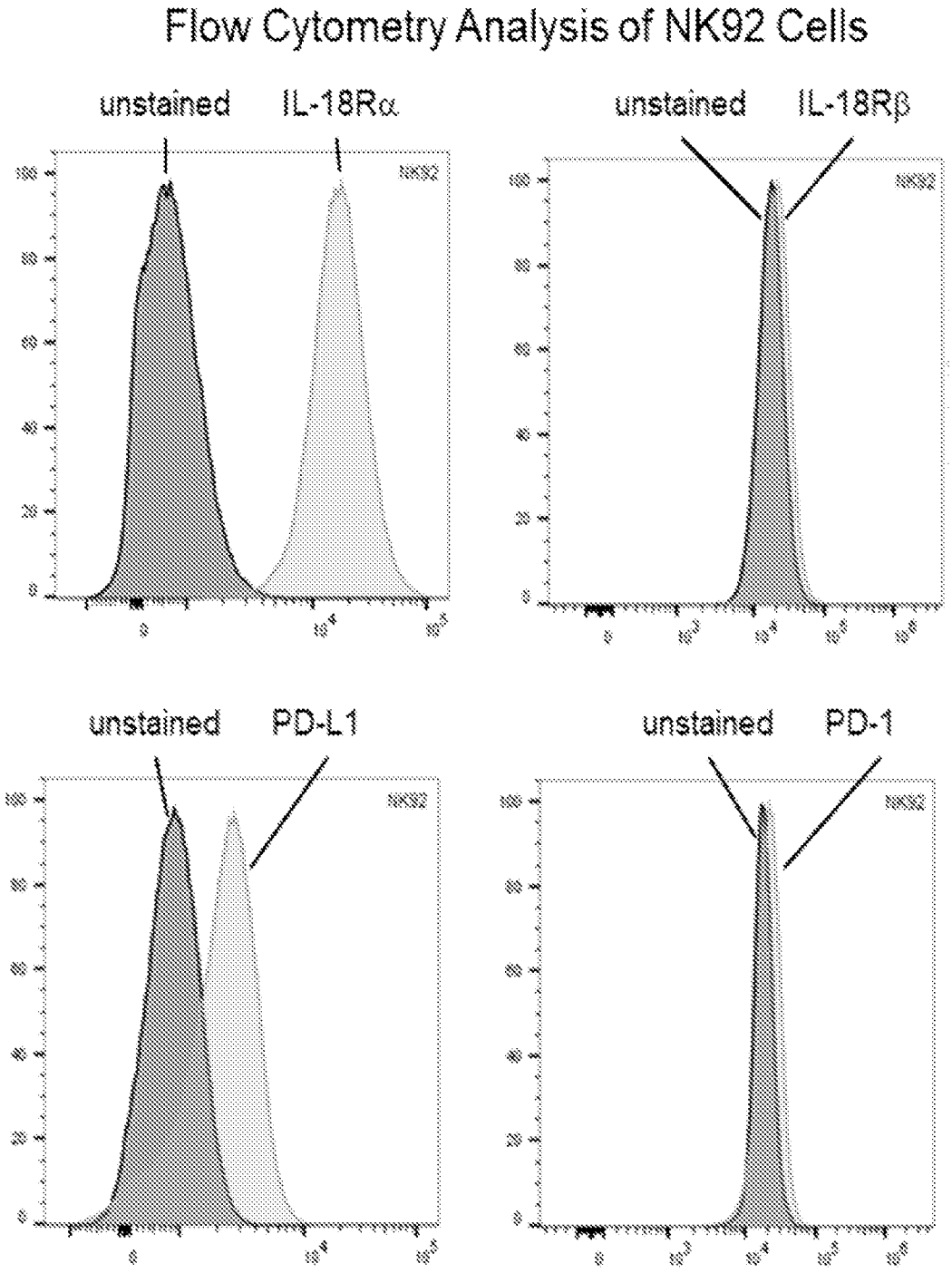

Flow Cytometry Analysis of NK92 Cells
unstained
IL-18Rα
NK92
unstained
IL-18Rβ
NK92
unstained
PD-L1
NK92
unstained
PD-1
NK92

FIGURE 20

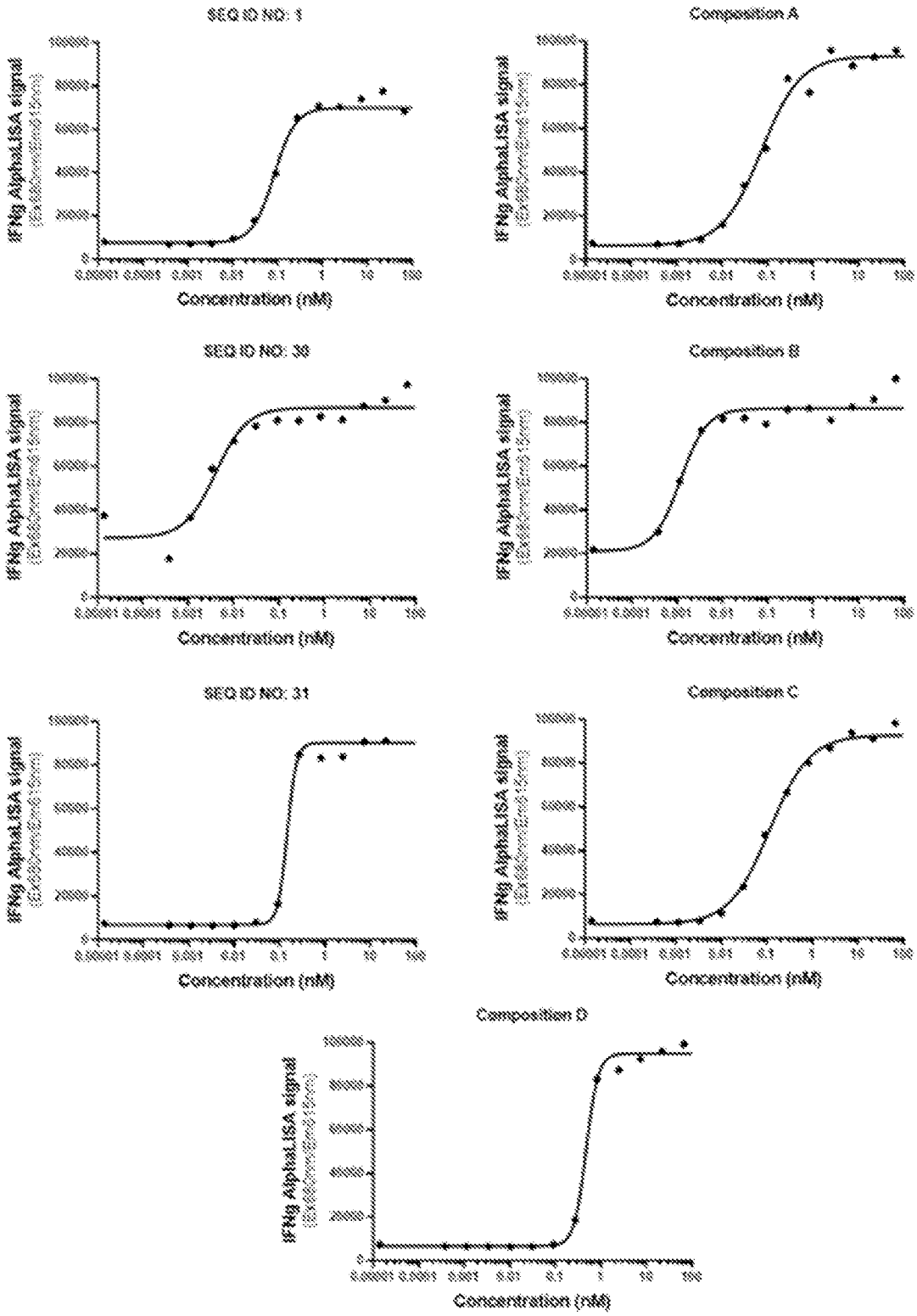

SEQ ID NO: 1
Composition A
SEQ ID NO: 39
Composition B
SEQ ID NO: 31
Composition C
Composition D
IFNg AlphaLISA signal
Concentration (nM)

FIGURE 21

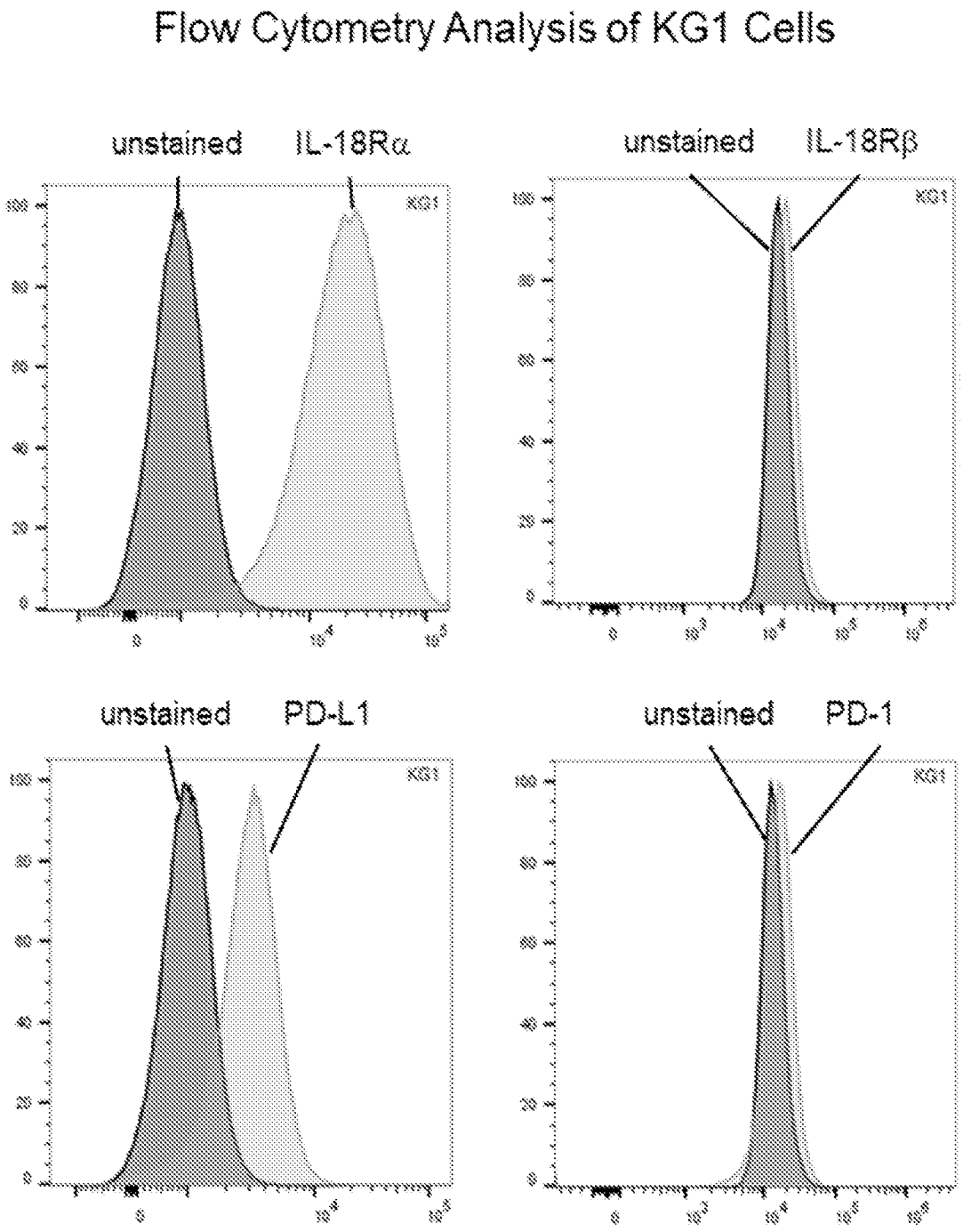

Flow Cytometry Analysis of KG1 Cells
unstained
IL-18Rα
KG1
unstained
IL-18Rβ
KG1
unstained
PD-L1
KG1
unstained
PD-1
KG1

FIGURE 22

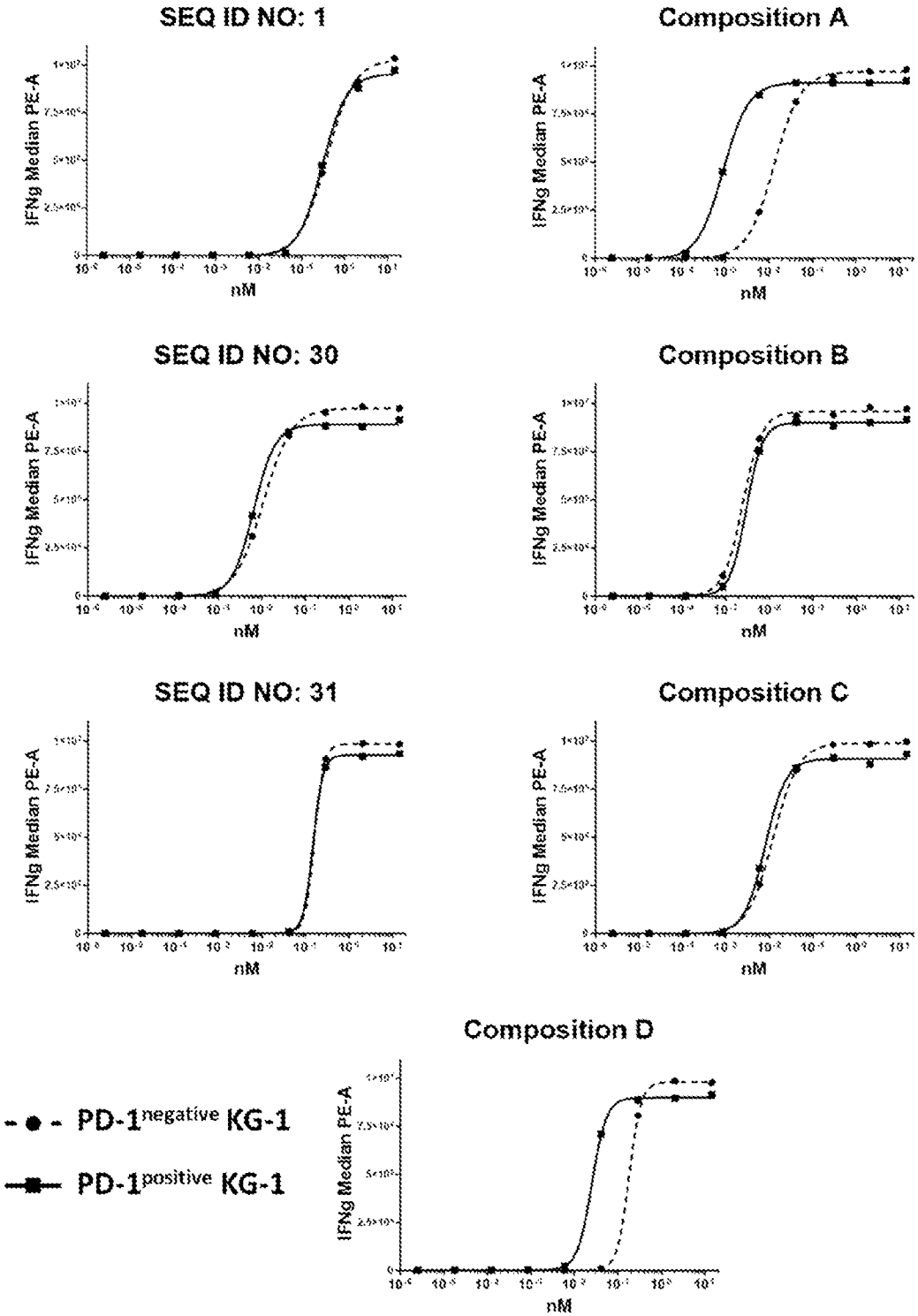

SEQ ID NO: 1
Composition A
SEQ ID NO: 30
Composition B
SEQ ID NO: 31
Composition C
Composition D
IFNg Median PE-A
nM
PD-1negative KG-1
PD-1positive KG-1

FIGURE 23

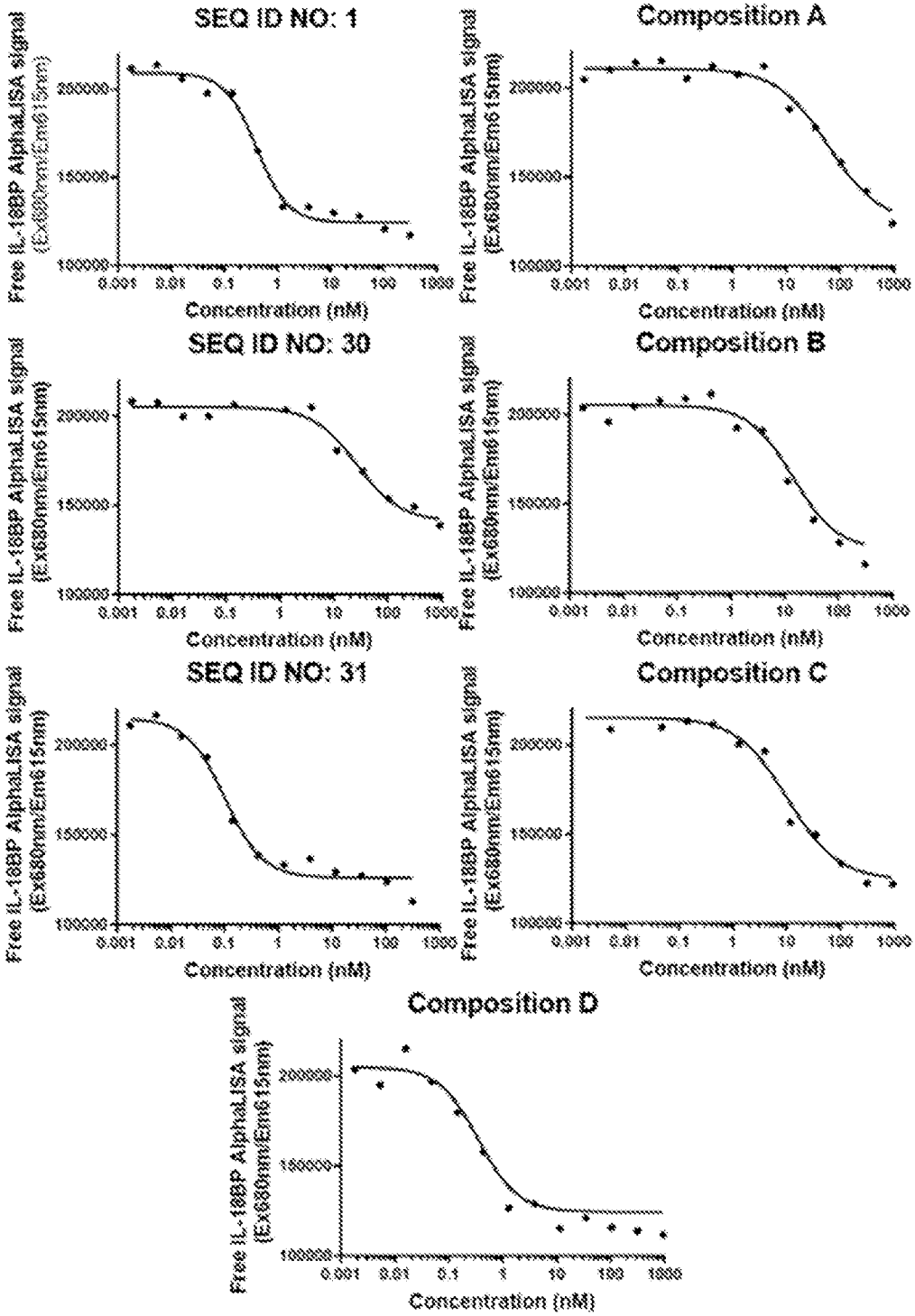

SEQ ID NO: 1
Composition A
SEQ ID NO: 30
Composition B
SEQ ID NO: 31
Composition C
Composition D
Free IL-18BP AlphaLISA signal
(Ex680nm/Em615nm)
Concentration (nM)

FIGURE 24

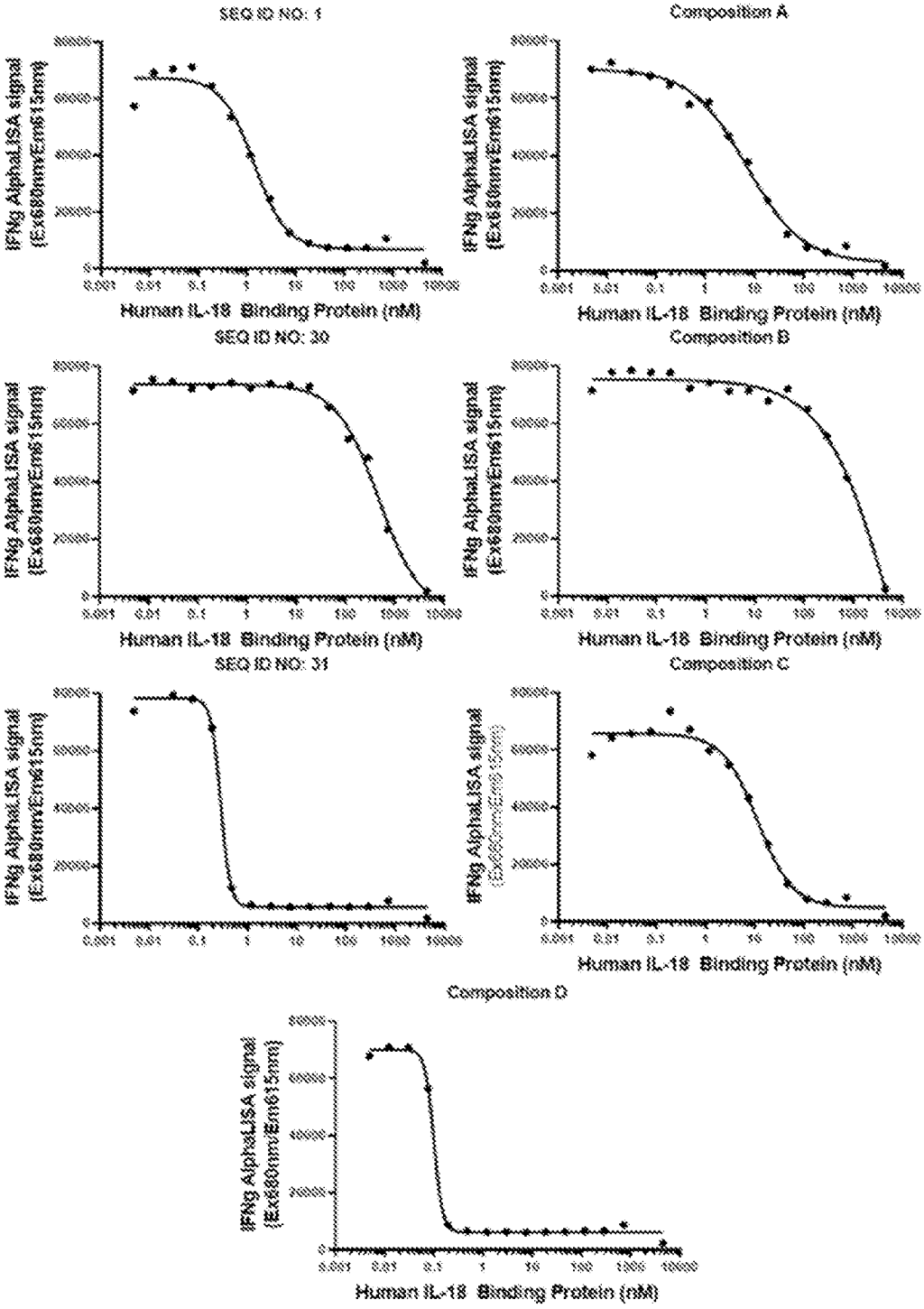

SEQ ID NO: 1
Composition A
SEQ ID NO: 30
Composition B
SEQ ID NO: 31
Composition C
Composition D
IFNg AlphaLISA signal
(Ex680nm/Em615nm)
Human IL-18 Binding Protein (nM)

FIGURE 25

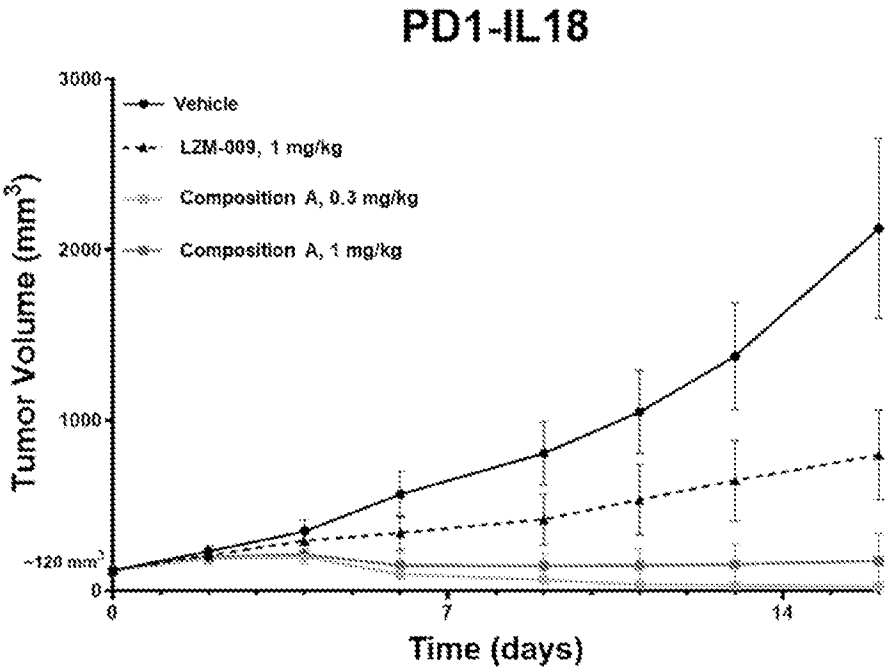


FIGURE 26A

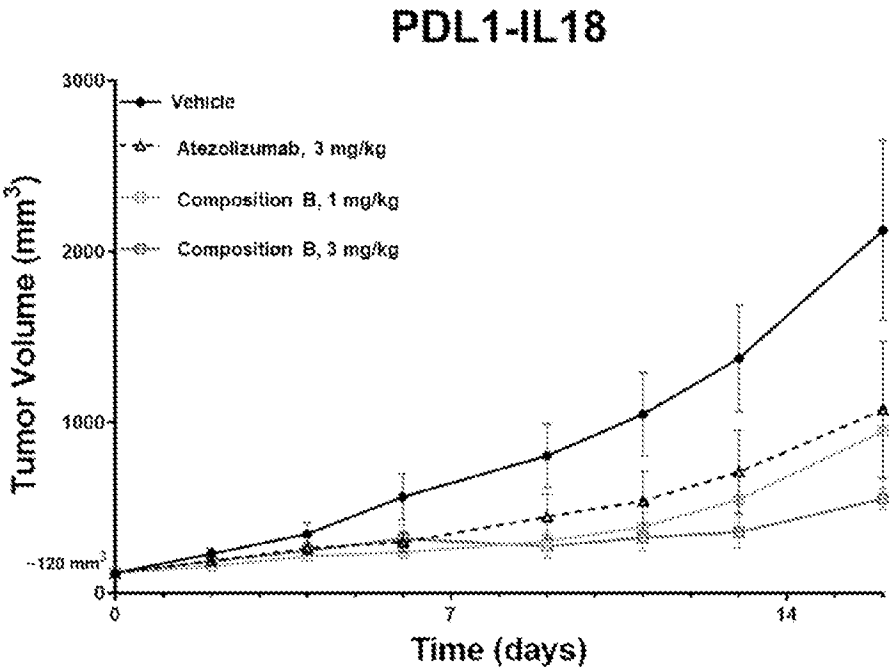


FIGURE 26B

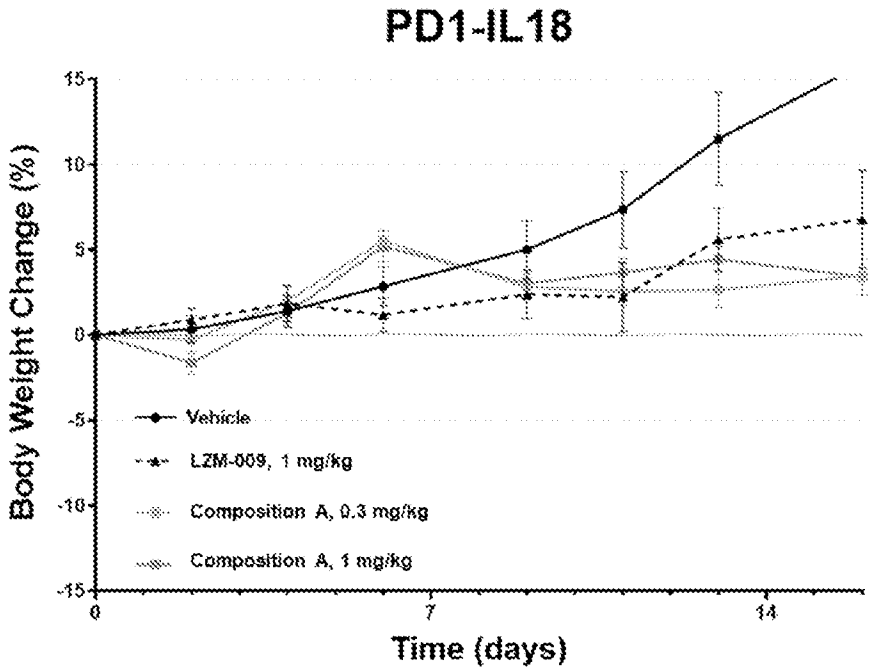


FIGURE 27A

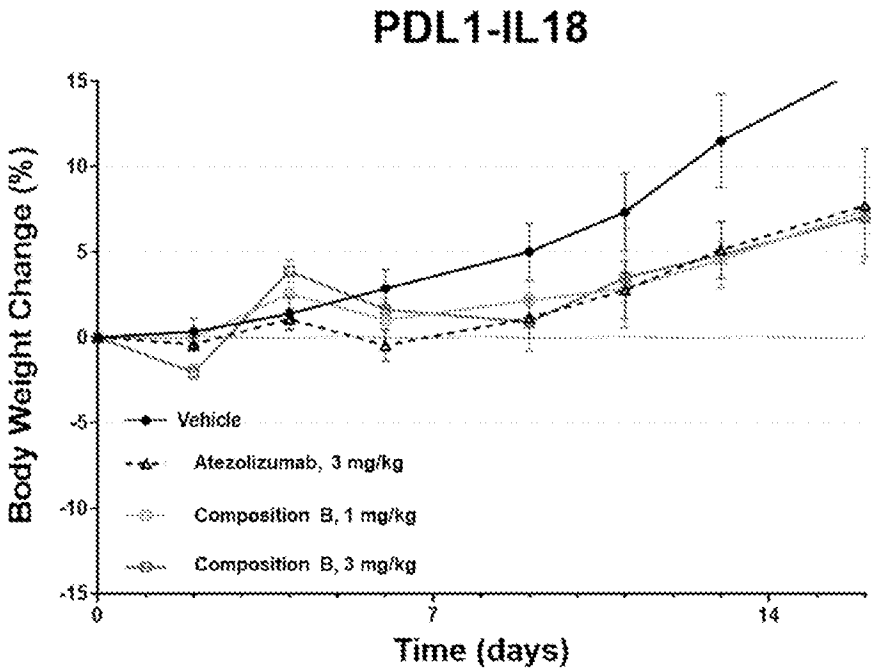


FIGURE 27B

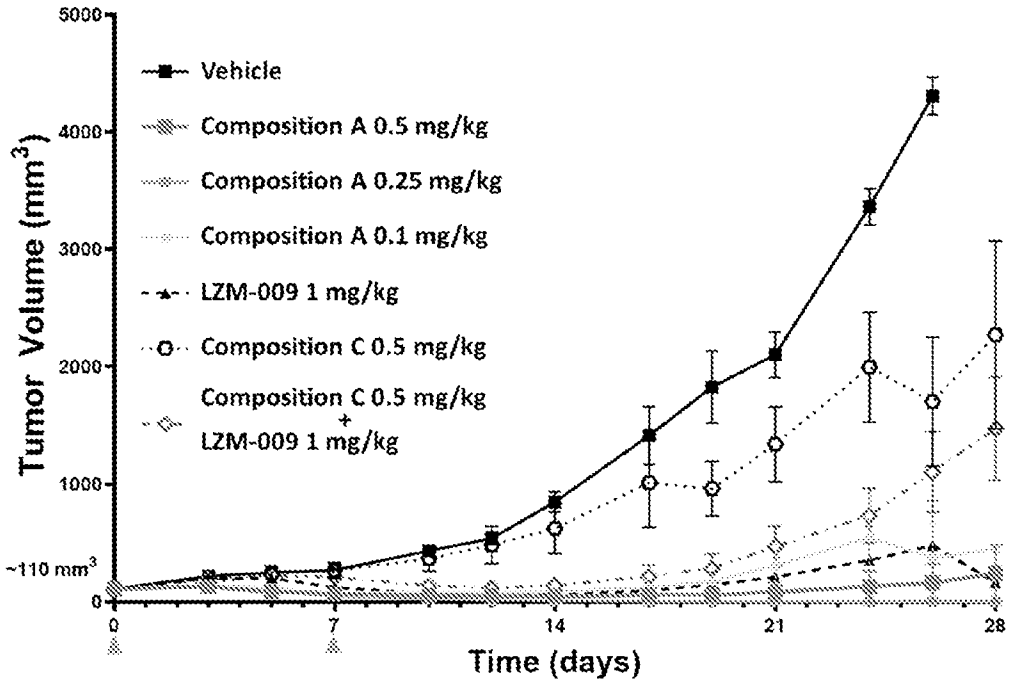


FIGURE 28A

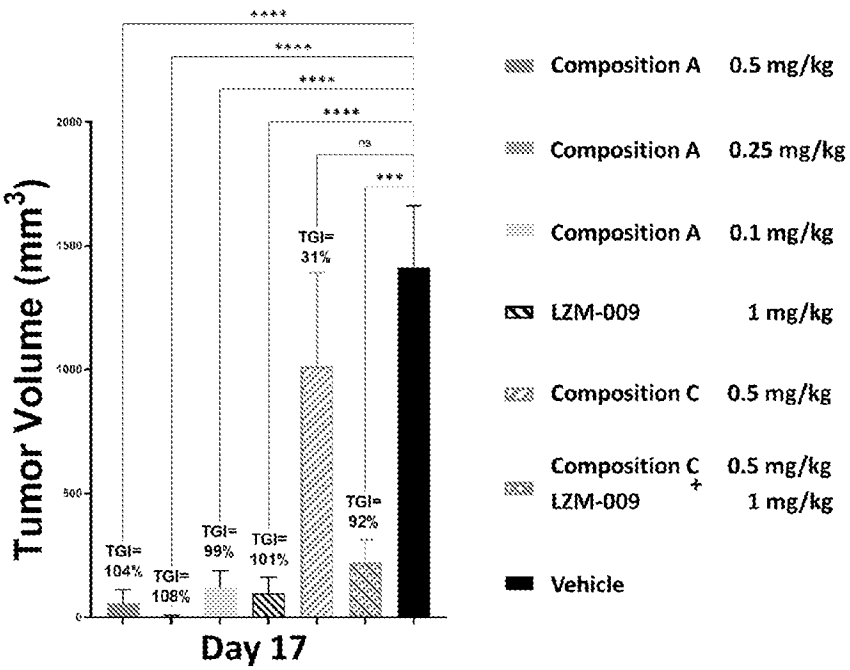


FIGURE 28B

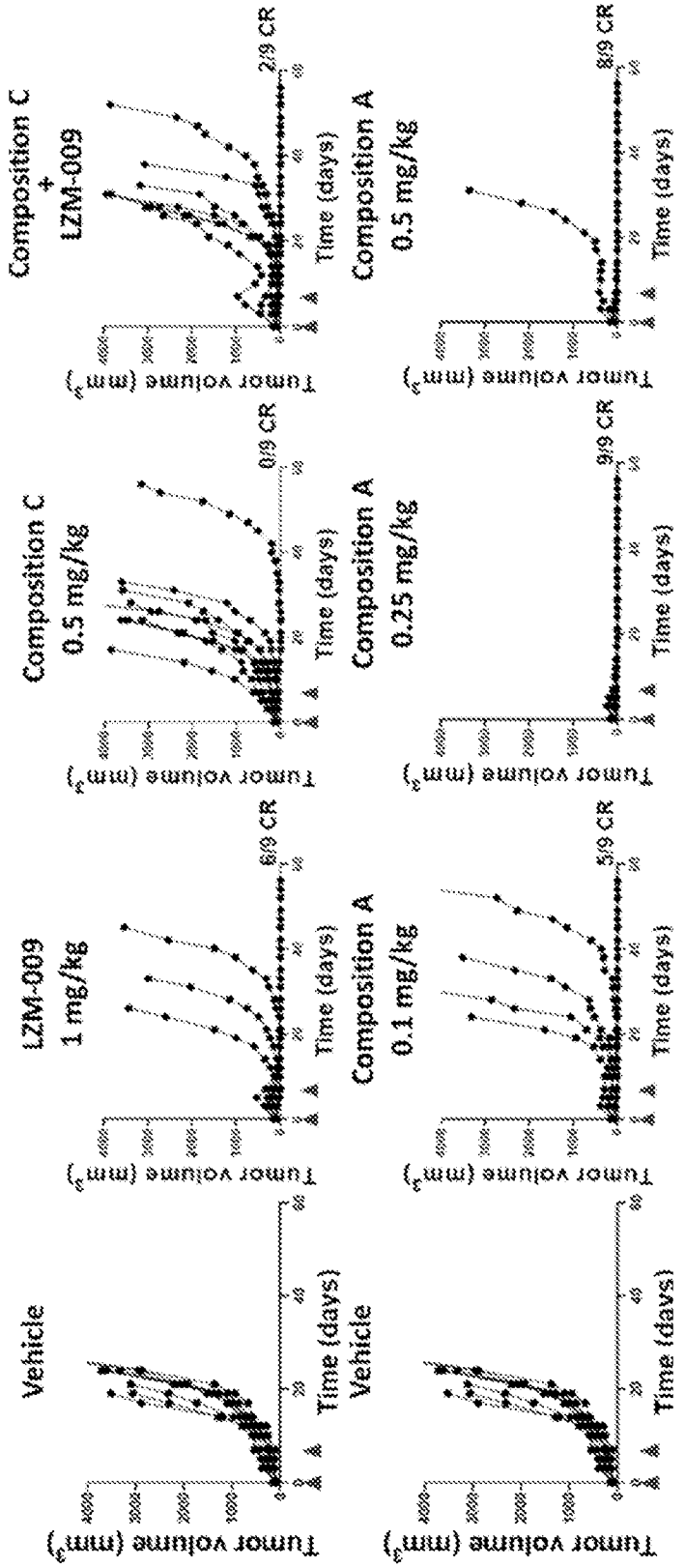

Vehicle
LZM-009
1 mg/kg
Composition C
0.5 mg/kg
Composition C
+
LZM-009
Composition A
0.1 mg/kg
Composition A
0.25 mg/kg
Composition A
0.5 mg/kg
Tumor volume (mm³)
Time (days)
6/9 CR
0/9 CR
2/9 CR
5/9 CR
9/9 CR
8/9 CR

FIGURE 28C

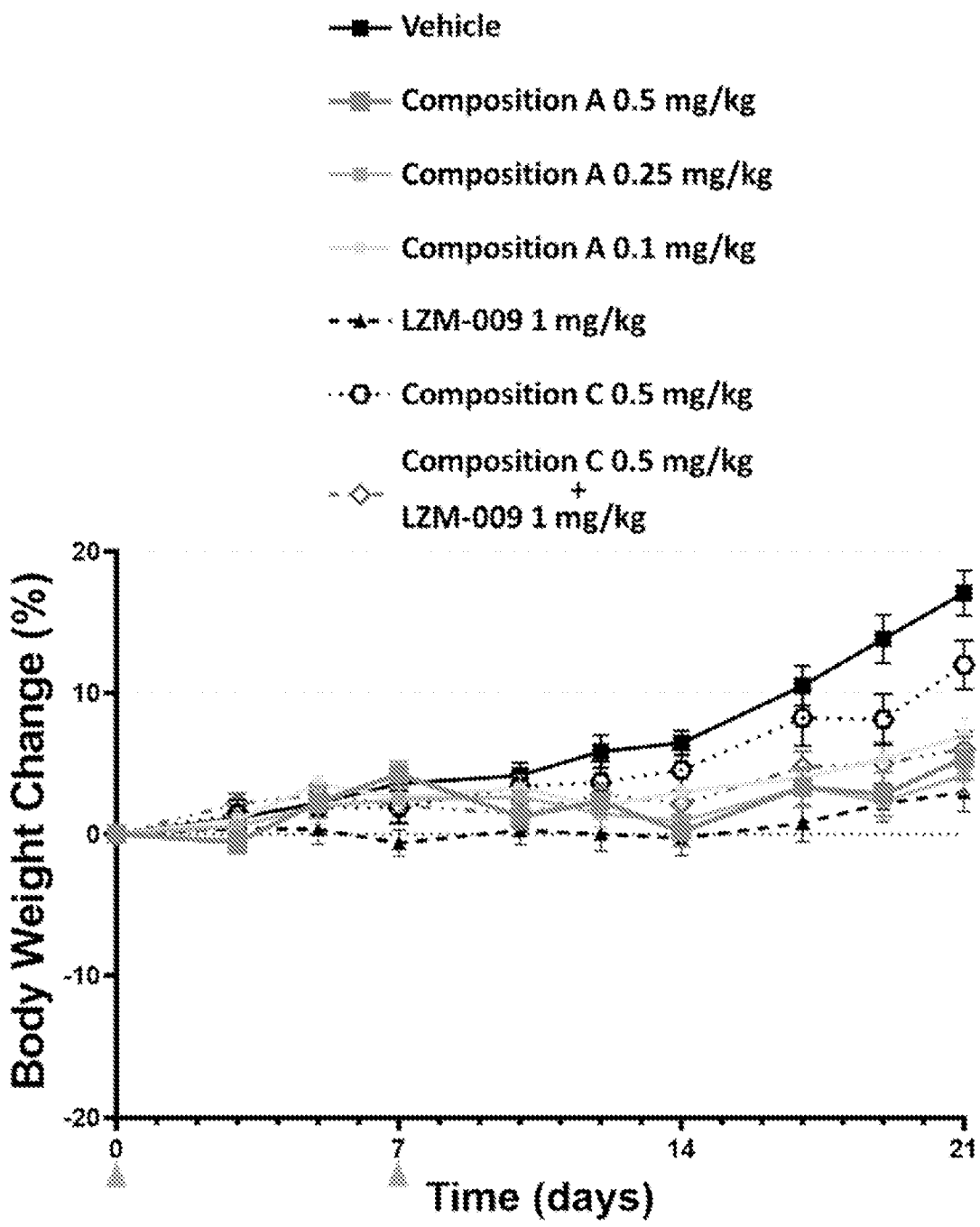

Vehicle
Composition A 0.5 mg/kg
Composition A 0.25 mg/kg
Composition A 0.1 mg/kg
LZM-009 1 mg/kg
Composition C 0.5 mg/kg
Composition C 0.5 mg/kg + LZM-009 1 mg/kg
Body Weight Change (%)
20
10
0
-10
-20
0
7
14
21
Time (days)

FIGURE 29

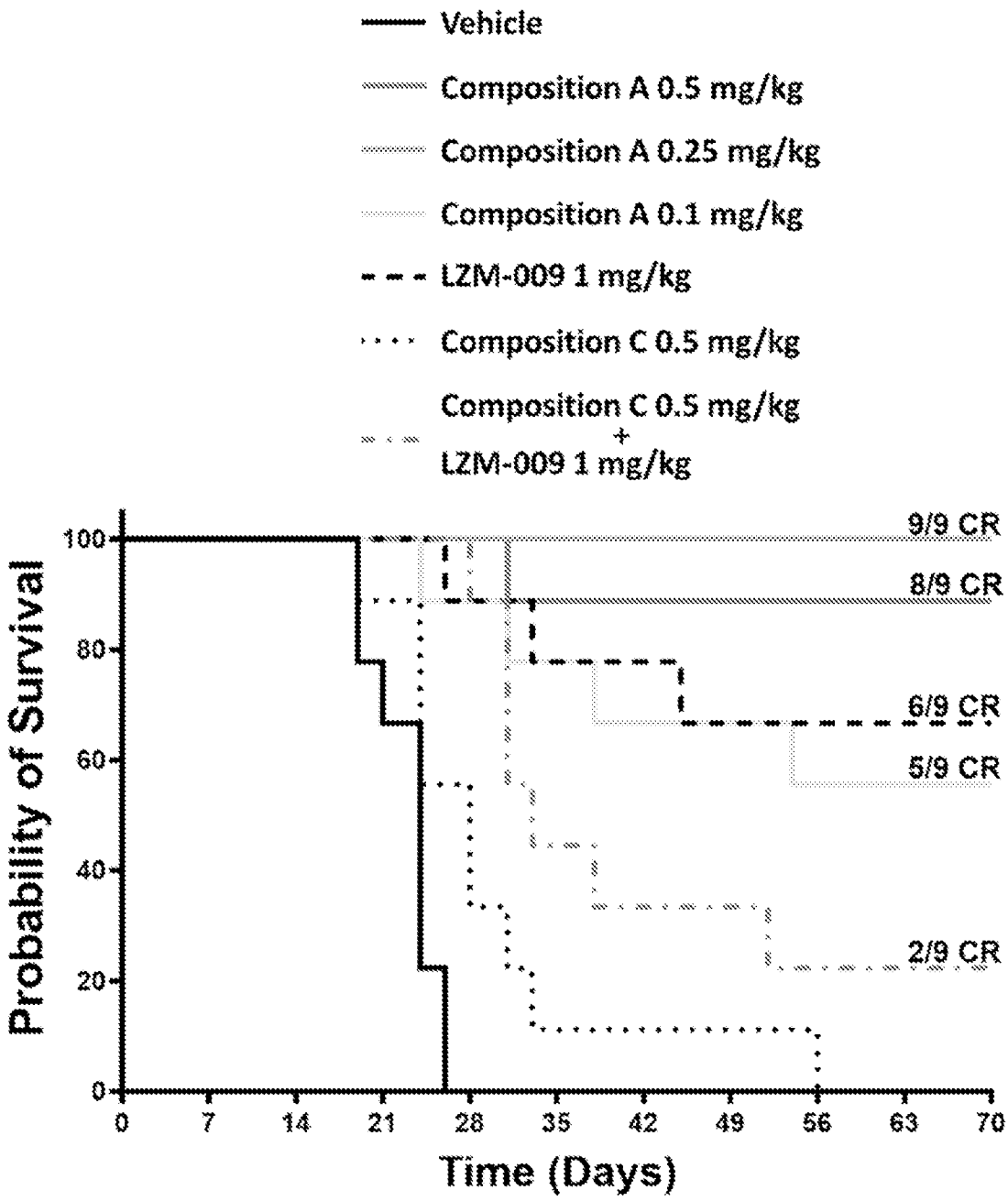

Vehicle
Composition A 0.5 mg/kg
Composition A 0.25 mg/kg
Composition A 0.1 mg/kg
LZM-009 1 mg/kg
Composition C 0.5 mg/kg
Composition C 0.5 mg/kg + LZM-009 1 mg/kg
9/9 CR
8/9 CR
6/9 CR
5/9 CR
2/9 CR
Probability of Survival
100
80
60
40
20
0
0
7
14
21
28
35
42
49
56
63
70
Time (Days)

FIGURE 30

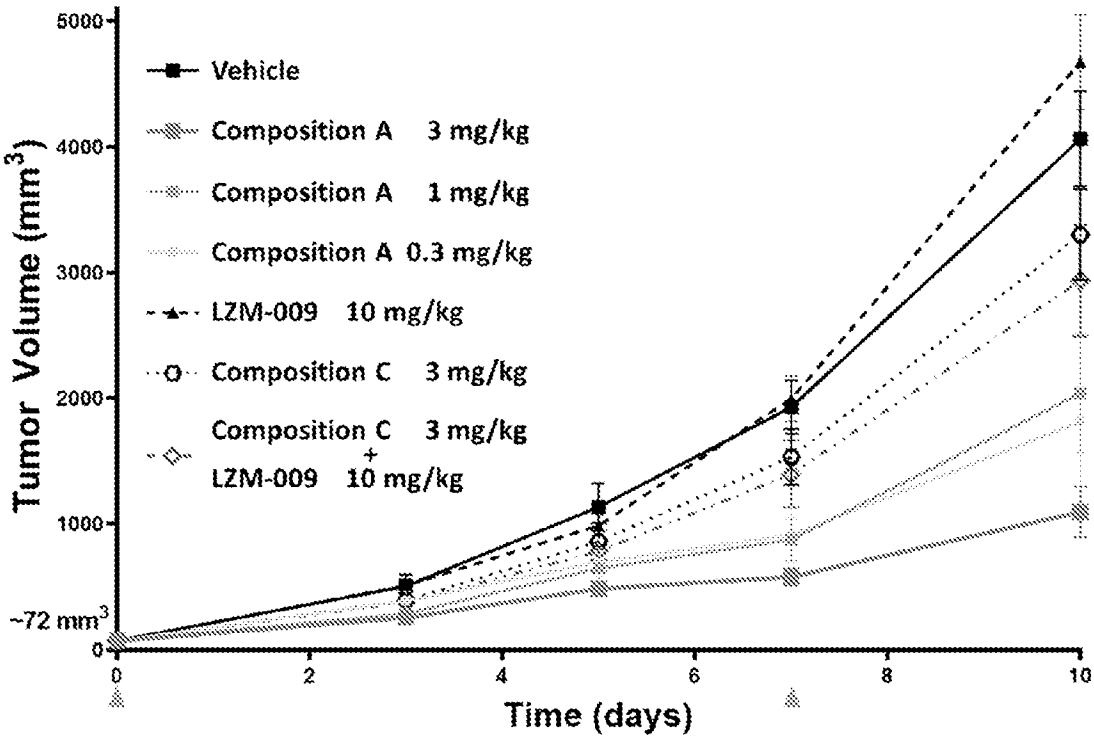

Tumor Volume (mm3)
5000
4000
3000
2000
1000
~72 mm3
0
0
2
4
6
8
10
Time (days)
Vehicle
Composition A 3 mg/kg
Composition A 1 mg/kg
Composition A 0.3 mg/kg
LZM-009 10 mg/kg
Composition C 3 mg/kg
Composition C 3 mg/kg
+
LZM-009 10 mg/kg

FIGURE 31A

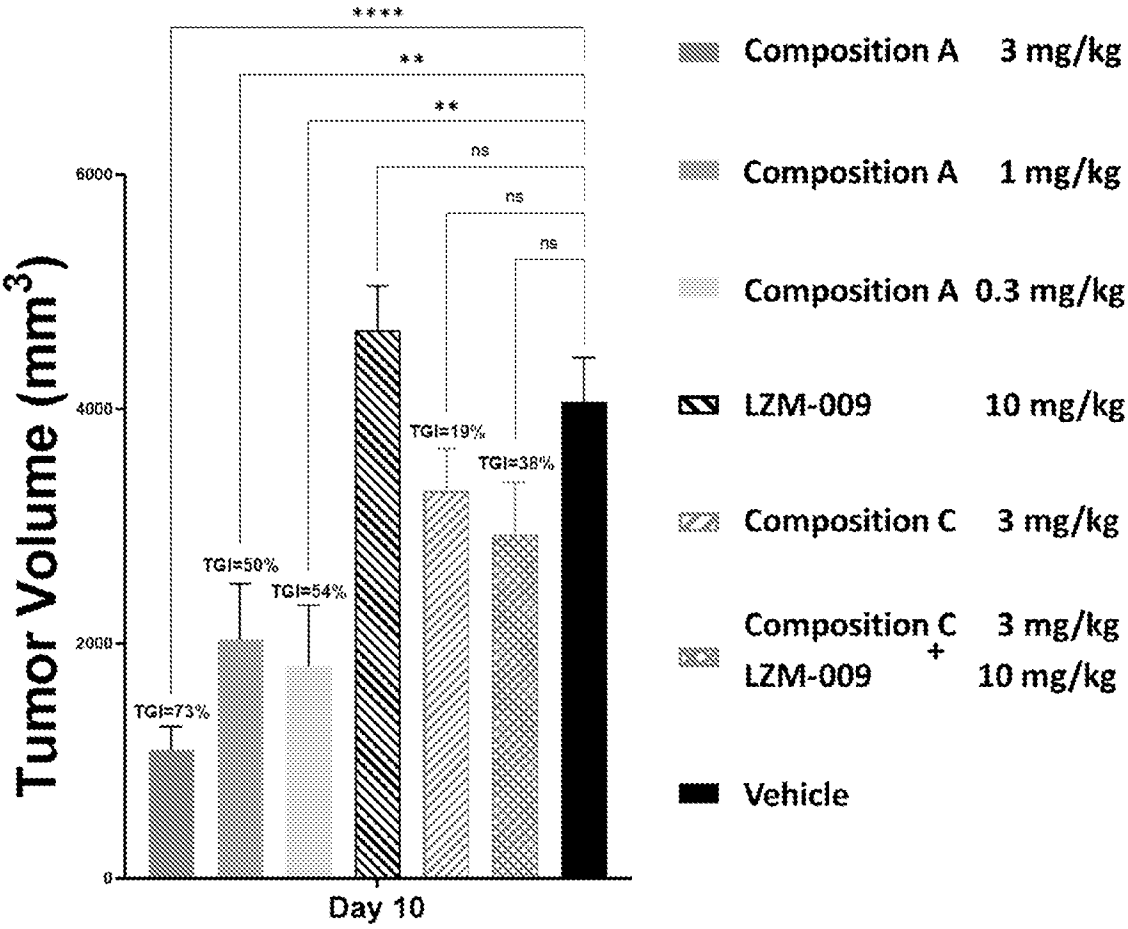

****
**
**
ns
ns
ns
6000
4000
2000
0
Tumor Volume (mm3)
TGI=73%
TGI=50%
TGI=54%
TGI=19%
TGI=38%
Day 10
Composition A 3 mg/kg
Composition A 1 mg/kg
Composition A 0.3 mg/kg
LZM-009 10 mg/kg
Composition C 3 mg/kg
Composition C 3 mg/kg
+
LZM-009 10 mg/kg
Vehicle

FIGURE 31B

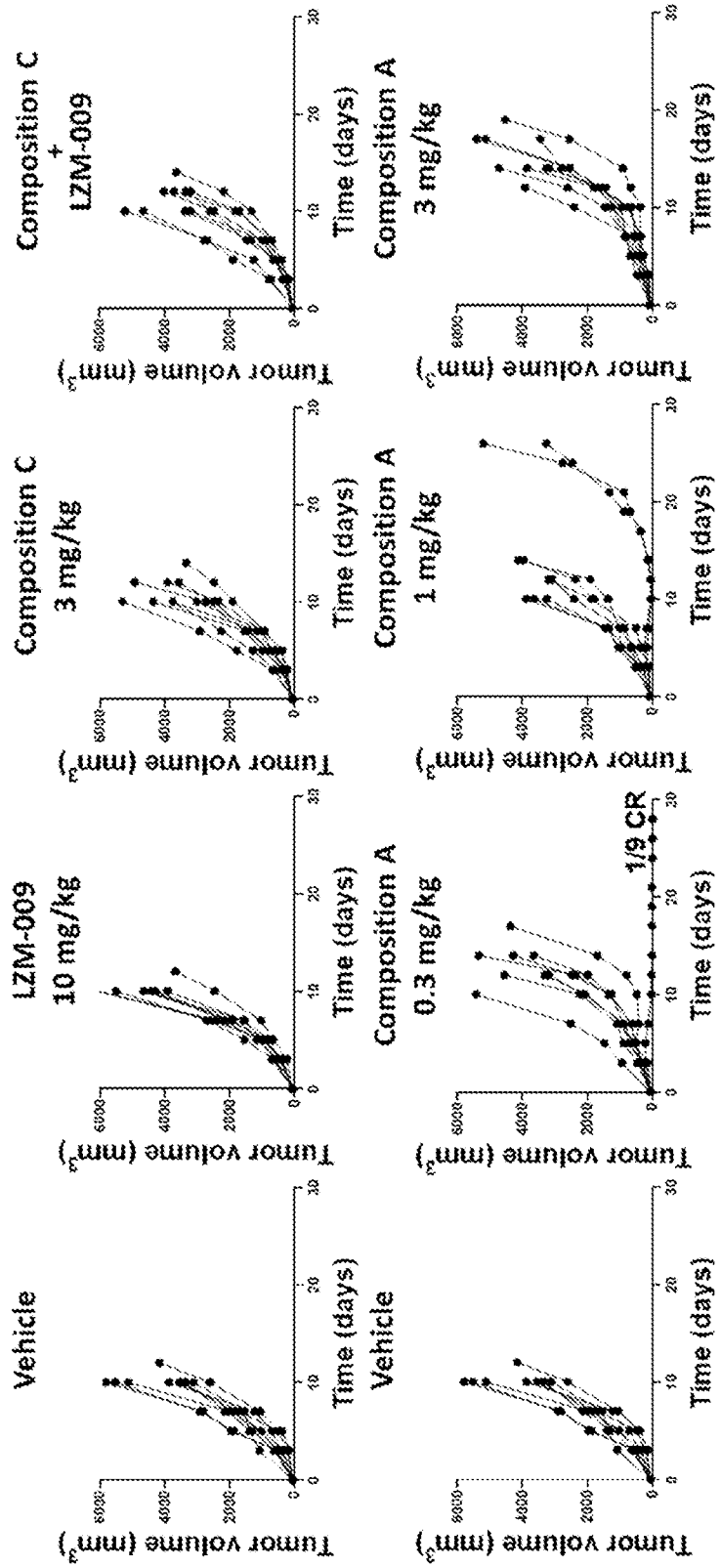

Vehicle
LZM-009
10 mg/kg
Composition A
0.3 mg/kg
1/9 CR
Composition A
1 mg/kg
Composition A
3 mg/kg
Composition C
3 mg/kg
Composition C
+
LZM-009
Tumor volume (mm³)
Time (days)

FIGURE 31C

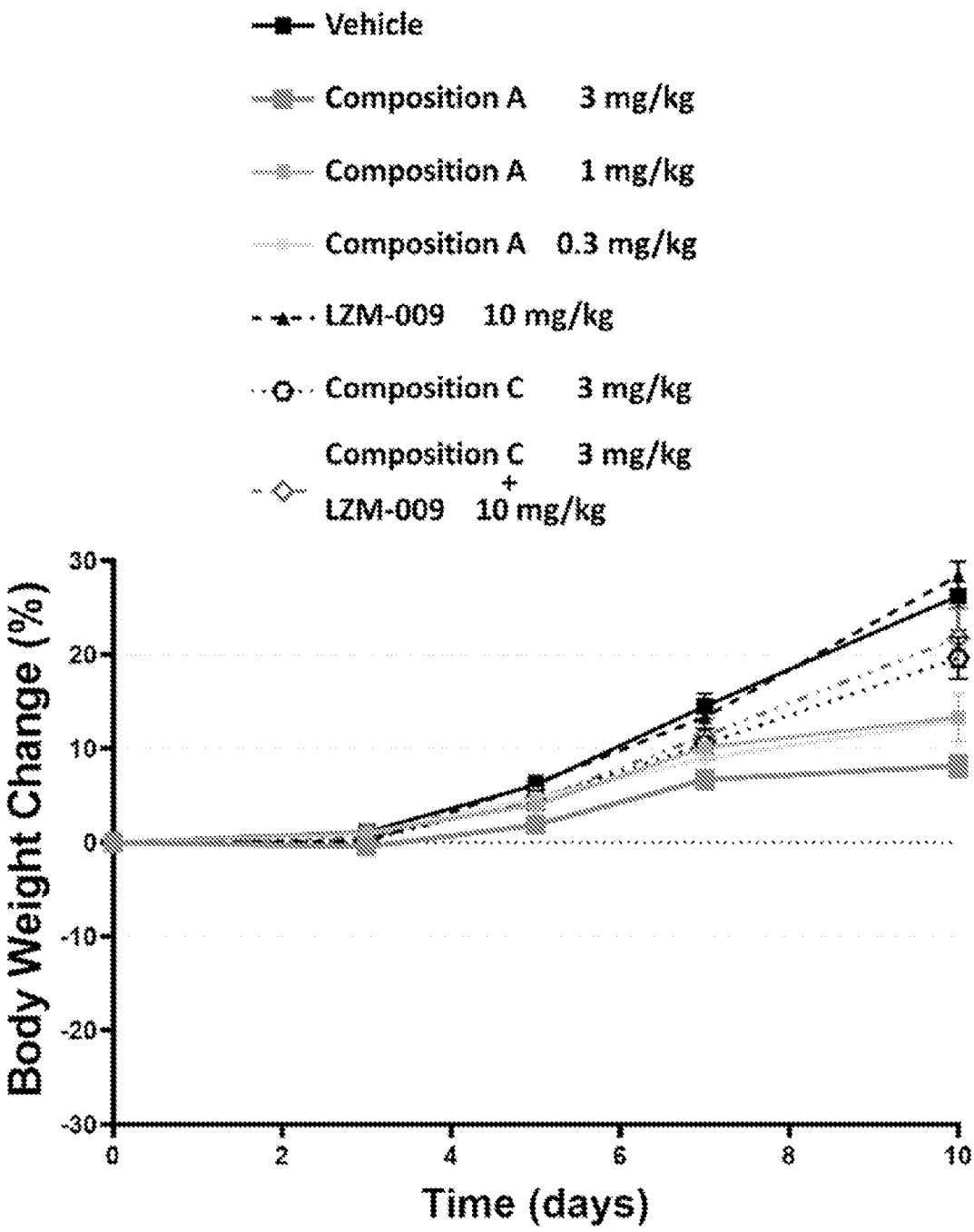

Vehicle
Composition A 3 mg/kg
Composition A 1 mg/kg
Composition A 0.3 mg/kg
LZM-009 10 mg/kg
Composition C 3 mg/kg
Composition C 3 mg/kg + LZM-009 10 mg/kg
Body Weight Change (%)
30
20
10
0
-10
-20
-30
0
2
4
6
8
10
Time (days)

FIGURE 32

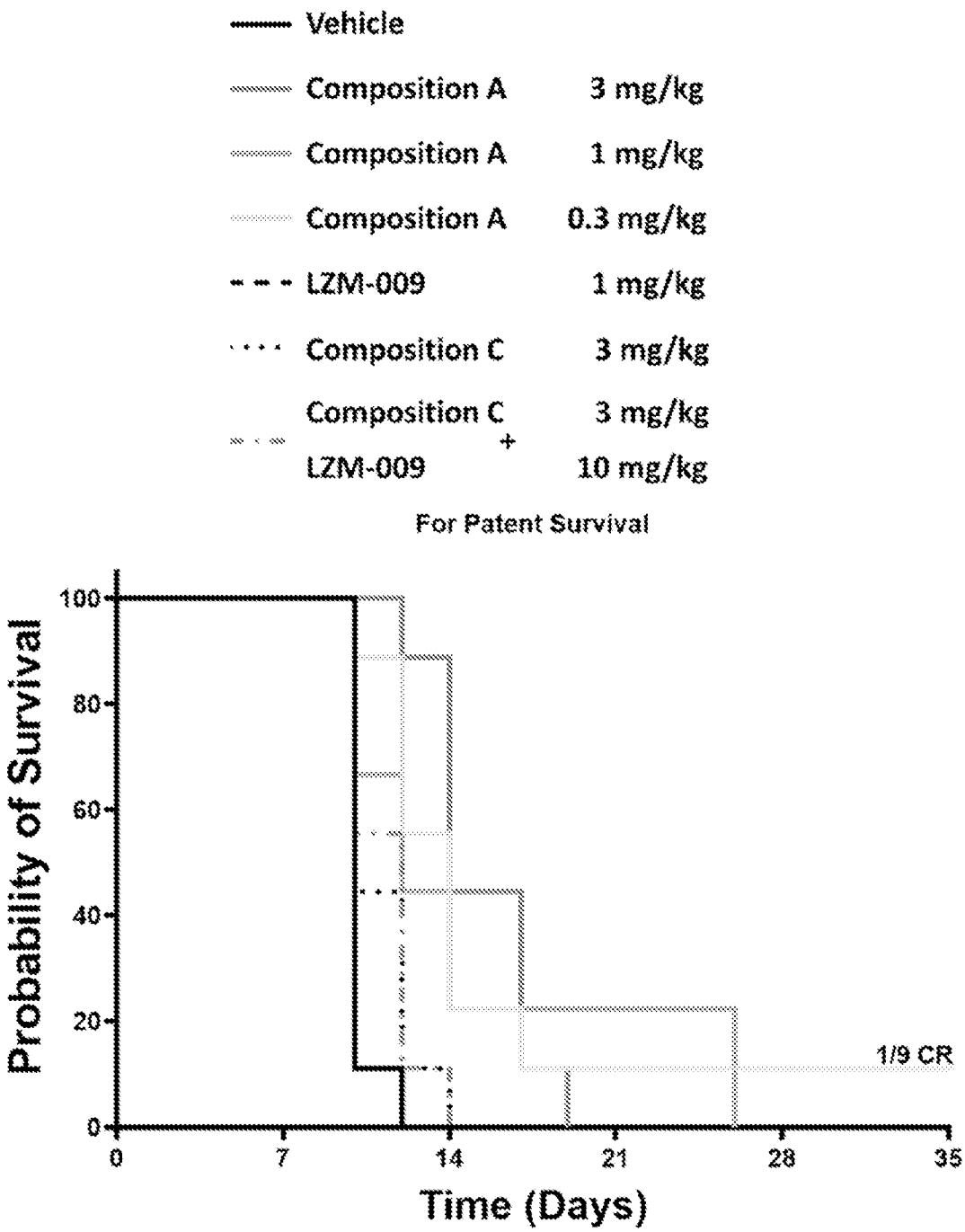

Vehicle
Composition A 3 mg/kg
Composition A 1 mg/kg
Composition A 0.3 mg/kg
LZM-009 1 mg/kg
Composition C 3 mg/kg
Composition C 3 mg/kg + LZM-009 10 mg/kg
For Patent Survival
Probability of Survival
100
80
60
40
20
0
0
7
14
21
28
35
Time (Days)
1/9 CR

FIGURE 33

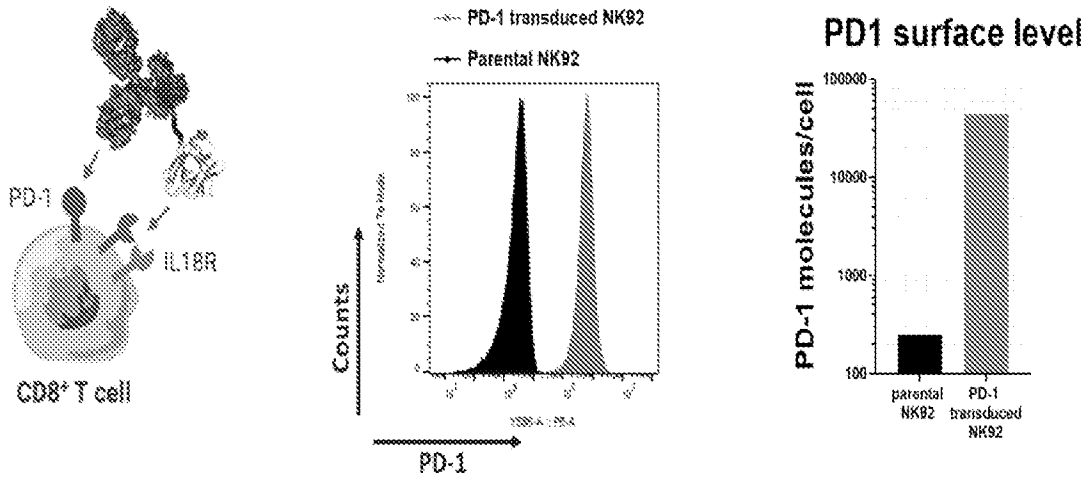

PD-1
IL18R
CD8+ T cell
PD-1 transduced NK92
Parental NK92
Counts
PD-1
PD1 surface level
PD-1 molecules/cell
100000
10000
1000
100
parental NK92
PD-1 transduced NK92

FIGURE 34

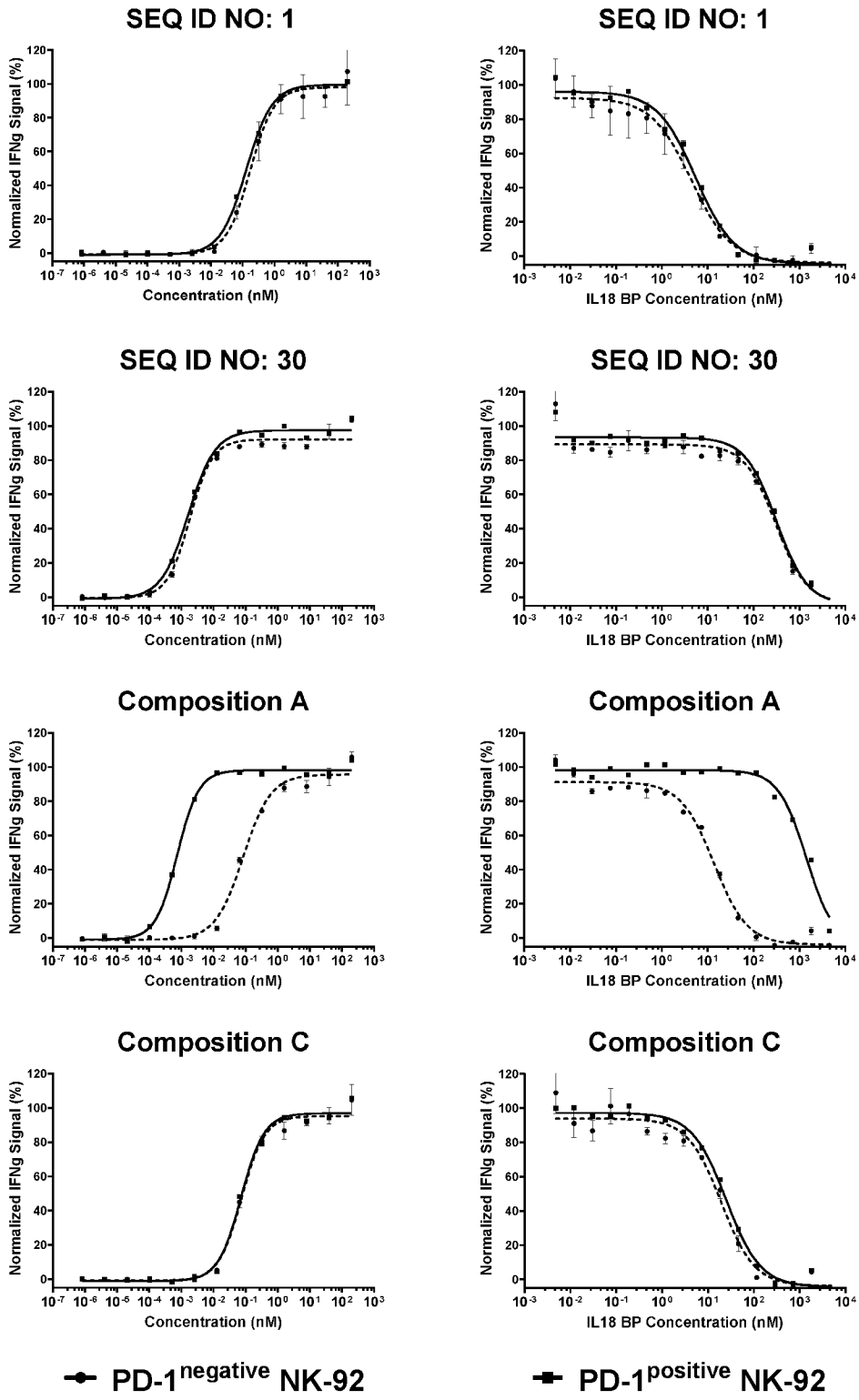

SEQ ID NO: 1
SEQ ID NO: 1
SEQ ID NO: 30
SEQ ID NO: 30
Composition A
Composition A
Composition C
Composition C
Normalized IFNg Signal (%)
Concentration (nM)
IL18 BP Concentration (nM)
PD-1negative NK-92
PD-1positive NK-92

FIGURE 35

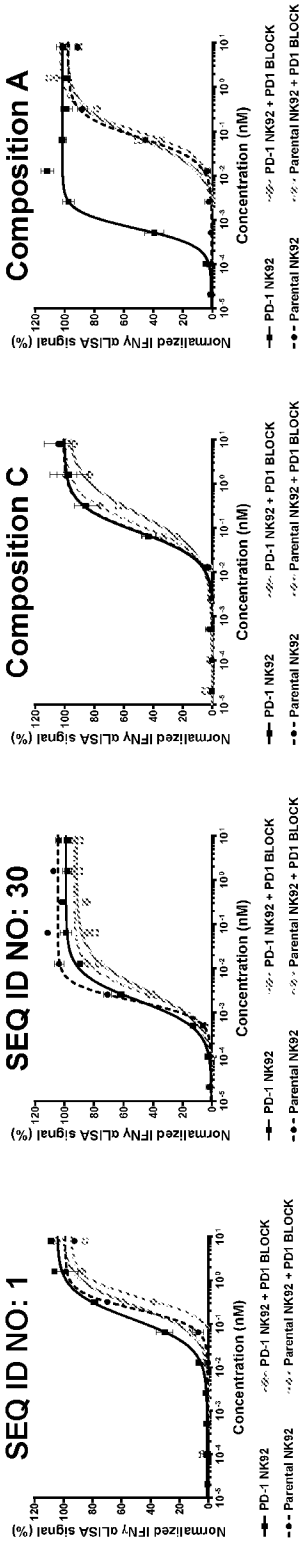

SEQ ID NO: 1
SEQ ID NO: 30
Composition C
Composition A
Normalized IFNγ qLISA signal (%)
Concentration (nM)
PD-1 NK92
PD-1 NK92 + PD1 BLOCK
Parental NK92
Parental NK92 + PD1 BLOCK

FIGURE 36

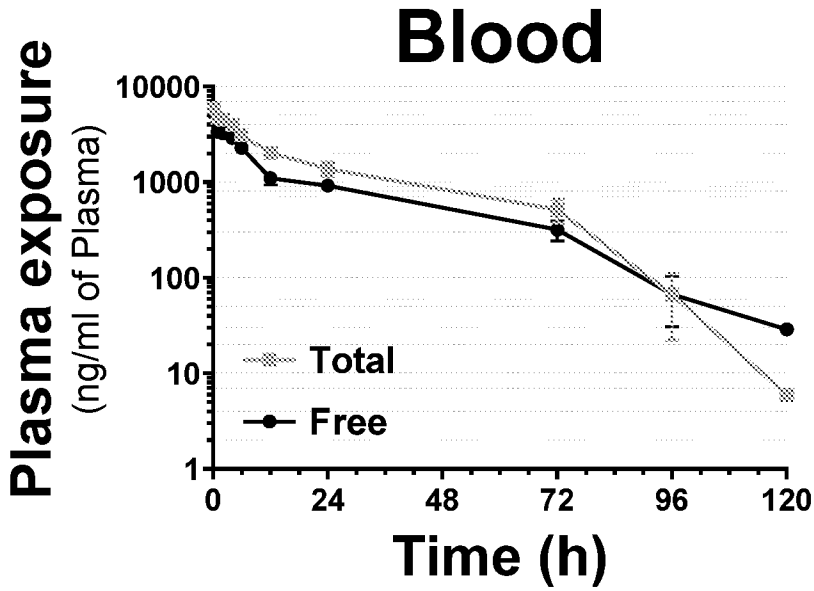


FIGURE 37A

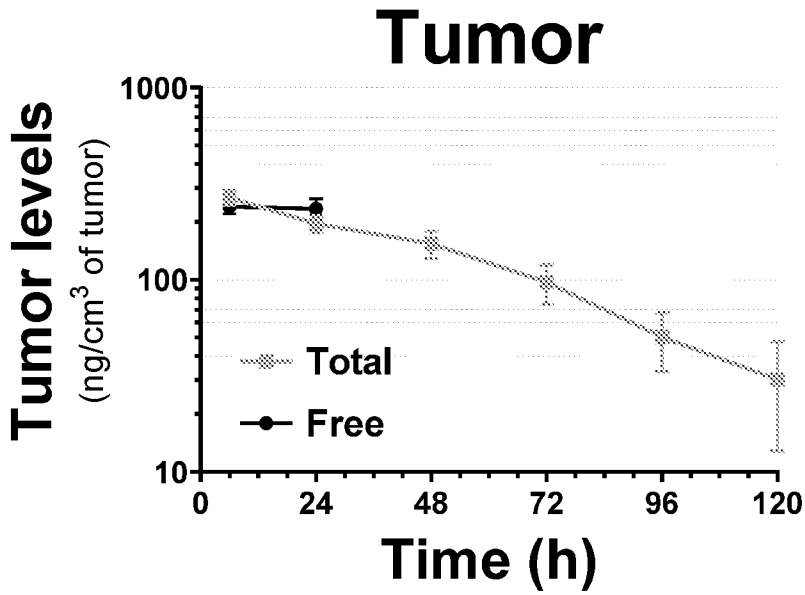


FIGURE 37B

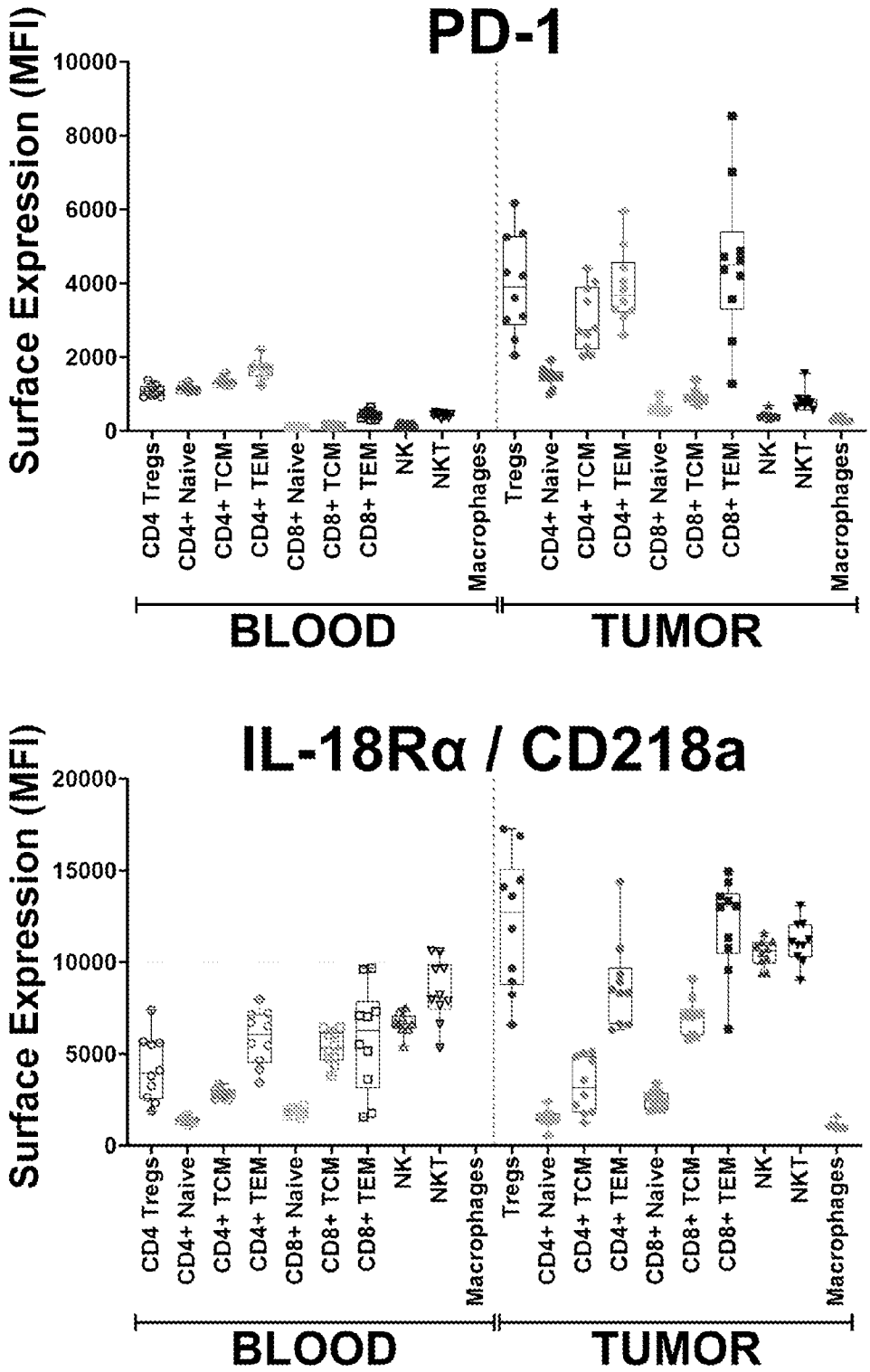

PD-1
Surface Expression (MFI)
10000
8000
6000
4000
2000
0
CD4 Tregs
CD4+ Naive
CD4+ TCM
CD4+ TEM
CD8+ Naive
CD8+ TCM
CD8+ TEM
NK
NKT
Macrophages
Tregs
CD4+ Naive
CD4+ TCM
CD4+ TEM
CD8+ Naive
CD8+ TCM
CD8+ TEM
NK
NKT
Macrophages
BLOOD
TUMOR
IL-18Rα / CD218a
Surface Expression (MFI)
20000
15000
10000
5000
0
CD4 Tregs
CD4+ Naive
CD4+ TCM
CD4+ TEM
CD8+ Naive
CD8+ TCM
CD8+ TEM
NK
NKT
Macrophages
Tregs
CD4+ Naive
CD4+ TCM
CD4+ TEM
CD8+ Naive
CD8+ TCM
CD8+ TEM
NK
NKT
Macrophages
BLOOD
TUMOR

FIGURE 38

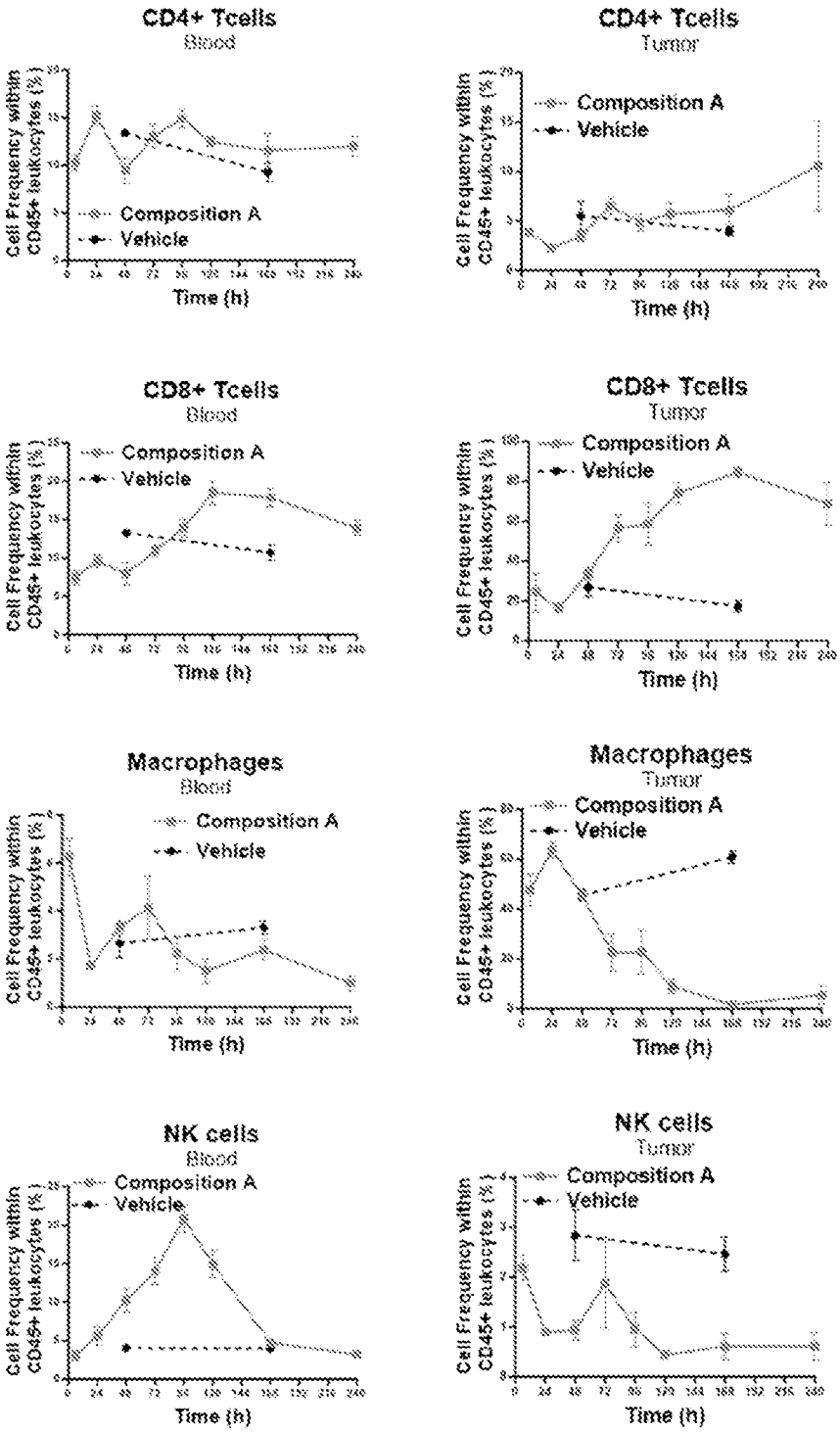

CD4+ Tcells
Blood
CD4+ Tcells
Tumor
CD8+ Tcells
Blood
CD8+ Tcells
Tumor
Macrophages
Blood
Macrophages
Tumor
NK cells
Blood
NK cells
Tumor
Composition A
Vehicle
Cell Frequency within CD45+ leukocytes (%)
Time (h)

FIGURE 39

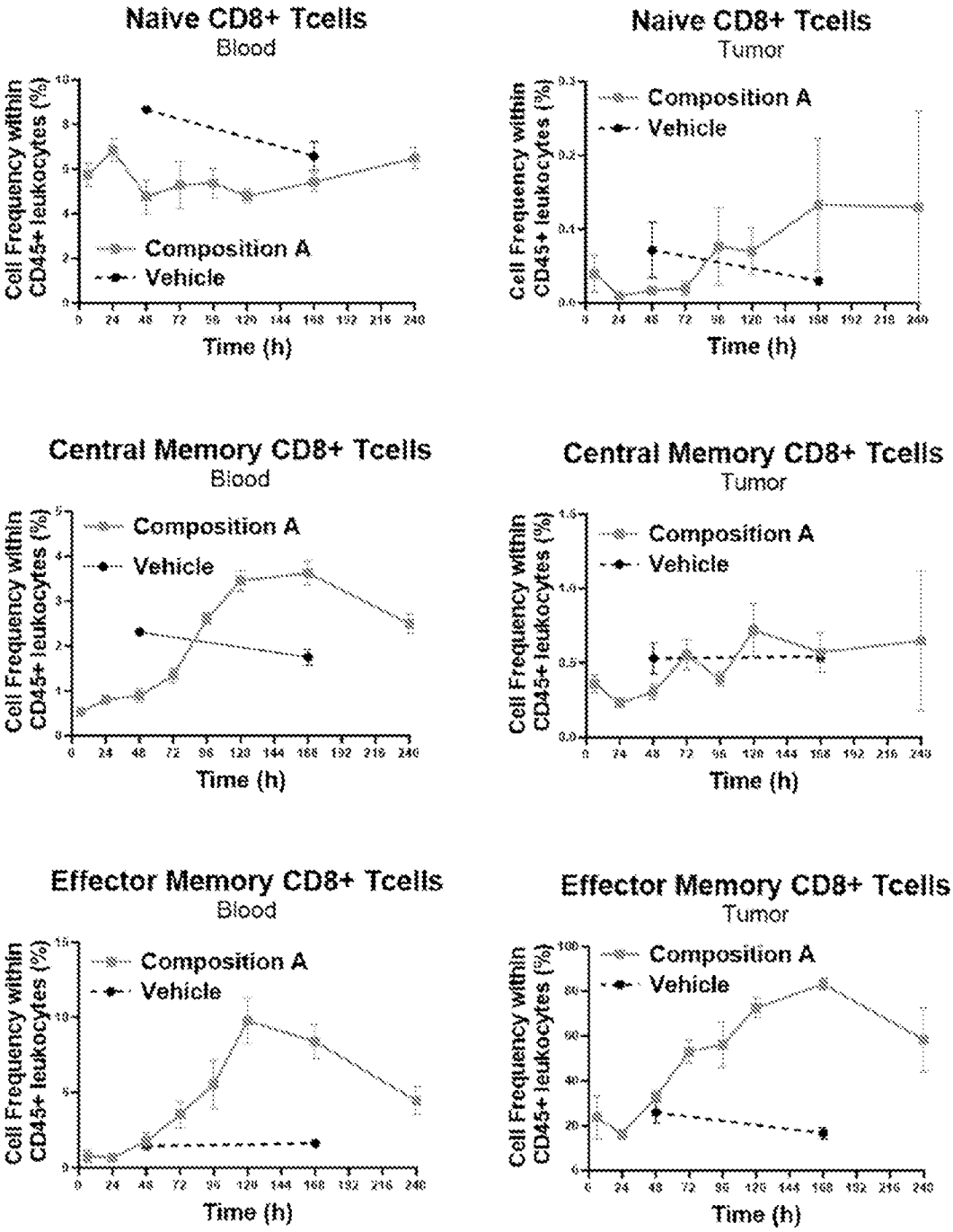

Naive CD8+ Tcells
Blood
Cell Frequency within CD45+ leukocytes (%)
Composition A
Vehicle
Time (h)
Naive CD8+ Tcells
Tumor
Cell Frequency within CD45+ leukocytes (%)
Composition A
Vehicle
Time (h)
Central Memory CD8+ Tcells
Blood
Cell Frequency within CD45+ leukocytes (%)
Composition A
Vehicle
Time (h)
Central Memory CD8+ Tcells
Tumor
Cell Frequency within CD45+ leukocytes (%)
Composition A
Vehicle
Time (h)
Effector Memory CD8+ Tcells
Blood
Cell Frequency within CD45+ leukocytes (%)
Composition A
Vehicle
Time (h)
Effector Memory CD8+ Tcells
Tumor
Cell Frequency within CD45+ leukocytes (%)
Composition A
Vehicle
Time (h)

FIGURE 40

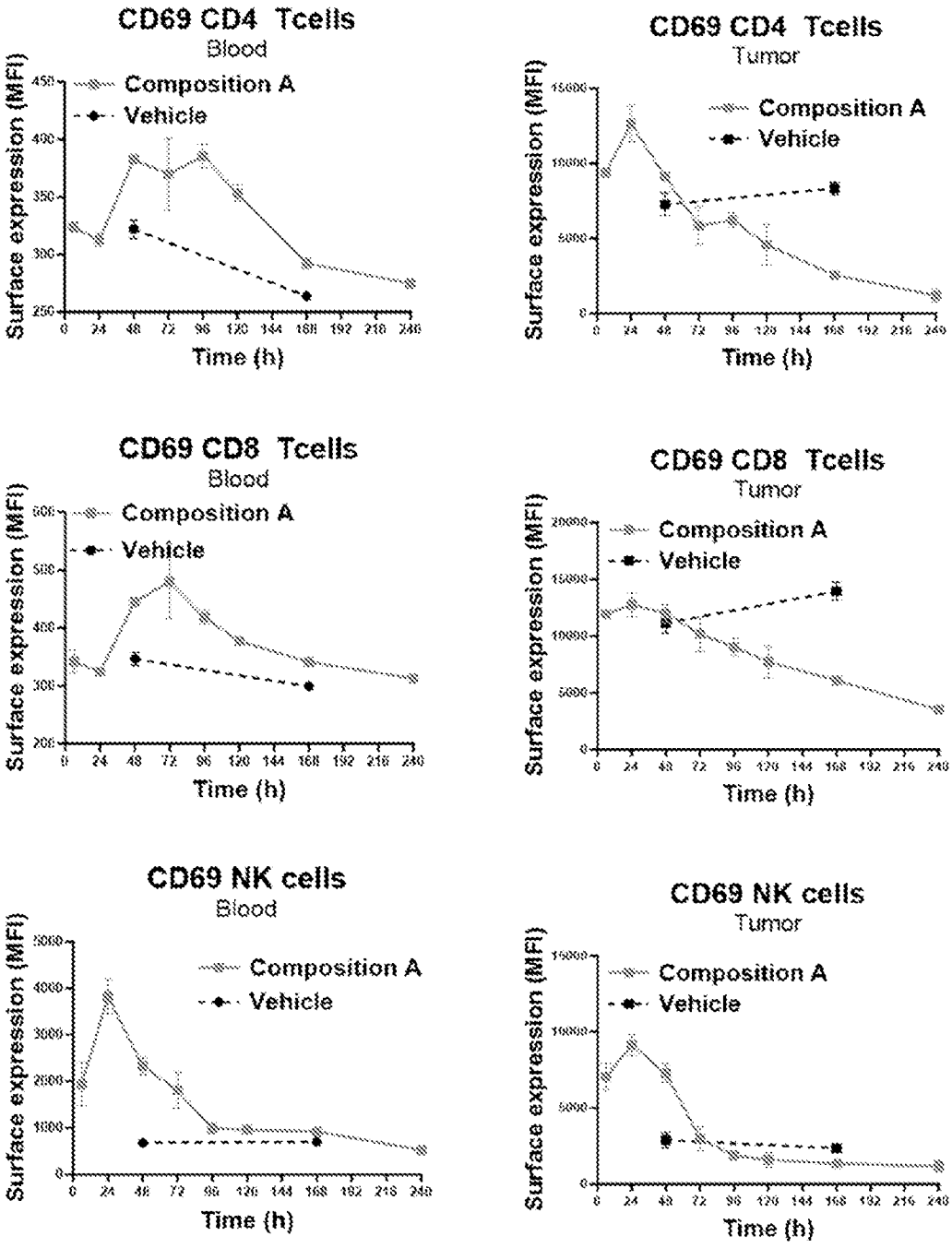

CD69 CD4 Tcells
Blood
Composition A
Vehicle
Surface expression (MFI)
Time (h)
CD69 CD4 Tcells
Tumor
Composition A
Vehicle
Surface expression (MFI)
Time (h)
CD69 CD8 Tcells
Blood
Composition A
Vehicle
Surface expression (MFI)
Time (h)
CD69 CD8 Tcells
Tumor
Composition A
Vehicle
Surface expression (MFI)
Time (h)
CD69 NK cells
Blood
Composition A
Vehicle
Surface expression (MFI)
Time (h)
CD69 NK cells
Tumor
Composition A
Vehicle
Surface expression (MFI)
Time (h)

FIGURE 41

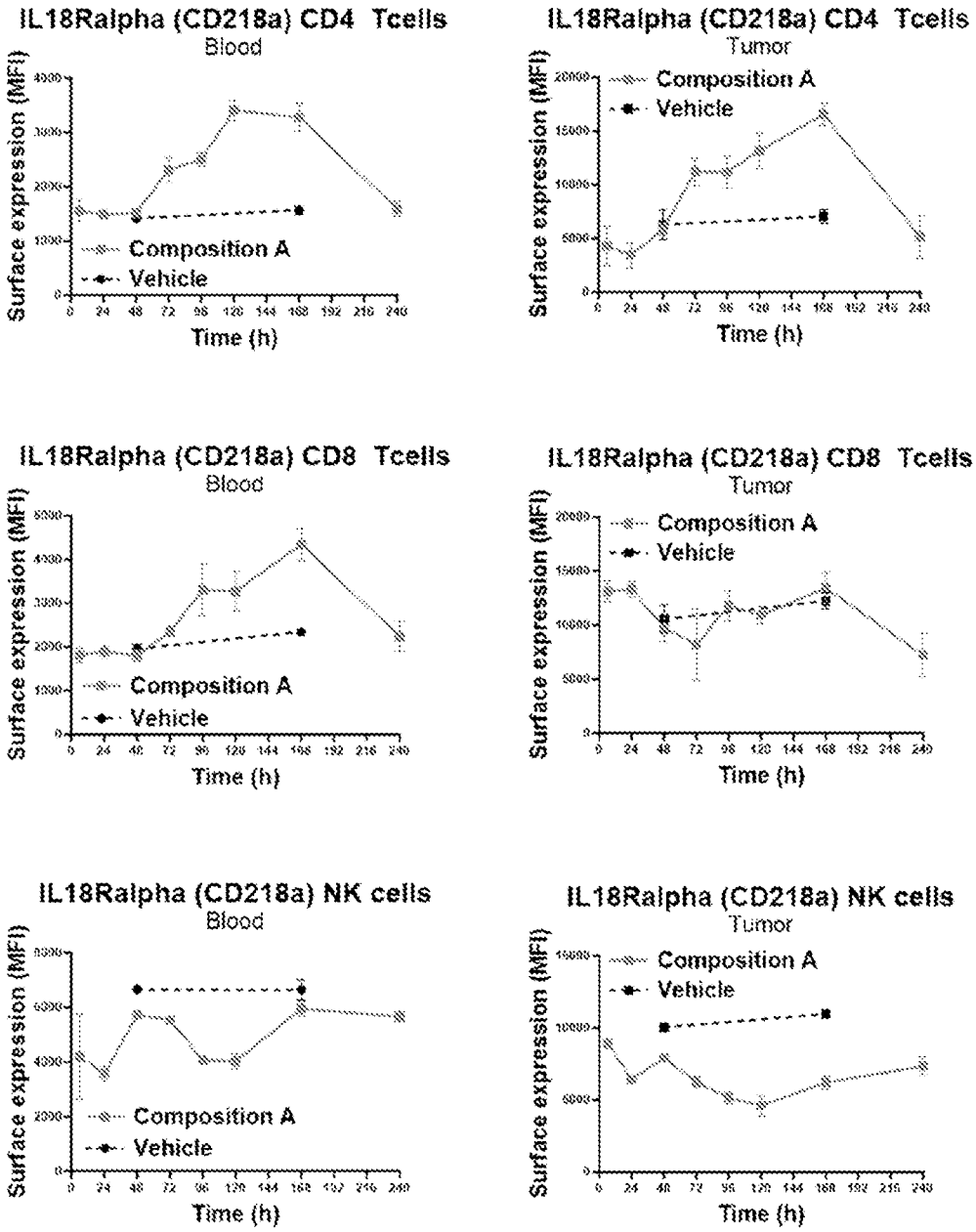

IL18Ralpha (CD218a) CD4 Tcells
Blood
Surface expression (MFI)
Composition A
Vehicle
Time (h)
IL18Ralpha (CD218a) CD4 Tcells
Tumor
Surface expression (MFI)
Composition A
Vehicle
Time (h)
IL18Ralpha (CD218a) CD8 Tcells
Blood
Surface expression (MFI)
Composition A
Vehicle
Time (h)
IL18Ralpha (CD218a) CD8 Tcells
Tumor
Surface expression (MFI)
Composition A
Vehicle
Time (h)
IL18Ralpha (CD218a) NK cells
Blood
Surface expression (MFI)
Composition A
Vehicle
Time (h)
IL18Ralpha (CD218a) NK cells
Tumor
Surface expression (MFI)
Composition A
Vehicle
Time (h)

FIGURE 42

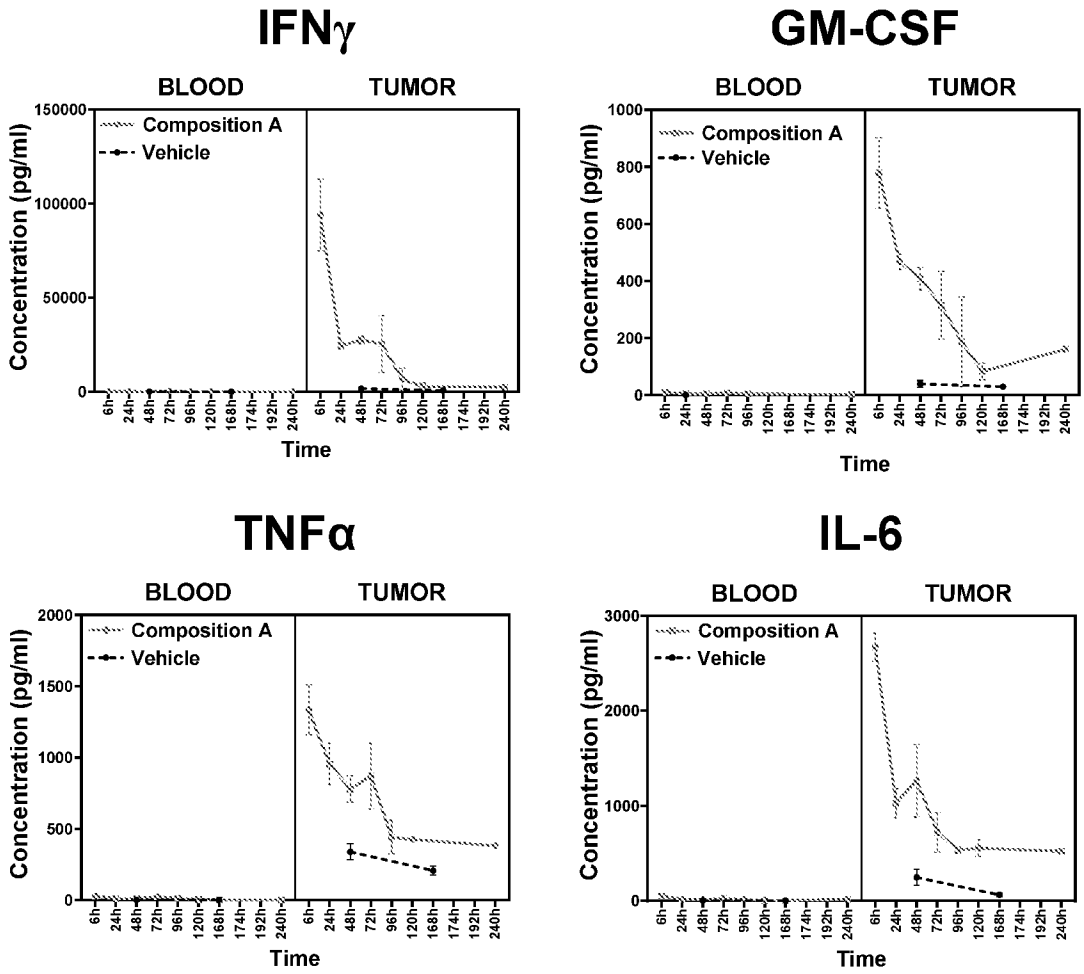

IFNγ
BLOOD
TUMOR
Composition A
Vehicle
Concentration (pg/ml)
150000
100000
50000
0
Time
GM-CSF
1000
800
600
400
200
TNFα
2000
1500
1000
500
IL-6
3000
2000
1000
6h
24h
48h
72h
96h
120h
168h
174h
192h
240h

FIGURE 43

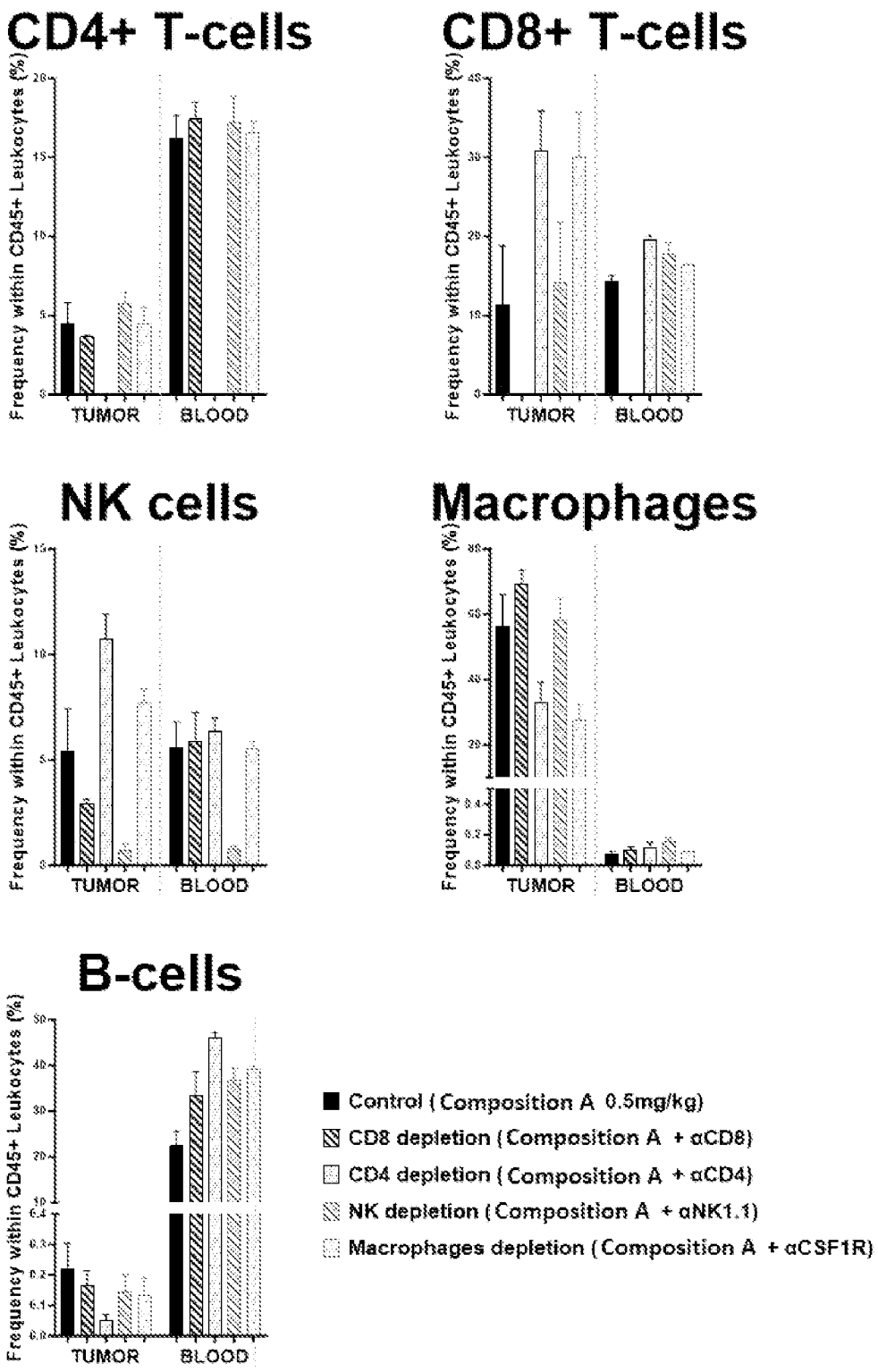

CD4+ T-cells
CD8+ T-cells
NK cells
Macrophages
B-cells
Frequency within CD45+ Leukocytes (%)
TUMOR
BLOOD
Control (Composition A 0.5mg/kg)
CD8 depletion (Composition A + αCD8)
CD4 depletion (Composition A + αCD4)
NK depletion (Composition A + αNK1.1)
Macrophages depletion (Composition A + αCSF1R)

FIGURE 44

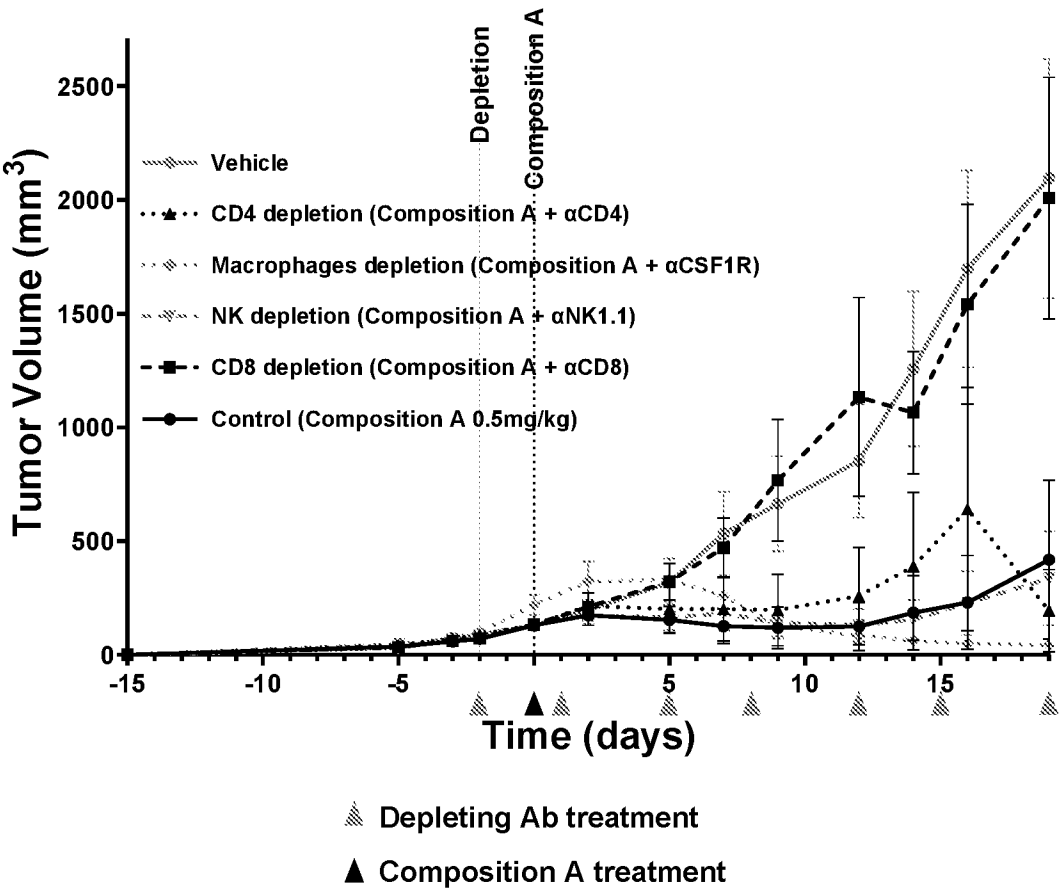

Depletion
Composition A
Vehicle
CD4 depletion (Composition A + αCD4)
Macrophages depletion (Composition A + αCSF1R)
NK depletion (Composition A + αNK1.1)
CD8 depletion (Composition A + αCD8)
Control (Composition A 0.5mg/kg)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
-15
-10
-5
5
10
15
Time (days)
Depleting Ab treatment
Composition A treatment

FIGURE 45

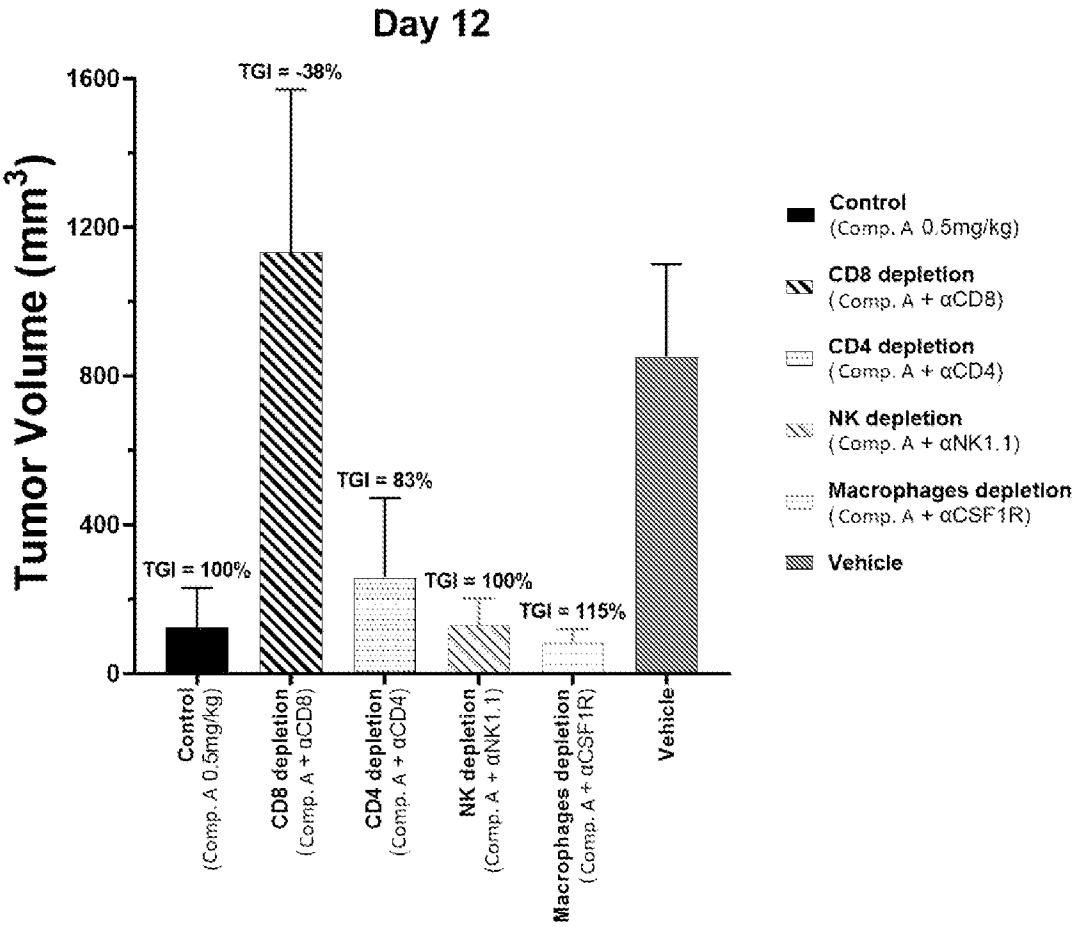

Day 12
Tumor Volume (mm3)
1600
1200
800
400
0
TGI = 100%
TGI = -38%
TGI = 83%
TGI = 100%
TGI = 115%
Control (Comp. A 0.5mg/kg)
CD8 depletion (Comp. A + αCD8)
CD4 depletion (Comp. A + αCD4)
NK depletion (Comp. A + αNK1.1)
Macrophages depletion (Comp. A + αCSF1R)
Vehicle
Control (Comp. A 0.5mg/kg)
CD8 depletion (Comp. A + αCD8)
CD4 depletion (Comp. A + αCD4)
NK depletion (Comp. A + αNK1.1)
Macrophages depletion (Comp. A + αCSF1R)
Vehicle

FIGURE 46

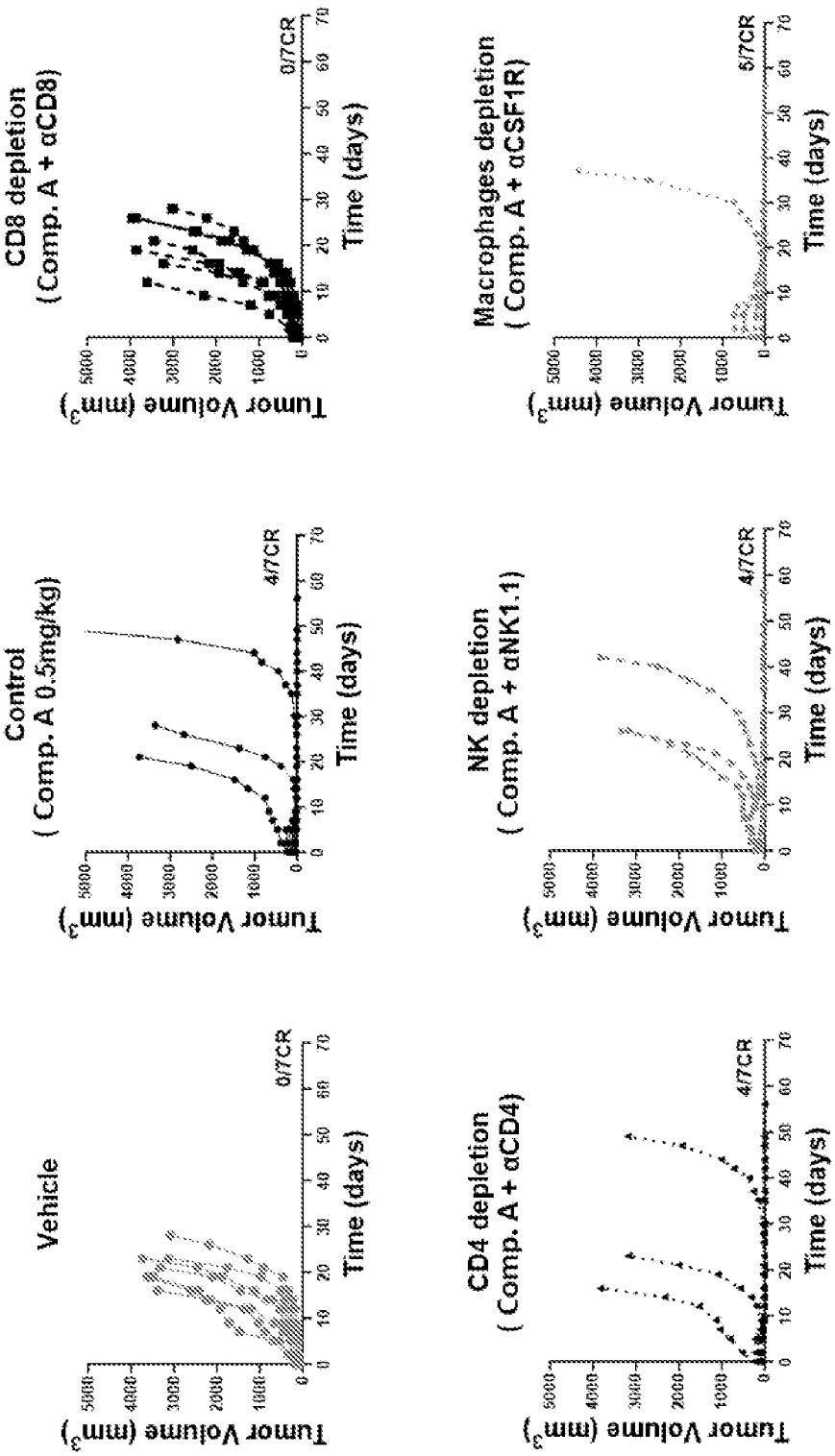

Vehicle
Control
(Comp. A 0.5mg/kg)
CD8 depletion
(Comp. A + αCD8)
CD4 depletion
(Comp. A + αCD4)
NK depletion
(Comp. A + αNK1.1)
Macrophages depletion
(Comp. A + αCSF1R)
Tumor Volume (mm3)
Time (days)
0/7CR
4/7CR
5/7CR

FIGURE 47

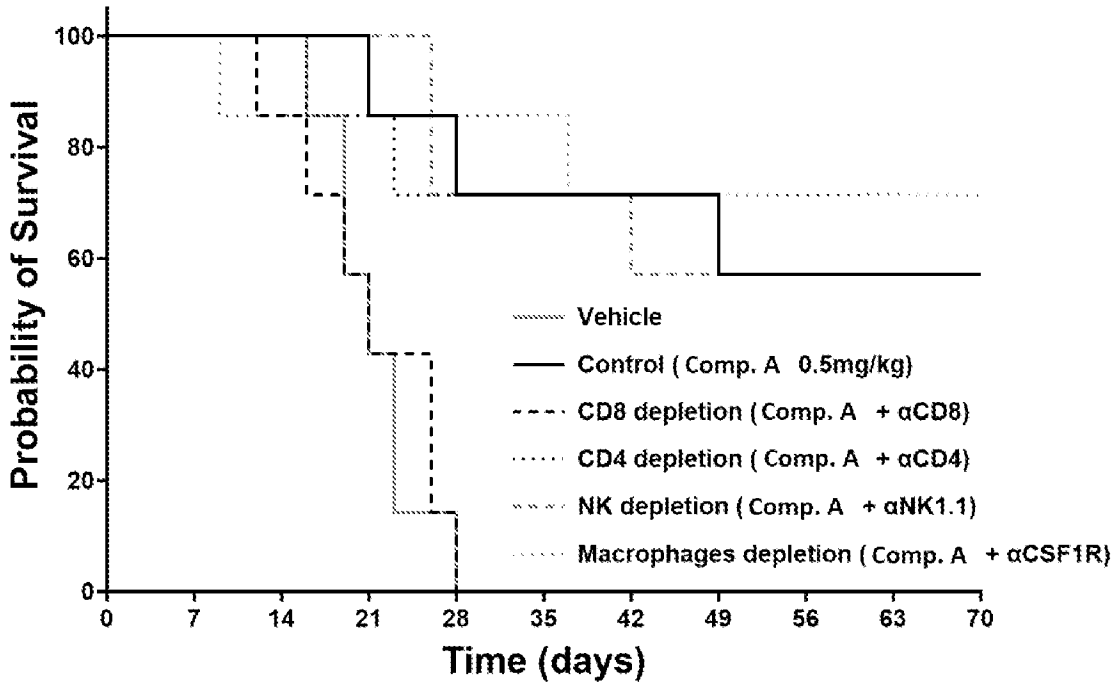


FIGURE 48

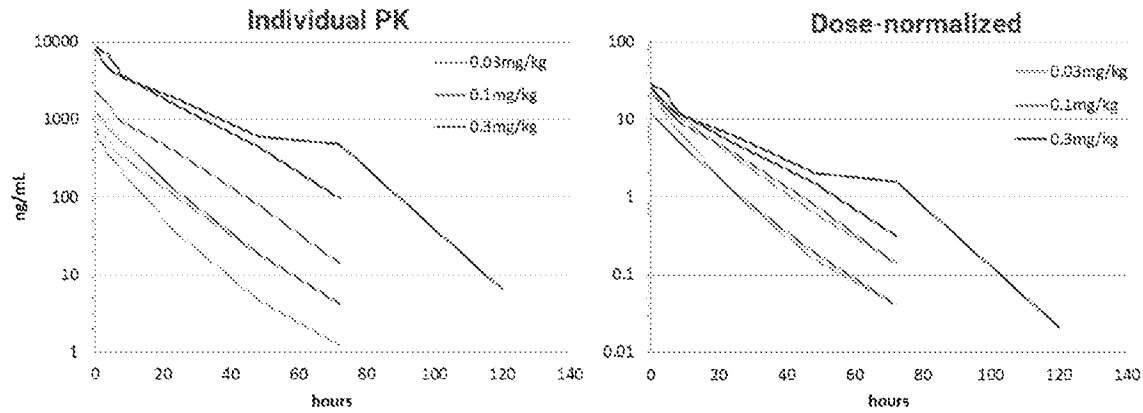


FIGURE 49

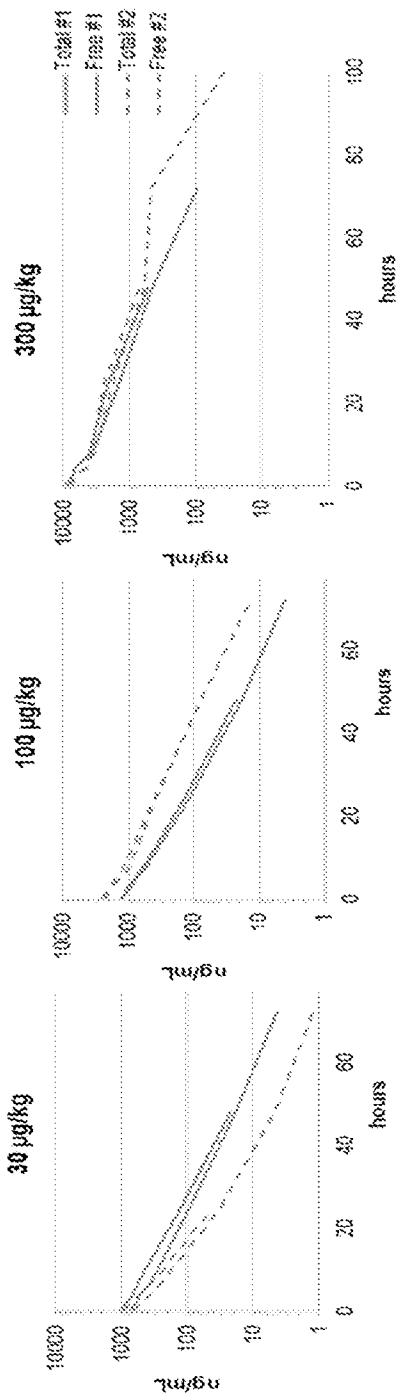

Total #1
Free #1
Total #2
Free #2
300 µg/kg
100 µg/kg
30 µg/kg
ng/mL
hours
10000
1000
100
10
1
0
20
40
60
80
100

FIGURE 50

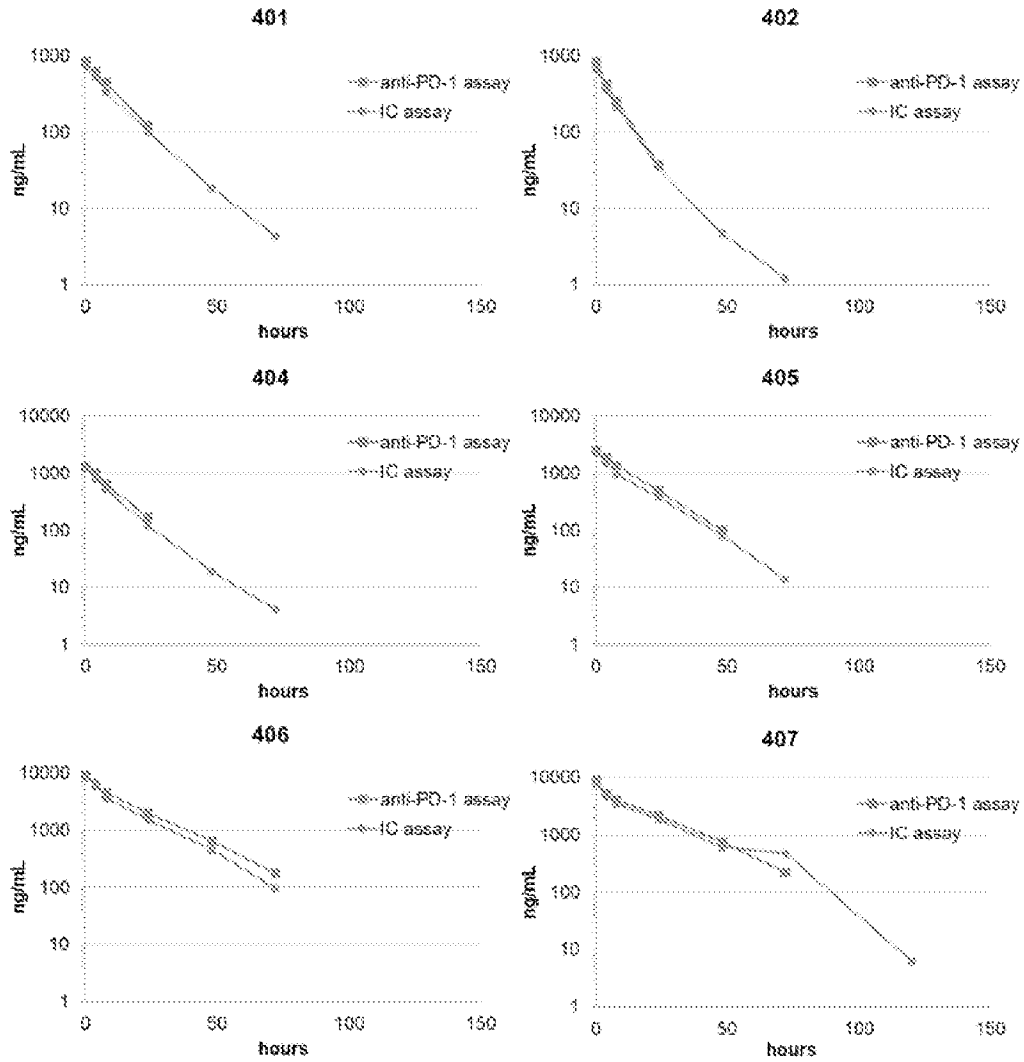

401
402
404
405
406
407
anti-PD-1 assay
IC assay
ng/mL
hours
0
50
100
150
1
10
100
1000
10000

FIGURE 51

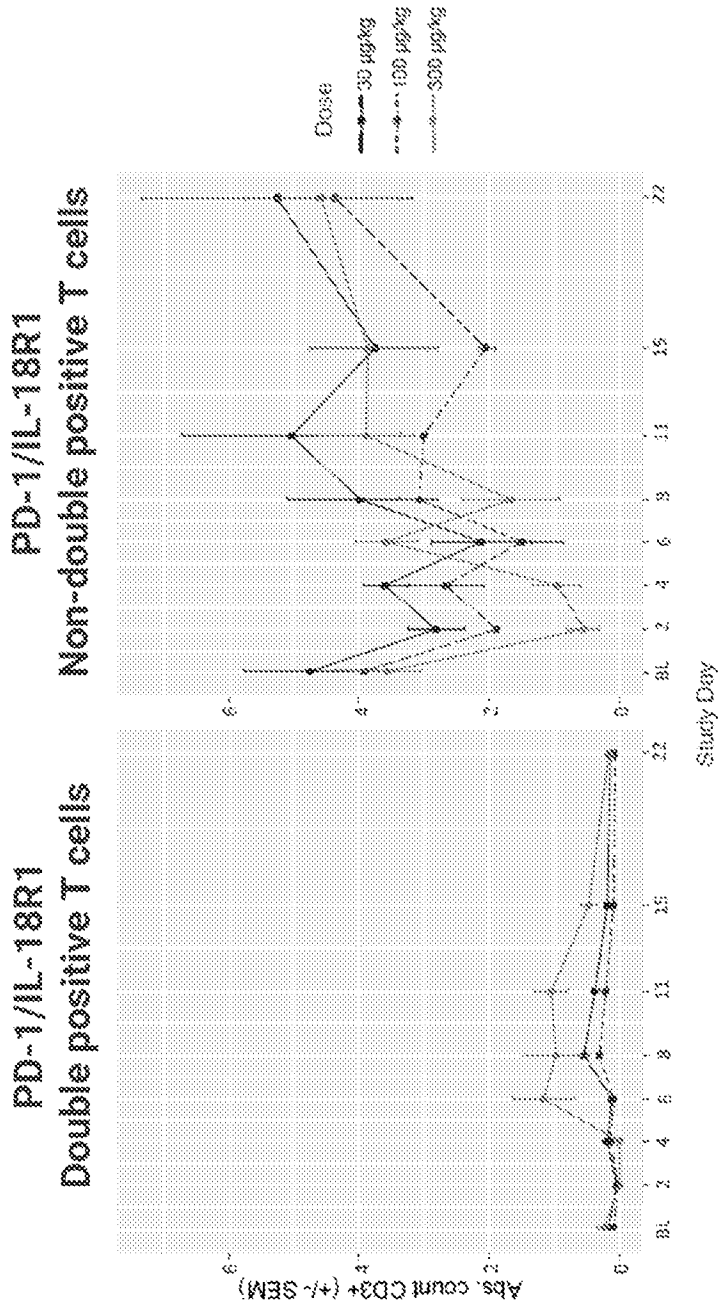

PD-1/IL-18R1
Double positive T cells
PD-1/IL-18R1
Non-double positive T cells
Abs. count CD3+ (+/- SEM)
Study Day
Dose
30 µg/kg
100 µg/kg
300 µg/kg

FIGURE 52

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/058236

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P35/00 A61K47/68 C07K14/54 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)

A61P 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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2023/281479 A1 (BRIGHT PEAK THERAPEUTICS AG [CH]) 12 January 2023 (2023-01-12)	1-36, 46-53, 57-79
Y	claims 65,67-79,82-86,90-99	37-45, 54-56

Y	US 2023/241172 A1 (RING AARON [US] ET AL) 3 August 2023 (2023-08-03) claims 1-24	37-45, 54-56

☐ 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
18 December 2024	20/01/2025

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	Lonnoy, Olivier

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2024/058236

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.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/058236

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2023281479 A1	12-01-2023	AU 2022307460 A1	04-01-2024
		CA 3222358 A1	12-01-2023
		EP 4366780 A1	15-05-2024
		EP 4366781 A1	15-05-2024
		JP 2024526298 A	17-07-2024
		KR 20240041379 A	29-03-2024
		US 2023250181 A1	10-08-2023
		US 2024158537 A1	16-05-2024
		WO 2023281479 A1	12-01-2023
		WO 2023281484 A1	12-01-2023
US 2023241172 A1	03-08-2023	AU 2018330444 A1	23-04-2020
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		BR 112020004389 A2	08-09-2020
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		JP 2023157959 A	26-10-2023
		KR 20200066623 A	10-06-2020
		SG 11202001384T A	30-03-2020
		US 2019070262 A1	07-03-2019
		US 2023241172 A1	03-08-2023
		US 2023321192 A1	12-10-2023
		US 2023355714 A1	09-11-2023
		US 2024325494 A1	03-10-2024
		WO 2019051015 A1	14-03-2019
		ZA 202307212 B	30-10-2024