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(54) Titre : IMMUNOCONJUGUE D'INTERLEUKINE -2, AGONISTE DE CD40 ET FACULTATIVEMENT UN ANTAGONISTE DE LIAISON DE L'AXE PD -1 DESTINE A ETRE UTILISE DANS DES METHODES DE TRAITEMENT DU CANCER

(54) Title: AN INTERLEUKIN-2 IMMUNOCONJUGATE, A CD40 AGONIST, AND OPTIONALLY A PD-1 AXIS BINDING ANTAGONIST FOR USE IN METHODS OF TREATING CANCER

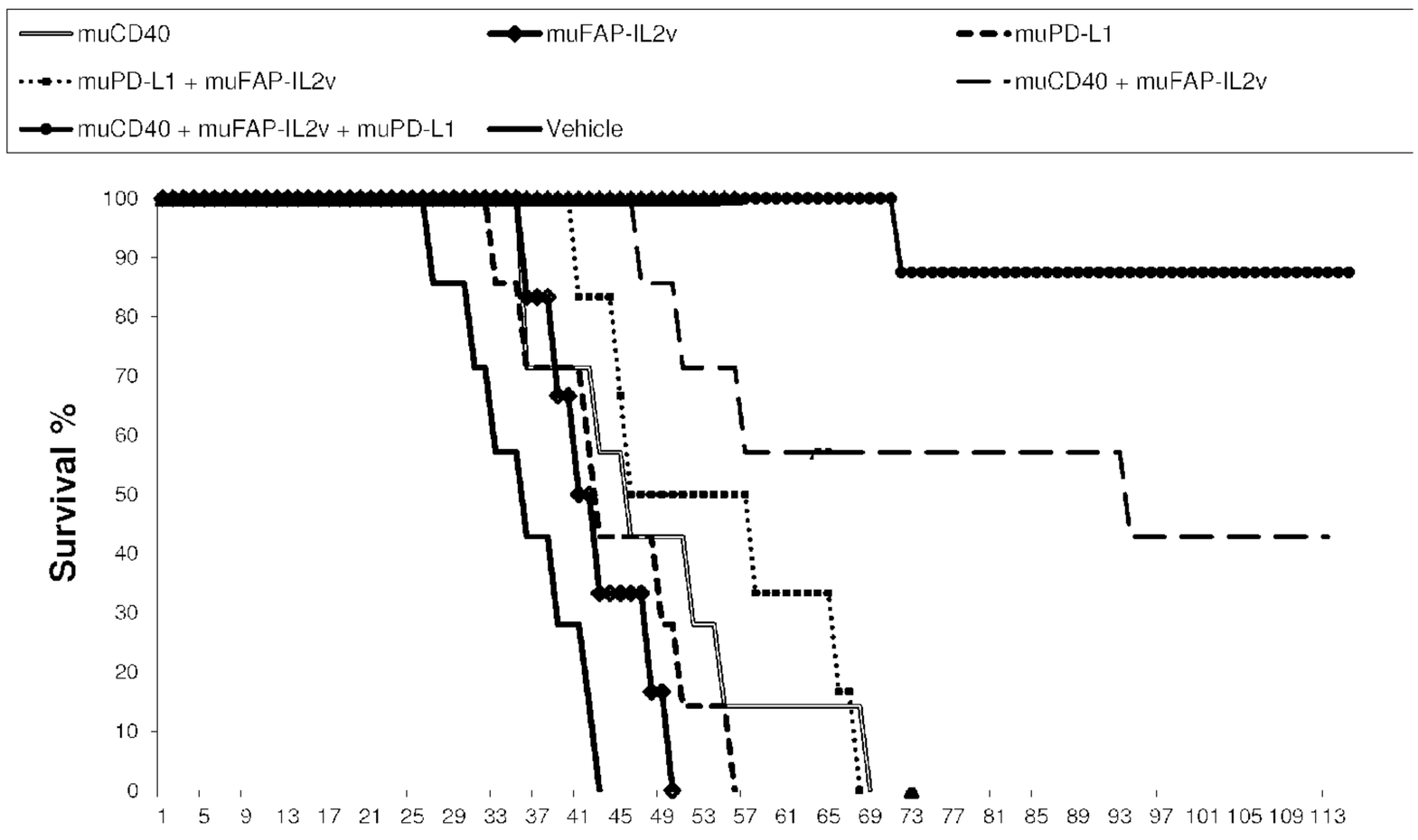

Fig.1A

(57) Abrégé/Abstract:
The invention provides compositions and methods for treating cancer, the method comprising administering an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-axis binding antagonist.

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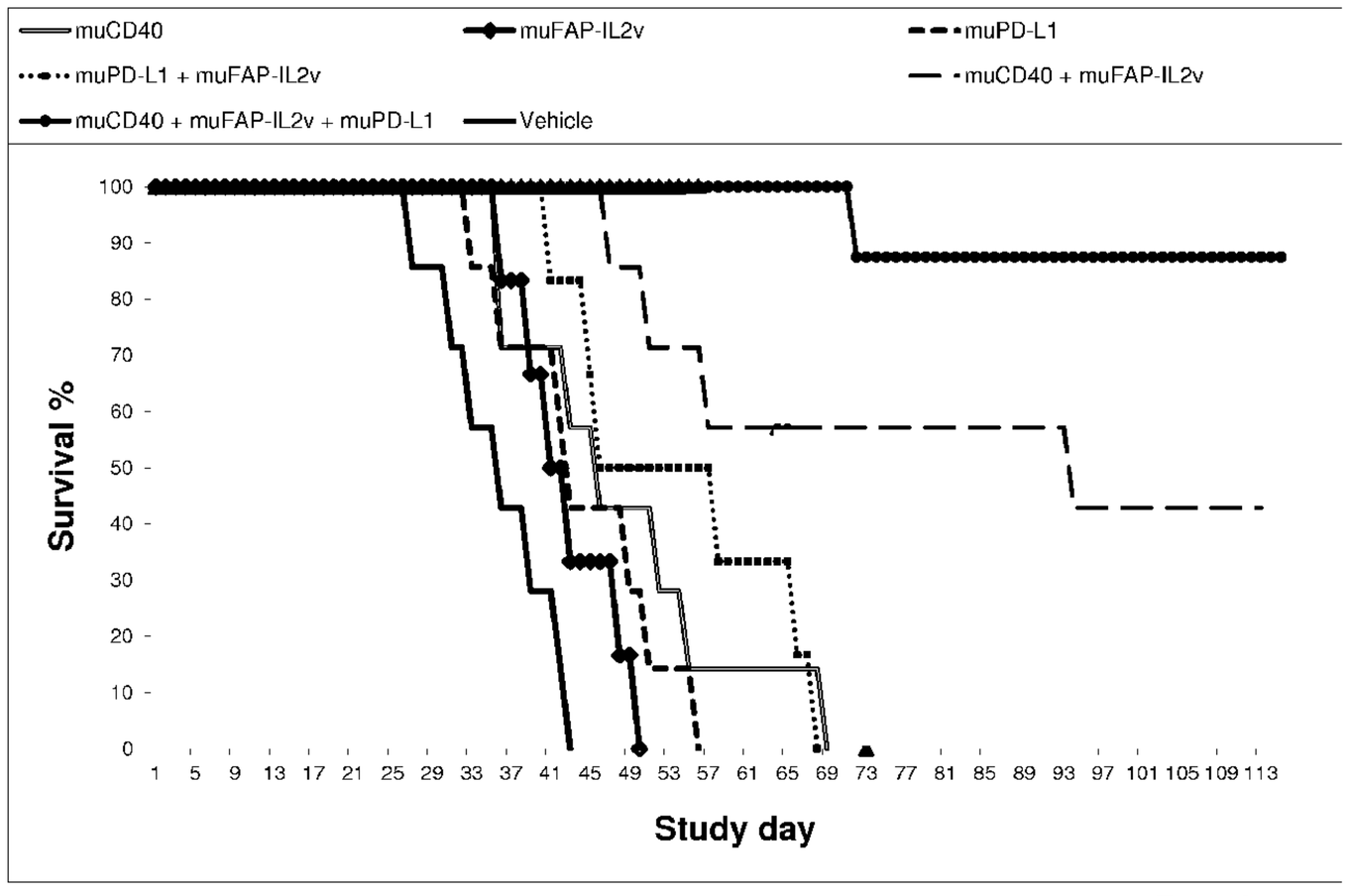

Fig.1A

(57) **Abstract:** The invention provides compositions and methods for treating cancer, the method comprising administering an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-axis binding antagonist.

[Continued on next page]

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AN INTERLEUKIN-2 IMMUNOCONJUGATE, A CD40 AGONIST, AND OPTIONALLY A PD-1 AXIS BINDING ANTAGONIST FOR USE IN METHODS OF TREATING CANCER

FIELD OF THE INVENTION

[0001] This invention relates to methods of treating cancer by administering an IL-2 immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

BACKGROUND OF THE INVENTION

[0002] Cancer is one of the leading causes of death worldwide. Despite advances in treatment options, prognosis of patients with advanced cancer remains poor. Consequently, there is a persisting and urgent medical need for optimal therapies to increase survival of cancer patients without causing unacceptable toxicity.

[0003] Recent results from clinical trials have shown that immune therapies, such as immune checkpoint inhibitors, can extend the overall survival of cancer patients and lead to durable responses. Despite these promising results, current immune-based therapies are only effective in a proportion of patients and combination strategies are needed to improve therapeutic benefit.

[0004] Interleukin-2 (IL-2), also known as T cell growth factor (TCGF), is a 15.5 kDa globular glycoprotein playing a central role in lymphocyte generation, survival and homeostasis. It stimulates the proliferation and differentiation of T cells, induces the generation of cytotoxic T lymphocytes (CTLs) and the differentiation of peripheral blood lymphocytes to cytotoxic cells and lymphokine-activated killer (LAK) cells, promotes cytokine and cytolytic molecule expression by T cells, facilitates the proliferation and differentiation of B-cells and the synthesis of immunoglobulin by B-cells, and stimulates the generation, proliferation and activation of natural killer (NK) cells (reviewed e.g. in Waldmann, Nat Rev Immunol 6, 595-601 (2009); Olejniczak and Kasprzak, Med Sci Monit 14, RA179-89 (2008); Malek, Annu Rev Immunol 26, 453-79 (2008)). Its ability to expand lymphocyte populations in vivo and to increase the effector functions of these cells confers antitumor effects to IL-2, and high-dose IL-2 treatment has been approved for use in patients with metastatic renal-cell carcinoma and malignant melanoma.

[0005] CD40, a member of the tumor necrosis factor receptor (TNFR) superfamily, is a critical regulator of the anti tumor immune response via its expression on antigen presenting cells (APCs) that include B lymphocytes, dendritic cells (DCs), and monocytes (see e.g. Grewal IS et al, *Ann Rev Immunol*, **1998**;16:111-35; Van Kooten C et al, *J Leukoc. Biol* , **2000**;67:2-17; or O'Sullivan B et al, *Crit Rev Immunol.* **2003**;23(1 2):83-107). CD40

stimulated DCs up regulate antigen processing and presentation pathways and migrate to lymph nodes to activate naive T cells. Agonist CD40 antibodies were shown to substitute the function of CD4+ lymphocytes resulting in cytotoxic T lymphocyte (CTL) expansion able to clear established lymphoma in murine models (see e.g. Sotomayor EM et al, *Nature Medicine*, **1999**;5(7):780-7; Gladue RP et al, *Cancer Immunol Immunother*, **2011**;60(7):1009-17). CD40 agonists trigger immune stimulation by activating host APCs, which then drive T cell responses directed against the tumor (see e.g. Vonderheide RH, *Clin Cancer Res*, **2007**;13:1083-8).

[0006] Programmed death-ligand 1 (PD-L1) is found on the surface of immune and tumor cells and its expression is induced by interferon gamma (IFNγ). It prevents the immune system from destroying cancer cells by interacting with the inhibitory programmed death-1 (PD-1) and B7.1 receptors on activated T cells, which results in a T-cell inhibitory signal.

[0007] As a result, therapeutic targeting of PD-1 and other molecules which signal through interactions with PD-1, such as programmed death-ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest. PD-L1 is overexpressed in many cancers and is often associated with poor prognosis (Okazaki T et al., Intern. Immun. 2007 19(7):813) (Thompson RH et al., Cancer Res 2006, 66(7):3381). Interestingly, the majority of tumor infiltrating T lymphocytes predominantly express PD-1, in contrast to T lymphocytes in normal tissues and peripheral blood T lymphocytes indicating that up-regulation of PD-1 on tumor-reactive T cells can contribute to impaired antitumor immune responses (Blood 2009 114(8):1537). This may be due to exploitation of PD-L1 signaling mediated by PD-L1 expressing tumor cells interacting with PD-1 expressing T cells to result in attenuation of T cell activation and evasion of immune surveillance (Sharpe et al., Nat Rev 2002) (Keir ME et al., 2008 Annu. Rev. Immunol. 26:677). Therefore, inhibition of the PD-L1/PD-1 interaction may enhance CD8+ T cell-mediated killing of tumors.

[0008] The inhibition of PD-L1 signaling has been proposed as a means to enhance T cell immunity for the treatment of cancer (e.g., tumor immunity) and infection, including both acute and chronic (e.g., persistent) infection. An optimal therapeutic treatment may combine blockade of PD-1 receptor/ligand interaction with one or more agent that enhances tumor immunity, e.g. by activating T cells.

[0009] As mentioned above, despite the availability of certain immune therapies, there remains a need for optimal (combination) therapies for treating, stabilizing, preventing, and/or delaying development of various cancers in patients.

SUMMARY OF THE INVENTION

[0010] In one aspect, provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of an interleukin-2 (IL-2) immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

[0011] In another aspect, provided herein is a method of enhancing immune function in an individual having cancer comprising administering an effective amount of an interleukin-2 (IL-2) immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

[0012] In another aspect, provided herein is the use of an IL-2 immunoconjugate in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and optionally further in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

[0013] In another aspect, provided herein is the use of a CD40 agonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the CD40 agonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and optionally further in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

[0014] In another aspect, provided herein is the use of a PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and further in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier.

[0015] In another aspect, provided herein is a composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the

second composition comprises a CD40 agonist and an optional pharmaceutically acceptable carrier, and optionally further in combination with third composition, wherein the third composition comprises a PD-1 axis antagonist and an optional pharmaceutically acceptable carrier.

[0016] In another aspect, provided herein is a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and optionally further in combination with a third composition, wherein the third composition comprises a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

[0017] In another aspect, provided herein is a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a CD40 agonist and an optional pharmaceutically acceptable carrier, and further in combination with third composition, wherein the third composition comprises an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier.

[0018] In another aspect, provided herein is a kit comprising a medicament comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

[0019] In another aspect, provided herein is a kit comprising a medicament comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

[0020] In another aspect, provided herein is a kit comprising a first medicament comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and a second medicament comprising a CD40 agonist and an optional pharmaceutically acceptable carrier. In some embodiments, the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament for treating or delaying progression of cancer in an individual.

[0021] In another aspect, provided herein is a kit comprising a medicament comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and further in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, for treating or delaying progression of cancer in an individual.

[0022] In another aspect, provided herein is a kit comprising a medicament comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and further in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, for treating or delaying progression of cancer in an individual.

[0023] In another aspect, provided herein is a kit comprising a first medicament comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, a second medicament comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and a third medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier. In some embodiments, the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament and the third medicament for treating or delaying progression of cancer in an individual.

[0024] In another aspect, provided herein is a kit comprising a medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and further in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, for treating or delaying progression of cancer in an individual.

[0025] In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist is a human PD-1 axis binding antagonist. In some embodiments the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is an antibody. In some embodiments,

the antibody is a humanized antibody, a chimeric antibody or a human antibody. In some embodiments, the antibody is an antigen binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of Fab, Fab', $F(ab')_2$, and Fv.

[0026] In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is an antibody. In some embodiments, the PD-1 binding antagonist is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108.

[0027] In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an anti-PD-L1 antibody. In some embodiments, the PD-L1 binding antagonist is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In particular embodiments, the anti-PD-L1 antibody is MPDL3280A (atezolizumab). In some embodiments, MPDL3280A is administered at a dose of about 800 mg to about 1500 mg every three weeks (e.g., about 1000 mg to about 1300 mg every three weeks, e.g., about 1100 mg to about 1200 mg every three weeks). In some embodiments, MPDL3280A is administered at a dose of about 1200 mg every three weeks. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and/or a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-PD-L1 antibody

comprises the three heavy chain HVR sequences of antibody YW243.55.S70 and/or the three light chain HVR sequences of antibody YW24355.S70 described in WO 2010/077634 and U.S. Patent No. 8,217,149, which are incorporated herein by reference. In some embodiments, the anti-PD-L1 antibody comprises the heavy chain variable region sequence of antibody YW243.55.S70 and/or the light chain variable region sequence of antibody YW24355.S70.

[0028] In some embodiments, the PD-1 axis binding antagonist is a PD-L2 binding antagonist. In some embodiments, the PD-L2 binding antagonist is an antibody. In some embodiments, the PD-L2 binding antagonist is an immunoadhesin.

[0029] In some embodiments, the PD-1 axis binding antagonist is an antibody (e.g., an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody) and comprises an aglycosylation site mutation. In some embodiments, the aglycosylation site mutation is a substitution mutation. In some embodiments, the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering). In some embodiments, the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A. In some embodiments, the substitution mutation is a D265A mutation and an N297G mutation. In some embodiments, the aglycosylation site mutation reduces effector function of the antibody. In some embodiments, the PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody) is a human IgG_1 having Asn to Ala substitution at position 297 according to EU numbering.

[0030] In some embodiments of the methods, uses, compositions, and kits described above and herein, the IL-2 immunoconjugate comprises an antibody that specifically binds to a tumor antigen, and an IL-2 polypeptide.

[0031] In some embodiments, the IL-2 immunoconjugate comprises an antibody that specifically binds to Carcinoembryonic Antigen (CEA). In some embodiments, the antibody that specifically binds to CEA comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 38, the HCDR2 of SEQ ID NO: 39, and the HCDR3 of SEQ ID NO: 40; and/or a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 41, the LCDR2 of SEQ ID NO: 42 and the LCDR3 of SEQ ID NO: 43. In some embodiments, the antibody that specifically binds to CEA comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:35. In some embodiments, the IL-2 immunoconjugate comprises an antibody that specifically binds to Fibroblast Activation Protein (FAP). In some embodiments, the antibody that specifically

binds to FAP comprises a heavy chain variable region comprising a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 47, and/or a light chain variable region comprising a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 48. In some embodiments, the antibody comprises a heavy chain variable region comprising the heavy chain complementarity determining region (HCDR) 1, HCDR 2 and HCDR 3 from the heavy chain variable region sequence of SEQ ID NO: 47, and/or a light chain variable region comprising the light chain complementarity determining region (LCDR) 1, LCDR 2 and LCDR 3 from the light chain variable region sequence of SEQ ID NO: 48. In some embodiments, the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 47 and/or a light chain variable region comprising the sequence of SEQ ID NO: 48.

[0032] In some embodiments, the antibody comprised in the IL-2 immunoconjugate is a full-length antibody. In some embodiments, the antibody is an IgG class antibody, particularly an IgG1 subclass antibody. In some embodiments, the antibody comprises an Fc domain, particularly an IgG Fc domain, more particularly an IgG1 Fc domain. In some embodiments, the Fc domain is a human Fc domain. In particular embodiments, the Fc domain is a human IgG1 Fc domain.

[0033] In some embodiments, the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain. In some embodiments, in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. In some embodiments, in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numbering according to Kabat EU index). In some embodiments, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second

subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numbering according to Kabat EU index).

[0034] In some embodiments, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, particularly an Fcγ receptor, and/or effector function, particularly antibody-dependent cell-mediated cytotoxicity (ADCC), as compared to a native IgG1 Fc domain. In some embodiments, the Fc domain comprises one or more amino acid substitution at one or more position selected from the group of L234, L235, and P329 (numbering according to Kabat EU index). In some embodiments, each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).

[0035] In some embodiments, the IL-2 polypeptide comprised in the IL-2 immunoconjugate is a human IL-2 polypeptide. In some embodiments, the IL-2 polypeptide is a mutant human IL-2 polypeptide comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 52). In some embodiments, the IL-2 polypeptide comprises the sequence of SEQ ID NO: 53.

[0036] In some embodiments, the IL-2 immunoconjugate comprises a single (i.e. no more than one) IL-2 polypeptide.

[0037] In one embodiment, the IL-2 immunoconjugate comprises a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 44, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 45, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 46. In one embodiment, the IL-2 immunoconjugate comprises a polypeptide comprising the sequence of SEQ ID NO: 44, a polypeptide comprising the sequence of SEQ ID NO: 45, and a polypeptide comprising the sequence of SEQ ID NO: 46. (CEA-IL2v)

[0038] In one embodiment, the IL-2 immunoconjugate comprises a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 49, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 50, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 51. In one embodiment, the IL-2 immunoconjugate comprises a polypeptide comprising the sequence of SEQ ID NO: 49, a

polypeptide comprising the sequence of SEQ ID NO: 50, and a polypeptide comprising the sequence of SEQ ID NO: 51. (FAP-IL2v)

[0039] In one embodiment, the IL-2 immunoconjugate comprises cergutuzumab amunaleukin.

[0040] In some embodiments of the methods, uses, compositions, and kits described above and herein, the CD40 agonist is an antibody that specifically binds to CD40. In some embodiments, the CD40 agonist is and antibody that specifically binds to and activates human CD40. In some embodiments, the antibody comprises a heavy chain variable region comprising a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 57, and/or a light chain variable region comprising a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a heavy chain variable region comprising the heavy chain complementarity determining region (HCDR) 1, HCDR 2 and HCDR 3 from the heavy chain variable region sequence of SEQ ID NO: 57, and/or a light chain variable region comprising the light chain complementarity determining region (LCDR) 1, LCDR 2 and LCDR 3 from the light chain variable region sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 57 and/or a light chain variable region comprising the sequence of SEQ ID NO: 58.

[0041] In some embodiments, the antibody that specifically binds to CD40 is a full-length antibody. In some embodiments, the antibody is an IgG class antibody, particularly an IgG2 subclass antibody, more particularly a human IgG2 subclass antibody.

[0042] In one embodiment, the antibody comprises a heavy chain polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 59, and a light chain polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 60. In one embodiment, the antibody comprises a heavy chain polypeptide comprising the sequence of SEQ ID NO: 59, and a light chain polypeptide comprising the sequence of SEQ ID NO: 60.

[0043] In some embodiments of the methods, uses, compositions and kits described above and herein, the cancer is a FAP-positive cancer. In some embodiments, the cancer is a CEA-positive cancer. In some embodiments, the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, prostate cancer, or other cancers described herein. In some

embodiments, the individual has cancer or has been diagnosed with cancer. In some embodiments, the individual has locally advanced or metastatic cancer or has been diagnosed with locally advanced or metastatic cancer. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the expression of PD-L1 may be determined by an immunohistochemistry (IHC) assay.

[0044] In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment or administration of the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist may result in a response in the individual. In some embodiments, the response is a complete response. In some embodiments, the response is a sustained response after cessation of the treatment. In some embodiments, the response is a complete response that is sustained after cessation of the treatment. In other embodiments, the response is a partial response. In some embodiments, the response is a partial response that is sustained after cessation of the treatment.

[0045] In some embodiments of the methods, uses, compositions, and kits described above and herein, the IL-2 immunoconjugate is administered before the CD40 agonist, simultaneous with the CD40 agonist, or after the CD40 agonist. The PD-1 axis binding antagonist may be administered before, in between, after or simultaneous with the IL-2 immunoconjugate and the CD40 agonist.

[0046] In some embodiments, the IL-2 immunoconjugate, the CD40 agonist, and optionally the PD-1 axis binding antagonist are in the same composition. In some embodiments, the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist are in separate compositions.

[0047] In some embodiments of the methods, uses, compositions, and kits described above and herein, the IL-2 immunoconjugate, the CD40 agonist and/or the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the IL-2 immunoconjugate, the CD40 agonist and/or the PD-1 axis binding antagonist is administered intravenously. In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment further comprises administering a chemotherapeutic agent for treating or delaying progression of cancer in an individual. In some embodiments, the individual has been treated with a chemotherapeutic agent before the combination treatment with the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist. In some embodiments, the individual treated with the combination of the IL-2

immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist is refractory to a chemotherapeutic agent treatment. In some embodiments, the individual treated with the combination of the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist is intolerant to a chemotherapeutic agent treatment. Some embodiments of the methods, uses, compositions, and kits described throughout the application, further comprise administering a chemotherapeutic agent for treating or delaying progression of cancer.

[0048] In some embodiments of the methods, uses, compositions and kits described above and herein, CD8 T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity relative to prior to the administration of the combination of the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist. In some embodiments, the number of CD8 T cells is elevated relative to prior to administration of the combination of the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist. In some embodiments, the CD8 T cell is an antigen-specific CD8 T cell. In some embodiments, Treg function is suppressed relative to prior to the administration of the combination of the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist. In some embodiments, T cell exhaustion is decreased relative to prior to the administration of the combination of the IL-2 immunoconjugate, the CD40 agonist and optionally the PD-1 axis binding antagonist.

[0049] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] Figure 1. Results of an efficacy experiment with FAP-IL2v , CD40 Mab and PD-L1 Mab as single agents and in a combination setting. The Panc02-H7-Fluc transfectant pancreatic carcinoma cell line was injected into the pancreas in Black 6 mice to study survival in a pancreatic orthotopic syngeneic model. The compounds were administered at the following doses: 2 mg/kg FAP-IL2v, 10 mg/kg CD40 Mab and 10 mg/kg PD-L1 Mab. The compounds were injected concomitantly ip once weekly for 3 weeks. (A) Survival curves. (B) Median and overall survival values.

[0051] Figure 2. Bioluminescence imaging of mice shown in Figure 1. Decrease of bioluminescence signal (photons/second) represents tumor inhibition.

.

DETAILED DESCRIPTION

[0052] The inventors of this application demonstrated that an IL-2 immunoconjugate, a CD40 agonist and optionally anti-PD-L1 immune therapy act synergistically in their anti-cancer properties and their combination could provide meaningful clinical benefit in patients with cancer. The data in the application show that the combination of an IL-2 immunoconjugate with a CD40 agonist, and optionally further with anti-PD-L1 immune therapy, resulted in enhanced median and overall survival as well as inhibition of tumor growth.

[0053] In one aspect, provided herein are methods, compositions and uses for treating or delaying progression of cancer in an individual comprising administering an effective amount of an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding antagonist.

[0054] In another aspect, provided herein are methods, compositions and uses for enhancing immune function in an individual having cancer comprising administering an effective amount of an IL-2 immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

I. Definitions

[0055] Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0056] As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a molecule” optionally includes a combination of two or more such molecules, and the like.

[0057] As used herein, the terms "*first*", "*second*", “*third*” etc. with respect to antigen binding domains etc., are used for convenience of distinguishing when there is more than one of each type of domain. Use of these terms is not intended to confer a specific order or orientation unless explicitly so stated.

[0058] The term "*about*" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0059] It is understood that aspects and embodiments of the invention described herein include "*comprising*," "*consisting*," and "*consisting essentially of*" aspects and embodiments.

[0060] The term "*PD-1 axis binding antagonist*" refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis – with a result being to restore or enhance T-cell function (*e.g.*, proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. A "*human*" PD-1 axis binding antagonist refers to a PD-1 axis binding antagonist which has the above-described effects on the human PD-1 signaling axis.

[0061] The term "*PD-1 binding antagonist*" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (pembrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is MEDI-0680 (AMP-514) described herein. In another specific aspect, a PD-1 binding antagonist is PDR001 described herein. In another specific aspect, a PD-1

binding antagonist is REGN2810 described herein. In another specific aspect, a PD-1 binding antagonist is BGB-108 described herein.

[0062] The term "*PD-L1 binding antagonist*" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is MPDL3280A (atezolizumab) described herein. In still another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 (durvalumab) described herein. In still another specific aspect, an anti-PD-L1 antibody is MSB0010718C (avelumab) described herein.

[0063] The term "*PD-L2 binding antagonist*" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T

lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

[0064] The term "*dysfunction*" in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both *exhaustion* and/or *anergy* in which antigen recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth.

[0065] The term "*dysfunctional*", as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into down-stream T-cell effector functions, such as proliferation, cytokine production (e.g., IL-2) and/or target cell killing.

[0066] The term "*anergy*" refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (*e.g.* increase in intracellular Ca^{2+} in the absence of ras-activation). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of costimulation. The unresponsive state can often be overriden by the presence of interleukin-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

[0067] The term "*exhaustion*" refers to T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Exhaustion can result from both extrinsic negative regulatory pathways (e.g., immunoregulatory cytokines) as well as cell intrinsic negative regulatory (costimulatory) pathways (PD-1, B7-H3, B7-H4, etc.).

[0068] "*Enhancing T-cell function*" means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include: increased secretion of γ-interferon from $CD8^+$ T-cells, increased proliferation, increased antigen responsiveness (*e.g.*, viral, pathogen, or tumor clearance) relative to such levels before the intervention. In one embodiment, the level of enhancement is as least 50%, alternatively 60%, 70%, 80%, 90%,

100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

[0069] A "*T cell dysfunctional disorder*" is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation. In a particular embodiment, a T-cell dysfunctional disorder is a disorder that is specifically associated with inappropriate increased signaling through PD-1. In another embodiment, a T-cell dysfunctional disorder is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In a specific aspect, the decreased responsiveness results in ineffective control of a pathogen or tumor expressing an immunogen. Examples of T cell dysfunctional disorders characterized by T-cell dysfunction include unresolved acute infection, chronic infection and tumor immunity.

[0070] *"Tumor immunity"* refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

[0071] *"Immunogenicity"* refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response. Examples of enhancing tumor immunogenicity include treatment with an IL-2 immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

[0072] "*Sustained response*" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the *beginning* of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5X, 2.0X, 2.5X, or 3.0X length of the treatment duration.

[0073] The term "*pharmaceutical composition*" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Preferably, such compositions are sterile.

[0074] A "*pharmaceutically acceptable carrier*" refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0075] As used herein, the term "*treatment*" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0076] As used herein, "*delaying progression of a disease*" means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0077] An "*effective amount*" is at least the minimum amount required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (*i.e.*, slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be

administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0078] As used herein, "*in conjunction with*" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0079] A "*disorder*" is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

[0080] The terms "*cell proliferative disorder*" and "*proliferative disorder*" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer. In one embodiment, the cell proliferative disorder is a tumor.

[0081] "*Tumor*", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "*cancer*", "*cancerous*", "cell *proliferative* disorder", "*proliferative* disorder" and "*tumor*" are not mutually exclusive as referred to herein.

[0082] The terms "*cancer*" and "*cancerous*" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer,

endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, glioblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma and breast carcinoma, including metastatic forms of those cancers. In some embodiments, the cancer is a CEA-positive cancer.

[0083] The term "*cytotoxic agent*" as used herein refers to any agent that is detrimental to cells (e.g., causes cell death, inhibits proliferation, or otherwise hinders a cellular function). Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Exemplary cytotoxic agents can be selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors,

immunotherapeutic agents, proapoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signalling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. In one embodiment the cytotoxic agent is a taxane. In one embodiment the taxane is paclitaxel or docetaxel. In one embodiment the cytotoxic agent is a platinum agent. In one embodiment the cytotoxic agent is an antagonist of EGFR. In one embodiment the antagonist of EGFR is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (*e.g.*, erlotinib). In one embodiment the cytotoxic agent is a RAF inhibitor. In one embodiment, the RAF inhibitor is a BRAF and/or CRAF inhibitor. In one embodiment the RAF inhibitor is vemurafenib. In one embodiment the cytotoxic agent is a PI3K inhibitor.

[0084] "*Chemotherapeutic agent*" includes compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, epigallocatechin gallate , salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX®, AstraZeneca), sunitib (SUTENT®, Pfizer/Sugen), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), finasunate (VATALANIB®, Novartis), oxaliplatin (ELOXATIN®, Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR®, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5α-reductases including finasteride and dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan,

novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (*Angew Chem. Intl. Ed. Engl.* **1994** 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® (docetaxel, doxetaxel; Sanofi-Aventis); chlorambucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as

cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0085] Chemotherapeutic agent also includes (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, iodoxyfene , 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide and goserelin; buserelin, tripterelin, medroxyprogesterone acetate, diethylstilbestrol, premarin, fluoxymesterone, all transretionic acid, fenretinide, as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (*e.g.*, ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; a topoisomerase 1 inhibitor such as LURTOTECAN®; ABARELIX® rmRH; and (ix) pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0086] Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab,

efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti–interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG_1 λ antibody genetically modified to recognize interleukin-12 p40 protein.

[0087] Chemotherapeutic agent also includes "EGFR inhibitors," which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an "EGFR antagonist." Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) *(see*, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (*see* WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto *et al. Eur. J. Cancer* 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3 and E7.6. 3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns *et al., J. Biol. Chem.* 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP659439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599,

6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimidine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3 fluorophenyl)methoxy]phenyl]-6[5[[[2methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine).

[0088] Chemotherapeutic agents also include "tyrosine kinase inhibitors" including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines,

such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules *(e.g.* those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); imatinib mesylate (GLEEVEC®); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Pfizer); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone), rapamycin (sirolimus, RAPAMUNE®); or as described in any of the following patent publications: US Patent No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

[0089] Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, amphotericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa, denileukin, dexrazoxane, epoetin alfa, elotinib, filgrastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methoxsalen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

[0090] Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine,

ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychloroquine, leflunomideminocycline, sulfasalazine, tumor necrosis factor alpha (TNFα) blockers such as etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers such as anakinra (Kineret), T cell costimulation blockers such as abatacept (Orencia), Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMRA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon alpha (IFN) blockers such as Rontalizumab; Beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTa3 and membrane bound heterotrimer LTa1/β2 blockers such as Anti-lymphotoxin alpha (LTa); radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18- OCH_3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scopolectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine; perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR^{TM}); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN^{TM}) combined with 5-FU and leucovorin.

[0091] Chemotherapeutic agents also include non-steroidal anti-inflammatory drugswith analgesic, antipyretic and anti-inflammatory effects. NSAIDs include non-selective inhibitors of the enzyme cyclooxygenase. Specific examples of NSAIDs include aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen,

acetic acid derivatives such as indomethacin, sulindac, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib. NSAIDs can be indicated for the symptomatic relief of conditions such as rheumatoid arthritis, osteoarthritis, inflammatory arthropathies, ankylosing spondylitis, psoriatic arthritis, Reiter's syndrome, acute gout, dysmenorrhoea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, ileus, and renal colic.

[0092] By "*radiation therapy*" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0093] A "*subject*" or an "*individual*" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human. An individual or subject may be a patient.

[0094] The term "*antibody*" herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0095] An "*isolated*" antibody is one which has been separated from a component of its natural environment, i.e. that is not in its natural milieu. No particular level of purification is required. For example, an isolated antibody can be removed from its native or natural environment. Recombinantly produced antibodies expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant antibodies which have been separated, fractionated, or partially or substantially purified by any suitable technique. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC) methods. For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

[0096] "*Native antibodies*" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is 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. Each light chain has a variable domain at one end (V_L) and a constant domain 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 chain and heavy chain variable domains.

[0097] The term "*constant domain*" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, CH) of the heavy chain and the or CL domain of the light chain.

[0098] The term "*variable region*" or "*variable domain*" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity. As used herein in connection with variable region sequences, "Kabat numbering" refers to the numbering system set forth by Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

[0099] The "*class*" of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[00100] The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. *Cellular and Mol. Immunology*, 4th ed. (W.B. Saunders, Co., 2000).

[00101] The terms "*full length antibody*," "*intact antibody*" and "*whole antibody*" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[00102] "*Antibody fragments*" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[00103] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0100] "*Fv*" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0101] The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments

originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0102] "*Single-chain Fv*" or "*scFv*" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Plückthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0103] The term "*diabodies*" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0104] The term "*monoclonal antibody*" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprised in the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, 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. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0105] The monoclonal antibodies herein specifically include "*chimeric*" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, *e.g.*, U.S. Pat. No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest.

[0106] "*Humanized*" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, *e.g.*, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0107] A "*human antibody*" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding

residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0108]

The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

[0109] "*Framework*" or "*FR*" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following order in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0110] The term "*variable domain residue numbering as in Kabat*" or "*amino acid position numbering as in Kabat*," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0111] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat *et al.*, *Sequences of Immunological Interest*. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "*EU numbering system*" or "*EU index*" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat *et al.*, *supra*). The "*EU index as in Kabat*" refers to the residue numbering of the human IgG1 EU antibody.

[0112] As use herein, the term "*binds*", "*specifically binds to*" or is "*specific for*" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets, i.e. the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.,* by surface plasmon resonance (SPR). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, or $\leq$ 0.1 nM. In certain embodiments, an antibody specifically binds

to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0113] The term "*antigen binding domain*" refers to the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

[0114] The term "*Fc domain*" or "*Fc region*" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain (also referred to herein as a "cleaved variant heavy chain"). This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to Kabat EU index). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (K447), of the Fc region may or may not be present. Amino acid sequences of heavy chains including Fc domains (or a subunit of an Fc domain as defined herein) are denoted herein without C-terminal glycine-lysine dipeptide if not indicated otherwise. In one embodiment of the invention, a heavy chain including a subunit of an Fc domain as specified herein, e.g. comprised in an immunoconjugate useful in the invention, comprises an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one embodiment of the invention, a heavy chain including a subunit of an Fc domain as specified herein, e.g. comprised in an immunoconjuate useful in the invention, comprises an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat). Compositions of the invention comprise a population of antibodies or immunoconjugates. The population of antibodies or immunoconjugates may comprise molecules having a full-length heavy chain and molecules having a cleaved variant heavy chain. The population of antibodies or immunoconjugates

may consist of a mixture of molecules having a full-length heavy chain and molecules having a cleaved variant heavy chain, wherein at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the antibodies or immunoconjugates have a cleaved variant heavy chain. In one embodiment of the invention, a composition comprising a population of antibodies or immunoconjugates comprises an antibody or immunoconjugate comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one embodiment of the invention, a composition comprising a population of antibodies or immunoconjugates comprises an antibody or immunoconjugate comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat). In one embodiment, such a composition comprises a population of antibodies or immunoconjugates comprised of molecules comprising a heavy chain including a subunit of an Fc domain as specified herein; molecules comprising a heavy chain including a subunit of a Fc domain as specified herein with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat); and molecules comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (see also above). A "subunit" of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain..

[0115] By "*fused*" is meant that the components (e.g. an antibody and an IL-2 polypeptide) are linked by peptide bonds, either directly or via one or more peptide linkers.

[0116] A "*modification promoting the association of the first and the second subunit of the Fc domain*" is a manipulation of the peptide backbone or the post-translational modifications of an Fc domain subunit that reduces or prevents the association of a polypeptide comprising the Fc domain subunit with an identical polypeptide to form a homodimer. A modification promoting association as used herein particularly includes separate modifications made to each of the two Fc domain subunits desired to associate (i.e. the first and the second subunit

of the Fc domain), wherein the modifications are complementary to each other so as to promote association of the two Fc domain subunits. For example, a modification promoting association may alter the structure or charge of one or both of the Fc domain subunits so as to make their association sterically or electrostatically favorable, respectively. Thus, (hetero)dimerization occurs between a polypeptide comprising the first Fc domain subunit and a polypeptide comprising the second Fc domain subunit, which might be non-identical in the sense that further components fused to each of the subunits (e.g. antigen binding moieties) are not the same. In some embodiments the modification promoting association comprises an amino acid mutation in the Fc domain, specifically an amino acid substitution. In a particular embodiment, the modification promoting association comprises a separate amino acid mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

[0117] An "*activating Fc receptor*" is an Fc receptor that following engagement by an Fc region of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32), and FcαRI (CD89).

[0118] The term "*effector functions*" when used in reference to antibodies refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

[0119] Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies or derivatives thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. As used herein, the term "reduced ADCC" is defined as either a reduction in the number of target cells that are lysed in a given time, at a given concentration of antibody in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or an increase in the concentration of antibody in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The reduction in ADCC is relative to the ADCC mediated by the same antibody produced by the same type of host cells, using the same standard production, purification, formulation and storage methods

(which are known to those skilled in the art), but that has not been engineered. For example the reduction in ADCC mediated by an antibody comprising in its Fc domain an amino acid substitution that reduces ADCC, is relative to the ADCC mediated by the same antibody without this amino acid substitution in the Fc domain. Suitable assays to measure ADCC are well known in the art (see e.g. PCT publication no. WO 2006/082515 or PCT publication no. WO 2012/130831).

[0120] As used herein, the terms "*engineer*", "*engineered*", "*engineering*", are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of the amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches.

[0121] As used herein, the term "*immunoconjugate*" refers to a polypeptide molecule that includes at least one IL-2 molecule and at least one antibody. The IL-2 molecule can be joined to the antibody by a variety of interactions and in a variety of configurations as described herein. In particular embodiments, the IL-2 molecule is fused to the antibody via a peptide linker. Particular immunoconjugates useful in the invention essentially consist of one IL-2 molecule and an antibody joined by one or more linker sequences.

[0122] "*Reduction*" (and grammatical variations thereof such as "*reduce*" or "*reducing*"), for example reduction of binding, refers to a decrease in the respective quantity, as measured by appropriate methods known in the art. For clarity the term includes also reduction to zero (or below the detection limit of the analytical method), i.e. complete abolishment or elimination. Conversely, "increased" refers to an increase in the respective quantity. "*Reduced binding*", for example, refers to a decrease in affinity for the respective interaction, as measured for example by SPR, and includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e. complete abolishment of the interaction. Conversely, "increased binding" refers to an increase in binding affinity for the respective interaction.

[0123] The term "*interleukin-2*" or "*IL-2*" as used herein, refers to any native IL-2 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses unprocessed IL-2 as well as any form of IL-2 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-2, e.g. splice variants or allelic variants. The amino acid sequence of an exemplary human IL-2 is shown in SEQ ID NO: 52. Unprocessed human IL-2

additionally comprises an N-terminal 20 amino acid signal peptide having the sequence of SEQ ID NO: 55, which is absent in the mature IL-2 molecule.

[0124] The term "*IL-2 mutant*" or "*mutant IL-2 polypeptide*" as used herein is intended to encompass any mutant forms of various forms of the IL-2 molecule including full-length IL-2, truncated forms of IL-2 and forms where IL-2 is linked to another molecule such as by fusion or chemical conjugation. "*Full-length*" when used in reference to IL-2 is intended to mean the mature, natural length IL-2 molecule. For example, full-length human IL-2 refers to a molecule that has 133 amino acids (see e.g. SEQ ID NO: 52). The various forms of IL-2 mutants are characterized in having a at least one amino acid mutation affecting the interaction of IL-2 with CD25. This mutation may involve substitution, deletion, truncation or modification of the wild-type amino acid residue normally located at that position. Mutants obtained by amino acid substitution are preferred. Unless otherwise indicated, an IL-2 mutant may be referred to herein as a mutant IL-2 peptide sequence, a mutant IL-2 polypeptide, a mutant IL-2 protein or a mutant IL-2 analog.

[0125] Designation of various forms of IL-2 is herein made with respect to the sequence shown in SEQ ID NO: 52. Various designations may be used herein to indicate the same mutation. For example a mutation from phenylalanine at position 42 to alanine can be indicated as 42A, A42, A_{42}, F42A, or Phe42Ala.

[0126] By a "*human IL-2 molecule*" as used herein is meant an IL-2 molecule comprising an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95% or at least about 96% identical to the human IL-2 sequence of SEQ ID NO:52. Particularly, the sequence identity is at least about 95%, more particularly at least about 96%. In particular embodiments, the human IL-2 molecule is a full-length IL-2 molecule.

[0127] The term "*amino acid mutation*" as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g. reduced binding to CD25. Amino acid sequence deletions and insertions include amino- and/or carboxy-terminal deletions and insertions of amino acids. An example of a terminal deletion is the deletion of the alanine residue in position 1 of full-length human IL-2. Preferred amino acid mutations are amino acid substitutions. For the purpose of altering e.g. the binding characteristics of an IL-2 polypeptide, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties, are

particularly preferred. Preferred amino acid substitions include replacing a hydrophobic by a hydrophilic amino acid. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids (e.g. 4-hydroxyproline, 3-methylhistidine, ornithine, homoserine, 5-hydroxylysine). Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful.

[0128] As used herein, a "*wild-type*" form of IL-2 is a form of IL-2 that is otherwise the same as the mutant IL-2 polypeptide except that the wild-type form has a wild-type amino acid at each amino acid position of the mutant IL-2 polypeptide. For example, if the IL-2 mutant is the full-length IL-2 (i.e. IL-2 not fused or conjugated to any other molecule), the wild-type form of this mutant is full-length native IL-2. If the IL-2 mutant is a fusion between IL-2 and another polypeptide encoded downstream of IL-2 (e.g. an antibody chain) the wild-type form of this IL-2 mutant is IL-2 with a wild-type amino acid sequence, fused to the same downstream polypeptide. Furthermore, if the IL-2 mutant is a truncated form of IL-2 (the mutated or modified sequence within the non-truncated portion of IL-2) then the wild-type form of this IL-2 mutant is a similarly truncated IL-2 that has a wild-type sequence. For the purpose of comparing IL-2 receptor binding affinity or biological activity of various forms of IL-2 mutants to the corresponding wild-type form of IL-2, the term wild-type encompasses forms of IL-2 comprising one or more amino acid mutation that does not affect IL-2 receptor binding compared to the naturally occurring, native IL-2, such as e.g. a substitution of cysteine at a position corresponding to residue 125 of human IL-2 to alanine. In some embodiments wild-type IL-2 for the purpose of the present invention comprises the amino acid substitution C125A (see SEQ ID NO: 54). In certain embodiments according to the invention the wild-type IL-2 polypeptide to which the mutant IL-2 polypeptide is compared comprises the amino acid sequence of SEQ ID NO: 52. In other embodiments the wild-type IL-2 polypeptide to which the mutant IL-2 polypeptide is compared comprises the amino acid sequence of SEQ ID NO: 54.

[0129] The term "*CD25*" or "*α-subunit of the IL-2 receptor*" as used herein, refers to any native CD25 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length", unprocessed CD25 as well as any form of CD25 that results from processing in the

cell. The term also encompasses naturally occurring variants of CD25, e.g. splice variants or allelic variants. In certain embodiments CD25 is human CD25. The amino acid sequence of human CD25 is found e.g. in UniProt entry no. P01589 (version 185).

[0130] The term "*high-affinity IL-2 receptor*" as used herein refers to the heterotrimeric form of the IL-2 receptor, consisting of the receptor γ-subunit (also known as common cytokine receptor γ-subunit, γ_c, or CD132, see UniProt entry no. P14784 (version 192)), the receptor β-subunit (also known as CD122 or p70, see UniProt entry no. P31785 (version 197)) and the receptor α-subunit (also known as CD25 or p55, see UniProt entry no. P01589 (version 185)). The term "intermediate-affinity IL-2 receptor" by contrast refers to the IL-2 receptor including only the γ-subunit and the β-subunit, without the α-subunit (for a review see e.g. Olejniczak and Kasprzak, Med Sci Monit 14, RA179-189 (2008)).

[0131] "*Affinity*" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., an antigen binding moiety and an antigen, or a receptor and its ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D), which is the ratio of dissociation and association rate constants (k_{off} and k_{on}, respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by well established methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR). The affinity of the mutant or wild-type IL-2 polypeptide for various forms of the IL-2 receptor can be determined in accordance with the method set forth in WO 2012/107417 by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare) and receptor subunits such as may be obtained by recombinant expression (see e.g. Shanafelt et al., Nature Biotechnol 18, 1197-1202 (2000)). Alternatively, binding affinity of IL-2 mutants for different forms of the IL-2 receptor may be evaluated using cell lines known to express one or the other such form of the receptor. Specific illustrative and exemplary embodiments for measuring binding affinity are described hereinafter.

[0132] By "*regulatory T cell*" or "*T_{reg} cell*" is meant a specialized type of $CD4^+$ T cell that can suppress the responses of other T cells. T_{reg} cells are characterized by expression of the α-subunit of the IL-2 receptor (CD25) and the transcription factor forkhead box P3 (FOXP3) (Sakaguchi, Annu Rev Immunol 22, 531-62 (2004)) and play a critical role in the induction

and maintenance of peripheral self-tolerance to antigens, including those expressed by tumors. T_{reg} cells require IL-2 for their function and development and induction of their suppressive characteristics.

[0133] As used herein, the term "*effector cells*" refers to a population of lymphocytes that mediate the cytotoxic effects of IL-2. Effector cells include effector T cells such as $CD8^+$cytotoxic T cells, NK cells, lymphokine-activated killer (LAK) cells and macrophages/monocytes.

[0134] "*Percent (%) amino acid sequence identity*" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, Clustal W, Megalign (DNASTAR) software or the FASTA program package. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the ggsearch program of the FASTA package version 36.3.8c or later with a BLOSUM50 comparison matrix. The FASTA program package was authored by W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448; W. R. Pearson (1996) "Effective protein sequence comparison" Meth. Enzymol. 266:227- 258; and Pearson et. al. (1997) Genomics 46:24-36, and is publicly available from http://fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml. Alternatively, a public server accessible at http://fasta.bioch.virginia.edu/fasta_www2/index.cgi can be used to compare the sequences, using the ggsearch (global protein:protein) program and default options (BLOSUM50; open: -10; ext: -2; Ktup = 2) to ensure a global, rather than local, alignment is performed. Percent amino acid identity is given in the output alignment header.

[0135] As used herein, term "*polypeptide*" refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein", "amino acid chain", or any other term used to refer to a chain of two or more amino acids, are

included within the definition of "polypeptide", and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded.

[0136] The term "*immunoglobulin molecule*" refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable region, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a light chain variable region, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called α (IgA), δ (IgD), ε (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. γ_1 (IgG_1), γ_2 (IgG_2), γ_3 (IgG_3), γ_4 (IgG_4), α_1 (IgA_1) and α_2 (IgA_2). The light chain of an immunoglobulin may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

[0137] A "*CD40 agonist*" as used herein includes any moiety that agonizes the CD40/CD40L interaction. Typically these moieties will be agonistic CD40 antibodies or agonistic CD40L polypeptides. An "*agonist*" combines with a receptor on a cell and initiates a reaction or activity that is similar to or the same as that initiated by a natural ligand of the receptor. A "*CD40 agonist*" may induce any or all of, but not limited to, the following responses: B cell proliferation and/or differentiation; upregulation of intercellular adhesion

via such molecules as ICAM- 1, E-selectin, VC AM, and the like; secretion of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, TNF, and the like; signal transduction through the CD40 receptor by such pathways as TRAF (e.g., TRAF2 and/or TRAF3), MAP kinases such as NIK (NF-kB inducing kinase), I-kappa B kinases (IKK /.beta.), transcription factor NF-kB, Ras and the MEK/ERK pathway, the PI3K AKT pathway, the P38 MAPK pathway, and the like; transduction of an anti-apoptotic signal by such molecules as XIAP, mcl-1, bcl-x, and the like; B and/or T cell memory generation; B cell antibody production; B cell isotype switching, up-regulation of cell-surface expression of MHC Class II and CD80/86, and the like. By agonist activity is intended an agonist activity of at least 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the agonist activity induced by a negative control as measured in an assay of a B cell response. In another embodiment an CD40 agonist has an agonist activity that is at least 2- fold greater or at least 3-fold greater than the agonist activity induced by a negative control as measured in an assay of a B cell response. Thus, for example, where the B cell response of interest is B cell proliferation, agonist activity would be induction of a level of B cell proliferation that is at least 2-fold greater or at least 3-fold greater than the level of B cell proliferation induced by a negative control.

II. IL-2 immunoconjugates

[00104] Examples of IL-2 immunoconjugates useful for the methods, uses, compositions and kits of the invention, and methods for making thereof, are described in PCT publication no. WO 2012/107417 and WO 2012/146628, each incorporated herein by reference in its entirety.

[00105] In some embodiments of the methods, uses, compositions, and kits described above and herein, the IL-2 immunoconjugate comprises an antibody that specifically binds to a tumor antigen, and an IL-2 polypeptide.

IL-2 polypeptides comprised in immunoconjugates

[00106] Immunoconjugates useful in the present invention comprise an IL-2 polypeptide. In some embodiments, the IL-2 polypeptide is a human IL-2 polypeptide. In some embodiments, the IL-2 polypeptide is a human IL-2 polypeptide wherein the cysteine at position 125 is replaced with a neutral amino acid such as serine (C125S), alanine (C125A), threonine (C125T) or valine (C125V).

[00107] Particularly useful immunoconjugates for the present invention comprise a mutant IL-2 polypeptide having advantageous properties for immunotherapy. In particular, pharmacological properties of IL-2 that contribute to toxicity but are not essential for efficacy of IL-2 are eliminated in the mutant IL-2 polypeptide. Such mutant IL-2 polypeptides are described in detail in WO 2012/107417, which is incorporated herein by reference in its entirety. As discussed above, different forms of the IL-2 receptor consist of different subunits and exhibit different affinities for IL-2. The intermediate-affinity IL-2 receptor, consisting of the β and γ receptor subunits, is expressed on resting effector cells and is sufficient for IL-2 signaling. The high-affinity IL-2 receptor, additionally comprising the α-subunit of the receptor, is mainly expressed on regulatory T (T_{reg}) cells as well as on activated effector cells where its engagement by IL-2 can promote T_{reg} cell-mediated immunosuppression or activation-induced cell death (AICD), respectively. Thus, without wishing to be bound by theory, reducing or abolishing the affinity of IL-2 to the α-subunit of the IL-2 receptor should reduce IL-2 induced downregulation of effector cell function by regulatory T cells and development of tumor tolerance by the process of AICD. On the other hand, maintaining the affinity to the intermediate-affinity IL-2 receptor should preserve the induction of proliferation and activation of effector cells like NK and T cells by IL-2.

[00108] The mutant interleukin-2 (IL-2) polypeptide comprised in the immunoconjugate useful in the present invention comprises at least one amino acid mutation that abolishes or reduces affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor and preserves affinity of the mutant IL-2 polypeptide to the intermediate-affinity IL-2 receptor each compared to a wild-type IL-2 polypeptide.

[00109] Mutants of human IL-2 (hIL-2) with decreased affinity to CD25 may for example be generated by amino acid substitution at amino acid position 35, 38, 42, 43, 45 or 72 or combinations thereof (numbering relative to the human IL-2 sequence SEQ ID NO: 52). Exemplary amino acid substitutions include K35E, K35A, R38A, R38E, R38N, R38F, R38S, R38L, R38G, R38Y, R38W, F42L, F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, F42K, K43E, Y45A, Y45G, Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R, Y45K, L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K. Particular IL-2 mutants useful in the immunoconjugates for the present invention comprise an amino acid mutation at an amino acid position corresponding to residue 42, 45, or 72 of human IL-2, or a combination thereof. In one embodiment said amino acid mutation is an amino acid substitution selected from the group of F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, F42K, Y45A, Y45G, Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R, Y45K, L72G,

L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K, more specifically an amino acid substitution selected from the group of F42A, Y45A and L72G. These mutants exhibit substantially similar binding affinity to the intermediate-affinity IL-2 receptor, and have substantially reduced affinity to the α-subunit of the IL-2 receptor and the high-affinity IL-2 receptor compared to a wild-type form of the IL-2 mutant.

[00110] Other characteristics of useful mutants may include the ability to induce proliferation of IL-2 receptor-bearing T and/or NK cells, the ability to induce IL-2 signaling in IL-2 receptor-bearing T and/or NK cells, the ability to generate interferon (IFN)-γ as a secondary cytokine by NK cells, a reduced ability to induce elaboration of secondary cytokines – particularly IL-10 and TNF-α – by peripheral blood mononuclear cells (PBMCs), a reduced ability to activate regulatory T cells, a reduced ability to induce apoptosis in T cells, and a reduced toxicity profile in vivo.

[00111] Particular mutant IL-2 polypeptides useful in the IL-2 immunoconjugates for the present invention comprise three amino acid mutations that abolish or reduce affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor but preserve affinity of the mutant IL-2 polypeptide to the intermediate affinity IL-2 receptor. In one embodiment said three amino acid mutations are at positions corresponding to residue 42, 45 and 72 of human IL-2. In one embodiment said three amino acid mutations are amino acid substitutions. In one embodiment said three amino acid mutations are amino acid substitutions selected from the group of F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, F42K, Y45A, Y45G, Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R, Y45K, L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K. In a specific embodiment said three amino acid mutations are amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence of SEQ ID NO: 52).

[00112] In certain embodiments said amino acid mutation reduces the affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor by at least 5-fold, specifically at least 10-fold, more specifically at least 25-fold. In embodiments where there is more than one amino acid mutation that reduces the affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor, the combination of these amino acid mutations may reduce the affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor by at least 30-fold, at least 50-fold, or even at least 100-fold. In one embodiment said amino acid mutation or combination of amino acid mutations abolishes the affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor so that no binding is detectable by surface plasmon resonance.

[00113] Substantially similar binding to the intermediate-affinity receptor, i.e. preservation of the affinity of the mutant IL-2 polypeptide to said receptor, is achieved when the IL-2 mutant exhibits greater than about 70% of the affinity of a wild-type form of the IL-2 mutant to the intermediate-affinity IL-2 receptor. IL-2 mutants useful in the invention may exhibit greater than about 80% and even greater than about 90% of such affinity.

[00114] Reduction of the affinity of IL-2 for the α-subunit of the IL-2 receptor in combination with elimination of the O-glycosylation of IL-2 results in an IL-2 protein with improved properties. For example, elimination of the O-glycosylation site results in a more homogenous product when the mutant IL-2 polypeptide is expressed in mammalian cells such as CHO or HEK cells.

[00115] Thus, in certain embodiments the mutant IL-2 polypeptide comprises an additional amino acid mutation which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2. In one embodiment said additional amino acid mutation which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2 is an amino acid substitution. Exemplary amino acid substitutions include T3A, T3G, T3Q, T3E, T3N, T3D, T3R, T3K, and T3P. In a specific embodiment, said additional amino acid mutation is the amino acid substitution T3A.

[00116] In certain embodiments the mutant IL-2 polypeptide is essentially a full-length IL-2 molecule. In certain embodiments the mutant IL-2 polypeptide is a human IL-2 molecule. In one embodiment the mutant IL-2 polypeptide comprises the sequence of SEQ ID NO: 52 with at least one amino acid mutation that abolishes or reduces affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor but preserve affinity of the mutant IL-2 polypeptide to the intermediate affinity IL-2 receptor, compared to an IL-2 polypeptide comprising SEQ ID NO: 52 without said mutation. In another embodiment, the mutant IL-2 polypeptide comprises the sequence of SEQ ID NO: 54 with at least one amino acid mutation that abolishes or reduces affinity of the mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor but preserve affinity of the mutant IL-2 polypeptide to the intermediate affinity IL-2 receptor, compared to an IL-2 polypeptide comprising SEQ ID NO: 54 without said mutation.

[00117] In a specific embodiment, the mutant IL-2 polypeptide can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated killer (LAK) antitumor cytotoxicity.

[00118] In one embodiment the mutant IL-2 polypeptide has a reduced ability to induce IL-2 signaling in regulatory T cells, compared to a wild-type IL-2 polypeptide. In one embodiment the mutant IL-2 polypeptide induces less activation-induced cell death (AICD) in T cells, compared to a wild-type IL-2 polypeptide. In one embodiment the mutant IL-2 polypeptide has a reduced toxicity profile in vivo, compared to a wild-type IL-2 polypeptide. In one embodiment the mutant IL-2 polypeptide has a prolonged serum half-life, compared to a wild-type IL-2 polypeptide.

[00119] A particular mutant IL-2 polypeptide useful in the IL-2 immunoconjugates for the present invention comprises four amino acid substitutions at positions corresponding to residues 3, 42, 45 and 72 of human IL-2. Specific amino acid substitutions are T3A, F42A, Y45A and L72G. As demonstrated in WO 2012/107417, said quadruple mutant IL-2 polypeptide exhibits no detectable binding to CD25, reduced ability to induce apoptosis in T cells, reduced ability to induce IL-2 signaling in T_{reg} cells, and a reduced toxicity profile in vivo. However, it retains ability to activate IL-2 signaling in effector cells, to induce proliferation of effector cells, and to generate IFN-γ as a secondary cytokine by NK cells.

[00120] Moreover, said mutant IL-2 polypeptide has further advantageous properties, such as reduced surface hydrophobicity, good stability, and good expression yield, as described in WO 2012/107417. Unexpectedly, said mutant IL-2 polypeptide also provides a prolonged serum half-life, compared to wild-type IL-2.

[00121] IL-2 mutants useful in the invention, in addition to having mutations in the region of IL-2 that forms the interface of IL-2 with CD25 or the glycosylation site, also may have one or more mutations in the amino acid sequence outside these regions. Such additional mutations in human IL-2 may provide additional advantages such as increased expression or stability. For example, the cysteine at position 125 may be replaced with a neutral amino acid such as serine, alanine, threonine or valine, yielding C125S IL-2, C125A IL-2, C125T IL-2 or C125V IL-2 respectively, as described in U.S. Patent no. 4,518,584. As described therein, one may also delete the N-terminal alanine residue of IL-2 yielding such mutants as des-A1 C125S or des-A1 C125A. Alternatively or conjunctively, the IL-2 mutant may include a mutation whereby methionine normally occurring at position 104 of wild-type human IL-2 is replaced by a neutral amino acid such as alanine (see U.S. Patent no. 5,206,344). The resulting mutants, e. g., des-A1 M104A IL-2, des-A1 M104A C125S IL-2, M104A IL-2, M104A C125A IL-2, des-A1 M104A C125A IL-2, or M104A C125S IL-2 (these and other mutants may be found in U.S. Patent No. 5,116,943 and in Weiger et al., Eur J Biochem 180,

295-300 (1989)) may be used in conjunction with the particular IL-2 mutations described hereinabove.

[00122] Thus, in certain embodiments the mutant IL-2 polypeptide comprises an additional amino acid mutation at a position corresponding to residue 125 of human IL-2. In one embodiment said additional amino acid mutation is the amino acid substitution C125A.

[00123] The skilled person will be able to determine which additional mutations may provide additional advantages for the purpose of the invention. For example, he will appreciate that amino acid mutations in the IL-2 sequence that reduce or abolish the affinity of IL-2 to the intermediate-affinity IL-2 receptor, such as D20T, N88R or Q126D (see e.g. US 2007/0036752), may not be suitable to include in the mutant IL-2 polypeptide.

[00124] In one embodiment, the mutant IL-2 polypeptide comprises no more than 12, no more than 11, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, or no more than 5 amino acid mutations as compared to the corresponding wild-type IL-2 sequence, e.g. the human IL-2 sequence of SEQ ID NO: 52. In a particular embodiment, the mutant IL-2 polypeptide comprises no more than 5 amino acid mutations as compared to the corresponding wild-type IL-2 sequence, e.g. the human IL-2 sequence of SEQ ID NO: 52.

[00125] In one embodiment the mutant IL-2 polypeptide comprises the sequence of SEQ ID NO: 53. In one embodiment the mutant IL-2 polypeptide consists of the sequence of SEQ ID NO: 53.

Immunoconjugate formats

[00126] Immunoconjugates useful in the present invention comprise an IL-molecule and an antibody. Such immunoconjugates significantly increase the efficacy of IL-2 therapy by directly targeting IL-2 e.g. into a tumor microenvironment. An antibody comprised in the immunoconjugate can be a whole antibody or immunoglobulin, or a portion or variant thereof that has a biological function such as antigen specific binding affinity.

[00127] The benefits of immunoconjugate therapy are readily apparent. For example, an antibody comprised in an immunoconjugate recognizes a tumor-specific epitope and results in targeting of the immunoconjugate molecule to the tumor site. Therefore, high concentrations of IL-2 can be delivered into the tumor microenvironment, thereby resulting in activation and proliferation of a variety of immune effector cells mentioned herein using a much lower dose of the immunoconjugate than would be required for unconjugated IL-2. Moreover, since application of IL-2 in form of immunoconjugates allows lower doses of the cytokine itself, the potential for undesirable side effects of IL-2 is restricted, and targeting the

IL-2 to a specific site in the body by means of an immunoconjugate may also result in a reduction of systemic exposure and thus less side effects than obtained with unconjugated IL-2. In addition, the increased circulating half-life of an immunoconjugate compared to unconjugated IL-2 contributes to the efficacy of the immunoconjugate. However, this characteristic of IL-2 immunoconjugates may again aggravate potential side effects of the IL-2 molecule: Because of the significantly longer circulating half-life of IL-2 immunoconjugate in the bloodstream relative to unconjugated IL-2, the probability for IL-2 or other portions of the fusion protein molecule to activate components generally present in the vasculature is increased. The same concern applies to other fusion proteins that contain IL-2 fused to another moiety such as Fc or albumin, resulting in an extended half-life of IL-2 in the circulation. Therefore an immunoconjugate comprising a mutant IL-2 polypeptide as described herein and in WO 2012/107417, with reduced toxicity compared to wild-type forms of IL-2, is particularly advantageous.

[00128] Accordingly, particularly useful in the invention is an IL-2 immunoconjugate comprising a mutant IL-2 polypeptide as described hereinbefore, and an antibody that binds to a target antigen. In one embodiment the (mutant) IL-2 polypeptide and the antibody form a fusion protein, i.e. the (mutant) IL-2 polypeptide shares a peptide bond with the antibody. In some embodiments, the antibody comprises an Fc domain composed of a first and a second subunit. In a specific embodiment the (mutant) IL-2 polypeptide is fused at its amino-terminal amino acid to the carboxy-terminal amino acid of one of the subunits of the Fc domain, optionally through a linker peptide. In some embodiments, the antibody is a full-length antibody. In some embodiments, the antibody is an immunoglobulin molecule, particularly an IgG class immunoglobulin molecule, more particularly an IgG_1 subclass immunoglobulin molecule. In one such embodiment, the (mutant) IL-2 polypeptide shares an amino-terminal peptide bond with one of the immunoglobulin heavy chains. In certain embodiments the antibody is an antibody fragment. In some embodiments the antibody is a Fab molecule or a scFv molecule. In one embodiment the antibody is a Fab molecule. In another embodiment the antibody is a scFv molecule. The immunoconjugate may also comprise more than one antibody. Where more than one antibody is comprised in the immunoconjugate, e.g. a first and a second antibody, each antibody can be independently selected from various forms of antibodies and antibody fragments. For example, the first antibody can be a Fab molecule and the second antibody can be a scFv molecule. In a specific embodiment each of said first and said second antibodies is a scFv molecule or each of said first and said second antibodies is a Fab molecule. In a particular embodiment each of said

first and said second antibodies is a Fab molecule. In one embodiment each of said first and said second antibodies binds to the same target antigen.

[00129] Exemplary immunoconjugate formats are described in PCT publication no. WO 2011/020783, which is incorporated herein by reference in its entirety. These immunoconjugates comprise at least two antibodies. Thus, in one embodiment, the immunoconjugate useful for the present invention comprises a (mutant) IL-2 polypeptide as described herein, and at least a first and a second antibody. In a particular embodiment, said first and second antibody are independently selected from the group consisting of an Fv molecule, particularly a scFv molecule, and a Fab molecule. In a specific embodiment, said (mutant) IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with said first antibody and said second antibody shares an amino- or carboxy-terminal peptide bond with either i) the (mutant) IL-2 polypeptide or ii) the first antibody. In a particular embodiment, the immunoconjugate consists essentially of a (mutant) IL-2 polypeptide and first and second antibodies, particularly Fab molecules, joined by one or more linker sequences. Such formats have the advantage that they bind with high affinity to the target antigen, but provide only monomeric binding to the IL-2 receptor, thus avoiding targeting the immunoconjugate to IL-2 receptor bearing immune cells at other locations than the target site. In a particular embodiment, a (mutant) IL-2 polypeptide shares a carboxy-terminal peptide bond with a first antibody, particularly a first Fab molecule, and further shares an amino-terminal peptide bond with a second antibody, particularly a second Fab molecule. In another embodiment, a first antibody, particularly a first Fab molecule, shares a carboxy-terminal peptide bond with a (mutant) IL-2 polypeptide, and further shares an amino-terminal peptide bond with a second antibody, particularly a second Fab molecule. In another embodiment, a first antibody, particularly a first Fab molecule, shares an amino-terminal peptide bond with a first (mutant) IL-2 polypeptide, and further shares a carboxy-terminal peptide with a second antibody, particularly a second Fab molecule. In a particular embodiment, a (mutant) IL-2 polypeptide shares a carboxy-terminal peptide bond with a first heavy chain variable region and further shares an amino-terminal peptide bond with a second heavy chain variable region. In another embodiment a (mutant) IL-2 polypeptide shares a carboxy-terminal peptide bond with a first light chain variable region and further shares an amino-terminal peptide bond with a second light chain variable region. In another embodiment, a first heavy or light chain variable region is joined by a carboxy-terminal peptide bond to a (mutant) IL-2 polypeptide and is further joined by an amino-terminal peptide bond to a second heavy or light chain variable region. In another embodiment, a first heavy or light chain variable region is joined by an

amino-terminal peptide bond to a (mutant) IL-2 polypeptide and is further joined by a carboxy-terminal peptide bond to a second heavy or light chain variable region. In one embodiment, a (mutant) IL-2 polypeptide shares a carboxy-terminal peptide bond with a first Fab heavy or light chain and further shares an amino-terminal peptide bond with a second Fab heavy or light chain. In another embodiment, a first Fab heavy or light chain shares a carboxy-terminal peptide bond with a (mutant) IL-2 polypeptide and further shares an amino-terminal peptide bond with a second Fab heavy or light chain. In other embodiments, a first Fab heavy or light chain shares an amino-terminal peptide bond with a (mutant) IL-2 polypeptide and further shares a carboxy-terminal peptide bond with a second Fab heavy or light chain. In one embodiment, the immunoconjugate comprises a (mutant) IL-2 polypeptide sharing an amino-terminal peptide bond with one or more scFv molecules and further sharing a carboxy-terminal peptide bond with one or more scFv molecules.

[00130] Particularly suitable formats for the immunoconjugates useful in the present invention, however comprise an immunoglobulin molecule as antibody. Such immunoconjugate formats are described in WO 2012/146628, which is incorporated herein by reference in its entirety.

[00131] Accordingly, in particular embodiments, the immunoconjugate comprises a (mutant) IL-2 polypeptide as described herein and an immunoglobulin molecule that binds to a target antigen, particularly an IgG molecule, more particularly an IgG_1 molecule. In one embodiment the immunoconjugate comprises not more than one (mutant) IL-2 polypeptide. In one embodiment the immunoglobulin molecule is human. In one embodiment, the immunoglobulin molecule comprises a human constant region, e.g. a human CH1, CH2, CH3 and/or CL domain. In one embodiment, the immunoglobulin comprises a human Fc domain, particularly a human IgG_1 Fc domain. In one embodiment the (mutant) IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with the immunoglobulin molecule. In one embodiment, the immunoconjugate essentially consists of a (mutant) IL-2 polypeptide and an immunoglobulin molecule, particularly an IgG molecule, more particularly an IgG_1 molecule, joined by one or more linker sequences. In a specific embodiment, the (mutant) IL-2 polypeptide is fused at its amino-terminal amino acid to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

[00132] The (mutant) IL-2 polypeptide may be fused to the antibody directly or through a linker peptide, comprising one or more amino acids, typically about 2-20 amino acids. Linker peptides are known in the art and are described herein. Suitable, non-immunogenic linker peptides include, for example, $(G_4S)_n$, $(SG_4)_n$, $(G_4S)_n$ or $G_4(SG_4)_n$ linker peptides. “n” is

generally an integer from 1 to 10, typically from 2 to 4. In one embodiment the linker peptide has a length of at least 5 amino acids, in one embodiment a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In a particular embodiment, the linker peptide has a length of 15 amino acids. In one embodiment the linker peptide is $(GxS)_n$ or $(GxS)_nG_m$ with G=glycine, S=serine, and (x=3, n= 3, 4, 5 or 6, and m=0, 1, 2 or 3) or (x=4, n=2, 3, 4 or 5 and m= 0, 1, 2 or 3), in one embodiment x=4 and n=2 or 3, in a further embodiment x=4 and n=3. In a particular embodiment the linker peptide is $(G_4S)_3$ (SEQ ID NO: 67). In one embodiment, the linker peptide has (or consists of) the amino acid sequence of SEQ ID NO: 67.

[00133] In a particular embodiment, the immunoconjugate comprises a (mutant) IL-2 molecule and an immunoglobulin molecule, particularly an IgG_1 subclass immunoglobulin molecule, that binds to a target antigen, wherein the (mutant) IL-2 molecule is fused at its amino-terminal amino acid to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains through the linker peptide of SEQ ID NO: 67.

[00134] In a particular embodiment, the immunoconjugate comprises a (mutant) IL-2 molecule and an antibody that binds to a target antigen, wherein the antibody comprises an Fc domain, particularly a human IgG_1 Fc domain, composed of a first and a second subunit, and the (mutant) IL-2 molecule is fused at its amino-terminal amino acid to the carboxy-terminal amino acid of one of the subunits of the Fc domain through the linker peptide of SEQ ID NO: 67.

Antibodies comprised in immunoconjugates

[00135] The antibody comprised in the immunoconjugate useful in the invention binds to a target antigen, particularly a human target antigen, and is able to direct the (mutant) IL-2 polypeptide to a target site where the antigen is expressed, particularly to a tumor.

[00136] In some embodiments, the IL-2 immunoconjugate comprises an antibody that specifically binds to Carcinoembryonic Antigen (CEA).

[00137] Alternative names for "CEA" include CEACAM5. The term "*CEA*" as used herein, refers to any native CEA from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length" and unprocessed CEA as well as any form of CEA that results from processing in the cell (e.g., mature protein). The term also encompasses naturally occurring variants and isoforms of CEA, e.g., splice variants or allelic variants. In one embodiment, CEA is human CEA. The amino acid

sequence of human CEA is shown in UniProt (www.uniprot.org) accession no. P06731, or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_004354.2.

[00138] Suitable CEA antibodies that may be used in the immunoconjugate for the invention are described in PCT publication no. WO 2012/117002, which is incorporated herein by reference in its entirety.

[00139] The immunoconjugate may comprise two or more antibodies, which may bind to the same or to different antigens. In particular embodiments, however, each of these antibodies binds to CEA. In one embodiment, the antibody comprised in the immunoconjugate of the invention is monospecific. In a particular embodiment, the immunoconjugate comprises a single, monospecific antibody, particularly a monospecific immunoglobulin molecule.

[00140] The antibody can be any type of antibody or fragment thereof that retains specific binding to CEA, particularly human CEA. Antibody fragments include, but are not limited to, Fv molecules, scFv molecule, Fab molecule, and $F(ab')_2$ molecules. In particular embodiments, however, the antibody is a full-length antibody. In some embodiments, the antibody comprises an Fc domain, composed of a first and a second subunit. In some embodiments, the antibody is an immunoglobulin, particularly an IgG class, more particularly an IgG_1 subclass immunoglobulin.

[00141] In some embodiments, the antibody is a monoclonal antibody.

[00142] In some embodiments, the antibody that specifically binds to CEA comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 38, the HCDR2 of SEQ ID NO: 39, and the HCDR3 of SEQ ID NO: 40; and/or a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 41, the LCDR2 of SEQ ID NO: 42 and the LCDR3 of SEQ ID NO: 43. In some embodiments, the heavy and/or light chain variable region is a humanized variable region. In some embodiments, the heavy and/or light chain variable region comprises human framework regions (FR).

[00143] In some embodiments, the antibody comprises a HCDR 1 comprising the amino acid sequence of SEQ ID NO:38, a HCDR 2 comprising the amino acid sequence of SEQ ID NO:39, a HCDR 3 comprising the amino acid sequence of SEQ ID NO:40, a LCDR 1 comprising the amino acid sequence of SEQ ID NO:41, a LCDR 2 comprising the amino acid sequence of SEQ ID NO:42, and a LCDR 3 comprising the amino acid sequence of SEQ ID NO:43.

[00144] In some embodiments, the antibody comprises (a) a heavy chain variable region (VH) comprising a HCDR 1 comprising the amino acid sequence of SEQ ID NO:38, a HCDR

2 comprising the amino acid sequence of SEQ ID NO:39, and a HCDR 3 comprising the amino acid sequence of SEQ ID NO:40, and (b) a light chain variable region (VL) comprising a LCDR 1 comprising the amino acid sequence of SEQ ID NO:41, a LCDR 2 comprising the amino acid sequence of SEQ ID NO:42, and a LCDR 3 comprising the amino acid sequence of SEQ ID NO:43. In some embodiments, the heavy and/or light chain variable region is a humanized variable region. In some embodiments, the heavy and/or light chain variable region comprises human framework regions (FR).

[00145] In some embodiments, the antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:34. In some embodiments, the antibody comprises a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the antibody comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:34, and (b) a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:35.

[00146] In a particular embodiment, the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 34, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 35.

[00147] In some embodiments, the antibody is a humanized antibody. In one embodiment, the antibody is an immunoglobulin molecule comprising a human constant region, particularly an IgG class immunoglobulin molecule comprising a human CH1, CH2, CH3 and/or CL domain. Exemplary sequences of human constant domains are given in SEQ ID NOs 68 and 69 (human kappa and lambda CL domains, respectively) and SEQ ID NO: 70 (human IgG1 heavy chain constant domains CH1-CH2-CH3). In some embodiments, the antibody comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 68 or SEQ ID NO: 69, particularly the amino acid sequence of SEQ ID NO: 68. In some embodiments, the antibody comprises a heavy chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 70. Particularly, the heavy chain constant region may comprise amino acid mutations in the Fc domain as described herein.

[00148] In some embodiments, the IL-2 immunoconjugate comprises an antibody that specifically binds to Fibroblast Activation Protein (FAP).

[00149] Alternative names for "FAP" include Seprase. The term "*FAP*" as used herein, refers to any native CEA from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length" and unprocessed FAP as well as any form of FAP that results from processing in the cell (e.g., mature protein). The term also encompasses naturally occurring variants and isoforms of FAP, e.g., splice variants or allelic variants. In one embodiment, FAP is human FAP. The amino acid sequence of human FAP is shown in UniProt (www.uniprot.org) accession no. Q12884, or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_004451.

[00150] Suitable FAP antibodies that may be used in the immunoconjugate for the invention are described in PCT publication no. WO 2012/020006, which is incorporated herein by reference in its entirety.

[00151] The immunoconjugate may comprise two or more antibodies, which may bind to the same or to different antigens. In particular embodiments, however, each of these antibodies binds to FAP. In one embodiment, the antibody comprised in the immunoconjugate is monospecific. In a particular embodiment, the immunoconjugate comprises a single, monospecific antibody, particularly a monospecific immunoglobulin molecule.

[00152] The antibody can be any type of antibody or fragment thereof that retains specific binding to FAP, particularly human FAP. Antibody fragments include, but are not limited to, Fv molecules, scFv molecule, Fab molecule, and $F(ab')_2$ molecules. In particular embodiments, however, the antibody is a full-length antibody. In some embodiments, the antibody comprises an Fc domain, composed of a first and a second subunit. In some embodiments, the antibody is an immunoglobulin, particularly an IgG class, more particularly an IgG_1 subclass immunoglobulin.

[00153] In some embodiments, the antibody is a monoclonal antibody.

[00154] In some embodiments, the antibody that specifically binds to FAP comprises a heavy chain variable region comprising a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 47, and/or a light chain variable region comprising a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 48. In some embodiments, the antibody comprises a heavy chain variable region comprising the heavy chain complementarity determining region (HCDR) 1, HCDR 2

and HCDR 3 from the heavy chain variable region sequence of SEQ ID NO: 47, and/or a light chain variable region comprising the light chain complementarity determining region (LCDR) 1, LCDR 2 and LCDR 3 from the light chain variable region sequence of SEQ ID NO: 48. In some embodiments, the heavy and/or light chain variable region is a human variable region. In some embodiments, the heavy and/or light chain variable region comprises human framework regions (FR).

[00155] In some embodiments, the antibody that specifically binds to FAP comprises a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 47, and a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 48. In some embodiments, the antibody comprises the heavy chain complementarity determining region (HCDR) 1, HCDR 2 and HCDR 3 from the heavy chain variable region sequence of SEQ ID NO: 47, and the light chain complementarity determining region (LCDR) 1, LCDR 2 and LCDR 3 from the light chain variable region sequence of SEQ ID NO: 48.

[00156] In some embodiments, the antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the antibody comprises a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:48. In some embodiments, the antibody comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:47, and (b) a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:48.

[00157] In a particular embodiment, the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 47, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 48.

[00158] In some embodiments, the antibody is a human antibody. In one embodiment, the antibody is an immunoglobulin molecule comprising a human constant region, particularly an IgG class immunoglobulin molecule comprising a human CH1, CH2, CH3 and/or CL domain. Exemplary sequences of human constant domains are given in SEQ ID NOs 68 and 69 (human kappa and lambda CL domains, respectively) and SEQ ID NO: 70 (human IgG1 heavy chain constant domains CH1-CH2-CH3). In some embodiments, the antibody comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO:

68 or SEQ ID NO: 69, particularly the amino acid sequence of SEQ ID NO: 68. In some embodiments, the antibody comprises a heavy chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 70. Particularly, the heavy chain constant region may comprise amino acid mutations in the Fc domain as described herein.

Fc domain

[00159] In particular embodiments, the antibody comprised in the immunconjugates useful in the invention comprises an Fc domain, composed of a first and a second subunit. The Fc domain of an antibody consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other. In one embodiment the immunoconjugate useful in the invention comprises not more than one Fc domain.

[00160] In one embodiment the Fc domain of the antibody comprised in the immunoconjugate is an IgG Fc domain. In a particular embodiment the Fc domain is an IgG_1 Fc domain. In another embodiment the Fc domain is an IgG_4 Fc domain. In a more specific embodiment, the Fc domain is an IgG_4 Fc domain comprising an amino acid substitution at position S228 (Kabat EU index numbering), particularly the amino acid substitution S228P. This amino acid substitution reduces *in vivo* Fab arm exchange of IgG_4 antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). In a further particular embodiment the Fc domain is a human Fc domain. In an even more particular embodiment, the Fc domain is a human IgG_1 Fc domain. An exemplary sequence of a human IgG_1 Fc region is given in SEQ ID NO: 66.

Fc domain modifications promoting heterodimerization

[00161] Immunoconjugates useful in the invention comprise a (mutant) IL-2 polypeptide, particularly a single (not more than one) IL-2 polypeptide, fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain are typically comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the immunoconjugate in recombinant

production, it will thus be advantageous to introduce in the Fc domain of the antibody a modification promoting the association of the desired polypeptides.

[00162] Accordingly, in particular embodiments, the Fc domain of the antibody comprised in the immunoconjugate comprises a modification promoting the association of the first and the second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

[00163] There exist several approaches for modifications in the CH3 domain of the Fc domain in order to enforce heterodimerization, which are well described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012058768, WO 2013157954, WO 2013096291. Typically, in all such approaches the CH3 domain of the first subunit of the Fc domain and the CH3 domain of the second subunit of the Fc domain are both engineered in a complementary manner so that each CH3 domain (or the heavy chain comprising it) can no longer homodimerize with itself but is forced to heterodimerize with the complementarily engineered other CH3 domain (so that the first and second CH3 domain heterodimerize and no homodimers between the two first or the two second CH3 domains are formed).

[00164] In a specific embodiment said modification promoting the association of the first and the second subunit of the Fc domain is a so-called "knob-into-hole" modification, comprising a "knob" modification in one of the two subunits of the Fc domain and a "hole" modification in the other one of the two subunits of the Fc domain.

[00165] The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine).

[00166] Accordingly, in a particular embodiment, in the CH3 domain of the first subunit of the Fc domain of the antibody comprised in the immunoconjugate an amino acid residue is

replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

[00167] Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W).

[00168] Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V).

[00169] The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis.

[00170] In a specific embodiment, in the CH3 domain of the first subunit of the Fc domain (the "knobs" subunit) the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the Fc domain (the "hole" subunit) the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In one embodiment, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index).

[00171] In yet a further embodiment, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (particularly the serine residue at position 354 is replaced with a cysteine residue), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index). Introduction of these two cysteine residues results in formation of a disulfide bridge between the two subunits of the Fc domain, further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

[00172] In a particular embodiment, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).

[00173] In some embodiments, the second subunit of the Fc domain additionally comprises the amino acid substitutions H435R and Y436F (numbering according to Kabat EU index).

[00174] In a particular embodiment the mutant IL-2 polypeptide is fused (optionally through a linker peptide) to the first subunit of the Fc domain (comprising the "knob" modification). Without wishing to be bound by theory, fusion of the mutant IL-2 polypeptide to the knob-containing subunit of the Fc domain will (further) minimize the generation of immunoconjugates comprising two mutant IL-2 polypeptides (steric clash of two knob-containing polypeptides).

[00175] Other techniques of CH3-modification for enforcing the heterodimerization are contemplated as alternatives and are described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954, WO 2013/096291.

[00176] In one embodiment the heterodimerization approach described in EP 1870459, is used alternatively. This approach is based on the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH3/CH3 domain interface between the two subunits of the Fc domain. A specific embodiment for the antibody comprised in the immunoconjugate are amino acid mutations R409D; K370E in one of the two CH3 domains (of the Fc domain) and amino acid mutations D399K; E357K in the other one of the CH3 domains of the Fc domain (numbering according to Kabat EU index).

[00177] In another embodiment, the antibody comprised in the immunoconjugate comprises amino acid mutation T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (numberings according to Kabat EU index).

[00178] In another embodiment, the antibody comprised in the immunoconjugate comprises amino acid mutations S354C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations Y349C, T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, or said antibody comprises amino acid mutations Y349C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations S354C, T366S, L368A, Y407V in the CH3 domains of the second subunit of the Fc domain and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit

of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (all numberings according to Kabat EU index).

[00179] In one embodiment, the heterodimerization approach described in WO 2013/157953 is used alternatively. In one embodiment, a first CH3 domain comprises amino acid mutation T366K and a second CH3 domain comprises amino acid mutation L351D (numberings according to Kabat EU index). In a further embodiment, the first CH3 domain comprises further amino acid mutation L351K. In a further embodiment, the second CH3 domain comprises further an amino acid mutation selected from Y349E, Y349D and L368E (preferably L368E) (numberings according to Kabat EU index).

[00180] In one embodiment, the heterodimerization approach described in WO 2012/058768 is used alternatively. In one embodiment, a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment, the second CH3 domain comprises a further amino acid mutation at position T411, D399, S400, F405, N390, or K392, e.g. selected from a) T411N, T411R, T411Q, T411K, T411D, T411E or T411W, b) D399R, D399W, D399Y or D399K, c) S400E, S400D, S400R, or S400K, d) F405I, F405M, F405T, F405S, F405V or F405W, e) N390R, N390K or N390D, f) K392V, K392M, K392R, K392L, K392F or K392E (numberings according to Kabat EU index). In a further embodiment, a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366V, K409F. In a further embodiment a first CH3 domain comprises amino acid mutation Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment, the second CH3 domain further comprises amino acid mutations K392E, T411E, D399R and S400R (numberings according to Kabat EU index).

[00181] In one embodiment the heterodimerization approach described in WO 2011/143545 is used alternatively, e.g. with the amino acid modification at a position selected from the group consisting of 368 and 409 (numbering according to Kabat EU index).

[00182] In one embodiment, the heterodimerization approach described in WO 2011/090762, which also uses the knobs-into-holes technology described above, is used alternatively. In one embodiment, a first CH3 domain comprises amino acid mutation T366W and a second CH3 domain comprises amino acid mutation Y407A. In one embodiment, a first CH3 domain comprises amino acid mutation T366Y and a second CH3 domain comprises amino acid mutation Y407T (numberings according to Kabat EU index).

[00183] In one embodiment, the antibody comprised in the immunoconjugate or its Fc domain is of IgG_2 subclass and the heterodimerization approach described in WO 2010/129304 is used alternatively.

[00184] In an alternative embodiment, a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable. In one such embodiment, a first CH3 domain comprises amino acid substitution of K392 or N392 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K392D or N392D) and a second CH3 domain comprises amino acid substitution of D399, E356, D356, or E357 with a positively charged amino acid (e.g. lysine (K) or arginine (R), preferably D399K, E356K, D356K, or E357K, and more preferably D399K and E356K). In a further embodiment, the first CH3 domain further comprises amino acid substitution of K409 or R409 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K409D or R409D). In a further embodiment, the first CH3 domain further or alternatively comprises amino acid substitution of K439 and/or K370 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D)) (all numberings according to Kabat EU index).

[00185] In yet a further embodiment, the heterodimerization approach described in WO 2007/147901 is used alternatively. In one embodiment, a first CH3 domain comprises amino acid mutations K253E, D282K, and K322D and a second CH3 domain comprises amino acid mutations D239K, E240K, and K292D (numberings according to Kabat EU index).

[00186] In still another embodiment, the heterodimerization approach described in WO 2007/110205 can be used alternatively.

[00187] In one embodiment, the first subunit of the Fc domain comprises amino acid substitutions K392D and K409D, and the second subunit of the Fc domain comprises amino acid substitutions D356K and D399K (numbering according to Kabat EU index).

Fc domain modifications reducing Fc receptor binding and/or effector function

[00188] The Fc domain confers to the immunoconjugate favorable pharmacokinetic properties, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however,

lead to undesirable targeting of the immunoconjugate to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Moreover, the co-activation of Fc receptor signaling pathways may lead to cytokine release which, in combination with the IL-2 polypeptide and the long half-life of the immunoconjugate, results in excessive activation of cytokine receptors and severe side effects upon systemic administration. In line with this, conventional IgG-IL-2 immunoconjugates have been described to be associated with infusion reactions (see e.g. King et al., J Clin Oncol 22, 4463-4473 (2004)).

[00189] Accordingly, in particular embodiments, the Fc domain of the antibody comprised in the immunoconjugate useful in the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG_1 Fc domain. In one such embodiment the Fc domain (or the antibody comprising said Fc domain) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG_1 Fc domain (or an antibody comprising a native IgG_1 Fc domain), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG_1 Fc domain domain (or an antibody comprising a native IgG_1 Fc domain). In one embodiment, the Fc domain domain (or an antibody comprising said Fc domain) does not substantially bind to an Fc receptor and/or induce effector function. In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one embodiment the effector function is one or more selected from the group of CDC, ADCC, ADCP, and cytokine secretion. In a particular embodiment the effector function is ADCC. In one embodiment the Fc domain domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG_1 Fc domain domain. Substantially similar binding to FcRn is achieved when the Fc domain (or an antibody comprising said Fc domain) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG_1 Fc domain (or an antibody comprising a native IgG_1 Fc domain) to FcRn.

[00190] In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In particular embodiments, the Fc domain of the antibody comprised in the immunoconjugate comprises one or more amino acid mutation that reduces the binding

affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the Fc domain to an Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the antibody comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to an antibody comprising a non-engineered Fc domain. In a particular embodiment the Fc receptor is an Fcγ receptor. In some embodiments the Fc receptor is a human Fc receptor. In some embodiments the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. Preferably, binding to each of these receptors is reduced. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or an antibody comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or an antibody comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or antibody comprised in the immunoconjugate comprising said Fc domain, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc domain of the antibody comprised in the immunoconjugate is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced crosslinking of target-bound antibodies, reduced dendritic cell maturation, or reduced T cell priming. In one embodiment the reduced

effector function is one or more selected from the group of reduced CDC, reduced ADCC, reduced ADCP, and reduced cytokine secretion. In a particular embodiment the reduced effector function is reduced ADCC. In one embodiment the reduced ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or an antibody comprising a non-engineered Fc domain).

[00191] In one embodiment the amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function is an amino acid substitution. In one embodiment the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In a more specific embodiment the Fc domain comprises an amino acid substitution at a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index). In some embodiments the Fc domain comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In one such embodiment, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In one embodiment the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (numberings according to Kabat EU index). In a more specific embodiment the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments the Fc domain comprises amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular embodiments the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA", "PGLALA" or "LALAPG"). Specifically, in particular embodiments, each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (Kabat EU index numbering), i.e. in each of the first and the second subunit of the Fc domain the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A) and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index). In one such embodiment, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor (as well as complement) binding of a human IgG_1 Fc domain, as described in PCT publication no. WO 2012/130831, which is incorporated herein by reference in its entirety. WO 2012/130831 also describes

methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

[00192] IgG_4 antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG_1 antibodies. Hence, in some embodiments the Fc domain of the antibody comprised in the immunoconjugate is an IgG_4 Fc domain, particularly a human IgG_4 Fc domain. In one embodiment the IgG_4 Fc domain comprises amino acid substitutions at position S228, specifically the amino acid substitution S228P (numberings according to Kabat EU index). To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment the IgG_4 Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E (numberings according to Kabat EU index). In another embodiment, the IgG_4 Fc domain comprises an amino acid substitution at position P329, specifically the amino acid substitution P329G (numberings according to Kabat EU index). In a particular embodiment, the IgG_4 Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G (numberings according to Kabat EU index). Such IgG_4 Fc domain mutants and their Fcγ receptor binding properties are described in PCT publication no. WO 2012/130831, incorporated herein by reference in its entirety.

[00193] In a particular embodiment, the Fc domain exhibiting reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG_1 Fc domain, is a human IgG_1 Fc domain comprising the amino acid substitutions L234A, L235A and optionally P329G, or a human IgG_4 Fc domain comprising the amino acid substitutions S228P, L235E and optionally P329G (numberings according to Kabat EU index).

[00194] In certain embodiments N-glycosylation of the Fc domain has been eliminated. In one such embodiment, the Fc domain comprises an amino acid mutation at position N297, particularly an amino acid substitution replacing asparagine by alanine (N297A) or aspartic acid (N297D) (numberings according to Kabat EU index).

[00195] In addition to the Fc domains described hereinabove and in PCT publication no. WO 2012/130831, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056) (numberings according to Kabat EU index). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[00196] Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

[00197] Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. Alternatively, binding affinity of Fc domains or antibodies comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcγIIIa receptor.

[00198] Effector function of an Fc domain, or an antibody comprising an Fc domain, can be measured by methods known in the art. Examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

[00199] In some embodiments, binding of the Fc domain to a complement component, specifically to C1q, is reduced. Accordingly, in some embodiments wherein the Fc domain is engineered to have reduced effector function, said reduced effector function includes reduced CDC. C1q binding assays may be carried out to determine whether the Fc domain, or antibody comprising the Fc domain, is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

[00200] FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006); WO 2013/120929).

Particular immunoconjugates

[00201] In one aspect, particularly useful in the invention is an immunoconjugate comprising a mutant IL-2 polypeptide and an antibody that binds to CEA,
wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 52); and
wherein the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:34, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:35.

[00202] In one aspect, particularly useful in the invention is an immunoconjugate comprising a mutant IL-2 polypeptide and an antibody that binds to CEA,
wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions T3A, F42A, Y45A, L72G and C125A (numbering relative to the human IL-2 sequence SEQ ID NO: 52); and
wherein the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:34, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:35.

[00203] In one aspect, particularly useful in the invention is an immunoconjugate comprising a mutant IL-2 polypeptide and an antibody that binds to CEA,
wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO: 53; and
wherein the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:34, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:35.

[00204] In one embodiment according to any of the above aspects of the invention, the antibody is an IgG class immunoglobulin, comprising a human IgG_1 Fc domain composed of a first and a second subunit,
wherein in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index),

and wherein further each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (Kabat EU index numbering).

[00205] In this embodiment, the mutant IL-2 polypeptide may be fused at its amino-terminal amino acid to the carboxy-terminal amino acid of the first subunit of the Fc domain, through a linker peptide of SEQ ID NO: 67.

[00206] In one aspect, particularly useful in the invention is an immunoconjugate comprising a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:44, a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:45, and a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:46.

[00207] A particularly useful immunoconjugate for the present invention is cergutuzumab amunaleukin (see WHO Drug Information (International Nonproprietary Names for Pharmaceutical Substances), Recommended INN: List 75, 2016, pre-publication copy" (incorporated herein by reference in its entirety).

[00208] In a further aspect, particularly useful in the invention is an immunoconjugate comprising a mutant IL-2 polypeptide and an antibody that binds to FAP,

wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 52); and

wherein the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:47, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:48.

[00209] In one aspect, particularly useful in the invention is an immunoconjugate comprising a mutant IL-2 polypeptide and an antibody that binds to FAP,

wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions T3A, F42A, Y45A, L72G and C125A (numbering relative to the human IL-2 sequence SEQ ID NO: 52); and

wherein the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:47, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:48.

[00210] In one aspect, particularly useful in the invention is an immunoconjugate comprising a mutant IL-2 polypeptide and an antibody that binds to FAP,

wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO: 53; and

wherein the antibody comprises (a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:47, and (b) a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:48.

[00211] In one embodiment according to any of the above aspects of the invention, the antibody is an IgG class immunoglobulin, comprising a human IgG_1 Fc domain composed of a first and a second subunit,

wherein in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index),

and wherein further each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (Kabat EU index numbering).

[00212] In this embodiment, the mutant IL-2 polypeptide may be fused at its amino-terminal amino acid to the carboxy-terminal amino acid of the first subunit of the Fc domain, through a linker peptide of SEQ ID NO: 67.

[00213] In one aspect, In one aspect, particularly useful in the invention is an immunoconjugate comprising a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:49, a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:50, and a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:51.

[00214] The IL-2 immunoconjugates useful in this invention, including compositions containing such IL-2 immunoconjugates, may be used in combination with a CD40 agonist and optionally a PD-1 axis binding antagonist to treat cancer.

III. CD40 Agonists

[00215] Examples of CD40 agonists useful for the methods, uses, compositions and kits of the invention, and methods for making thereof, are described in PCT publication no. WO 2003/040170, incorporated herein by reference in its entirety.

[00216] In some embodiments of the methods, uses, compositions, and kits described above and herein, the CD40 agonist is an antibody that specifically binds to CD40. In some embodiments, the CD40 agonist is an antibody that specifically binds to and activates human CD40.

[00217] CD40 is also referred to in the art as "tumor necrosis factor superfamily member 5", TNFRSF5, B-cell surface antigen 40, CD40L receptor, CDw40 and p50. The term "*CD40*" as used herein, refers to any native CD40 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length" and unprocessed CD40 as well as any form of CD40 that results from processing in the cell (e.g., mature protein). The term also encompasses naturally occurring variants and isoforms of CD40, e.g., splice variants or allelic variants. In one embodiment, CD40 is human CD40. The amino acid sequence of human CD40 is shown in UniProtKB/Swiss-Prot Accession No.P25942.

[00218] In some embodiments, the antibody comprises a heavy chain variable region comprising a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 57, and/or a light chain variable region comprising a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises a heavy chain variable region comprising the heavy chain complementarity determining region (HCDR) 1, HCDR 2 and HCDR 3 from the heavy chain variable region sequence of SEQ ID NO: 57, and/or a light chain variable region comprising the light chain complementarity determining region (LCDR) 1, LCDR 2 and LCDR 3 from the light chain variable region sequence of SEQ ID NO: 58. In some embodiments, the heavy and/or light chain variable region is a human variable region. In some embodiments, the heavy and/or light chain variable region comprises human framework regions (FR).

[00219] In some embodiments, the antibody comprises a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 57, and a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 58. In some embodiments, the antibody comprises the heavy chain complementarity determining region (HCDR) 1, HCDR 2 and HCDR 3 from the heavy chain variable region sequence of SEQ ID

NO: 57, and the light chain complementarity determining region (LCDR) 1, LCDR 2 and LCDR 3 from the light chain variable region sequence of SEQ ID NO: 58.

[00220] In some embodiments, the antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:57. In some embodiments, the antibody comprises a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:58. In some embodiments, the antibody comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:57, and (b) a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:58.

[00221] In some embodiments, the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 57 and a light chain variable region comprising the sequence of SEQ ID NO: 58.

[00222] In some embodiments, the antibody that specifically binds to CD40 is a full-length antibody. In some embodiments, the antibody is an IgG class antibody, particularly an IgG2 subclass antibody, more particularly a human IgG2 subclass antibody. In some embodiments, the antibody that specifically binds to CD40 is a fully human antibody of the IgG2 subclass. In one embodiment, the antibody is a fully human antibody of the IgG2 subclass which binds to human CD40 with a K_D of 4×10^{-10} M or less.

[00223] In one embodiment, the antibody that specifically binds to CD40 comprises a heavy chain polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 59, and a light chain polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 60. In one embodiment, the antibody comprises a heavy chain polypeptide comprising the sequence of SEQ ID NO: 59, and a light chain polypeptide comprising the sequence of SEQ ID NO: 60.

[00224] In some embodiments said CD40 agonist is any of the anti-CD40 antibodies as specifically disclosed in WO2003/040170. In some embodiments, the CD40 agonist is selected from the group of antibodies designated 3.1.1, 7.1.2, 10.8.3, 15. 1.1, 21.4.1, 21.2.1, 22.1.1, 23.5.1, 23.25.1, 23.29.1 and 24.2.1 according to WO2003/040170. Hybridomas secreting those antibodies have been deposited in accordance with the Budapest Treaty. Deposit Numbers can be found in para [0250] of WO2003/040170. In one embodiment, the

CD40 agonist is antibody 21.4.1 of WO 2003/040170. In one embodiment, the CD40 agonist is an antibody comprising the heavy and light chain variable domain amino acid sequences of antibody 21.4.1 of WO 2003/040170. In yet another embodiment, the CD40 agonist is an antibody comprising the heavy and light chain amino acid sequences of antibody 21.4.1 of WO 2003/040170.

[00225] The CD40 agonists useful in this invention, including compositions containing such CD40 agonists, may be used in combination with an IL-2 immunoconjugate and optionally a PD-1 axis binding antagonist to treat cancer.

IV. PD-1 Axis Binding Antagonists

[0138] A PD-1 axis binding antagonist may optionally be used in the methods, uses, compositions and kits of the inventions. For example, a PD-1 axis binding antagonist that may be used includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. PD-1 (programmed death 1) is also referred to in the art as "programmed cell death 1", PDCD1, CD279 and SLEB2. An exemplary human PD-1 is shown in UniProtKB/Swiss-Prot Accession No. Q15116. PD-L1 (programmed death ligand 1) is also referred to in the art as "programmed cell death 1 ligand 1", PDCD1LG1, CD274, B7-H, and PDL1. An exemplary human PD-L1 is shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7. PD-L2 (programmed death ligand 2) is also referred to in the art as "programmed cell death 1 ligand 2", PDCD1LG2, CD273, B7-DC, Btdc, and PDL2. An exemplary human PD-L2 is shown in UniProtKB/Swiss-Prot Accession No. Q9BQ51. In some embodiments, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2.

[0139] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, the PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0140] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX 1106 (nivolumab), MK-

3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. In some embodiments, the PD-L1 binding antagonist is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody is selected from the group consisting of YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In a preferred embodiment, the anti-PD-L1 antibody is atezolizumab. Antibody YW243.55.S70 is an anti-PD-L1 described in WO 2010/077634. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874. MEDI4736, is an anti-PD-L1 monoclonal antibody described in WO2011/066389 and US2013/034559. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0141] In some embodiments, the PD-1 axis binding antagonist is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PD-L1 antibody is a monoclonal antibody. In some embodiments, the anti-PD-L1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the anti-PD-L1 antibody is a humanized antibody. In some embodiments, the anti-PD-L1 antibody is a human antibody.

[0142] Examples of anti-PD-L1 antibodies useful for the methods, uses, compositions and kits of this invention, and methods for making thereof are described in PCT patent application WO 2010/077634, WO2007/005874, WO2011/066389, and US2013/034559, which are incorporated herein by reference in their entirety. The anti-PD-L1 antibodies useful in this invention, including compositions containing such antibodies, may be used in combination with an IL-2 immunoconjugate and a CD40 agonist to treat cancer.

Anti-PD1 antibodies

[0143] In some embodiments, the anti-PD-1 antibody is MDX-1106. Alternative names for "MDX-1106" include MDX-1106-04, ONO-4538, BMS-936558 or nivolumab. In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4). In a still further embodiment, useful is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:1 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:2. In a still further embodiment, useful is an isolated anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has 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%, at least 99% or 100% sequence identity to the heavy chain sequence:

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDG
SKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVT
VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE
FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:1),

and

(b) the light chain sequences has 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%, at least 99% or 100% sequence identity to the light chain sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGI
PARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:2).

Anti-PD-L1 antibodies

[0144] Anti-PD-L1 antibodies described in WO 2010/077634 A1 and US 8,217,149 may be used in the methods, uses, compositions and kits described herein. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain variable region sequence of SEQ ID NO:3 and/or a light chain variable region sequence of SEQ ID NO:4. In a still further embodiment,

useful is an isolated anti-PD-L1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has 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%, at least 99% or 100% sequence identity to the heavy chain sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSA (SEQ ID NO:3), and

(b) the light chain sequences has 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%, at least 99% or 100% sequence identity to the light chain sequence: DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

[0145] In one embodiment, the anti-PD-L1 antibody comprises a heavy chain variable region polypeptide comprising an HVR-H1, HVR-H2 and HVR-H3 sequence, wherein:

(a) the HVR-H1 sequence is GFTFSX$_1$SWIH (SEQ ID NO:5);

(b) the HVR-H2 sequence is AWIX$_2$PYGGSX$_3$YYADSVKG (SEQ ID NO:6);

(c) the HVR-H3 sequence is RHWPGGFDY (SEQ ID NO:7);

further wherein: X$_1$ is D or G; X$_2$ is S or L; X$_3$ is T or S. In one specific aspect, X$_1$ is D; X$_2$ is S and X$_3$ is T.

[0146] In another aspect, the polypeptide further comprises variable region heavy chain framework sequences juxtaposed between the HVRs according to the formula: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the framework sequences are VH subgroup III consensus framework. In a still further aspect, at least one of the framework sequences is the following:

HC-FR1 is EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:8)

HC-FR2 is WVRQAPGKGLEWV (SEQ ID NO:9)

HC-FR3 is RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)

HC-FR4 is WGQGTLVTVSA (SEQ ID NO:11).

[0147] In a still further aspect, the heavy chain polypeptide is further combined with a variable region light chain comprising an HVR-L1, HVR-L2 and HVR-L3, wherein:

(a) the HVR-L1 sequence is RASQX$_4$X$_5$X$_6$TX$_7$X$_8$A (SEQ ID NO:12);

(b) the HVR-L2 sequence is $SASX_9LX_{10}S$, (SEQ ID NO:13);

(c) the HVR-L3 sequence is $QQX_{11}X_{12}X_{13}X_{14}PX_{15}T$ (SEQ ID NO:14);

wherein: X_4 is D or V; X_5 is V or I; X_6 is S or N; X_7 is A or F; X_8 is V or L; X_9 is F or T; X_{10} is Y or A; X_{11} is Y, G, F, or S; X_{12} is L, Y, F or W; X_{13} is Y, N, A, T, G, F or I; X_{14} is H, V, P, T or I; X_{15} is A, W, R, P or T. In a still further aspect, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H; X_{15} is A.

[0148] In a still further aspect, the light chain further comprises variable region light chain framework sequences juxtaposed between the HVRs according to the formula: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the framework sequences are VL kappa I consensus framework. In a still further aspect, at least one of the framework sequence is the following:

LC-FR1 is DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:15)

LC-FR2 is WYQQKPGKAPKLLIY (SEQ ID NO:16)

LC-FR3 is GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:17)

LC-FR4 is FGQGTKVEIKR (SEQ ID NO:18).

[0149] In another embodiment, useful is an isolated anti-PD-L1 antibody or antigen binding fragment comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises and HVR-H1, HVR-H2 and HVR-H3, wherein further:

(i) the HVR-H1 sequence is $GFTFSX_1SWIH$; (SEQ ID NO:5)

(ii) the HVR-H2 sequence is $AWIX_2PYGGSX_3YYADSVKG$ (SEQ ID NO:6)

(iii) the HVR-H3 sequence is RHWPGGFDY, and (SEQ ID NO:7)

(b) the light chain comprises and HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is $RASQX_4X_5X_6TX_7X_8A$ (SEQ ID NO:12)

(ii) the HVR-L2 sequence is $SASX_9LX_{10}S$; and (SEQ ID NO:13)

(iii) the HVR-L3 sequence is $QQX_{11}X_{12}X_{13}X_{14}PX_{15}T$; (SEQ ID NO:14)

wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S; X_4 is D or V; X_5 is V or I; X_6 is S or N; X_7 is A or F; X_8 is V or L; X_9 is F or T; X_{10} is Y or A; X_{11} is Y, G, F, or S; X_{12} is L, Y, F or W; X_{13} is Y, N, A, T, G, F or I; X_{14} is H, V, P, T or I; X_{15} is A, W, R, P or T. In a specific aspect, X_1 is D; X_2 is S and X_3 is T. In another aspect, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H; X_{15} is A. In yet another aspect, X_1 is D; X_2 is S and X_3 is T, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H and X_{15} is A.

[0150] In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0151] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0152] In yet another embodiment, useful is an anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, or

(b) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

[0153] In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0154] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0155] In another further embodiment, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSS (SEQ ID NO:25), and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSS (SEQ ID NO:27).

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0156] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or

minimal effector function. In a still further specific aspect, the minimal effector function results from production in prokaryotic cells. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0157] In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences is the following:

HC-FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	(SEQ ID NO:29)
HC-FR2	WVRQAPGKGLEWVA	(SEQ ID NO:30)
HC-FR3	RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR	(SEQ ID NO:10)
HC-FR4	WGQGTLVTVSS	(SEQ ID NO:27).

[0158] In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences is the following:

LC-FR1	DIQMTQSPSSLSASVGDRVTITC	(SEQ ID NO:15)
LC-FR2	WYQQKPGKAPKLLIY	(SEQ ID NO:16)
LC-FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	(SEQ ID NO:17)
LC-FR4	FGQGTKVEIK	(SEQ ID NO:28).

[0159] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function

results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0160] In yet another embodiment, useful is an anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(c) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, and/or

(d) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

[0161] In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSSASTK (SEQ ID NO:31).

[0162] In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18. In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from

the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0163] In a still further embodiment, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSS (SEQ ID NO:25), or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

[0164] In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:4. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:25. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least

85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:25. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

[0165] In a still further embodiment, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSSASTK (SEQ ID NO:26), or

(b) the light chain sequence has at least 85% sequence identity to the light chain sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

[0166] In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:4. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

[0167] In a still further embodiment, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:32), and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33)

In a still further embodiment, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP

PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:56), and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33).

[0168] In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% sequence identity to the amino acid sequence of SEQ ID NO:33. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% sequence identity to the amino acid sequence of SEQ ID NO:32 or 56. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% sequence identity to the amino acid sequence of SEQ ID NO:32 or 56. In some embodiments, useful is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% sequence identity to the amino acid sequence of SEQ ID NO:32.

[0169] In some embodiments, the isolated anti-PD-L1 antibody is aglycosylated. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites form an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or threonine residue within the glycosylation site another amino acid residue (e.g., glycine, alanine or a conservative substitution).

[0170] In any of the embodiments herein, the isolated anti-PD-L1 antibody can bind to a human PD-L1, for example a human PD-L1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof.

IV. Pharmaceutical Compositions and Formulations

[0171] Also provided herein are pharmaceutical compositions and formulations comprising an IL-2 immunoconjugate, a CD40 agonist, and/or a PD-1 axis binding antagonist as described herein, and a pharmaceutically acceptable carrier.

[0172] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (e.g. an IL-2 immunoconjugate, a CD40 agonist, and/or a PD-1 axis binding antagonist) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-

pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0173] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0174] The composition and formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0175] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0176] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

IV. Methods of Treatment

[0177] Provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding. In some embodiments, the treatment results in a response in the individual after treatment. In some embodiments, the response is a partial response. In some embodiments, the response is a complete response. In some embodiments, the treatment results in a sustained response (e.g., a sustained partial response or complete response) in the individual after cessation of the treatment. The methods described herein may find use in treating conditions where enhanced immunogenicity is desired such as increasing tumor immunogenicity for the treatment of cancer. Also provided herein are methods of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of an IL-2 immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

[0178] In some instances, the methods provided herein include administration of an effective amount of a PD-1 axis binding antagonist selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist, and a PD-L2 binding antagonist. In some instances, the PD-L1 binding antagonist is an antibody, such as an antibody that is capable of inhibiting PD-L1 binding to PD-1 and B7.1, but does not disrupt binding of PD-1 to PD-L2. In some instances, the PD-L1 binding antagonist antibody is MPDL3280A, which may be administered at a dose of about 800 mg to about 1500 mg every three weeks (e.g., about 1000 mg to about 1300 mg every three weeks, e.g., about 1100 mg to about 1200 mg every three weeks). In some embodiments, MPDL3280A is administered at a dose of about 1200 mg every three weeks.

[0179] As a general proposition, the therapeutically effective amount of a PD-1 axis binding antagonist (e.g., an anti-PD-L1 antibody, e.g., MPDL3280A) may be administered to a human will be in the range of about 0.01 to about 50 mg/kg of patient body weight whether by one or more administrations. In some embodiments, for example, the antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) is administered in a dose of about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In some embodiments, the antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, a PD-1 axis binding antagonist (e.g., anti-PD-

L1 antibody, e.g., MPDL3280A) is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, or about 1500 mg. In some embodiments, a PD-1 axis binding antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) is administered at a dose of about 1150 mg to about 1250 mg every three weeks. In some embodiments, a PD-1 axis binding antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) is administered at a dose of about 1200 mg every three weeks. The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. In some embodiments, for example, the method for treating or delaying progression of cancer in an individual comprises a dosing regimen comprising treatment cycles, wherein the individual is administered, on days 1 of each cycle, a PD-1 axis binding antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) at a dose of about 1200 mg, wherein each cycle is 21 days (i.e., each cycle is repeated every 21 days). The progress of this therapy is easily monitored by conventional techniques.

[0180] In some instances, the methods provided herein include administration of an effective amount of an IL-2 immunconjugate (e.g., CEA IL2v, FAP IL2v). In some instances, the IL-2 immunconjugate is administered to the individual at a dose of about 5 mg to about 100 mg every week (e.g., about 10 mg to about 60 mg every week, e.g., about 10 mg to about 40 mg every week). In some embodiments, the IL-2 immunconjugate is administered at a dose of about 10 mg every week. As a general proposition, the therapeutically effective amount of an IL-2 immunconjugate administered to a human will be in the range of about 5 to about 100 mg (e.g., about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg about 85 mg, about 90 mg, about 95 mg, or about 100 mg), whether by one or more administrations. For example, in some embodiments, about 10 mg of IL-2 immunconjugate is administered. In some embodiments, IL-2 immunconjugate is administered at 10 mg once a week. In some embodiments, the IL-2 immunconjugate may be administered weekly, every 2 weeks, every 3 weeks, every 4 weeks, on days 1, 8 and 15 of each 21-day cycle, or on days 1, 8, and 15 of each 28-day cycle.

[0181] In some instances, the methods provided herein include administration of an effective amount of a CD40 agonist. In some instances, the CD40 agonist is administered to the individual at a dose of about 2 mg to about 100 mg every week (e.g., about 4 mg to about

60 mg every week, e.g., about 4 mg to about 20 mg every week). In some embodiments, the CD40 agonist is administered at a dose of about 8 mg every week. As a general proposition, the therapeutically effective amount of a CD40 agonist administered to a human will be in the range of about 2 to about 100 mg (e.g., about 2 mg, about 4 mg, about 5 mg, about 8 mg, about 10 mg, about 12 mg, about 15 mg, about 16 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, or about 100 mg), whether by one or more administrations. For example, in some embodiments, about 8 mg of CD40 agonist is administered. In some embodiments, the CD40 agonist is administered at 8 mg once a week. In some embodiments, the CD40 agonist may be administered weekly, every 2 weeks, every 3 weeks, every 4 weeks, on days 1, 8 and 15 of each 21-day cycle, or on days 1, 8, and 15 of each 28-day cycle.

[0182] In some instances, the IL-2 immunconjugate (e.g., CEA IL2v or FAP IL2v), the CD40 agonist, and optionally the PD-1 axis binding antagonist (e.g., an anti-PD-L1 antibody, e.g., MPDL3280A) are administered in a single dosing regimen. The administration of these agents may be concurrent or separate within the context of the dosing regimen. For example, in some instances, the methods provided herein include a dosing regimen comprising treatment cycles, wherein the individual is administered, on days 1 of each cycle, a PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8, and 15 of each cycle an IL-2 immunoconjugate at a dose of about 10 mg, and on day 1 of each cycle a CD40 agonist at a dose of about 16 mg, each cycle being repeated every 21 days.

[0183] In some embodiments, the individual is a human. In some embodiments, the individual is suffering from locally advanced or metastatic cancer. In some embodiments, the individual has CEA positive cancer. In some embodiments, the individual has a FAP positive cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer. In some embodiments, the breast cancer is a breast carcinoma or a breast adenocarcinoma. In some embodiments, the breast carcinoma is an invasive ductal carcinoma. In some embodiments, the lung cancer is a lung adenocarcinoma. In some embodiments, the colon cancer is a colorectal adenocarcinoma. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the cancer cells in the individual express CEA protein at a level that is detectable (e.g., detectable using methods known in the art). In some embodiments, the cancer cells (particularly stromal cells of the cancer, such as

fibroblasts) in the individual express FAP protein at a level that is detectable (e.g., detectable using methods known in the art).

[0184] In some embodiments, the individual has been treated with a cancer therapy before the combination treatment with an IL-2 immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist. In some embodiments, the individual has cancer that is resistant to one or more cancer therapies. In some embodiments, resistance to cancer therapy includes recurrence of cancer or refractory cancer. Recurrence may refer to the reappearance of cancer, in the original site or a new site, after treatment. In some embodiments, resistance to a cancer therapy includes progression of the cancer during treatment with the anti-cancer therapy. In some embodiments, resistance to a cancer therapy includes cancer that does not response to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment. In some embodiments, the cancer is at early stage or at late stage.

[0185] In some embodiments, the combination therapy of the invention comprises administration of an IL-2 immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist. The IL-2 immunoconjugate, CD40 agonist, and PD-1 axis binding antagonist may be administered in any suitable manner known in the art. For example, the IL-2 immunoconjugate, CD40 agonist, and PD-1 axis binding antagonist may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, the IL-2 immunoconjugate, CD40 agonist and PD-1 axis binding antagonist are each in a separate composition. In some embodiments, the IL-2 immunoconjugate is in the same composition as CD40 agonist and/or the PD-1 axis binding antagonist.

[0186] The IL-2 immunoconjugate, the CD40 agonist, and the PD-1 axis binding antagonist may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the CD40 agonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. An effective amount of the IL-2 immunoconjugate, the CD40 agoinst and optionally the PD-1 axis binding antagonist may be administered for prevention or treatment of disease. The appropriate dosage of the IL-2 immunoconjugate, the CD40 agonist and/or the PD-1 axis binding antagonist may be determined based on the type

of disease to be treated, the type of IL-2 immunoconjugate, CD40 agonist and PD-1 axis binding antagonist, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[0187] In some embodiments, the methods may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (*e.g.*, agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PI3K/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents described herein.

Other Combination Therapies

[0188] Also provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) in conjunction with another anti-cancer agent or cancer therapy.

[0189] In some embodiments, an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding antagonist may be administered in conjunction with a chemotherapy or chemotherapeutic agent. In some embodiments, an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding antagonist may be administered in conjunction with a radiation therapy or radiotherapeutic agent. In some embodiments, an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding antagonist may be administered in conjunction with a targeted therapy or targeted therapeutic agent. In some embodiments, an IL-2 immunoconjugate, a CD40 agonist and optionally a PD-1 axis binding

antagonist may be administered in conjunction with an immunotherapy or immunotherapeutic agent, for example a monoclonal antibody.

V. Articles of Manufacture or Kits

[0190] In another embodiment of the invention, an article of manufacture or a kit is provided comprising an IL-2 immunoconjugate, a CD40 agonist, and/or a PD-1 axis binding antagonist. In some embodiments, the article of manufacture or kit further comprises a package insert comprising instructions for using an IL-2 immunoconjugate in conjunction with a CD40 agonist and optionally a PD-1 axis binding antagonist to treat or delay progression of cancer in an individual or to enhance immune function of an individual having cancer. Any of the IL-2 immunoconjugates, CD40 agonists and/or PD-1 axis binding antagonists described herein may be included in the article of manufacture or kit.

[0191] In some embodiments, the IL-2 immunoconjugate, the CD40 agonist, and the PD-1 axis binding antagonist are in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, the article of manufacture further includes one or more of another agent (e.g., a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

[0192] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0193] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: *In vivo* Efficacy of Targeted-IL2v Immunoconjugate against FAP in syngeneic model of Mouse Tumor Cell Lines alone and in combination with anti-CD40 Mab and anti-PD-L1 Mab

[0194] Targeted-IL2v immunoconjugate against FAP was tested alone and in combination with CD40 mab and PD-L1 Mab for their anti-tumoral efficacy in a syngeneic mouse model.

Panc02-Fluc pancreatic Syngeneic Model

[0195] The murine surrogate FAP-targeted FAP-IL2v immunoconjugate was tested in the mouse pancreatic Panc02-Fluc transfectant cell line intra-pancreatically injected into Black 6 mice.

[0196] Panc02-H7 cells (mouse pancreatic carcinoma) were originally obtained from the MD Anderson cancer center (Texas, USA) and after expansion deposited in the Roche-Glycart internal cell bank. Panc02-H7-Fluc cell line was produced in house by calcium transfection and sub-cloning techniques. Panc02-H7-Fluc were cultured in RPMI medium containing 10% FCS (Sigma), 500 µg/ml hygromicin and 1% of Glutamax. The cells were cultured at 37°C in a water-saturated atmosphere at 5 % CO_2. Passage 14 was used for transplantation. Cell viability was 92.8%. $1x10^5$ cells per animal were injected into the pancreas of the mice using a 0.3 ml tuberculin syringe (BD Biosciences, Germany). For this a small incision was made at the left abdominal site of anesthetized Black 6 mouse. The peritoneal wall was opened and the pancreas carefully isolated with forceps. Ten microliters ($1x10^5$ Panc02-H7-Fluc cells in RPMI medium) cell suspension was injected in the tail of the pancreas. Peritoneal wall and skin wounds were closed using 5/0 resolvable sutures.

[0197] Female Black 6 mice aged 8-9 weeks at the start of the experiment (Charles River, Lyon, France) were maintained under specific-pathogen-free condition with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (ZH193/2014). After arrival, animals were maintained for one week to get accustomed to the

new environment and for observation. Continuous health monitoring was carried out on a regular basis.

[0198] Mice were injected intra-pancreatically on study day 0 with $1x10^5$ Panc02-Fluc cells, randomized and weighed. One week after the tumor cell injection mice were injected i.v. with FAP-IL-2v (40 µg), PD-L1-Mab (200 µg), CD40 Mab (200 µg) and their combinations; FAP-IL-2v + PD-L1 Mab, FAP-IL-2v + CD40 Mab, FAP-IL-2v + PD-L1 Mab + CD40 Mab, once weekly for three weeks. The mice in the vehicle group were injected with histidine buffer.

[0199] Figure 1 shows that the combination FAP-IL-2v + CD40 Mab + PD-L1 Mab mediated superior efficacy in terms of enhanced median and overall survival compared to all other single agents and combinations tested.

[0200] For Bioluminescence imaging by IVIS® SPECTRUM, the mice are injected intra-peritoneally with 150 mg/kg of D-Luciferin 10 minutes before bioluminescence imaging acquisition (BLI) and later anesthetized with 4% isoflurane. Subsequently the mice are transferred into an isolation chamber, which is positioned into the IVIS® spectrum. In vivo BLI acquisitions are performed by acquiring the luminescence signal for 10-50 seconds. Data is stored as Radiance (photons)/sec/cm^2/sr. In vivo BLI data analysis is performed with the Living Image® 4.4 software and represented by a tumor inhibition curve.

[0201] Figure 2 shows that the combination FAP-IL-2v + CD40 Mab + PD-L1 Mab mediated superior efficacy in terms of decreasing the bioluminescence signal (photons/second) compared to all other single agents and combinations tested.

[0202] An anti-mouse PD-L1 antibody based on the YW243.55.S70 PD-L1 antibody described in WO 2010/077634 (sequence shown in Figure 11) was used in the *in vivo* tumor models. This antibody contained a DAPG mutation to abolish FcγR interaction. The variable region of YW243.55.S70 was attached to a murine IgG1 constant domain with DAPG Fc mutations.

[0203] A murinized chimeric version of the FAP-targeted IL-2 variant immunocytokine FAP-IL2v, termed muFAP-muIL2v, was used in the *in vivo* tumor models in fully immunocompetent mice in order to reduce the formation of anti-drug antibodies (ADA). In the murinized surrogate molecule, the Fc domain knob-into-holes mutations were replaced by DDKK mutations on muIgG1 and the LALA P329G mutations were replaced by DAPG mutations on muIgG1.

[0204] An anti-mouse CD40 antibody was used in the *in vivo* tumor models.

[0205] The polypeptide sequences of the molecules used in the *in vivo* tumor models are as follows:

Construct	Amino Acid Sequence	SEQ ID NO
YW243.55.S70 PD-L1 muIgG1 DAPG HC	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQA PGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVT CNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPK PKDVLTITLTPKVTCVVVDISKDAPEVQFSWFVDDVEVHTA QTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAF GAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMIT DFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKL NVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSP	36
YW243.55.S70 PD-L1 muIgG1 LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQYLYHPATFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWT DQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC	37
CD40 muIgG1 (FGK4.5) HC	EVQLVESDGGLVQPGRSLKLPCAASGFTFSDYYMAWVRQ APTKGLEWVASISYDGSSTYYRDSVKGRFTISRDNAKSTLY LQMDSLRSEDTATYYCGRHSSYFDYWGQGVMVTVSSAKT TPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNS GSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCNV AHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKD VLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQT KPREEQINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPA PIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITNF FPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNV QKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSP	61
CD40 muIgG1 (FGK4.5) LC	DTVLTQSPALAVSPGERVTISCRASDSVSTLMHWYQQKPG QQPKLLIYLASHLESGVPARFSGSGSGTDFTLTIDPVEADDT ATYYCQQSWNDPWTFGGGTKLELKRADAAPTVSIFPPSSE QLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSW TDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSP IVKSFNRNEC	62
FAP muIgG1 DAPG DDKK-muIL2v HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA PGKGLEWVSAIIGSGASTYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSAK TTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWN SGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCN VAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPK DVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQ TKPREEQINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFG APIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITN FFPEDITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLN VQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGGGGGS	63

	GGGGSGGGGSAPASSSTSSSTAEAQQQQQQQQQQQQHLEQ LLMDLQELLSRMENYRNLKLPRMLTAKFALPKQATELKDL QCLEDELGPLRHVLDGTQSKSFQLEDAENFISNIRVTVVKL KGSDNTFECQFDDESATVVDFLRRWIAFAQSIISTSPQ	
FAP muIgG1 DAPG DDKK HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA PGKGLEWVSAIIGSGASTYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSAK TTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWN SGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCN VAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPK DVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQ TKPREEQINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFG APIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMIT NFFPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKL NVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSP	64
FAP muIgG1 DAPG DDKK LC	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKP GQAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQGIMLPPTFGQGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTD QDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIV KSFNRNEC	65

CLAIMS

1. A method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of an interleukin-2 (IL-2) immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

2. A method of enhancing immune function in an individual having cancer comprising administering an effective amount of an interleukin-2 (IL-2) immunoconjugate, a CD40 agonist, and optionally a PD-1 axis binding antagonist.

3. Use of an IL-2 immunoconjugate in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and optionally further in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

4. Use of a CD40 agonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the CD40 agonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and optionally further in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

5. Use of a PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and further in combination with a composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier.

6. A composition comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual,

wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a CD40 agonist and an optional pharmaceutically acceptable carrier, and optionally further in combination with third composition, wherein the third composition comprises a PD-1 axis antagonist and an optional pharmaceutically acceptable carrier.

7. A composition comprising a CD40 agonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and optionally further in combination with a third composition, wherein the third composition comprises a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

8. A composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a CD40 agonist and an optional pharmaceutically acceptable carrier, and further in combination with third composition, wherein the third composition comprises an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier.

9. A kit comprising a first medicament comprising an IL-2 immunoconjugate and an optional pharmaceutically acceptable carrier, and a second medicament comprising a CD40 agonist and an optional pharmaceutically acceptable carrier, and optionally a third medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

10. The kit of claim 9, wherein the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament and optionally the third medicament for treating or delaying progression of cancer in an individual.

11. The method, use, composition or kit of any one of the preceding claims, wherein the IL-2 immunoconjugate comprises an antibody that specifically binds to a tumor antigen, and an IL-2 polypeptide.

12. The method, use, composition or kit of any one of the preceding claims, wherein the IL-2 immunoconjugate comprises an antibody that specifically binds to Carcinoembryonic Antigen (CEA).

13. The method, use, composition or kit of claim 12, wherein the antibody comprises a heavy chain variable region comprising the heavy chain CDR (HCDR) 1 of SEQ ID NO: 38, the HCDR2 of SEQ ID NO: 39, and the HCDR3 of SEQ ID NO: 40; and/or a light chain variable region comprising the light chain CDR (LCDR) 1 of SEQ ID NO: 41, the LCDR2 of SEQ ID NO: 42 and the LCDR3 of SEQ ID NO: 43.

14. The method, use, composition or kit of claim 12 or 13, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:35.

15. The method, use, composition or kit of any one of the preceding claims, wherein the IL-2 immunoconjugate comprises an antibody that specifically binds to Fibroblast Activation Protein (FAP).

16. The method, use, composition or kit of claim 15, wherein the antibody comprises a heavy chain variable region comprising a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 47, and/or a light chain variable region comprising a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 48.

17. The method, use, composition or kit of claim 15 or 16, wherein the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 47 and/or a light chain variable region comprising the sequence of SEQ ID NO: 48.

18. The method, use, composition or kit of any one of claims 11 to 17, wherein the antibody is a full-length antibody.

19. The method, use, composition or kit of any one of claims 11 to 18, wherein the antibody is an IgG class antibody, particularly an IgG1 subclass antibody.

20. The method, use, composition or kit of any one of claims 11 to 19, wherein the antibody comprises an Fc domain, particularly an IgG Fc domain, more particularly an IgG1 Fc domain, most particularly a human IgG1 Fc domain.

21. The method, use, composition or kit of claim 20, wherein the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain.

22. The method, use, composition or kit of claim 20 or 21, wherein in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

23. The method, use, composition or kit of any one of claims 20 to 22, wherein in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numbering according to Kabat EU index).

24. The method, use, composition or kit of claim 23, wherein in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numbering according to Kabat EU index).

25. The method, use, composition or kit of any one of claims 20 to 24, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, particularly an Fcγ receptor, and/or effector function, particularly antibody-dependent cell-mediated cytotoxicity (ADCC), as compared to a native IgG1 Fc domain.

26. The method, use, composition or kit of any one of claims 20 to 25, wherein the Fc domain comprises one or more amino acid substitution at one or more position selected from the group of L234, L235, and P329 (numbering according to Kabat EU index).

27. The method, use, composition or kit of any one claims 20 to 26, wherein the each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).

28. The method, use, composition or kit of any one of claims 11 to 27, wherein the IL-2 polypeptide is a human IL-2 polypeptide.

29. The method, use, composition or kit of any one of claims 11 to 28, wherein the IL-2 polypeptide is a mutant human IL-2 polypeptide comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 52).

30. The method, use, composition or kit of any one of claims 11 to 29, wherein the IL-2 polypeptide comprises the sequence of SEQ ID NO: 53.

31. The method, use, composition or kit of any one of the preceding claims, wherein the CD40 agonist is an antibody that specifically binds to CD40.

32. The method, use, composition or kit of claim 31, wherein the antibody comprises a heavy chain variable region comprising a HVR-H1, HVR-H2 and HVR-H3 from the heavy chain variable region sequence of SEQ ID NO: 57, and/or a light chain variable region comprising a HVR-L1, HVR-L2 and HVR-L3 from the light chain variable region sequence of SEQ ID NO: 58.

33. The method, use, composition or kit of claim 31 or 32, wherein the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 57 and/or a light chain variable region comprising the sequence of SEQ ID NO: 58.

34. The method, use, composition or kit of any one of the claims 31 to 33, wherein the antibody is a full-length antibody.

35. The method, use, composition or kit of any one of claims 31 to 34, wherein the antibody is an IgG class antibody, particularly an IgG2 subclass antibody, more particularly a human IgG2 subclass antibody.

36. The method, use, composition or kit of any one of the preceding claims, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

37. The method, use, composition or kit of any one of the preceding claims, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

38. The method, use, composition or kit claim 37, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1 and/or to B7-1.

39. The method, use, composition or kit of claim 37 or 38, wherein the PD-L1 binding antagonist is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

40. The method, use, composition or kit of any one of claims 37 to 39, wherein the PD-L1 binding antagonist MPDL3280A (atezolizumab).

41. The method, use, composition or kit of claim 37 or 38, wherein the PD-L1 binding antagonist is an antibody.

42. The method, use, composition or kit of claim 41, wherein the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24.

43. The method, use, composition or kit of claim 41 or 42, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

44. The method, use, composition or kit of any one of the preceding claims, wherein the PD-1 axis binding antagonist is an antibody and comprises an aglycosylation site mutation.

45. The method, use, composition or kit of claim 44, wherein the aglycosylation site mutation is a substitution mutation.

46. The method, use, composition or kit of claim 45, wherein the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering).

47. The method, use, composition or kit of claim 45 or 46, wherein the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A.

48. The method, use, composition or kit of any one of the claims 45 to 47, wherein the substitution mutation is a D265A mutation and an N297G mutation.

49. The method, use, composition or kit of any one of the preceding claims, wherein the cancer is a CEA-positive cancer and/or a FAP-positive cancer.

50. The method, use, composition or kit of any one of the preceding claims, wherein the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer.

51. The method, use, composition or kit of any one of the preceding claims, wherein the cancer expresses PD-L1.

52. The invention as described hereinbefore.

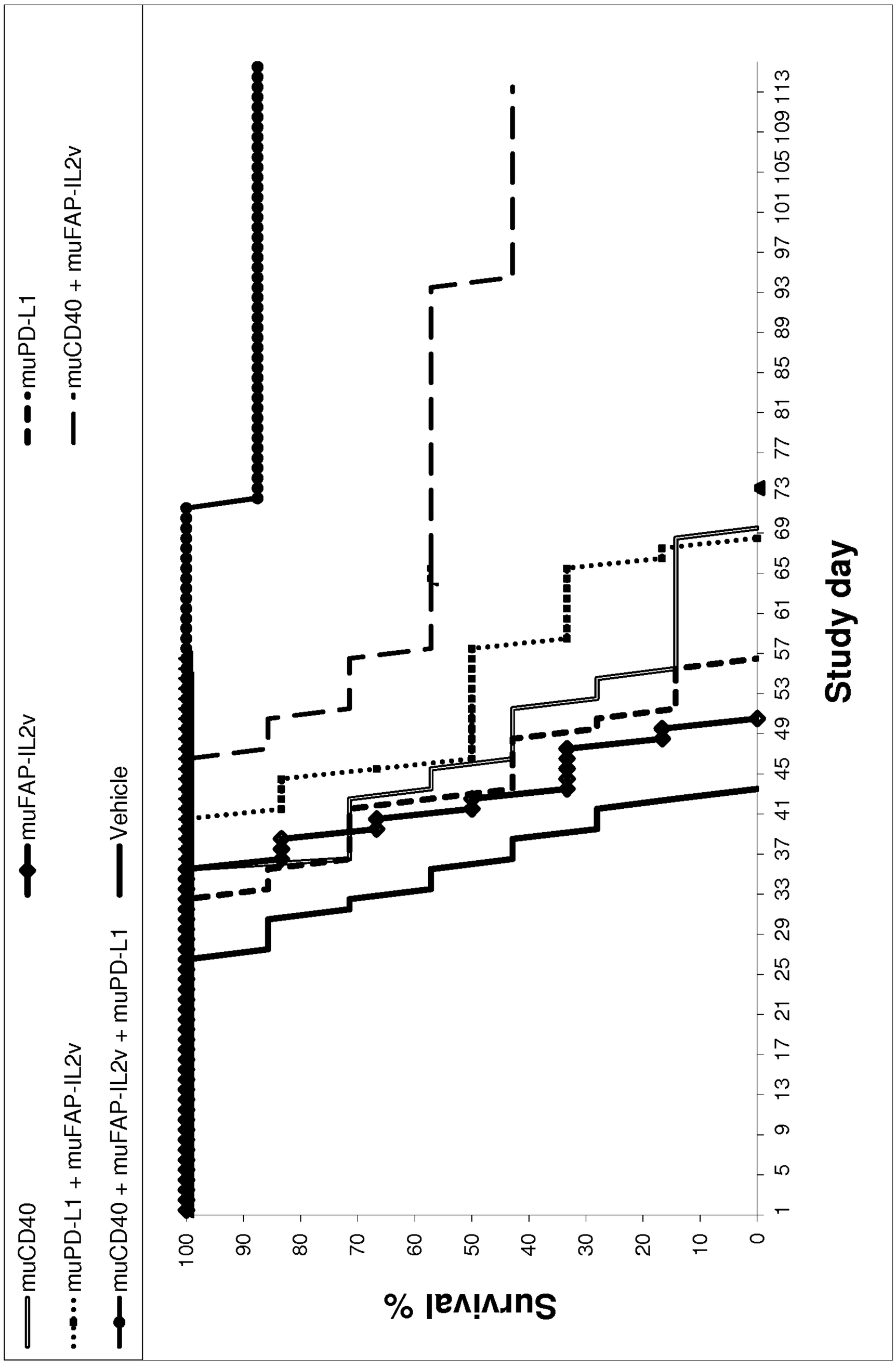
muPD-L1
muCD40 + muFAP-IL2v
muFAP-IL2v
Vehicle
muCD40
muPD-L1 + muFAP-IL2v
muFAP-IL2v + muPD-L1
muCD40 + muFAP-IL2v + muPD-L1
Survival %
100
90
80
70
60
50
40
30
20
10
0
Study day
1
5
9
13
17
21
25
29
33
37
41
45
49
53
57
61
65
69
73
77
81
85
89
93
97
101
105
109
113

Fig.1A

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Groups	Median Survival in days	p-value vs control	Overall survival
muFAP-IL2v	40	0.2470	0/7
muPD-L1	42	0.0585	0/7
muPD-L1 + muFAP-IL2v	45	0.0392*	0/6
muCD40 + muFAP-IL2v	93	0.0005**	3/7
muCD40 + muFAP-IL2v + muPD-L1	Not reached	<0.0001***	7/8
Vehicle	35	1	0/7

Fig.1B

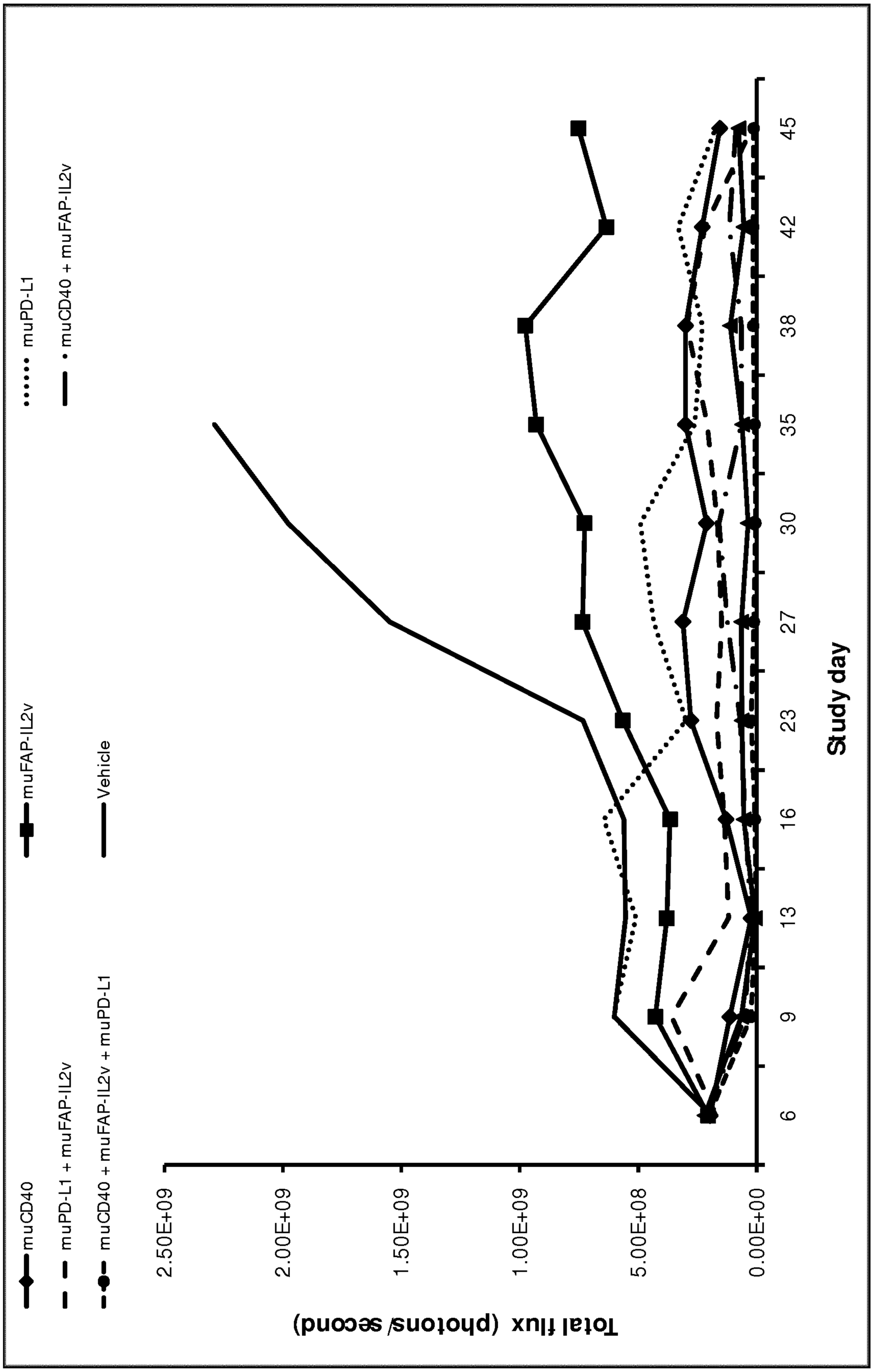
Total flux (photons/second)
2.50E+09
2.00E+09
1.50E+09
1.00E+09
5.00E+08
0.00E+00
6
9
13
16
23
27
30
35
38
42
45
Study day
muCD40
muPD-L1 + muFAP-IL2v
muCD40 + muFAP-IL2v + muPD-L1
muFAP-IL2v
Vehicle

Fig.2

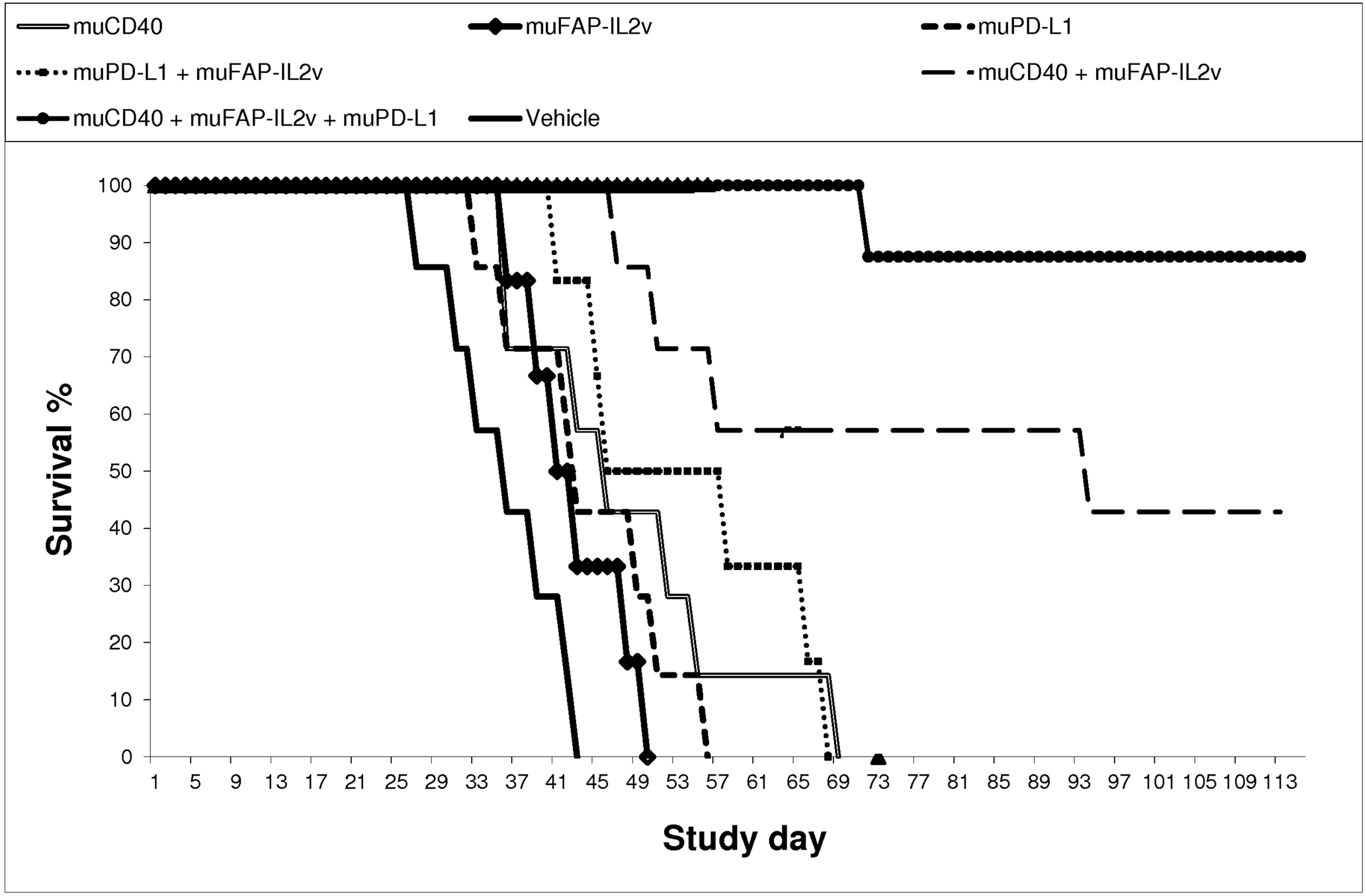
muCD40
muFAP-IL2v
muPD-L1
muPD-L1 + muFAP-IL2v
muCD40 + muFAP-IL2v
muCD40 + muFAP-IL2v + muPD-L1
Vehicle
Survival %
100
90
80
70
60
50
40
30
20
10
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101 105 109 113
Study day

Fig.1A