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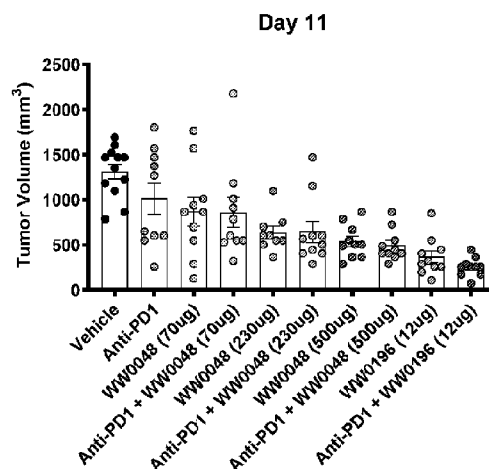
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(54) Title: INDUCIBLE CYTOKINE PRODRUG AND PD-1/PD-L1 COMBINATION THERAPY

FIG. 1E



(57) **Abstract:** Prodrugs typically include a native cytokine polypeptide that is attached to a cytokine blocking domain and typically a half-life extension domain, through a protease cleavable linker. This disclosure relates to methods and compositions for treating cancer including lymphoma using an inducible cytokine prodrug, and to a combination therapy comprising an inducible cytokine prodrug in combination with anti-PD-1 and anti-PD-L1 antibodies.

INDUCIBLE CYTOKINE PRODRUG AND PD-1/PD-L1 COMBINATION THERAPY

1. RELATED APPLICATIONS

[01] The present application claims the benefit of U.S. Provisional Application No.: 63/234,001, filed on August 17, 2021, and U.S. Provisional Application No.: 63/275,714, filed on November 4, 2021, each of which are incorporated by reference in their entirety.

2. BACKGROUND

[02] Inducible cytokine prodrugs, that are conditionally activated in the tumor microenvironment through protease cleavage to release the fully active, native cytokine within the tumor to stimulate a potent anti-tumor immune response, are described in International Publication Nos.: WO2019/222294, WO2019/222295, WO2019/222296, WO2021/097376. These prodrugs typically include a native cytokine polypeptide that is attached to a cytokine blocking domain and typically a half-life extension domain, through a protease cleavable linker. For example, IL-2 prodrugs can include a native IL-2 molecule attached through a protease cleavable linker to a half-life extension domain (e.g., anti-human serum albumin antibody binding fragment such as a VH domain) and an IL-2 blocking element (e.g., anti-IL-2 antibody binding fragment, such as a Fab) to block binding of IL-2 to IL-2 β / γ receptors on normal tissue in the periphery. Upon cleavage of the protease cleavable linker in the tumor microenvironment, fully active native IL-2 is released within the tumor to stimulate a potent anti-tumor immune response.

[03] Immune checkpoint inhibitors are proteins that regulate T cell functions. T cell effector function is important for immunotherapeutic approaches to treating tumors. But immunosuppression, and decreased effector function, is often seen as tumors grow and cancer progresses. One mechanism behind this phenomenon is the activation of immune checkpoint inhibitors by cancer cells, leading to suppression of the anti-tumor immune response. This typically occurs when cancer cells express proteins on their surface that can interact with immune checkpoint proteins on the surface of T cells in the tumor microenvironment to suppress the activity of the T cells. Immune checkpoint proteins include, for example, PD-1 which binds ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), CTLA-4 (CD152) which binds B7-1 (CD80) and B7-2 (CD86), LAG 3 (CD223) which binds Galectin3, LSECtin and FGL1;

TIM3 (HAVCR2) which binds ligands Ceacam1 and Galectin9; TIGIT (VSTM3, WUCAM) which binds CD112 and CD155; BTLA (CD272) which binds HVEM (TNFRSF14), B7-H3 (CD276), B7-H4 (VTCN1), VISTA (B7-H5), KIR, CD44 (2B4), CD160 (BY55) which bind HVEM; CD134 (TNFRSF4, OX40) which binds CD252 (OX-40L).

[04] Therapeutic agents, such as antibodies, that bind immune checkpoint proteins and inhibit their immunosuppressive activity have been developed as anti-tumor agents. Several such agents are now commercially available for cancer therapy, including the anti-PD1 antibodies pembrolizumab (KEYTRUDA), dostarlimab (JEMPERLI), cemiplimab-rwlc (LIBATYO), nivolumab (OPDIVO), camrelizumab, tislelizumab, toripalimab, and sintilimab (TYVYT); the anti-PD-L1 antibodies avelumab (BAVENCIO), durvalumab (IMFINZI), and atezolizumab (TECENTRIQ); the anti-CTLA-4 antibody ipilimumab (YERVOY). While therapy with such immune checkpoint inhibitors provide advantages for cancer therapy, the overall success remains low, relapse occurs and resistance to checkpoint inhibition develops.

[05] Lymphomas are a heterogenous group of hematological malignancies originating from lymphoid cells. In lymphoma the normal balance between activation and suppression of the immune system is frequently shifted by the tumor cells toward profound immune suppression, which can protect tumor cells from T-cell mediated killing. (Ansell, SM Hematology Am Soc, Hematol Educ Program (2017), 2017(1):618-621.) Immune modulation using agonist antibodies (e.g., anti-CD27, anti-CD40, anti-CD137) has been used in attempts to treat lymphomas, but with only modest therapeutic benefit seen in clinical studies, highlighting the challenge of overcoming the immunosuppressive tumor microenvironment in lymphoma. (Id.)

[06] There is an unmet medical need for improved methods for treating cancer.

3. SUMMARY

[07] This disclosure relates to methods of treating lymphoma in a subject comprising administering an effective amount of an inducible cytokine prodrug optionally in combination with an additional therapeutic, such as a chemotherapeutic agent and/or an immune checkpoint inhibitor (e.g. an anti-PD-1 antibody or an anti-PD-L1 antibody). The disclosure also relates to a combination comprising an inducible cytokine prodrug and an additional therapeutic, such as a chemotherapeutic agent and/or an immune checkpoint inhibitor (e.g. an anti-PD-1 antibody or an anti-PD-L1 antibody) that can be used for treating lymphoma.

[08] This disclosure relates to methods of treating cancer in a subject comprising administering an effective amount of an inducible cytokine prodrug and an anti-PD-1 antibody or an anti-PD-L1 antibody. The disclosure also relates to a combination comprising an inducible cytokine prodrug and an anti-PD-1 antibody or an anti-PD-L1 antibody that can be used for treating cancer. In some instances, an anti-PD-1 antibody will be combined with the inducible cytokine prodrug. In some instances, an anti-PD-L1 antibody will be combined with the inducible cytokine prodrug.

[09] The inducible cytokine prodrug comprises cytokine polypeptide [A], a blocking element [D], optionally a half-life extension element [H] and a protease-cleavable polypeptide linker. The cytokine polypeptide and the cytokine blocking element and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the inducible cytokine prodrug has attenuated cytokine receptor activating activity. The cytokine-receptor activating activity of the inducible cytokine prodrug is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

[10] The cytokine polypeptide can be IL-2, IL-12, interferon alpha, interferon beta, interferon gamma, or a mutein, or an active fragment of any of the foregoing. The cytokine polypeptide can be IL-2, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing. The cytokine polypeptide can be IL-12, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing. The cytokine polypeptide can be interferon alpha, interferon beta, interferon gamma, or a mutein, or an active fragment of any of the foregoing.

[11] The inducible cytokine prodrug can have the formula:

[A]-[L1]-[H]-[L2]-[D]

[D]-[L2]-[H]-[L1]-[A]

[A]-[L1]-[D]-[L2]-[H]

[H]-[L2]-[D]-[L1]-[A]

[H]-[L1]-[A]-[L2']-[D]

[D]-[L1]-[A]-[L2']-[H]

[12] A is a cytokine polypeptide, D is a blocking moiety, H is a half-life extension moiety, L1 is a protease-cleavable polypeptide linker, L2 is an polypeptide linker that is optionally protease-cleavable, and L2' is a protease-cleavable polypeptide linker.

[13] The inducible cytokine prodrug can be a single polypeptide chain. The inducible cytokine prodrug can comprise at least two polypeptide chains. The inducible cytokine prodrug can comprise at least three polypeptide chains.

[14] The cytokine prodrug can comprise Compound 1, Compound 2, Compound 3, Compound 4, or an amino acid sequence variant of the foregoing. Compound 1 can comprise a first polypeptide chain of SEQ ID NO:1 and a second polypeptide chain of SEQ ID NO:5, and the amino acid sequence variant of Compound 1 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO:1 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5. Compound 2 can comprise a first polypeptide chain of SEQ ID NO:2 and a second polypeptide chain of SEQ ID NO:5, and the amino acid sequence variant of Compound 2 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO:2 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5. Compound 3 can comprise a first polypeptide chain of SEQ ID NO:3 and a second polypeptide chain of SEQ ID NO:5, and the amino acid sequence variant of Compound 3 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO:3 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5. Compound 4 can comprise a first polypeptide chain of SEQ ID NO:1 and a second polypeptide chain of SEQ ID NO:4, and the amino acid sequence variant of Compound 4 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO:4 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5.

[15] The inducible cytokine prodrug can be Compound 5, Compound 6, Compound 7, Compound 8, Compound 9, or Compound 10, or an amino acid sequence variant of the foregoing. Compound 5 can comprise a first polypeptide chain of SEQ ID NO: 6 and a second polypeptide chain of SEQ ID NO:12, and the amino acid sequence variant of Compound 5 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 6 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 12. Compound 6 can comprise a first polypeptide chain of SEQ ID NO: 7 and a second polypeptide chain of SEQ

ID NO: 12, and the amino acid sequence variant of Compound 6 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO:7 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 12. Compound 7 can comprise a first polypeptide chain of SEQ ID NO: 8 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 7 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 8 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 13. Compound 8 can comprise a first polypeptide chain of SEQ ID NO: 9 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 8 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 9 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 13. Compound 9 can comprise a first polypeptide chain of SEQ ID NO: 10 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 9 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 10 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 13. Compound 10 can comprise a first polypeptide chain of SEQ ID NO: 11 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 10 can comprise a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 11 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 1.

[16] The inducible cytokine prodrug can comprise the amino acid selected from 14-20, or an amino acid sequence that has at least about 80% identity to SEQ ID NOs 14-20.

[17] The half-life extension element can be a human serum albumin, an antigen binding polypeptide that binds human serum albumin, or an immunoglobulin Fc or fragment thereof.

[18] The protease cleavable linker can comprise a sequence that is capable of being cleaved by a protease selected from kallikrein, thrombin, chymase, carboxypeptidase A, cathepsin, elastase, PR-3, granzyme M, a calpain, a matrix metalloproteinase (MMP), an ADAM, a FAP, a plasminogen activator, a caspase, a tryptase, or a tumor protease.

[19] In particular, the protease can be selected from cathepsin B, cathepsin C, cathepsin D, cathepsin E, cathepsin K, cathepsin L, or cathepsin G. In particular, the protease can be selected from matrix metalloprotease (MMP) is MMP1, MMP2, MMP3, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, or MMP14.

[20] The blocking element can bind the cytokine polypeptide. The blocking element can comprise a ligand-binding domain or fragment of a cognate receptor for the cytokine polypeptide, an antibody or antigen-binding fragment of an antibody that binds to the cytokine polypeptide. The antibody or antigen-binding fragment can be a single domain antibody, a Fab, or a scFv that binds the cytokine polypeptide.

[21] The inducible cytokine prodrug can be administered before, concurrently with or after the additional therapeutic agent, e.g. chemotherapeutic agent, immune checkpoint inhibitor (such as anti-PD-1 antibody or anti-PD-L1 antibody).

[22] The anti-PD-1 antibody can be selected from the group consisting of AMP-224 (AstraZeneca), 609A (3SBio), 704 (3SBio), 705 (3SBio), ABBV-181 (AbbVie), ADU-1503 / bion-004 (Chinook Therapeutics), AGEN2034 / balstilimab (Agenus), AK103 (Akeso), AK104 (Akeso), AK112 (Akeso), AK123 (Akeso), AMG 256 (Amgen), AMG 404 (Amgen), ANB030 (AnaptysBio), ANKEBIO Anti-PD1 product (Anhui Anke Biotechnology), Anti PD-1 / Anti-CD47 (DiNonA), ASKG915 (Ask Gene Pharmaceuticals), AV-MEL-1 (Aivita Biomedical), BCD-100 (Biocad CJSC), BI 754091 (Boehringer Ingelheim), BiCKI-IL-7 (OSE Immunotherapeutics), Boehringer-PD-1-unknown (Boehringer Ingelheim), BSK-050K01 (Biosion), Camrelizumab (Jiangsu Hengrui Medicine), CB201 (Crescendo Biologics), CB213 (Crescendo Biologics), CC-90006 (AnaptysBio), cetrelimab (J&J), chPD1 (Kiromic Biopharma), CMAB819 (Mabpharm), CS1003 (CStone Pharmaceuticals), CS17938 (Shenzhen Chipscreen Biosciences), CTX-8371 (Compass Therapeutics), CX-072 (CytomX Therapeutics), CX-188 (CytomX Therapeutics), cypalizumab (Harbin Gloria Pharmaceuticals), DB004 (DotBio), EMB02 (EpimAb Biotherapeutics), Geptanblimab / genolimzumab (Apollomics), GS19 (Suzhou Zelgen Biopharmaceuticals), HLX10 (Shanghai Henlius Biotech), HX008 (Taizhou HanZhong Pharmaceuticals), HY003 (Juventas Cell Therapy), IBI315 / BH2950 (Innovent Biologics), IBI318 (Innovent Biologics), IBI319 (Innovent Biologics), IMM1802 (ImmuneOnco Biopharma), IMT200 (TrueBinding), Jemperli / dostarlimab (AnaptysBio), JTX-4014 (Jounce Therapeutics), Keytruda / pembrolizumab (Merck), LBL-006 (Nanjing Leads Biolabs), Libtayo / cemiplimab-rwlc (Regeneron Pharmaceuticals), LVGN3616 (Lyvgen Biopharma), LXF821 (Novartis), LY01015 (Luye Pharma Group), LY3462817 (Eli Lilly), MCLA-134 (Merus N.V.), MEDI5752 (AstraZeneca), NIR178 (Novartis), ONCR-177 (Oncorus), ONO-4685 (Ono Pharmaceutical), Opdivo / nivolumab (Ono Pharmaceutical), MGD019 (MacroGenius), PD1-

GDT CAR-T (Kiromic Biopharma), penpulimab (Akeso), PSB205 (Qilu Puget Sound Biotherapeutics), PT-001 (Merck), PT627 (Merck), RB-M1 (Refuge Biotechnologies), Retifanimab (MacroGenics), RG6139 (Roche), RG6279 (Roche), RTX-002 (RubrYc Therapeutics), sasanlimab (Pfizer), Servier-PD1xLAG3-unknown (Servier), SL-279252 / TAK-252 (Shattuck Labs), Sofusa anti-PD1 (Sorrento Therapeutics), spartalizumab (Novartis), SSI-361 (Lyvgen Biopharma), Sym021 (Servier), Tebotelimab (MacroGenics), tislelizumab (BeiGene), TSR-075 (AnaptsBio), Tuhura-DO/PD-1-unknown (Tuhura Biopharma), toripalimab (Shanghai Junshi Biosciences), sintilimab (Innovent Biologics), Unicar-CAR-T&PD-1-unknown (Shanghai Unicar-Therapy Bio-Medicine Technology), Xdivane (Xbrane Biopharma), XmAb20717 (Xencor), XmAb23104 (Xencor), YBL-006 (Y-Biologics), zimberelimab (Arcus Biosciences).

[23] Pembrolizumab, dostarlimab, cemiplimab-rwlc, nivolumab, camrelizumab, tislelizumab, toripalimab, sintilimab or a biosimilar of any of the foregoing are preferred anti-PD-L1 antibodies.

[24] The anti-PD-L1 antibody can be chosen from the group consisting of A167 (Sichuan Kelun), ABL501 (ABL Bio), ABL503 (ABL Bio), ABSK041 (Abbisko Therapeutics), ACE1708 (Acepodia), ACE-NK-PDL1 (Acepodia), ADG104 (Adagene), AK106 (Akeso), ALPN-202 (Alpine Immune Sciences), AN4005 (Adlai Nortye Biopharma), BMS-936559 / MDX-1105 (BMS), APL-502 / TQB2450 (Apollomics), Arbutus-PD-L1-unknown (Arbutus Biopharma), ASC22 (Ascletis Pharma), ATG-101 (Antengene), AVA-004 (Avacta Group), AVA021 (Avacta Group), AVA027 (Avacta Group), AVA-040-100 (Avacta Group), AVA04-Vbp (Avacta Group), Bavencio / avelumab (Merck), BCD-135 (Biocad CJSC), BGB-A333 (BeiGene), Bintrafusp alfa / GSK4045154 (Merck), CA-170 / aupm-170 (Dr. Reddy's Laboratories), CCX559 (ChemoCentryx), CDR101 (CDR-Life), cosibelimab (Checkpoint Therapeutics), CTX-8371 (Compass Therapeutics), DiNonA-Solid Tumors-unknown (DiNonA), DR30207 (Zhejiang Doer Biologics), DuoBody-PD-L1x4-1BB (Ligand Pharmaceuticals), envafolimab (Alphamab Oncology), EPIM-001 (Elpis Biopharmaceuticals), ES101 (Elpiscience Biopharma), INBRX-105 (Inhibrx), FAZ053 (Novartis), FS118 (F-star Therapeutics), GB262 (Genor Biopharma), GS-4224 (Gilead), GT900008 (Kintor Pharmaceuticals), GX-P2 (Genexine), Hamni-PS-L1/CD47-Unknown (Hanmi Pharmaceutical), HBM7015 (HBM Holdings), HBM9167 (HBM Holdings), HLX20 (Shanghai Henlius Biotech), HTI-1088 (Jiangsu Hengrui Medicine), IBI318 (Innovent

Biologics), IBI322 (Innovent Biologics), IBI323 (Innovent Biologics), IGM-7354 (IGM Biosciences), IMC-001 (Sorrento Therapeutics), Imfinzi / durvalumab (AstraZenica), IMM25 (ImmuneOnco Biopharma), IMM2502 (ImmuneOnco Biopharma), IMM2503 (ImmuneOnco Biopharma), IMM2504 (ImmuneOnco Biopharma), INCB86550 (Incyte), IO103 (IO Biotech), JS003 (Shanghai Junshi Biosciences), Jubilant-PD-L1-unknown (Jubilant Therapeutics), KD033 (Kadmon Holdings), KN046 (Alphamab Oncology), KY1003 (Sanofi), KY1043 (Sanofi), LY3300054 (Eli Lilly), LY3415244 (Eli Lilly), MRNA-6981 (Moderna), MSB2311 (Transcenta Holding), MT-6035 (Molecular Templates), ND021 / NM21-1480 (Numab Therapeutics), OX001R (Oxford BioTherapeutics), PD-L1 based BsAbs (I-Mab), PD-L1 Boltbody ISAC (Bolt Biotherapeutics), PDL-GEX (Glycotope GmbH), PMC-122 (PharmAbcine), PMI06 (D&D Pharmatech), Protheragen-RV-scFv-PDL1-unknown (Protheragen), PRS-344 (Pieris Pharmaceuticals), Q-1802 (Merck), RC98 (Yantai Rongchang Pharmaceutical), RV-scFv-PDL1 (Protheragen), SenI_TAAx22P (Hebei Senlang Biotechnology), SHC020 (Nanjing Sanhome Pharmaceutical), sugemalimab (Ligand Pharmaceuticals), atezolizumab (Roche), TST005 (Transcenta Holding), TT-01 (Topmunnity Therapeutics), TTX-siPDL1 (TransCode Therapeutics), UniCAR-T-PD-L1 (GEMoaB monoclonals), Vaximm (VXM10), and YBL-013 (Y-Biologics).

[25] Avelumab, durvalumab, atezolizumab or a biosimilar of any of the foregoing are preferred anti-PD-1 antibodies.

[26] The chemotherapeutic agent can be cyclophosphamide, mechlorethamine, melphalan, chlorambucil, ifosfamide, busulfan, N-Nitroso-N-methylurea (MNU), carmustine (BCNU), lomustine (CCNU), semustine (MeCCNU), fotemustine, streptozotocin, dacarbazine, mitozolomide, temozolomide, thiotepe, mitomycin, diaziquone (AZQ), cisplatin, carboplatin, oxaliplatin, procarbazine, hexamethylmelamine, methotrexate, pemetrexed, fluorouracil (e.g. 5-fluorouracil), capecitabine, cytarabine, gemcitabine, decitabine, azacitidine, fludarabine, nelarabine, cladribine, clofarabine, pentostatin, thioguanine, mercaptopurine, vincristine, vinblastine, vinorelbine, vindesine, vinflunine, paclitaxel, docetaxel, etoposide, teniposide, doxorubicin, daunorubicin, epirubicin, idarubicin, pirarubicin, aclarubicin, mitoxantrone, actinomycin, bleomycin, bisantrene, gemcitabine and/or cytarabine, and any combinations thereof, for example.

[27] The methods disclosed herein can be suitable for any cancer. Exemplary cancers include, but are not limited to, adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, brain tumor, bile duct cancer, bladder cancer, bone cancer, breast cancer, bronchial tumor, carcinoma of unknown primary origin, cardiac tumor, cervical cancer, chordoma, colon cancer, colorectal cancer, craniopharyngioma, ductal carcinoma, embryonal tumor, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, fibrous histiocytoma, Ewing sarcoma, eye cancer, germ cell tumor, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gestational trophoblastic disease, glioma, head and neck cancer, hepatocellular cancer, histiocytosis, hypopharyngeal cancer, intraocular melanoma, islet cell tumor, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ, lung cancer, macroglobulinemia, malignant fibrous histiocytoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, midline tract carcinoma involving NUT gene, mouth cancer, multiple endocrine neoplasia syndrome, mycosis fungoides, myelodysplastic syndrome, myelodysplastic/myeloproliferative neoplasm, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytomas, pituitary tumor, pleuropulmonary blastoma, prostate cancer, rectal cancer, renal cell cancer, renal pelvis and ureter cancer, retinoblastoma, rhabdoid tumor, salivary gland cancer, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cord tumor, stomach cancer, T-cell lymphoma, teratoid tumor, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms tumor. The methods are preferably useful for colon cancer, lung cancer, melanoma, renal cell carcinoma, or breast cancer. The cancer can be melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell cancer (HNSCC), classical Hodgkin lymphoma (cHL), primary mediastinal large B cell lymphoma (PMBCL), microsatellite instability high or mismatch repair deficient cancer, microsatellite instability high or mismatch repair deficient colorectal cancer, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma (HCC), merkel cell carcinoma (MCC), renal cell carcinoma (RCC), endometrial carcinoma, tumor mutational burden high

cancer, cutaneous squamous cell carcinoma (cSCC), triple negative breast cancer (TNBC), urothelial carcinoma, colorectal cancer or oesophageal carcinoma. The cancer can be metastatic renal clear cell carcinoma or metastatic cutaneous malignant melanoma.

[28] In certain embodiments, the cancer is a lymphoma, such as a B cell lymphoma, a T cell lymphoma, Non-Hodgkin Lymphoma, Hodgkin Lymphoma, diffuse large B-cell lymphoma, primary mediastinal B cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, marginal zone lymphoma, mantle cell lymphoma, Waldenstrom's macroglobulinemia, Burkitt lymphoma, peripheral T cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T cell lymphoma, central nervous system lymphoma, grey zone lymphoma, double hit lymphoma, triple hit lymphoma, high grade B cell lymphomas not otherwise specified, lymphoblastic lymphoma, lymphoplasmacytic lymphoma, MALT lymphoma, monocytoid B cell lymphoma, natural killer (NK) cell lymphoma, mycosis fungoides, Sezary syndrome, enteropathy-type T cell lymphoma, hepatosplenic gamma/delta T cell lymphoma, and the like.

[29] In particular embodiments, the cancer is selected from the group consisting of: melanoma, non-small cell lung cancer, head and neck squamous cell cancer, classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, urothelial carcinoma, microsatellite instability-high or mismatch repair deficient cancer, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma, endometrial carcinoma, a cancer characterized by a tumor having a high mutational burden, cutaneous squamous cell carcinoma, and triple negative breast cancer.

[30] The disclosure also relates to pharmaceutical compositions comprising the inducible cytokine prodrug an additional therapeutic, such as a chemotherapeutic agent and/or an immune checkpoint inhibitor (e.g. an anti-PD-1 antibody or an anti-PD-L1 antibody) and a suitable carrier. The disclosure also relates to pharmaceutical compositions comprising the inducible cytokine prodrug, an anti-PD-1 or an anti-PD-L1 antibody, and a suitable carrier. The pharmaceutical composition can be a liquid composition for intravenous administration. The pharmaceutical composition can be a lyophilized composition. The pharmaceutical composition can be a lyophilized composition is for reconstitution using water for formulation is suitable of intravenous administration.

4. BRIEF DESCRIPTION OF DRAWINGS

[31] **FIGs. 1A-1F** shows data (tumor volume and/or body weight) for mice treated with inducible IL-2 prodrugs, anti-PD1 antibody, or inducible IL-2 prodrugs and anti-PD1 antibody.

[32] **FIG. 2A-2B.** shows data (tumor volume and/or body weight) for mice treated with inducible IL-2 prodrugs, anti-PD1 antibody, or inducible IL-2 prodrugs and anti-PD1 antibody.

[33] **FIG. 3A** is a graph showing tumor volume progression in a B16F10 tumor model in which mice were treated with 100 μg of compound 1, 200 μg of compound 1, or only vehicle. 1×10^5 tumor cells were implanted in the flank of animals and tumor growth was monitored. Once tumors reached an average volume of 30-60 mm^3 , the animals were randomized and dosed. Tumor volumes and body weights were recorded three times per week.

[34] **FIG. 3B** is a graph showing tumor volume progression in a B16F10 tumor model in which mice were treated with 100 μg of compound 1, 200 μg of anti-PD-1 antibody (RMP1-14) alone, 100 μg of compound 1 and 200 μg of anti-PD-1 antibody (RMP1-14), or only vehicle. 1×10^5 tumor cells were implanted in the flank of animals and tumor growth was monitored. Once tumors reached an average volume of 30-60 mm^3 , the animals were randomized and dosed. Tumor volumes and body weights were recorded three times per week.

[35] **FIG. 4** is a graph showing body weight progression in a B16F10 tumor model in which mice were treated with compound 1, compound 1 and RMP1-14, or only vehicle. 1×10^5 tumor cells were implanted in the flank of animals and tumor growth was monitored. Once tumors reached an average volume of 30-60 mm^3 , the animals were randomized and dosed. Body weights were recorded three times per week.

[36] **FIG. 5A** is a graph showing results of analyzing inducible IL-12 prodrug (WW0757/636) in a syngeneic A20 mouse tumor model. The graph shows average tumor volume over time in mice treated with 30 μg WW0757/636 (diamond), 100 μg WW0757/636 (square) and 300 μg WW0757/636 (circle). Vehicle alone is indicated by a black circle (top line with largest tumor volume). The data show tumor volume increase was inhibited over time in a dose-dependent manner in mice treated with WW0757/636 at the higher concentrations.

[37] **FIG. 5B** is a graph showing results of analyzing inducible IFN α prodrug WW0610 in a syngeneic A20 mouse tumor model. It shows average tumor volume over time in mice treated with 45 μg WW0610 (diamond), and 133 μg WW0610 (square). Vehicle alone is indicated by a black circle (top line with largest tumor volume). The data show tumor volume increase was

inhibited over time in a dose-dependent manner in mice treated with WW0610 at the higher concentrations.

[38] FIG. 6A is a graph showing results of analyzing inducible IL-12 prodrug (WW0757/636) in a syngeneic EG7.OVA mouse tumor model. It shows average tumor volume over time in mice treated with 30 μ g WW0757/636 (diamond), 100 μ g WW0757/636 (square) and 300 μ g WW0757/636 (circle). Vehicle alone is indicated by a black circle (top line with largest tumor volume data through day 18). The data show tumor volume increase was inhibited over time in a dose-dependent manner in mice treated with WW0757/636 at the higher concentrations.

[39] FIG. 6B is a graph showing results of analyzing inducible IFN α prodrug (WW0610) in a syngeneic EG7.OVA mouse tumor model. It shows average tumor volume over time in mice treated with 45 μ g WW0610 (diamond), and 133 μ g WW0610 (square). Vehicle alone is indicated by a black circle (top line with largest tumor volume data through day 18). The data show tumor volume increase was inhibited over time in a dose-dependent manner in mice treated with WW0610 at the higher concentrations.

[40] FIG. 7A is a graph showing average tumor volumes in mice treated with vehicle (PBS, circles), inducible IL-2 prodrug (WW0621/WW0523, squares), anti-PD-1 (triangles), or WW0621/WW0523 and anti-PD-1 (diamonds). The dosing schedule is also shown (arrows).

[41] FIGs. 7B-7E are graphs of tumor volume in individual mice treated with vehicle (FIG. 7B), WW0621/WW0523 (FIG. 7C), anti-PD-1 (FIG. 7D), or WW0621/WW0523 and anti-PD-1 (FIG. 7E).

[42] FIG. 7F is a graph showing average body weights of mice treated with vehicle (PBS circles), inducible IL-2 prodrug (WW0621/WW0523, squares), anti-PD-1 (triangles), and WW0621/WW0523 and anti-PD-1 (diamonds).

5. DETAILED DESCRIPTION

A. Inducible Cytokines

[43] The disclosure relates to inducible cytokine prodrugs that contain at least one polypeptide chain, and can contain two or more polypeptides, if desired. The inducible cytokine prodrug comprises a cytokine (e.g., IL-2, IL-12, or IFN), a blocking element, a protease cleavable linker, and a half-life extension element. Any cytokine of interest can be suitable for the inducible cytokine polypeptide prodrugs of this disclosure. Exemplary cytokines include, but are not

limited to, interleukins such as IL-2, IL-7, IL-12, IL-15, IL-18, IL-21 IL-23, IFN-alpha (e.g., human IFN-alpha1, human IFN-alpha2, human IFN-alpha4, human IFN-alpha5, human IFN-alpha6, human IFN-alpha7, human IFN-alpha8, human IFN-alpha10, human IFN-alpha13, human IFN-alpha14, human IFN-alpha16, human IFN-alpha17, human IFN-alpha2), IFN-beta, IFN-kappa, or IFN-epsilon, lymphotoxin, TGF-beta1, TGFbeta2, TGFbeta3, GM-CSF, CXCL10, CCL19, CCL20, CCL21 and functional fragments or muteins of any of the foregoing. Preferred cytokines for use in the inducible cytokine prodrugs disclosed herein are IL-2, IL-12, IFN, muteins, functional variants, and functional fragments, or subunits of any of the foregoing.

[44] Inducible cytokine (e.g., IL-2, IL-12, or IFN) prodrugs of this disclosure have attenuated cytokine receptor agonist activity and the circulating half-life is extended. The inducible cytokine receptor agonist activity is attenuated through the blocking element. The half-life extension element can also contribute to attenuation, for example through steric effects. The blocking element is capable of blocking all or some of the receptor agonist activity of the cytokine by noncovalently binding to the cytokine (e.g., to IL-2, IL-12, or IFN) and/or sterically blocking receptor binding. Upon cleavage of the protease cleavable linker a form of the cytokine is released that is active (e.g., more active than the cytokine polypeptide prodrug). Typically, the released cytokine is at least 10 x more active than the cytokine polypeptide prodrug. Preferably, the released cytokine is at least 20 x, at least 30 x, at least 50 x, at least 100 x, at least 200 x, at least 300 x, at least 500 x, at least 1000 x, at least about 10,000X or more active than the cytokine polypeptide complex.

[01] The form of cytokine that is released upon cleavage of the inducible cytokine prodrug typically has a short half-life, which is often substantially similar to the half-life of naturally occurring cytokine. Even though the half-life of the inducible cytokine prodrug is extended, toxicity is reduced or eliminated because the agonist activity of the circulating inducible cytokine prodrug is attenuated and active cytokine is targeted to the desired site of activity (e.g., tumor microenvironment).

[02] It will be appreciated by those skilled in the art, that the number of polypeptide chains, and the location of the elements, the half-life extension element, the protease cleavable linker(s), and the blocking element (and components of such elements, such as a VH or VL domain) on the polypeptide chains can vary and is often a matter of design preference. All such variations are encompassed by this disclosure.

[03] The inducible cytokine prodrug can comprise a single polypeptide chain. Typically, the single polypeptide complex comprises a cytokine polypeptide [A], a blocking element [D], a half-life extension element [H], and a protease cleavable linker [L]. The cytokine [A] polypeptide can be operably linked to the blocking element, the half-life extension element or both the blocking element and the half-life extension element by a protease cleavable linker.

[04] For example, the polypeptide can be of any of Formulas (I)-(VI).

[A]-[L1]-[H]-[L2]-[D] (I);

[D]-[L2]-[H]-[L1]-[A] (II);

[A]-[L1]-[D]-[L2]-[H] (III);

[H]-[L2]-[D]-[L1]-[A] (IV);

[H]-[L1]-[A]-[L2']-[D] (V);

[D]-[L1]-[A]-[L2']-[H] (VI);

[05] In Formulas (I) – (VI), [A] is a cytokine polypeptide, [D] is a blocking element, [H] is a half-life extension element, [L1] is a protease-cleavable polypeptide linker, [L2] is an polypeptide linker that is optionally protease-cleavable, and [L2'] is a protease-cleavable polypeptide linker. [L1] and [L2] or [L1] and [L2'] can be have the same or different amino acid sequence and or protease-cleavage site (when L2 is protease-cleavable) as desired.

[06] SEQ ID NOs: 21-30 are specific examples of inducible IL-2 prodrugs encompassed by Formulas (I)-(VI) and for use according to this disclosure. SEQ ID NOs. 21-30 and additional details regarding their activity is disclosed in International Publication No.: WO2021/097376.

[07] SEQ ID NOs: 31-40 are specific examples of inducible IL-12 prodrugs encompassed by Formulas (I)-(VI) and for use according to this disclosure. SEQ ID NOs.: 31-40 and additional details regarding their activity is disclosed in International Application No.: PCT/US2021/33014 and International Publication No.: WO2019/222295.

[08] In some instances, the single polypeptide complex comprises a cytokine polypeptide [A], a blocking element (i.e., a steric blocking polypeptide) [D], a protease cleavable linker [L], and an optional half-life extension element. The blocking element [D] can be, for example, HSA or an antibody or antibody fragment (e.g. scFv) that binds HSA.

[09] As an example, the polypeptide can be of Formula (VII): [D]-[L1]-[A]-[L2]-[D]. SEQ ID NOs: 14-20 are specific examples, of inducible IFN prodrugs for use according to this disclosure.

SEQ ID NOs: 14-20 and additional details regarding their activity is disclosed in International Application No.: PCT/US2020/060624.

[010] In some instances, the inducible IFN prodrug can comprise IFN polypeptide [A], a blocking element [D], a half-life extension element [H], and a protease cleavable linker [L]. The IFN [A] polypeptide can be operably linked to the blocking element, the half-life extension element or both the blocking element, the half-life extension element by a protease cleavable linker.

[011] IFN polypeptide and the blocking element and the half-life extension element are operably linked by the protease-cleavable polypeptide. For example, the polypeptide can be of any of Formulas (I)-(IX).

[A]-[L1]-[H]-[L2]-[D] (I);

[D]-[L2]-[H]-[L1]-[A] (II);

[A]-[L1]-[D]-[L2]-[H] (III);

[H]-[L2]-[D]-[L1]-[A] (IV);

[H]-[L1]-[A]-[L2']-[D] (V);

[D]-[L1]-[A]-[L2']-[H] (VI);

[H]-[L]-[D]-[L2]-[A]-[L3]-[D'] (VII);

[D]-[L]-[A]-[L2]-[D']-[L3]-[H] (VIII);

[D]-[L]-[H]-[L2]-[D']-[L3]-[A] (IX);

[012] In Formulas (I) – (IX), [A] is a IFN polypeptide, [D] is a IFN blocking element (e.g., extracellular portion of the INFalpha receptor 1 (IFNAR1) or IFNalpha receptor 2 (IFNAR2)), [D'] is either the INFalpha receptor 1 (IFNAR1) or the IFNalpha receptor 2 (IFNAR2) that is not present in [D], [H] is a half-life extension element, [L1] is a protease-cleavable polypeptide linker, [L2] is an polypeptide linker that is optionally protease-cleavable, and [L2'] is a protease-cleavable polypeptide linker. [L1] and [L2] or [L1] and [L2'] can be have the same or different amino acid sequence and or protease-cleavage site (when L2 is protease-cleavable) as desired.

[013] While the inducible cytokine prodrugs disclosed herein preferably contain one half-life extension element and one blocking element, such elements can contain two or more components that are present on the same polypeptide chain or on different polypeptide chains. Illustrative of this, and as disclosed and exemplified herein, components of the blocking element can present on separate polypeptide chains. For example, a first polypeptide chain can include an antibody light

chain (VL+CL) or light chain variable domain (VL) and a second polypeptide can include an antibody heavy chain Fab fragment (VH + CH1) or heavy chain variable domain (VH) that is complementary to the VL+ CL or VL on the first polypeptide. In such situations, these components can associate in the peptide complex to form an antigen-binding site, such as a Fab that binds to the cytokine (e.g., IL-2, IL-12, or IFN) and attenuates the cytokine activity.

[014] For example, the inducible cytokine prodrug can have a first polypeptide of Formulas (X-XI). Formula X: [D]-[L]-[A]-[L2]-[H] or Formula XI: [H]-[L]-[A]-[L2]-[D]. In Formulas (X) – (XI), [A] is a cytokine polypeptide, [D] is an antibody heavy chain Fab fragment (VH + CH1) or heavy chain variable domain (VH), [H] is a half-life extension element, [L1] is a protease-cleavable polypeptide linker, [L2] is an polypeptide linker that is optionally protease-cleavable, and [L2'] is a protease-cleavable polypeptide linker. [L1] and [L2] or [L1] and [L2'] can be have the same or different amino acid sequence and or protease-cleavage site (when L2 is protease-cleavable) as desired. A second polypeptide antibody light chain (VL+CL) or light chain variable domain (VL) that is complementary to the VH + CH1 or VH.

[015] For instances, an inducible cytokine prodrug can comprise two polypeptide chains. The first polypeptide chain can comprise from the cytokine polypeptide, a protease cleavable linker, half-life extension element (e.g., an anti-human serum albumin (HSA) binding single antibody variable domain), and a VH and CH1 of an antibody that binds the cytokine (e.g., IL-2, a IL-12 subunit i.e., p35, p40, or the p35p40 heterodimeric complex, or IFN). The second polypeptide chain can comprise a VL and CL of an antibody that binds the cytokine (e.g., IL-2, IL-12 subunit (i.e., p35, p40, or the p35p40 heterodimeric complex), or IFN) and that together with the VH and CH1 of the first polypeptide chain form a Fab that binds the cytokine (e.g., IL-2, IL-12 subunit (i.e., p35, p40, or the p35p40 heterodimeric complex), or IFN) polypeptide.

[016] Compounds 1, 2, 3 and 4 are specific examples of inducible IL-2 prodrugs that comprise two polypeptide chains for use according to this disclosure. Compounds 1, 2, 3, and 4 and additional details regarding their activity is disclosed in WO2021/097376.

Table 1. Inducible IL-2 prodrugs

IL-2 Prodrug	First Polypeptide	Second Polypeptide
Compound 1	SEQ ID NO:1	SEQ ID NO:5
Compound 2	SEQ ID NO:2	SEQ ID NO:5

Compound 3	SEQ ID NO:3	SEQ ID NO:5
Compound 4	SEQ ID NO:4	SEQ ID NO:5

[017] Cytokines that comprise two subunits, such as IL-12, can also comprise two or more different polypeptides. For instance, the first polypeptide can comprise an IL-12 subunit, and optionally a blocking element. The blocking element when present can be operably linked to the IL-12 subunit through a first protease cleavable linker. The second polypeptide chain can comprise an IL-12 subunit operably linked to a half-life extension element through a second protease cleavable linker, and optionally a IL-12 blocking element. The IL-12 blocking element when present can be operably linked to the IL-12 subunit through a protease cleavable linker or can be operably linked to the half-life extension element through a linker that is optionally protease cleavable. Only one of the first and second polypeptide contains the IL-12 blocking element. When the IL-12 subunit in the first polypeptide is p35, the IL-12 subunit in the second polypeptide is p40, and when the IL-12 subunit in the first polypeptide is p40, the IL-12 subunit in the second polypeptide is p35. A blocking element can be a single chain antibody that binds IL-12 or an antigen binding fragment thereof. The cleavable linkers in this complex can be the same or different.

[018] The inducible cytokine polypeptide prodrug can comprise three different polypeptides. For example, one polypeptide chain comprises either the p35 or p40 IL-12 subunit, but not both, and a second polypeptide comprises the other IL-12 subunit and the third polypeptide comprises at least a portion (component) of the blocking element. The first polypeptide can comprise an IL-12 subunit, and optionally a half-life extension element. The half-life extension element when present can be operably linked to the IL-12 subunit through a protease cleavable linker.

[019] The second polypeptide can comprise a IL-12 subunit, at least an antigen binding portion of an antibody light chain or an antigen binding portion of an antibody heavy chain, and optionally a half-life extension element. When the half-life extension element is present, it can be operably linked to the IL-12 subunit through a protease cleavable linker and the antibody heavy chain or light chain is either a) operably linked to the IL-12 subunit through a second protease cleavable linker, or b) operably linked to the half-life extension element through an optionally cleavable linker.

[020] The third polypeptide can comprise an antigen binding portion of an antibody heavy chain that is complementary to the light chain in the second polypeptide, or an antibody light chain that is complementary to the heavy chain in the second polypeptide and together with said light chain forms an IL-12 binding site. When the IL-12 subunit in the first polypeptide is p35, the IL-12 subunit in the second polypeptide is p40, and when the IL-12 subunit in the first polypeptide is p40, the IL-12 subunit in the second polypeptide is p35. In this complex, the IL-12 blocking element is preferably an antigen binding fragment of an antibody. The antigen binding fragment comprises as separate components, at least an antigen-binding portion of an antibody light chain and at least an antigen-binding portion of a complementary antibody heavy chain. The protease cleavable linkers in this inducible IL-12 polypeptide complex can be the same or different.

[021] The inducible polypeptide complex can comprise two different polypeptides wherein p35 and p40 are located on the same polypeptide chain. A first polypeptide chain can comprise p35, p40, a half-life extension element and at least an antigen binding portion of an antibody light chain. p35 and p40 can be operably linked, and the half-life extension element can be operably linked to p40 through a first protease cleavable linker and the antigen binding portion of an antibody light chain can be operably linked to p35 through a protease cleavable linker.

[022] Alternatively, the half-life extension element can be operably linked to p35 through a protease cleavable linker and the antigen binding portion of an antibody light chain is operably linked to p40 through a protease cleavable linker. The second polypeptide comprises at least an antigen binding portion of an antibody heavy chain that is complementary to the light chain in the second polypeptide and together with said light chain forms an IL-12 binding site. The protease cleavable linkers in this complex can be the same or different.

[023] In an alternative format, a first polypeptide chain can comprise p35, p40, a half-life extension element and at least an antigen binding portion of an antibody heavy chain. p35 and p40 can be operably linked, and the half-life extension element can be operably linked to p40 or through a protease cleavable linker and the antigen binding portion of an antibody heavy chain can be operably linked to p35 through a protease cleavable linker. Alternatively, the half-life extension element can be operably linked to p35 through a protease cleavable linker and the antigen binding portion of an antibody heavy chain can be operably linked to p40 through a second protease cleavable linker. A second polypeptide comprises at least an antigen binding

portion of an antibody light chain that is complementary to the heavy chain in the second polypeptide and together with said light chain forms and IL-12 binding site. The protease cleavable linkers in this complex can be the same or different.

[024] In an example, the IL-12 polypeptide complex comprises a first polypeptide does not comprise a blocking element and the second polypeptide has the formula: [A]-[L1]-[B]-[L3]-[D] or [D]-[L3]-[B]-[L1]-[A] or [B]-[L1]-[A]-[L2]-[D] or [D]-[L1]-[A]-[L2]-[B], wherein, A is the IL-12 subunit; L1 is the first protease-cleavable linker; L2 is the second protease cleavable linker; L3 is the optionally cleavable linker; B is the half-life extension element; and D is the blocking element.

[025] In another example, the first polypeptide comprises the formula: [A]-[L1]-[D] or [D]-[L1]-[A]; and the second polypeptide has the formula: [A']-[L2]-[B] or [B]-[L2]-[A'], wherein A is either p35 or p40, wherein when A is p35, A' is p40 and when A is p40, A' is p35; A' is either p35 or p40; L1 is the first protease cleavable linker; L2 is the second protease cleavable linker; B is the half-life extension element; and D is the blocking element.

[026] Compounds 5, 6, 7, 8, 9, and 10 are specific examples of inducible IL-12 prodrugs that comprise two polypeptide chains for use according to this disclosure. Compounds 5, 6, 7, 8, 9, and 10 and additional details regarding their activity is disclosed in International Application No.: PCT/US2021/33014.

Table 2. Inducible IL-12 prodrugs

IL-12 Prodrug	First Polypeptide	Second Polypeptide
Compound 5	SEQ ID NO: 6	SEQ ID NO: 12
Compound 6	SEQ ID NO: 7	SEQ ID NO: 12
Compound 7	SEQ ID NO: 8	SEQ ID NO:13
Compound 8	SEQ ID NO: 9	SEQ ID NO:13
Compound 9	SEQ ID NO: 10	SEQ ID NO:13
Compound 10	SEQ ID NO: 11	SEQ ID NO:13

[027] As described above, the cytokine can be a mutein, if desired. The cytokine mutein retains activity, for example intrinsic IL-12/IL-2/IFN receptor agonist activity.

B. Half-Life Extension Element

[028] The half-life extension element increases the in vivo half-life and provides altered pharmacodynamics and pharmacokinetics of the inducible cytokine prodrugs. Without being bound by theory, the half-life extension element alters pharmacodynamics properties including alteration of tissue distribution, penetration, and diffusion of the inducible cytokine prodrug. In some embodiments, the half-life extension element can improve tissue targeting, tissue penetration, diffusion within the tissue, and enhanced efficacy as compared with a protein without a half-life extension element. Without being bound by theory, an exemplary way to improve the pharmacokinetics of a polypeptide is by expression of an element in the polypeptide chain that binds to receptors that are recycled to the plasma membrane of cells rather than degraded in the lysosomes, such as the FcRn receptor on endothelial cells and transferrin receptor. Three types of proteins, e.g., human IgGs, HSA (or fragments), and transferrin, persist for much longer in human serum than would be predicted just by their size, which is a function of their ability to bind to receptors that are recycled rather than degraded in the lysosome. These proteins, or fragments retain FcRn binding and are routinely linked to other polypeptides to extend their serum half-life. HSA may also be directly bound to the pharmaceutical compositions or bound via a short linker. Fragments of HSA may also be used. HSA and fragments thereof can function as both a blocking element and a half-life extension element. Human IgGs and Fc fragments can also carry out a similar function.

[029] The serum half-life extension element can also be antigen-binding polypeptide that binds to a protein with a long serum half-life such as serum albumin, transferrin and the like. Examples of such polypeptides include antibodies and fragments thereof including, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody a single chain variable fragment (scFv), single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain of camelid-type nanobody (VHH), a dAb and the like. Other suitable antigen-binding domain include non-immunoglobulin proteins that mimic antibody binding and/or structure such as, anticalins, affilins, affibody molecules, affimers, affitins, alphabodies, avimers, DARPins, fynomers, kunitz domain peptides, monobodies, and binding domains based on other engineered scaffolds such as SpA, GroEL, fibronectin, lipocalin and CTLA4 scaffolds. Further examples of antigen-binding polypeptides include a ligand for a

desired receptor, a ligand-binding portion of a receptor, a lectin, and peptides that binds to or associates with one or more target antigens.

[030] The half-life extension element as provided herein is preferably a human serum albumin (HSA) binding domain, and antigen binding polypeptide that binds human serum albumin or an immunoglobulin Fc or fragment thereof.

[031] The half-life extension element of an inducible cytokine prodrug extends the half-life of the inducible cytokine prodrug by at least about two days, about three days, about four days, about five days, about six days, about seven days, about eight days, about nine days, about 10 days or more.

C. Blocking Element

[032] The blocking element can be any element that binds to the cytokine and inhibits the ability of the cytokine polypeptide to bind and activate its receptor. The blocking element can inhibit the ability of the cytokine (e.g. IL-2, IL-12, or IFN) to bind and/or activate its receptor e.g., by sterically blocking and/or by noncovalently binding to the cytokine polypeptide. The blocking element disclosed herein can bind to IL-2, IL-12 (e.g. p35, p40, or the heterodimer), or IFN (e.g., IFN-alpha (e.g., human IFN-alpha1, human IFN-alpha2, human IFN-alpha4, human IFN-alpha5, human IFN-alpha6, human IFN-alpha7, human IFN-alpha8, human IFN-alpha10, human IFN-alpha13, human IFN-alpha14, human IFN-alpha16, human IFN-alpha17, human IFN-alpha2) IFN-beta, IFN-gamma).

[033] Examples of suitable blocking elements include the full length or a cytokine-binding fragment or mutein of the cognate receptor of a cytokine (e.g. IL-2, IL-12, or IFN). The cognate receptor for IL-2 can be the IL-2 alpha chain, the IL-2 beta chain, the IL-2 gamma chain, or combinations thereof. The cognate receptor for IL-12 can be IL-12R β 1 and/or IL-12R β 2. The cognate receptor for IFN can be the IFNGR receptor or a portion thereof. For instance, when the interferon polypeptide is an IFNalpha, such as INFalpha2a, the blocking element can be the extracellular portion of the INFalpha receptor 1 (IFNAR1) or interferon binding portion or mutein thereof, or the extracellular portion of the IFNalpha receptor 2 (IFNAR2) or interferon binding portion or mutein thereof. When the interferon polypeptide is IFNgamma, the blocking element can be the extracellular portion of the IFNgamma receptor 1 (IFNGR1) or interferon binding portion or mutein thereof, or the extracellular portion of the IFNgamma receptor 2 (IFNGR2) or interferon binding portion or mutein thereof.

[034] Antibodies and antigen-binding fragments thereof including, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody a single chain variable fragment (scFv), single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain of camelid-type nanobody (VHH), a dAb and the like that bind to a cytokine (e.g., IL-2, IL-12, or IFN) can also be used. Other suitable antigen-binding domain that bind to the cytokine polypeptide can also be used, include non-immunoglobulin proteins that mimic antibody binding and/or structure such as, anticalins, affilins, affibody molecules, affimers, affitins, alphabodies, avimers, DARPins, fynomers, kunitz domain peptides, monobodies, and binding domains based on other engineered scaffolds such as SpA, GroEL, fibronectin, lipocalin and CTLA4 scaffolds. Further examples of suitable blocking polypeptides include polypeptides that sterically inhibit or block binding of the to its cognate receptor. Advantageously, such moieties can also function as half-life extending elements. For example, a peptide that is modified by conjugation to a water-soluble polymer, such as PEG, can sterically inhibit or prevent binding of the cytokine to its receptor. Polypeptides, or fragments thereof, that have long serum half-lives can also be used, such as serum albumin (human serum albumin), immunoglobulin Fc, transferrin and the like, as well as fragments and muteins of such polypeptides.

[035] Blocking elements that are particularly suitable are single chain variable fragments (scFv) or Fab fragments.

[036] Also disclosed herein is an inducible cytokine prodrug e that contains a blocking element having specificity for a cytokine and further contains a half-life extension element.

[037] The blocking element can contain two or more components that are present on the same polypeptide chain or on separate polypeptide chains. A first polypeptide chain can include an antibody light chain (VL+CL) or light chain variable domain (VL) and a second polypeptide can include an antibody heavy chain Fab fragment (VH + CH1) or heavy chain variable domain (VH) that is complementary to the VL+ CL or VL on the first polypeptide. In such situations, these components can associate in the peptide complex to form an antigen-binding site, such as a Fab that binds the cytokine (e.g., IL-2, IL-12, IFN) and attenuates cytokine activity.

D. Protease Cleavable Linker

[038] As disclosed herein, the inducible cytokine prodrug comprises one or more linker sequences. A linker sequence serves to provide flexibility between the polypeptides, such that,

for example, the blocking element is capable of inhibiting the activity of the cytokine. The linker can be located between the cytokine subunit, the half-life extension element, and/or the blocking element. As described herein the inducible cytokine prodrug comprises a protease cleavable linker. The protease cleavable linker can comprise one or more cleavage sites for one or more desired protease. Preferably, the desired protease is enriched or selectively expressed at the desired target site of the cytokine activity (e.g., the tumor microenvironment). Thus, the inducible cytokine prodrug is preferentially or selectively cleaved at the target site of desired cytokine activity.

[039] Suitable linkers are typically less than about 100 amino acids. Such linkers can be of different lengths, such as from 1 amino acid (e.g., Gly) to 30 amino acids, from 1 amino acid to 40 amino acids, from 1 amino acid to 50 amino acids, from 1 amino acid to 60 amino acids, from 1 to 70 amino acids, from 1 to 80 amino acids, from 1 to 90 amino acids, and from 1 to 100 amino acids. In some embodiments, the linker is at least about 1, about 2, about 3, about 4, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, or about 100 amino acids in length. Preferred linkers are typically from about 5 amino acids to about 30 amino acids.

[040] Preferably the lengths of linkers vary from 2 to 30 amino acids, optimized for each condition so that the linker does not impose any constraints on the conformation or interactions of the linked domain. In a preferred embodiment, the linker is cleavable by a cleaving agent, e.g., an enzyme. Preferably, the linker comprises a protease cleavage site. In some cases, the linker comprises one or more cleavage sites. The linker can comprise a single protease cleavage site. The linker can also comprise 2 or more protease cleavage sites. For example, 2 cleavage sites, 3 cleavage sites, 4, cleavage sites, 5 cleavage sites, or more. In cases the linker comprises 2 or more protease cleavage sites, the cleavage sites can be cleaved by the same protease or different proteases. A linker comprising two or more cleavage sites is referred to as a “tandem linker.” The two or more cleavage sites can be arranged in any desired orientation, including, but not limited to one cleavage site adjacent to another cleavage site, one cleavage site overlapping another cleavage site, or one cleavage site following by another cleavage site with intervening amino acids between the two cleavage sites.

[041] Of particular interest in the present invention are disease specific protease-cleavable linkers. Also preferred are protease-cleavable linkers that are preferentially cleaved at a desired location in the body, such as the tumor microenvironment, relative to the peripheral circulation. For example, the rate at which the protease-cleavable linker is cleaved in the tumor microenvironment can be at least about 10 times, at least about 100 times, at least about 1000 times or at least about 10,000 times faster in the desired location in the body, e.g., the tumor microenvironment, in comparison to in the peripheral circulation (e.g., in plasma).

[042] Proteases known to be associated with diseased cells or tissues include but are not limited to serine proteases, cysteine proteases, aspartate proteases, threonine proteases, glutamic acid proteases, metalloproteases, asparagine peptide lyases, serum proteases, cathepsins, Cathepsin B, Cathepsin C, Cathepsin D, Cathepsin E, Cathepsin G, Cathepsin K, Cathepsin L, kallikreins, hK1, hK10, hK15, plasmin, collagenase, Type IV collagenase, stromelysin, Factor Xa, chymotrypsin-like protease, trypsin-like protease, elastase-like protease, subtilisin-like protease, actinidain, bromelain, calpain, caspases, caspase-3, Mirt-CP, papain, HIV-1 protease, HSV protease, CMV protease, chymosin, renin, pepsin, matriptase, legumain, plasmepsin, nepenthesin, metalloexopeptidases, metalloendopeptidases, matrix metalloproteases (MMP), MMP1, MMP2, MMP3, MMP8, MMP9, MMP13, MMP11, MMP14, urokinase plasminogen activator (uPA), enterokinase, prostate-specific antigen (PSA, hK3), interleukin-1 β converting enzyme, thrombin, FAP (FAP α), dipeptidyl peptidase, meprins, granzymes and dipeptidyl peptidase IV (DPPIV/CD26). Proteases capable of cleaving linker amino acid sequences (which can be encoded by the chimeric nucleic acid sequences provided herein) can, for example, be selected from the group consisting of a prostate specific antigen (PSA), a matrix metalloproteinase (MMP), an A Disintegrin and a Metalloproteinase (ADAM), a plasminogen activator, a cathepsin, a caspase, a tumor cell surface protease, and an elastase. The MMP can, for example, be matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), matrix metalloproteinase 14 (MMP14). In addition, or alternatively, the linker can be cleaved by a cathepsin, such as, Cathepsin B, Cathepsin C, Cathepsin D, Cathepsin E, Cathepsin G, Cathepsin K and/or Cathepsin L. Preferably, the linker can be cleaved by MMP14 or Cathepsin L.

[043] Proteases useful for cleavage of linkers and for use in the inducible cytokine prodrug disclosed herein are presented in Table 3, and exemplary proteases and their cleavage site are presented in Table 4.

Table 3. Proteases relevant to inflammation and cancer

Protease	Specificity	Other aspects
<i>Secreted by killer T cells:</i>		
Granzyme B (grB)	Cleaves after Asp residues (asp-ase)	Type of serine protease; strongly implicated in inducing perforin-dependent target cell apoptosis
Granzyme A (grA)	trypsin-like, cleaves after basic residues	Type of serine protease;
Granzyme H (grH)	Unknown substrate specificity	Type of serine protease; <i>Other granzymes are also secreted by killer T cells, but not all are present in humans</i>
Caspase-8	Cleaves after Asp residues	Type of cysteine protease; plays essential role in TCR-induced cellular expansion-exact molecular role unclear
Mucosa-associated lymphoid tissue (MALT1)	Cleaves after arginine residues	Type of cysteine protease; likely acts both as a scaffold and proteolytically active enzyme in the CBM-dependent signaling pathway
Tryptase	Targets: angiotensin I, fibrinogen, prourokinase, TGF β ; preferentially cleaves proteins after lysine or arginine residues	Type of mast cell-specific serine protease; trypsin-like; resistant to inhibition by macromolecular protease inhibitors expressed in mammals due to their tetrameric structure, with all sites facing narrow central pore; <i>also associated with inflammation</i>
<i>Associated with inflammation:</i>		
Thrombin	Targets: FGF-2, HB-EGF, Osteo-pontin, PDGF, VEGF	Type of serine protease; modulates activity of vascular growth factors, chemokines and extracellular proteins; strengthens VEGF-induced proliferation; induces cell migration; angiogenic factor; regulates hemostasis
Chymase	Exhibit chymotrypsin-like specificity, cleaving proteins after aromatic amino acid residues	Type of mast cell-specific serine protease
Carboxypeptidase A (MC-CPA)	Cleaves amino acid residues from C-terminal end of peptides and proteins	Type of zinc-dependent metalloproteinase
Kallikreins	Targets: high molecular weight kininogen, pro-urokinase	Type of serine protease; modulate relaxation response; contribute to inflammatory response; fibrin degradation
Elastase	Targets: E-cadherin, GM-CSF, IL-1, IL-2, IL-6, IL8, p38 ^{MAPK} , TNF α , VE-cadherin	Type of neutrophil serine protease; degrades ECM components; regulates inflammatory response; activates pro-apoptotic signaling

Protease	Specificity	Other aspects
Cathepsin G	Targets: EGF, ENA-78, IL-8, MCP-1, MMP-2, MT1-MMP, PAI-1, RANTES, TGF β , TNF α	Type of serine protease; degrades ECM components; chemo-attractant of leukocytes; regulates inflammatory response; promotes apoptosis
PR-3	Targets: ENA-78, IL-8, IL-18, JNK, p38 ^{MAPK} , TNF α	Type of serine protease; promotes inflammatory response; activates pro-apoptotic signaling
Granzyme M (grM)	Cleaves after Met and other long, unbranched hydrophobic residues	Type of serine protease; only expressed in NK cells
Calpains	Cleave between Arg and Gly	Family of cysteine proteases; calcium-dependent; activation is involved in the process of numerous inflammation-associated diseases

Table 4. Exemplary Proteases and Protease Recognition Sequences

Protease	Cleavage Domain Sequence	SEQ ID NO:
MMP7	KRALGLPG	375
MMP7	(DE) ₈ RPLALWRS(DR) ₈	376
MMP9	PR(S/T)(L/I)(S/T)	
MMP9	LEATA	378
MMP11	GGAANLVRGG	379
MMP14	SGRIGFLRTA	380
MMP	PLGLAG	381
MMP	PLGLAX	382
MMP	PLGC(me)AG	383
MMP	ESPAYYTA	384
MMP	RLQLKL	385
MMP	RLQLKAC	386
MMP2, MMP9, MMP14	EP(Cit)G(Hof)YL	387
Urokinase plasminogen activator (uPA)	SGRSA	388
Urokinase plasminogen activator (uPA)	DAFK	389
Urokinase plasminogen activator (uPA)	GGGRR	390
Lysosomal Enzyme	GFLG	391
Lysosomal Enzyme	ALAL	392
Lysosomal Enzyme	FK	

Protease	Cleavage Domain Sequence	SEQ ID NO:
Cathepsin B	NLL	
Cathepsin D	PIC(Et)FF	395
Cathepsin K	GGPRGLPG	396
Prostate Specific Antigen	HSSKLQ	397
Prostate Specific Antigen	HSSKLQL	398
Prostate Specific Antigen	HSSKLQEDA	399
Herpes Simplex Virus Protease	LVLASSSFGY	400
HIV Protease	GVSQNYPIVG	401
CMV Protease	GVVQASCRLA	402
Thrombin	F(Pip)RS	
Thrombin	DPRSFL	404
Thrombin	PPRSFL	405
Caspase-3	DEVD	406
Caspase-3	DEVDP	407
Caspase-3	KGSGDVEG	408
Interleukin 1 β converting enzyme	GWEHDG	409
Enterokinase	EDDDDKA	410
FAP	KQEQNPGST	411
Kallikrein 2	GKAFRR	412
Plasmin	DAFK	413
Plasmin	DVLK	414
Plasmin	DAFK	415
TOP	ALLLALL	416
	GPLGVRG	417
	IPVSLRSG	418
	VPLSLYSG	419
	SGESPAYTA	420

[044] Exemplary protease cleavable linkers include, but are not limited to kallikrein cleavable linkers, thrombin cleavable linkers, chymase cleavable linkers, carboxypeptidase A cleavable linkers, cathepsin cleavable linkers, elastase cleavable linkers, FAP cleavable linkers, ADAM cleavable linkers, PR-3 cleavable linkers, granzyme M cleavable linkers, a calpain cleavable linkers, a matrix metalloproteinase (MMP) cleavable linkers, a plasminogen activator cleavable

linkers, a caspase cleavable linkers, a tryptase cleavable linkers, or a tumor cell surface protease. Specifically, MMP9 cleavable linkers, ADAM cleavable linkers, CTSL1 cleavable linkers, FAP α cleavable linkers, and cathepsin cleavable linkers. Some preferred protease-cleavable linkers are cleaved by a MMP and/or a cathepsin.

[045] The separation moieties disclosed herein are typically less than 100 amino acids. Such separation moieties can be of different lengths, such as from 1 amino acid (e.g., Gly) to 30 amino acids, from 1 amino acid to 40 amino acids, from 1 amino acid to 50 amino acids, from 1 amino acid to 60 amino acids, from 1 to 70 amino acids, from 1 to 80 amino acids, from 1 to 90 amino acids, and from 1 to 100 amino acids. In some embodiments, the linker is at least about 1, about 2, about 3, about 4, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, or about 100 amino acids in length. Preferred linkers are typically from about 5 amino acids to about 30 amino acids.

[046] Preferably the lengths of linkers vary from 2 to 30 amino acids, optimized for each condition so that the linker does not impose any constraints on the conformation or interactions of the linked domains.

[047] In some embodiments, the linker comprises the sequence GPAGLYAQ (SEQ ID NO: 195); GPAGMKGL (SEQ ID NO: 196); PGGPAGIG (SEQ ID NO: 197); ALFKSSFP (SEQ ID NO: 198); ALFFSSPP (SEQ ID NO: 199); LAQRLRSS (SEQ ID NO: 200); LAQKLKSS (SEQ ID NO: 201); GALFKSSFPSSGGGPAGLYAQGGSGKGGSGK (SEQ ID NO: 202); RSGGGPAGLYAQSGGGPAGLYAQGGSGK (SEQ ID NO: 203); KGGGPAGLYAQGPAGLYAQGPAGLYAQGSR (SEQ ID NO: 204); RGGPAGLYAQGGPAGLYAQGGGPAGLYAQK (SEQ ID NO: 205); KGGALFKSSFPGGPAGIGPLAQKLKSSGGS (SEQ ID NO: 206); SGGPAGIGALFKSSFPPLAQKLKSSGGG (SEQ ID NO: 207); RGPLAQKLKSSALFKSSFPGGPAGIGGGGK (SEQ ID NO: 208); GGGALFKSSFPPLAQKLKSSPGGPAGIGGGR (SEQ ID NO: 209); RGPAGPAGIGPLAQKLKSSALFKSSFPGGG (SEQ ID NO: 210); RGGPLAQKLKSSPGGPAGIGALFKSSFPKG (SEQ ID NO: 211); RSGGPAGLYAQALFKSSFPPLAQKLKSSGGG (SEQ ID NO: 212); GGPLAQKLKSSALFKSSFPGPAGLYAQGGGR (SEQ ID NO: 213);

GGALFKSSFPGPAGLYAQPLAQKLKSSGGK (SEQ ID NO: 214);
 RGGALFKSSFPLAQKLKSSGPAGLYAQGGK (SEQ ID NO: 215);
 RGGGPAGLYAQPLAQKLKSSALFKSSFPGG (SEQ ID NO: 216);
 SGPLAQKLKSSGPAGLYAQALFKSSFPGSK (SEQ ID NO: 217);
 KGGPGGPAGIGPLAQRLRSSALFKSSFPGR (SEQ ID NO: 218);
 KSGPGGPAGIGALFFSSPPLAQKLKSSGGR (SEQ ID NO: 219); or
 SGGFPRSGGSFNPRTFGSKRKRGRSGGGG (SEQ ID NO: 220)

[048] Certain preferred separation moieties comprises the sequence GPAGLYAQ (SEQ ID NO: 195) or ALFKSSFP (SEQ ID NO: 198). The separation moieties disclosed herein can comprise one or more cleavage motif or functional variants that are the same or different. The separation moieties can comprise 1, 2, 3, 4, 5, or more cleavage motifs or functional variants. Separation moieties comprising 30 amino acids can contain 2 cleavage motifs or functional variants, 3 cleavage motifs or functional variants or more. A “functional variant” of a linker retains the ability to be cleaved with high efficiency at a target site (e.g., a tumor microenvironment that expresses high levels of the protease) and are not cleaved or cleaved with low efficiency in the periphery (e.g., serum). For example, the functional variants retain at least about 50%, about 55%, about 60%, about 70%, about 80%, about 85%, about 95% or more of the cleavage efficiency of a linker comprising any one of SEQ ID NOs: 195-220 or 447-448.

[049] The separation moieties comprising more than one cleavage motif can be selected from SEQ ID NOs: 195-201 or 447-448 and combinations thereof. Preferred separation moieties comprising more than one cleavage motif comprise the amino acids selected from SEQ ID NO: 202-220.

[050] The linker can comprise both ALFKSSFP (SEQ ID NO: 198) and GPAGLYAQ (SEQ ID NO: 195). The linker can comprise two cleavage motifs that each have the sequence GPAGLYAQ (SEQ ID NO: 195). Alternatively or additionally, the linker can comprise two cleavage motifs that each have the sequence ALFKSSFP (SEQ ID NO: 198). The linker can comprise a third cleavage motif that is the same or different.

[051] In some embodiments, the linker comprises an amino acid sequence that is at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 99% identical to SEQ ID NOs: 195 to SEQ ID NO: 220 or 447-448 over the full length of SEQ ID NO: 195-220 or SEQ ID NOS 447-448.

[052] The disclosure also relates to functional variants of separation moieties comprising SEQ ID NOs: 195-220 or 447-448. The functional variants of separation moieties comprising SEQ ID NOs: 195-220 or 447-448 generally differ from SEQ ID NOs: 195-220 or 447-448 by one or a few amino acids (including substitutions, deletions, insertions, or any combination thereof), and substantially retain their ability to be cleaved by a protease.

[053] The functional variants can contain at least one or more amino acid substitutions, deletions, or insertions relative to the separation moieties comprising SEQ ID NOs: 195-220 or 447-448. The functional variant can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid alterations compared to the separation moieties comprising SEQ ID NOs: 195-220 or 447-448. In some preferred embodiments, the functional variant differs from the linker comprising SEQ ID NOs: 195-220 by less than 10, less than 8, less than 5, less than 4, less than 3, less than 2, or one amino acid alterations, e.g., amino acid substitutions or deletions. In other embodiments, the functional variant may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions compared to SEQ ID NOs: 195-220 or 447-448. The amino acid substitution can be a conservative substitution or a non-conservative substitution, but preferably is a conservative substitution.

[054] In other embodiments, the functional variants of the separation moieties may comprise 1, 2, 3, 4, or 5 or more non-conservative amino acid substitutions compared the separation moieties comprising SEQ ID NOs: 195-220 or 447-448. Non-conservative amino acid substitutions could be recognized by one of skill in the art. The functional variant of the linker preferably contains no more than 1, 2, 3, 4, or 5 amino acid deletions.

[055] The amino acid sequences disclosed in the separation moieties can be described by the relative linear position in the linker with respect to the scissile bond. As will be well-understood by persons skilled in the art, separation moieties comprising 8 amino acid protease substrates (e.g., SEQ ID Nos: 195-201 or 447-448) contain amino acid at positions P4, P3, P2, P1, P1', P2', P3', P4', wherein the scissile bond is between P1 and P1'. For example, amino acid positions for the linker comprising the sequence GPAGLYAQ (SEQ ID NO: 195) can be described as follows:

G	P	A	G	L	Y	A	Q
P4	P3	P2	P1	P1'	P2'	P3'	P4'

"GPAGLYAQ" disclosed as SEQ ID NO: 195.

[056] Amino acids positions for the linker comprising the sequence ALFKSSFP (SEQ ID NO: 198) can be described as follows:

A	L	F	K	S	S	F	P
P4	P3	P2	P1	P1'	P2'	P3'	P4'

"ALFKSSFP" disclosed as SEQ ID NO: 198.

[057] Preferably, the amino acids surrounding the cleavage site (e.g., positions P1 and P1' for SEQ ID NOs: 195-201 or 447-448) are not substituted.

[058] In embodiments, the linker comprises the sequence GPAGLYAQ (SEQ ID NO: 195) or ALFKSSFP (SEQ ID NO: 198) or a functional variant of SEQ ID NO: 195 or a function variant of SEQ ID NO: 198. As described herein, a functional variant of PAGLYAQ (SEQ ID NO: 447) or ALFKSSFP (SEQ ID NO: 198) can comprise one or more amino acid substitutions, and substantially retain their ability to be cleaved by a protease. Specifically, the functional variants of GPAGLYAQ (SEQ ID NO: 195) is cleaved by MMP14, and the functional variant of ALFKSSFP (SEQ ID NO: 198) is cleaved by Cathepsin L (CTSL1). The functional variants also retain their ability to be cleaved with high efficiency at a target site (e.g., a tumor microenvironment that expresses high levels of the protease). For example, the functional variants of GPAGLYAQ (SEQ ID NO: 195) or ALFKSSFP (SEQ ID NO: 198) retain at least about 50%, about 55%, about 60%, about 70%, about 80%, about 85%, about 95% or more of the cleavage efficiency of a linker comprising amino acid sequence GPAGLYAQ (SEQ ID NO: 195) or ALFKSSFP (SEQ ID NO: 198), respectively.

[059] Preferably, the functional variant of GPAGLYAQ (SEQ ID NO: 195) or ALFKSSFP (SEQ ID NO: 198) comprise no more than 1, 2, 3, 4, or 5 conservative amino acid substitutions compared to GPAGLYAQ (SEQ ID NO: 195) or ALFKSSFP (SEQ ID NO: 198). Preferably, the amino acids at position P1 and P1' are not substituted. The amino acids at positions P1 and P1' in SEQ ID NO: 195 are G and L, and the amino acids at positions P1 and P1' in SEQ ID NO: 198 are K and S.

[060] The functional variant of GPAGLYAQ (SEQ ID NO: 195) can preferably comprise one or more of the following: a) an arginine amino acid substitution at position P4, b) a leucine, valine, asparagine, or proline amino acid substitution at position P3, c) a asparagine amino acid substitution at position P2, d) a histidine, asparagine, or glycine amino acid substitution at

position P1, e) a asparagine, isoleucine, or leucine amino acid substitution at position P1', f) a tyrosine or arginine amino acid substitution at position P2', g) a glycine, arginine, or alanine amino acid substitution at position P3', h) or a serine, glutamine, or lysine amino acid substitution at position P4'. The following amino acid substitutions are disfavored in functional variants of GPAGLYAQ (SEQ ID NO: 195): a) arginine or isoleucine at position P3, b) alanine at position P2, c) valine at position P1, d) arginine, glycine, asparagine, or threonine at position P1', e) aspartic acid or glutamic acid at position P2', f) isoleucine at position P3', g) valine at position P4'. In some embodiments, the functional variant of GPAGLYAQ (SEQ ID NO: 195) does not comprise an amino acid substitution at position P1 and/or P1'.

[061] The amino acid substitution of the functional variant of GPAGLYAQ (SEQ ID NO: 195) preferably comprises an amino acid substitution at position P4 and/or P4'. For example, the functional variant of GPAGLYAQ (SEQ ID NO: 195) can comprise a leucine at position P4, or serine, glutamine, lysine, or phenylalanine at position P4. Alternatively or additionally, the functional variant of GPAGLYAQ (SEQ ID NO: 195) can comprise a glycine, phenylalanine, or a proline at position P4'.

[062] In some embodiments, the amino acid substitutions at position P2 or P2' of GPAGLYAQ (SEQ ID NO: 195) are not preferred.

[063] In some embodiments, the functional variant of GPAGLYAQ (SEQ ID NO: 195) comprises the amino acid sequence selected from SEQ ID NOs: 221- 295. Specific functional variants of GPAGLYAQ (SEQ ID NO: 195) include GPLGLY AQ (SEQ ID NO: 259), and GPAGLKGA (SEQ ID NO: 249).

[064] The functional variants of LFKSSFP (SEQ ID NO: 448) preferably comprises hydrophobic amino acid substitutions. The functional variant of LFKSSFP (SEQ ID NO: 448) can preferably comprise one or more of the following: (a) lysine, histidine, serine, glutamine, leucine, proline, or phenylalanine at position P4; (b) lysine, histidine, glycine, proline, asparagine, phenylalanine at position P3; (c) arginine, leucine, alanine, glutamine, or histidine at position P2; (d) phenylalanine, histidine, threonine, alanine, or glutamine at position P1; (e) histidine, leucine, lysine, alanine, isoleucine, arginine, phenylalanine, asparagine, glutamic acid, or glycine at position P1', (f) phenylalanine, leucine, isoleucine, lysine, alanine, glutamine, or proline at position P2'; (g) phenylalanine, leucine, glycine, serine, valine, histidine, alanine, or

asparagine at position P3'; and phenylalanine, histidine, glycine, alanine, serine, valine, glutamine, lysine, or leucine.

[065] The inclusion of aspartic acid and/or glutamic acid in functional variants of SEQ ID NO: 448 are generally disfavored and avoided. The following amino acid substitutions are also disfavored in functional variants of LFKSSFP (SEQ ID NO: 448): (a) alanine, serine, or glutamic acid at position P3; (b) proline, threonine, glycine, or aspartic acid at position P2; (c) proline at position P1; (d) proline at position P1'; (e) glycine at position P2'; (f) lysine or glutamic acid at position P3'; (g) aspartic acid at position P4'.

[066] The amino acid substitution of the functional variant of LFKSSFP (SEQ ID NO: 448) preferably comprises an amino acid substitution at position P4 and/or P1. In some embodiments, an amino acid substitution of the functional variant of LFKSSFP (SEQ ID NO: 448) at position P4' is not preferred.

[067] In some embodiments, the functional variant of LFKSSFP (SEQ ID NO: 448) comprises the amino acid sequence selected from SEQ ID NOs: 296- 374. Specific functional variants of LFKSSFP (SEQ ID NO: 448) include ALFFSSPP (SEQ ID NO: 199), ALFKSFPP (SEQ ID NO: 346), ALFKSLPP (SEQ ID NO: 347); ALFKHSPP (SEQ ID NO: 335); ALFKSIPP (SEQ ID NO: 348); ALFKSSLP (SEQ ID NO: 356); or SPFRSSRQ (SEQ ID NO: 297).

[068] The separation moieties disclosed herein can form a stable prodrug under physiological conditions with the amino acid sequences (e.g. domains) that they link, while being capable of being cleaved by a protease. For example, the linker is stable (e.g., not cleaved or cleaved with low efficiency) in the circulation and cleaved with higher efficiency at a target site (i.e. a tumor microenvironment). Accordingly, fusion polypeptides that include the linkers disclosed herein can, if desired, have a prolonged circulation half-life and/or lower biological activity in the circulation in comparison to the components of the fusion polypeptide as separate molecular entities. Yet, when in the desired location (e.g., tumor microenvironment) the linkers can be efficiently cleaved to release the components that are joined together by the linker and restoring or nearly restoring the half-life and biological activity of the components as separate molecular entities.

[069] The linker desirably remains stable in the circulation for at least 2 hours, at least 5, hours, at least 10 hours, at least 15 hours, at least 20 hours, at least 24 hours, at least 30 hours, at least

35 hours, at least 40 hours, at least 45 hours, at least 50 hours, at least 60 hours, at least 65 hours, at least 70 hours, at least 80 hours, at least 90 hours, or longer.

[070] In some embodiments, the linker is cleaved by less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 20%, 5%, or 1% in the circulation as compared to the target location. The linker is also stable in the absence of an enzyme capable of cleaving the linker. However, upon exposure to a suitable enzyme (i.e., a protease), the linker is cleaved resulting in separation of the linked domain.

Additional Anti-cancer Agents

[071] This disclosure relates to a therapeutic combination of any of the inducible cytokine prodrugs disclosed herein (e.g., inducible cytokines that comprise an IL-2 polypeptide, and IL-12 polypeptide, or an IFN polypeptide) in combination with one or more additional agents to treat cancer (such as lymphoma), such as chemotherapeutic agents (e.g., cyclophosphamide, mechlorethamine, melphalan, chlorambucil, ifosfamide, busulfan, N-Nitroso-N-methylurea (MNU), carmustine (BCNU), lomustine (CCNU), semustine (MeCCNU), fotemustine, streptozotocin, dacarbazine, mitozolomide, temozolomide, thiotepa, mitomycin, diaziquone (AZQ), cisplatin, carboplatin, oxaliplatin, procarbazine, hexamethylmelamine, methotrexate, pemetrexed, fluorouracil (e.g. 5-fluorouracil), capecitabine, cytarabine, gemcitabine, decitabine, azacitidine, fludarabine, nelarabine, cladribine, clofarabine, pentostatin, thioguanine, mercaptopurine, vincristine, vinblastine, vinorelbine, vindesine, vinflunine, paclitaxel, docetaxel, etoposide, teniposide, doxorubicin, daunorubicin, epirubicin, idarubicin, pirarubicin, aclarubicin, mitoxantrone, actinomycin, bleomycin, bisantrene, gemcitabine, cytarabine, and the like), immuno-oncology agents and immune checkpoint inhibitors (e.g., anti-PD-L1, anti-CTLA4, anti-PD-1, anti-CD47, anti-GD2), oncolytic viruses and the like.

[072] The inducible cytokine (e.g., IL-2, IL-12, and IFN) prodrugs disclosed herein can be combined with any desired additional anti-cancer agent. The inducible cytokine (e.g., IL-2, IL-12, and IFN) prodrugs disclosed herein can be combined with any desired anti-PD-1 antibody or any desired anti-PD-L1 antibody.

[073] Exemplary anti-PD-1 antibodies that can be combined with the inducible cytokine prodrugs include, but are not limited to, AMP-224 (AstraZenica), 609A (3SBio), 704 (3SBio), 705 (3SBio), ABBV-181 (AbbVie), ADU-1503 / bion-004 (Chinook Therapeutics), AGEN2034 / balstilimab (Agenus), AK103 (Akeso), AK104 (Akeso), AK112 (Akeso), AK123 (Akeso),

AMG 256 (Amgen), AMG 404 (Amgen), ANB030 (AnaptysBio), ANKEBIO Anti-PD1 product (Anhui Anke Biotechnology), Anti PD-1 / Anti-CD47 (DiNonA), ASKG915 (Ask Gene Pharmaceuticals), AV-MEL-1 (Aivita Biomedical), BCD-100 (Biocad CJSC), BI 754091 (Boehringer Ingelheim), BiCKI-IL-7 (OSE Immunotherapeutics), Boehringer-PD-1-unknown (Boehringer Ingelheim), BSK-050K01 (Biosion), Camrelizumab (Jiangsu Hengrui Medicine), CB201 (Crescendo Biologics), CB213 (Crescendo Biologics), CC-90006 (AnaptysBio), cetrelimab (J&J), chPD1 (Kiromic Biopharma), CMAB819 (Mabpharm), CS1003 (CStone Pharmaceuticals), CS17938 (Shenzhen Chipscreen Biosciences), CTX-8371 (Compass Therapeutics), CX-072 (CytomX Therapeutics), CX-188 (CytomX Therapeutics), cypalizumab (Harbin Gloria Pharmaceuticals), DB004 (DotBio), EMB02 (EpimAb Biotherapeutics), Geptanblimab / genolimzumab (Apollomics), GS19 (Suzhou Zelgen Biopharmaceuticals), HLX10 (Shanghai Henlius Biotech), HX008 (Taizhou HanZhong Pharmaceuticals), HY003 (Juventas Cell Therapy), IBI315 / BH2950 (Innovent Biologics), IBI318 (Innovent Biologics), IBI319 (Innovent Biologics), IMM1802 (ImmuneOnco Biopharma), IMT200 (TrueBinding), Jemperli / dostarlimab (AnaptysBio), JTX-4014 (Jounce Therapeutics), Keytruda / pembrolizumab (Merck), LBL-006 (Nanjing Leads Biolabs), Libtayo / cemiplimab-rwlc (Regeneron Pharmaceuticals), LVGN3616 (Lyvgen Biopharma), LXF821 (Novartis), LY01015 (Luye Pharma Group), LY3462817 (Eli Lilly), MCLA-134 (Merus N.V.), MEDI5752 (AstraZenica), NIR178 (Novartis), ONCR-177 (Oncorus), ONO-4685 (Ono Pharmaceutical), Opdivo / nivolumab (Ono Pharmaceutical), MGD019 (MacroGenius), PD1-GDT CAR-T (Kiromic Biopharma), penpulimab (Akeso), PSB205 (Qilu Puget Sound Biotherapeutics), PT-001 (Merck), PT627 (Merck), RB-M1 (Refuge Biotechnologies), Retifanlimab (MacroGenics), RG6139 (Roche), RG6279 (Roche), RTX-002 (RubrYc Therapeutics), sasanlimab (Pfizer), Servier-PD1xLAG3-unknown (Servier), SL-279252 / TAK-252 (Shattuck Labs), Sofusa anti-PD1 (Sorrento Therapeutics), spartalizumab (Novartis), SSI-361 (Lyvgen Biopharma), Sym021 (Servier), Tebotelimab (MacroGenics), tislelizumab (BeiGene), TSR-075 (AnaptysBio), Tuhura-DO/PD-1-unknown (Tuhura Biopharma), toripalimab (Shanghai Junshi Biosciences), sintilimab (Innovent Biologics), Unicar-CAR-T&PD-1-unknown (Shanghai Unicar-Therapy Bio-Medicine Technology), Xdivane (Xbrane Biopharma), XmAb20717 (Xencor), XmAb23104 (Xencor), YBL-006 (Y-Biologics), and zimberelimab (Arcus Biosciences).

[074] The anti-PD-1 antibody that can be combined with the inducible cytokine prodrugs is typically an approved anti-PD-1 antibody. Approved anti-PD-1 antibodies include, but are not limited to, pembrolizumab (KEYTRUDA), dostarlimab (JEMPERLI), cemiplimab-rwlc (LIBATYO), nivolumab (OPDIVO), camrelizumab, tislelizumab, toripalimab, and sintilimab (TYVYT).

[075] Exemplary anti-PD-L1 antibodies that can be combined with the inducible cytokine prodrugs include, but are not limited to, A167 (Sichuan Kelun), ABL501 (ABL Bio), ABL503 (ABL Bio), ABSK041 (Abbisko Therapeutics), ACE1708 (Acepodia), ACE-NK-PDL1 (Acepodia), ADG104 (Adagene), AK106 (Akeso), ALPN-202 (Alpine Immune Sciences), AN4005 (Adlai Nortye Biopharma), BMS-936559 / MDX-1105 (BMS), APL-502 / TQB2450 (Apollomics), Arbutus-PD-L1-unknown (Arbutus Biopharma), ASC22 (Ascletis Pharma), ATG-101 (Antengene), AVA-004 (Avacta Group), AVA021 (Avacta Group), AVA027 (Avacta Group), AVA-040-100 (Avacta Group), AVA04-Vbp (Avacta Group), Bavencio / avelumab (Merck), BCD-135 (Biocad CJSC), BGB-A333 (BeiGene), Bintrafusp alfa / GSK4045154 (Merck), CA-170 / aupm-170 (Dr. Reddy's Laboratories), CCX559 (ChemoCentryx), CDR101 (CDR-Life), cosibelimab (Checkpoint Therapeutics), CTX-8371 (Compass Therapeutics), DiNonA-Solid Tumors-unknown (DiNonA), DR30207 (Zhejiang Doer Biologics), DuoBody-PD-L1x4-1BB (Ligand Pharmaceuticals), envafolimab (Alphamab Oncology), EPIM-001 (Elpis Biopharmaceuticals), ES101 (Elpiscience Biopharma), INBRX-105 (Inhibrx), FAZ053 (Novartis), FS118 (F-star Therapeutics), GB262 (Genor Biopharma), GS-4224 (Gilead), GT900008 (Kintor Pharmaceuticals), GX-P2 (Genexine), Hamni-PS-L1/CD47-Unknown (Hanmi Pharmaceutical), HBM7015 (HBM Holdings), HBM9167 (HBM Holdings), HLX20 (Shanghai Henlius Biotech), HTI-1088 (Jiangsu Hengrui Medicine), IBI318 (Innovent Biologics), IBI322 (Innovent Biologics), IBI323 (Innovent Biologics), IGM-7354 (IGM Biosciences), IMC-001 (Sorrento Therapeutics), Imfinzi / durvalumab (AstraZenica), IMM25 (ImmuneOnco Biopharma), IMM2502 (ImmuneOnco Biopharma), IMM2503 (ImmuneOnco Biopharma), IMM2504 (ImmuneOnco Biopharma), INCB86550 (Incyte), IO103 (IO Biotech), JS003 (Shanghai Junshi Biosciences), Jubilant-PD-L1-unknown (Jubilant Therapeutics), KD033 (Kadmon Holdings), KN046 (Alphamab Oncology), KY1003 (Sanofi), KY1043 (Sanofi), LY3300054 (Eli Lilly), LY3415244 (Eli Lilly), MRNA-6981 (Moderna), MSB2311 (Transcenta Holding), MT-6035 (Molecular Templates), ND021 / NM21-1480 (Numab Therapeutics),

OX001R (Oxford BioTherapeutics), PD-L1 based BsAbs (I-Mab), PD-L1 Boltbody ISAC (Bolt Biotherapeutics), PDL-GEX (Glycotope GmbH), PMC-122 (PharmAbcine), PMI06 (D&D Pharmatech), Protheragen-RV-scFv-PDL1-unknown (Protheragen), PRS-344 (Pieris Pharmaceuticals), Q-1802 (Merck), RC98 (Yantai Rongchang Pharmaceutical), RV-scFv-PDL1 (Protheragen), SenI_TAAx22P (Hebei Senlang Biotechnology), SHC020 (Nanjing Sanhome Pharmaceutical), sugemalimab (Ligand Pharmaceuticals), atezolizumab (Roche), TST005 (Transcenta Holding), TT-01 (Topmunnity Therapeutics), TTX-siPDL1 (TransCode Therapeutics), UniCAR-T-PD-L1 (GEMoaB monoclonals), Vaximm (VXM10), and YBL-013 (Y-Biologics).

[076] The anti-PD-L1 antibody that can be combined with the inducible cytokine prodrugs is typically an approved anti-PD-L1 antibody. Approved anti-PD-1 antibodies include, but are not limited to, avelumab (BAVENCIO), durvalumab (IMFINZI), and atezolizumab (TECENTRIQ).

E. Therapy and Pharmaceutical Compositions

[077] This disclosure relates to methods for treating cancer (lymphoma) using an inducible cytokine prodrug (e.g., IL-2, IL-12, or IFN), and to pharmaceutical compositions for use in such methods, including pharmaceutical compositions that contain an inducible cytokine prodrug (e.g., IL-2, IL-12, or IFN) in combination an excipient as described herein.

[078] This disclosure further relates to methods for treating cancer (lymphoma) using a combination therapy comprising an inducible cytokine prodrug (e.g., IL-2, IL-12, or IFN) in combination with one or more additional agents to treat cancer (such as lymphoma) as disclosed herein, and to pharmaceutical compositions for use in such methods, including pharmaceutical compositions that contain an inducible cytokine prodrug (e.g., IL-2, IL-12, or IFN) in combination an one or more additional agents to treat cancer (such as lymphoma) as described herein.

[079] This disclosure further relates to methods for treating cancer using a combination therapy comprising an inducible cytokine prodrug (e.g., IL-2, IL-12, or IFN) in combination with an anti-PD-1 antibody or an anti-PD-L1 antibody as disclosed herein, and to pharmaceutical compositions for use in such methods, including pharmaceutical compositions that contain an inducible cytokine prodrug (e.g., IL-2, IL-12, or IFN) in combination an anti-PD-1 antibody or an anti-PD-L1 antibody.

[080] The disclosure relates to methods for treating cancer comprising administering to a subject in need thereof an effective amount of a combination therapy that includes an inducible cytokine prodrug and an anti-PD-1 antibody or an anti-PD-L1 antibody. The inducible an inducible cytokine prodrug and an anti-PD-1 antibody or an anti-PD-L1 antibody are administered to the subject so that there is overlap of the pharmacological activities of the two therapeutic agents. Accordingly, the inducible cytokine prodrug can be administered before, after, concurrently, or periprocedurally with an anti-PD-1 antibody or an anti-PD-L1 antibody. In some practices of the methods, an anti-PD-1 antibody or an anti-PD-L1 antibody is administered before the inducible cytokine prodrug. In some practices of the methods, the anti-PD-1 antibody or the anti-PD-L1 antibody is administered, then after administration is completed, the inducible cytokine prodrug is administered.

[081] The inducible cytokine prodrug and the anti-PD-1/anti-PD-L1 antibody are typically administered systemically, for example by intravenous injection or preferably intravenous infusion. Other types of administration can be used, such as orally, parenterally, intravenous, intravenously, intra-articularly, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, intrahepatically, intracranially, nebulization/inhalation, by installation via bronchoscopy, or intratumorally.

[082] The methods and compositions disclosed herein can be used to treat any suitable cancer, in particular solid tumors, such as sarcomas and carcinomas. For examples, the methods and compositions disclosed herein can be used to treat acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, brain tumor, bile duct cancer, bladder cancer, bone cancer, breast cancer, bronchial tumor, carcinoma of unknown primary origin, cardiac tumor, cervical cancer, chordoma, colon cancer, colorectal cancer, craniopharyngioma, ductal carcinoma, embryonal tumor, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, fibrous histiocytoma, Ewing sarcoma, eye cancer, germ cell tumor, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gestational trophoblastic disease, glioma, head and neck cancer, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumor, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ, lung cancer, macroglobulinemia, malignant fibrous histiocytoma, melanoma,

Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, midline tract carcinoma involving NUT gene, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis fungoides, myelodysplastic syndrome, myelodysplastic/myeloproliferative neoplasm, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytomas, pituitary tumor, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell cancer, renal pelvis and ureter cancer, retinoblastoma, rhabdoid tumor, salivary gland cancer, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cord tumor, stomach cancer, T-cell lymphoma, teratoid tumor, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms tumor.

[083] In certain embodiments, the methods and compositions disclosed herein can be used to treat adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, brain tumor, bile duct cancer, bladder cancer, bone cancer, breast cancer, bronchial tumor, carcinoma of unknown primary origin, cardiac tumor, cervical cancer, chordoma, colon cancer, colorectal cancer, craniopharyngioma, ductal carcinoma, embryonal tumor, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, fibrous histiocytoma, Ewing sarcoma, eye cancer, germ cell tumor, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gestational trophoblastic disease, glioma, head and neck cancer, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumor, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ, lung cancer, malignant fibrous histiocytoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, midline tract carcinoma involving NUT gene, mouth cancer, multiple endocrine neoplasia syndrome, mycosis fungoides, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytomas, pituitary tumor, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal

cancer, renal cell cancer, renal pelvis and ureter cancer, retinoblastoma, rhabdoid tumor, salivary gland cancer, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cord tumor, stomach cancer, T-cell lymphoma, teratoid tumor, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, non-Hodgkin lymphoma, squamous carcinoma of the head and neck, malignant pleural mesothelioma, and Wilms tumor.

[084] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell cancer (HNSCC), classical Hodgkin lymphoma (cHL), primary mediastinal large B cell lymphoma (PMBCL), urothelial carcinoma, microsatellite instability high or mismatch repair deficient cancer, microsatellite instability high or mismatch repair deficient colorectal cancer, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma (HCC), merkel cell carcinoma (MCC), renal cell carcinoma (RCC), endometrial carcinoma, tumor mutational burden high cancer, cutaneous squamous cell carcinoma (cSCC), triple negative breast cancer (TNBC), urothelial carcinoma, colorectal cancer or oesophageal carcinoma.

[085] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat Merkel cell carcinoma (MCC), urothelial carcinoma (UC), renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), triple negative breast cancer (TNBC), endometrial cancer, cutaneous squamous cell carcinoma (CSCC), basal cell carcinoma (BCC), melanoma, malignant pleural mesothelioma, classical Hodgkin lymphoma (cHL), squamous cell carcinoma of the head and neck (SCCHN), hepatocellular carcinoma (HCC), esophageal squamous cell carcinoma (ESCC), non-squamous non-small cell lung cancer, or nasopharyngeal carcinoma (NPC).

[086] Preferably, the methods and compositions disclosed herein are used to treat colon cancer, lung cancer, melanoma, renal cell carcinoma, or breast cancer.

[087] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat melanoma. As an example, the methods and compositions disclosed herein can be used to treat melanoma in subjects with unresectable or metastatic melanoma. As another example, the methods and compositions disclosed herein can be used for the adjuvant treatment of subjects with melanoma with involvement of lymph node(s) following complete resection.

[088] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat non-small cell lung cancer (NSCLC). As an example, the methods and compositions disclosed herein can be used to treat NSCLC in subjects with NSCLC expressing PD-L1 (e.g., Tumor Proportion Score (TPS) $\geq 1\%$) as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations, and is: stage III where subjects are not candidates for surgical resection or definitive chemoradiation, or metastatic. As another example, the methods and compositions disclosed herein can be used to treat NSCLC in patients with metastatic NSCLC whose tumors express PD-L1 (TPS $\geq 1\%$) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. As another example, the methods and compositions disclosed herein can be used in combination with pemetrexed and platinum chemotherapy, as first-line treatment of patients with metastatic nonsquamous NSCLC, with no EGFR or ALK genomic tumor aberrations. As another example, the methods and compositions disclosed herein can be used in combination with carboplatin and either paclitaxel or paclitaxel protein-bound, as first-line treatment of patients with metastatic squamous NSCLC.

[089] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat SCLC. As an example, the methods and compositions disclosed herein can be used to treat SCLC in subjects with metastatic SCLC with disease progression on or after platinum-based chemotherapy and at least one other prior line of therapy.

[090] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat HNSCC. As an example, the methods and compositions disclosed herein can be used to treat HNSCC in subjects with metastatic or with unresectable, recurrent HNSCC whose tumors express PD-L1 (e.g., Combined Positive Score (CPS) ≥ 1) as determined by an FDA-approved test. As another example, the methods and compositions disclosed herein can be used to treat HNSCC in subjects with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy. As another example, the methods and compositions disclosed herein can be used in combination with platinum and fluorouracil for the first-line treatment of patients with metastatic or with unresectable, recurrent HNSCC.

[091] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat cHL. As an example, the methods and compositions disclosed herein can be used to treat cHL in subjects with relapsed or refractory cHL. As another example, the methods and

compositions disclosed herein can be used to treat cHL in pediatric subjects with refractory cHL, or cHL that has relapsed after 2 or more lines of therapy.

[092] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat PMBCL. As an example, the methods and compositions disclosed herein can be used to treat PMBCL in subjects with refractory PMBCL, or in subjects who have relapsed after 2 or more prior lines of therapy.

[093] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat urothelial carcinoma. As an example, the methods and compositions disclosed herein can be used to treat urothelial carcinoma in subjects with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 (e.g., Combined Positive Score (CPS) ≥ 10) as determined by an FDA-approved test, or in subjects who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status. As another example, the methods and compositions disclosed herein can be used to treat urothelial carcinoma in subjects with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. As another example, the methods and compositions disclosed herein can be used to treat urothelial carcinoma in subjects with Bacillus Calmette-Guerin (BCG)-unresponsive, high-risk, non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumors who are ineligible for or have elected not to undergo cystectomy.

[094] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat Microsatellite Instability-High (MSI-H) or Mismatch Repair Deficient (dMMR) Cancer. As an example, the methods and compositions disclosed herein can be used to treat MSI-H or dMMR cancer in subjects with unresectable or metastatic MSI-H or dMMR cancer wherein the solid tumors have progressed following prior treatment and the subject has no satisfactory alternative treatment options, or wherein the colorectal cancer has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.

[095] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat Microsatellite Instability-High (MSI-H) or Mismatch Repair Deficient (dMMR) Colorectal Cancer. As an example, the methods and compositions disclosed herein can be used to

treat MSI-H or dMMR colorectal cancer in subjects with unresectable or metastatic MSI-H or dMMR colorectal cancer.

[096] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat gastric cancer. As an example, the methods and compositions disclosed herein can be used to treat gastric cancer in subjects with recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma whose tumors express PD-L1 (e.g., Combined Positive Score (CPS) ≥ 1) as determined by an FDA-approved test, with disease progression on or after 2 or more prior lines of therapy including fluoropyrimidine- and platinum-containing chemotherapy and if appropriate, HER2/neu-targeted therapy.

[097] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat esophageal cancer. As an example, the methods and compositions disclosed herein can be used to treat esophageal cancer in subjects with locally advanced or metastatic esophageal or gastroesophageal junction (GEJ) (e.g., tumors with epicenter 1 to 5 centimeters above the GEJ) carcinoma that is not amenable to surgical resection or definitive chemoradiation, in combination with platinum- and fluoropyrimidine-based chemotherapy. As another example, the methods and compositions disclosed herein can be used to treat esophageal cancer in subjects with locally advanced or metastatic esophageal or gastroesophageal junction (GEJ) (e.g., tumors with epicenter 1 to 5 centimeters above the GEJ) carcinoma that is not amenable to surgical resection or definitive chemoradiation, after one or more prior lines of systemic therapy for patients with tumors of squamous cell histology that express PD-L1 (CPS ≥ 10) as determined by an FDA-approved test.

[098] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat cervical cancer. As an example, the methods and compositions disclosed herein can be used to treat cervical cancer in subjects with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (e.g., Combined Positive Score (CPS) ≥ 1) as determined by an FDA-approved test.

[099] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat HCC. As an example, the methods and compositions disclosed herein can be used to treat HCC in subjects who have been previously treated with sorafenib.

[0100] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat MCC. As an example, the methods and compositions disclosed herein can be used to treat MCC in subjects with recurrent locally advanced or metastatic MCC.

[0101] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat RCC. As an example, the methods and compositions disclosed herein can be used in combination with axitinib, for the first-line treatment of patients with advanced RCC.

[0102] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat endometrial carcinoma. As an example, the methods and compositions disclosed herein can be used in combination with lenvatinib, for the treatment of subjects with advanced endometrial carcinoma that is not MSI-H or dMMR, who have disease progression following prior systemic therapy and are not candidates for curative surgery or radiation.

[0103] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat Tumor Mutational Burden-High (TMB-H) Cancer. As an example, the methods and compositions disclosed herein can be used to treat TMB-H cancer in subjects with unresectable or metastatic tumor mutational burden-high (e.g., ≥ 10 mutations/megabase (mut/Mb)) solid tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options.

[0104] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat Cutaneous Squamous Cell Carcinoma (cSCC). As an example, the methods and compositions disclosed herein can be used to treat cSCC in subjects with recurrent or metastatic cutaneous squamous cell carcinoma that is not curable by surgery or radiation.

[0105] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat Triple-Negative Breast Cancer (TNBC). As an example, the methods and compositions disclosed herein can be used in combination with chemotherapy, for the treatment of subjects with locally recurrent unresectable or metastatic TNBC whose tumors express PD-L1 (e.g., Combined Positive Score (CPS) ≥ 10) as determined by an FDA approved test.

[0106] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat Merkel cell carcinoma (MCC). As an example, a combination comprising Avelumab can be used to treat MCC in subjects with metastatic MCC.

[0107] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat Urothelial Carcinoma (UC). As an example, a combination comprising avelumab

can be used to treat UC in subjects with locally advanced or metastatic UC who have disease progression during or following platinum-containing chemotherapy. As another example, a combination comprising avelumab can be used to treat UC in subjects with locally advanced or metastatic UC who have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

[0108] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat Renal Cell Carcinoma (RCC). As an example, a combination comprising avelumab and axitinib can be used in a subject with advanced RCC.

[0109] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat urothelial carcinoma (UC). As an example, a combination comprising Durvalumab can be used to treat UC in subjects with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy. As another example, a combination comprising Durvalumab can be used to treat UC in subjects with locally advanced or metastatic urothelial carcinoma who have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

[0110] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat non-small cell lung cancer (NSCLC). As an example, a combination comprising Durvalumab can be used to treat NSCLC in subjects with unresectable, Stage III non-small cell lung cancer (NSCLC) whose disease has not progressed following concurrent platinum-based chemotherapy and radiation therapy.

[0111] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat small cell lung cancer (SCLC). As an example, a combination comprising Durvalumab can be used in combination with etoposide and either carboplatin or cisplatin, as first-line treatment of adult subjects with extensive-stage small cell lung cancer (ES-SCLC).

[0112] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat urothelial carcinoma (UC). As an example, a combination comprising Atezolizumab can be used to treat UC in adult subjects with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 (e.g., PD-L1 stained tumor-infiltrating immune cells [IC] covering $\geq 5\%$ of the tumor area), as determined by an FDA-approved test, or are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status, or have disease progression during or following any

platinum-containing chemotherapy, or within 12 months of neoadjuvant or adjuvant chemotherapy.

[0113] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat NSCLC. As an example, a combination comprising Atezolizumab can be used to treat NSCLC in adult subjects with metastatic NSCLC whose tumors have high PD-L1 expression (e.g., PD-L1 stained $\geq 50\%$ of tumor cells [TC $\geq 50\%$] or PD-L1 stained tumor-infiltrating immune cells [IC] covering $\geq 10\%$ of the tumor area [IC $\geq 10\%$]), as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations. As another example, a combination comprising Atezolizumab can be used in combination with bevacizumab, paclitaxel, and carboplatin, for the first-line treatment of adult subjects with metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations. As another example, a combination comprising Atezolizumab can be used in combination with paclitaxel protein-bound and carboplatin for the first-line treatment of adult subjects with metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations. As another example, a combination comprising Atezolizumab can be used to treat NSCLC in adult subjects with metastatic NSCLC who have disease progression during or following platinum-containing chemotherapy.

[0114] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat triple negative breast cancer (TNBC). As an example, a combination comprising Atezolizumab can be used in combination with paclitaxel protein-bound for the treatment of adult subjects with unresectable locally advanced or metastatic TNBC whose tumors express PD-L1 (e.g., PD-L1 stained tumor-infiltrating immune cells [IC] of any intensity covering $\geq 1\%$ of the tumor area), as determined by an FDA approved test.

[0115] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat Small cell lung cancer (SCLC). As an example, a combination comprising Atezolizumab can be used in combination with carboplatin and etoposide, for the first-line treatment of adult subjects with extensive-stage small cell lung cancer (ES-SCLC).

[0116] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat endometrial cancer. As an example, a combination comprising Dostarlimab can be used to treat endometrial cancer in adult subjects with mismatch repair deficient (dMMR) recurrent or advanced endometrial cancer, as determined by an FDA-approved test, that has progressed on or following prior treatment with a platinum-containing regimen.

[0117] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat cutaneous squamous cell carcinoma (CSCC). As an example, a combination comprising Cemiplimab-rwlc can be used to treat CSCC in subjects with metastatic cutaneous squamous cell carcinoma (mCSCC) or locally advanced CSCC (laCSCC) who are not candidates for curative surgery or curative radiation.

[0118] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat basal cell carcinoma (BCC). As an example, a combination comprising Cemiplimab-rwlc can be used to treat BCC in subjects with locally advanced BCC (laBCC) previously treated with a hedgehog pathway inhibitor or for whom a hedgehog pathway inhibitor is not appropriate.

[0119] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat NSCLC. As an example, a combination comprising Cemiplimab-rwlc can be used to treat NSCLC in subjects whose tumors have high PD-L1 expression (e.g., Tumor Proportion Score (TPS) $\geq 50\%$) as determined by an FDA-approved test, with no EGFR, ALK or ROS1 aberrations, and is locally advanced where subjects are not candidates for surgical resection or definitive chemoradiation, or metastatic.

[0120] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat melanoma. As an example, a combination comprising Nivolumab can be used to treat melanoma in subjects with unresectable or metastatic melanoma, as a single agent or in combination with ipilimumab. As another example, a combination comprising Nivolumab can be used to treat melanoma in subjects with melanoma with lymph node involvement or metastatic disease who have undergone complete resection, in the adjuvant setting.

[0121] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat NSCLC. As an example, a combination comprising Nivolumab can be used to treat NSCLC in adult subjects with metastatic non-small cell lung cancer expressing PD-L1 ($\geq 1\%$) as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations, as first-line treatment in combination with ipilimumab. As another example, a combination comprising NSCLC can be used to treat melanoma in adult subjects with metastatic or recurrent non-small cell lung cancer with no EGFR or ALK genomic tumor aberrations as first-line treatment, in combination with ipilimumab and 2 cycles of platinum-doublet chemotherapy. As another example, a combination comprising NSCLC can be used to treat melanoma in subjects

with metastatic non-small cell lung cancer and progression on or after platinum-based chemotherapy.

[0122] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat malignant pleural mesothelioma. As an example, a combination comprising Nivolumab can be used to treat malignant pleural mesothelioma in adult subjects with unresectable malignant pleural mesothelioma, as first-line treatment in combination with ipilimumab.

[0123] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat RCC. As an example, a combination comprising Nivolumab can be used to treat RCC in subjects with intermediate or poor risk advanced renal cell carcinoma, as a first-line treatment in combination with ipilimumab. As another example, a combination comprising Nivolumab can be used to treat RCC in subjects with advanced renal cell carcinoma, as a first-line treatment in combination with cabozantinib. As another example, a combination comprising Nivolumab can be used to treat RCC in subjects with advanced renal cell carcinoma who have received prior anti-angiogenic therapy.

[0124] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat classical Hodgkin lymphoma (cHL). As an example, a combination comprising Nivolumab can be used to treat cHL in adult subjects with cHL that has relapsed or progressed after autologous hematopoietic stem cell transplantation (HSCT) and brentuximab vedotin, or 3 or more lines of systemic therapy that includes autologous HSCT.

[0125] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat squamous cell carcinoma of the head and neck (SCCHN). As an example, a combination comprising Nivolumab can be used to treat SCCHN in subjects with recurrent or metastatic squamous cell carcinoma of the head and neck with disease progression on or after a platinum-based therapy.

[0126] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat urothelial carcinoma (UC). As an example, a combination comprising Nivolumab can be used to treat UC in subjects with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

[0127] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat colorectal cancer. As an example, a combination comprising Nivolumab can be used to treat colorectal cancer in subjects with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan, as a single agent or in combination with ipilimumab.

[0128] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat hepatocellular carcinoma (HCC). As an example, a combination comprising Nivolumab can be used to treat HCC in subjects with HCC who have been previously treated with sorafenib, as a single agent or in combination with ipilimumab.

[0129] In certain preferred embodiments, the methods and compositions disclosed herein can be used to treat esophageal squamous cell carcinoma (ESCC). As an example, a combination comprising Nivolumab can be used to treat ESCC in subjects with unresectable advanced, recurrent or metastatic esophageal squamous cell carcinoma after prior fluoropyrimidine- and platinum-based chemotherapy.

[0130] In certain preferred embodiments, a combination comprising Camrelizumab can be used to treat cHL.

[0131] In certain preferred embodiments, a combination comprising Tislelizumab can be used to treat non-squamous non-small cell lung cancer. In certain preferred embodiments, a combination comprising Tislelizumab can be used to treat hepatocellular carcinoma (HCC).

[0132] In certain preferred embodiments, a combination comprising Toripalimab can be used to treat urothelial carcinoma. In certain preferred embodiments, a combination comprising Toripalimab can be used to treat melanoma. In certain preferred embodiments, a combination comprising Toripalimab can be used to treat nasopharyngeal carcinoma (NPC).

[0133] In certain preferred embodiments, a combination comprising Sintilimab can be used to treat non-squamous non-small cell lung cancer. In certain preferred embodiments, a combination comprising Sintilimab can be used to treat cHL.

[0134] The cancer to be treated using the methods and compositions of this disclosure can be metastatic cancer. The methods and compositions disclosed herein can be used to treat metastatic renal clear cell carcinoma or metastatic cutaneous malignant melanoma.

[0135] In certain embodiments, the cancer is a lymphoma, such as Non-Hodgkin Lymphoma, Hodgkin Lymphoma, diffuse large B-cell lymphoma, primary mediastinal B cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, marginal zone lymphoma, mantle cell lymphoma, Waldenstrom's macroglobulinemia, Burkitt lymphoma, peripheral T cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T cell lymphoma, central nervous system lymphoma, grey zone lymphoma, double hit lymphoma, triple hit lymphoma, high grade B cell lymphomas not otherwise specified, lymphoblastic lymphoma, lymphoplasmacytic lymphoma, MALT lymphoma, monocytoid B cell lymphoma, natural killer (NK) cell lymphoma, mycosis fungoides, Sezary syndrome, enteropathy-type T cell lymphoma, hepatosplenic gamma/delta T cell lymphoma, and the like.

[0136] If desired, additional therapeutic agents can be administered to the subject as described herein. Typically such additional therapeutic agents are anti-cancer agents such as chemotherapeutic agents (e.g., adriamycin, cerubidine, bleomycin, alkeran, velban, oncovin, fluorouracil, thiotepa, methotrexate, bisantrene, noantrone, thiguanine, cytaribine, procarbazine), other immune-check point inhibitors (e.g., anti-CTLA4 (ipilimumab (YERVOY)), other cytokines (such as IL-12, inducible IL-12 prodrugs, inducible IFN, inducible IFN prodrugs, IL-2 or IL-2 prodrugs), angiogenesis inhibitors, antibody-drug conjugates (e.g., trastuzumab emtansine (KADCYLA), trastuzumab deruxtecan (ENHERTU), enfortumab vedotin (PADCEV), sacituzumab govitecan (TRODELVY), cellular therapies (e.g., CAR-T, TCT-T, T-cell therapy, such as tumor infiltrating lymphocyte (TIL) therapy), oncolytic viruses, radiation therapy and/or small molecules.

[0137] An additional therapeutic agent can be, for example, pemetrexed, a platinum chemotherapeutic agent, carboplatin, paclitaxel, protein-bound paclitaxel, fluorouracil, a fluoropyrimidine-based chemotherapeutic agent, or axitinib.

[0138] This disclosure also relates to pharmaceutical compositions that contain an inducible cytokine prodrug and an anti-PD-1 or an anti-PD-L1 antibody, and to the use of such pharmaceutical compositions to treat cancer.

[0139] The pharmaceutical compositions can take a variety of forms, e.g., liquid, lyophilized, and typically contain a suitable pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers (or excipients) are the non-active ingredient components of the pharmaceutical composition and are not biologically or otherwise undesirable, i.e., the material is administered

to a subject without causing undesirable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical formulation or composition in which it is contained. Carriers are frequently selected to minimize degradation of the active ingredient and to minimize adverse side effects in the subject.

[0140] Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy, 21st Edition, David B. Troy, ed., Lippicott Williams & Wilkins (2005). Examples of the pharmaceutically-acceptable carriers include, but are not limited to, sterile water, saline, buffered solutions like Ringer's solution, and dextrose solution. Other carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the immunogenic polypeptides. Matrices are in the form of shaped articles, e.g., films, liposomes, or microparticles. Certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered. Carriers are those suitable for administration of the chimeric polypeptides or nucleic acid sequences encoding the chimeric polypeptides to humans or other subjects.

[0141] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives are optionally present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic, although the formulation can be hypertonic or hypotonic if desired. The pH of the solution is generally about 5 to about 8 or from about 7 to 7.5.

[0142] Formulations for topical administration include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder, or oily bases, thickeners and the like are optionally necessary or desirable.

[0143] Compositions for oral administration include powders or granules, suspension or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders are optionally desirable.

[0144] This disclosure also relates to a kit that includes a pharmaceutical composition that contains an a) inducible cytokine prodrug composition, for example as a liquid composition or a lyophilized composition, in a suitable container (e.g., a vial, bag or the like), and b) a pembrolizumab composition, for example as a liquid composition or a lyophilized composition, in a suitable container (e.g., a vial, bag or the like). The kit can further include other components, such as sterile water or saline for reconstitution of lyophilized compositions.

F. Definitions

[0145] Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodologies by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 4th ed. (2012) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer-defined protocols and conditions unless otherwise noted.

[0146] “Cytokine” is a well-known term of art that refers to any of a class of immunoregulatory proteins (such as interleukin or interferon) that are secreted by cells especially of the immune system and that are modulators of the immune system. Cytokine polypeptides that can be used in the fusion proteins disclosed herein include, but are not limited to transforming growth factors, such as TGF- α and TGF- β (e.g., TGF β 1, TGF β 2, TGF β 3); interferons, such as interferon- α , interferon- β , interferon- γ , interferon-kappa and interferon-omega; interleukins, such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-21 and IL-25; tumor necrosis factors, such as tumor necrosis factor alpha and lymphotoxin; chemokines (e.g., C-X-C motif chemokine 10 (CXCL10), CCL19,

CCL20, CCL21), and granulocyte macrophage-colony stimulating factor (GM-CS), as well as fragments of such polypeptides that active the cognate receptors for the cytokine (i.e., functional fragments of the foregoing). “Chemokine” is a term of art that refers to any of a family of small cytokines with the ability to induce directed chemotaxis in nearby responsive cells.

[0147] As used herein, the terms “inducible” refer to the ability of a protein, i.e. IL-2, IL-12, or IFN, that is part of a prodrug, to bind its receptor and effectuate activity upon cleavage of the prodrug in the tumor microenvironment. The inducible cytokine prodrugs disclosed herein have attenuated or no cytokine agonist activity, but upon cleavage in the tumor microenvironment release active cytokine.

[0148] “Attenuated” activity, means that biological activity and typically cytokine (i.e., IL-2, IL-12 or IFN) agonist activity is decreased as compared to the activity of the natural cytokine (i.e., IL-2, IL-12 or IFN). The inducible cytokine prodrugs disclosed herein have attenuated cytokine receptor agonists activity, that is at least about 10X, at least about 50X, at least about 100X, at least about 250X, at least about 500X, at least about 1000X or less agonist activity as compared to natural cytokine (i.e., IL-2, IL-12 or IFN). Upon cleavage in the tumor microenvironment, cytokine is released that is active. Typically, the cytokine that is released has cytokine receptor agonist activity that is at least about 10X, at least about 50X, at least about 100X, at least about 250X, at least about 500X, or at least about 1000x greater than the IL-2 receptor activating activity of the prodrug.

[0149] As used herein, the terms “peptide”, “polypeptide”, or “protein” are used broadly to mean two or more amino acids linked by a peptide bond. Protein, peptide, and polypeptide are also used herein interchangeably to refer to amino acid sequences. It should be recognized that the term polypeptide is not used herein to suggest a particular size or number of amino acids comprising the molecule and that a peptide of the invention can contain up to several amino acid residues or more.

[0150] As used throughout, “subject” can be a vertebrate, more specifically a mammal (e.g. a human, horse, cat, dog, cow, pig, sheep, goat, mouse, rabbit, rat, and guinea pig), birds, reptiles, amphibians, fish, and any other animal. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered. As used herein, “patient” or “subject” may be used interchangeably and can refer to a subject with a disease or disorder (e.g. cancer). The term patient or subject includes human and veterinary subjects.

[0151] As used herein the terms “treatment”, “treat”, or “treating” refers to a method of reducing the effects of a disease or condition or symptom of the disease or condition. Thus, in the disclosed methods, treatment can refer to at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or substantially complete reduction in the severity of an established disease or condition or symptom of the disease or condition, such as reduction in tumor volume, reduction in tumor burden, reduction in death. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus, the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

[0152] As used herein, the terms “prevent”, “preventing”, and “prevention” of a disease or disorder refers to an action, for example, administration of the chimeric polypeptide or nucleic acid sequence encoding the chimeric polypeptide, that occurs before or at about the same time a subject begins to show one or more symptoms of the disease or disorder, which inhibits or delays onset or exacerbation of one or more symptoms of the disease or disorder.

[0153] As used herein, references to “decreasing”, “reducing”, or “inhibiting” include a change of at least about 10%, of at least about 20%, of at least about 30%, of at least about 40%, of at least about 50%, of at least about 60%, of at least about 70%, of at least about 80%, of at least about 90% or greater as compared to a suitable control level. Such terms can include but do not necessarily include complete elimination of a function or property, such as agonist activity.

[0154] The term “sequence variant” refers to an amino acid sequence of a polypeptide that has substantially similar biological activity as a reference polypeptide but differs in amino acid sequence or to the nucleotide sequence of a nucleic acid that has substantially similar biological activity (e.g., encodes a protein with substantially similar activity) as a reference sequence but differs in nucleotide sequence. Typically the amino acid or nucleotide sequence of a “sequence variant” is highly similar (e.g. at least about 80% similar) to that of a reference sequence. Those of skill in the art readily understand how to determine the identity of two polypeptides or two nucleic acids. For example, the identity can be calculated after aligning the two sequences so that the identity is at its highest level over a defined number of nucleotides or amino acids. Optimal

alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman Adv. Appl. Math. 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection.

[01] The term “conservative amino acid substitution” is a term of art that refers to the replacement of an amino acid in a polypeptide with another amino acid that has similar biochemical properties, such as size, charge and hydrophobicity as a reference amino acid. It is well-known that conservative amino acid replacements in the amino acid sequence of a polypeptide frequently do not significantly alter the overall structure or function of the polypeptide. Conservative substitutions of amino acids are known to those skilled in the art. Conservative substitutions of amino acids can include, but not limited to, substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D. For instance, a person of ordinary skill in the art reasonably expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological activity of the resulting molecule.

[0155] The term “effective amount,” as used herein, refers to the amount of agent (inducible cytokine prodrug and an anti-PD-1 or anti-PD-L1 antibody) that is administered to achieve the desired effect under the conditions of administration, such an amount that reduces tumor size, reduces tumor burden, extends progression free survival or extends overall survival. The actual effective amount selected will depend on the particular cancer being treated and its stage and other factors, such as the subject’s age, gender, weight, ethnicity, prior treatments and response to those treatments and other factors. Suitable amounts of inducible cytokine prodrug and pembrolizumab to be administered, and dosage schedules for a particular patient can be determined by a clinician of ordinary skill based on these and other considerations.

[0156] Preferably, the methods and compositions disclosed herein are used to treat colon cancer, lung cancer, melanoma, renal cell carcinoma, breast cancer, squamous carcinoma of the head and neck.

[0157] In certain preferred embodiments, the methods and compositions disclosed herein are used to treat melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell cancer (HNSCC), classical Hodgkin lymphoma (cHL), primary mediastinal large B cell lymphoma (PMBCL), urothelial carcinoma, microsatellite instability high or mismatch repair deficient cancer, microsatellite instability high or mismatch repair deficient colorectal cancer, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma (HCC), merkel cell carcinoma (MCC), renal cell carcinoma (RCC), endometrial carcinoma, tumor mutational burden high cancer, cutaneous squamous cell carcinoma (cSCC), triple negative breast cancer (TNBC), urothelial carcinoma, colorectal cancer or oesophageal carcinoma.

6. EQUIVALENTS

[0158] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the disclosure or the embodiments. Having now described certain compounds and methods in detail, the same will be more clearly understood by reference to the following examples, which are introduced for illustration only and not intended to be limiting.

7. EXAMPLES

[0159] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided herein.

Example 1. CT26 experiments - Treatment with ACP16 alone or in combination with anti-PD1 antibody

[0160] The CT26 cell line, a rapidly growing colon adenocarcinoma cell line that expresses MMP9 in vitro, was used. Using this tumor model, the ability of fusion proteins to affect tumor growth was examined.

Table 5.

Gr.	N	Agent	Formulation dose	Route	Schedule
1 [#]	12	vehicle 1 // vehicle 2	na // na	ip //ip	days 1,4,8,11 // days 3,6,10,13
2	10	vehicle 1 // ACP16	na // 70 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
3	10	vehicle 1 // ACP16	na // 232 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
4	10	vehicle 1 // ACP16	na // 500 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
5	10	anti-PD-1 RMP1-14 // vehicle 2	200 µg/animal // na	ip //ip	days 1,4,8,11 // days 3,6,10,13
6	10	anti-PD-1 RMP1-14 // ACP16	200 µg/animal // 70 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
7	10	anti-PD-1 RMP1-14 // ACP16	200 µg/animal // 232 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
8	10	anti-PD-1 RMP1-14 // ACP16	200 µg/animal // 500 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
9	10	vehicle 1 // IL-2	na // 12 µg/animal	ip //ip	days 1,4,8,11 // bid x 5 first day 1 dose per week x 2
10	10	anti-PD-1 RMP1-14 // IL-2	200 µg/animal // 12 µg/animal	ip //ip	days 1,4,8,11 // bid x 5 first day 1 dose per week x 2

[0161] Mice were anaesthetized with isoflurane for implant of cells to reduce the ulcerations. CR female BALB/c mice were set up with 3x10⁵ CT26 tumor cells in 0% Matrigel sc in flank. Cell Injection Volume was 0.1 mL/mouse. Mouse age at start date was 8 to 12 weeks. Pair matches were performed when tumors reach an average size of 100 - 150 mm³ and begin treatment.

ACP16 was dosed at 70, 230 or 500 µg/animal with or without anti-PD-1 antibody (RMP1-14) at 200 µg/animal (see Table 5). Body weights were taken at initiation and then biweekly to the end. Caliper measurements were taken biweekly to the end. Any adverse reactions were to be reported immediately. Any individual animal with a single observation of > than 30% body weight loss or three consecutive measurements of >25% body weight loss was euthanized. Any group with a mean body weight loss of >20 % or >10% mortality stopped dosing; the group was not euthanized and recovery is allowed. Within a group with >20% weight loss, individuals hitting the individual body weight loss endpoint were euthanized. If the group treatment related body weight loss is recovered to within 10% of the original weights, dosing resumed at a lower dose or less frequent dosing schedule. Exceptions to non-treatment body weight % recovery were allowed on a case-by-case basis. Endpoint was tumor growth delay (TGD). Animals were monitored individually. The endpoint of the experiment was a tumor volume of 1500 mm³ or 45 days, whichever comes first. Responders were followed longer. When the endpoint was reached, the animals are to be euthanized. Results are shown in FIGs. 1A-1F and FIGs. 2A-2B.

Example 2. IL-2 in Combination with an Anti-PD1 Antibody

[0162] Inducible IL-2 prodrug was tested in a B16F10 syngeneic tumor model with or without the addition of an anti-PD1 antibody (RMP1-14). 1×10^5 tumor cells were implanted in the flank of animals and tumor growth was monitored. Once tumors reached an average volume of 30-60 mm³, the animals were randomized and dosed as described in Table 6.

Table 6.

Group	N	Agent 1	Dose	Agent 2	Dose	Schedule
1	10	Vehicle	N/A	N/A	100 µL	Biwk x 2
2	10	Inducible IL-2 prodrug	100 µg/mouse	N/A	N/A	Biwk x 2
3	10	Inducible IL-2 prodrug	200 µg/mouse	N/A	N/A	Biwk x 2
4	10	N/A	N/A	Anti-PD-1	200 µg/mouse	Biwk x 2
5	10	Inducible IL-2 prodrug	100 µg/mouse	Anti-PD-1	200 µg/mouse	Biwk x 2

6	10	Inducible IL-2 prodrug	200 µg/mouse	Anti-PD-1	200 µg/mouse	Biwk x 2
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[45] Tumor volumes and body weights were recorded three times per week with a gap of 2-3 days in between two measurements. Treatment with Inducible IL-2 prodrug showed dose dependent efficacy as a monotherapy. Monotherapy with anti-PD1 in this model had no efficacy and tumor volumes in anti-PD1 treated mice were similar to those in mice treated with vehicle only. But the combination therapy using Inducible IL-2 prodrug and anti-PD1 synergistically improved tumor control, and was more effective than either Inducible IL-2 prodrug or anti-PD-1. The results are shown in FIGs. 3A, 3B, and 4.

Example 3. CT26 experiments - Treatment with inducible IFN prodrug alone or in combination with anti-PD1 antibody

[0163] The CT26 cell line, a rapidly growing colon adenocarcinoma cell line that expresses MMP9 in vitro, will be used. Using this tumor model, the ability of fusion proteins to affect tumor growth will be examined.

Table 7.

Gr.	N	Agent	Formulation dose	Route	Schedule
1 [#]	12	vehicle 1 // vehicle 2	na // na	ip //ip	days 1,4,8,11 // days 3,6,10,13
2	10	vehicle 1 // inducible IFN prodrug	na // 70 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
3	10	vehicle 1 // inducible IFN prodrug	na // 232 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
4	10	vehicle 1 // inducible IFN prodrug	na // 500 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
5	10	anti-PD-1 RMP1-14 // vehicle 2	200 µg/animal // na	ip //ip	days 1,4,8,11 // days 3,6,10,13
6	10	anti-PD-1 RMP1-14 // inducible IFN prodrug	200 µg/animal // 70 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13

7	10	anti-PD-1 RMP1-14 // inducible IFN prodrug	200 µg/animal // 232 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
8	10	anti-PD-1 RMP1-14 // ACP16	200 µg/animal // 500 µg/animal	ip //ip	days 1,4,8,11 // days 3,6,10,13
9	10	vehicle 1 // IL-2	na // 12 µg/animal	ip //ip	days 1,4,8,11 // bid x 5 first day 1 dose per week x 2
10	10	anti-PD-1 RMP1-14 // IL-2	200 µg/animal // 12 µg/animal	ip //ip	days 1,4,8,11 // bid x 5 first day 1 dose per week x 2

[0164] Mice will be anaesthetized with isoflurane for implant of cells to reduce the ulcerations. CR female BALB/c mice will be set up with 3×10^5 CT26 tumor cells in 0% Matrigel sc in flank. Cell Injection Volume was 0.1 mL/mouse. Mouse age at start date will be 8 to 12 weeks. Pair matches will be performed when tumors reach an average size of 100 - 150 mm³ and begin treatment as shown in Table 7. ACP16 will be dosed at 70, 230 or 500 µg/animal with or without anti-PD-1 antibody (RMP1-14) at 200 µg/animal. Body weights will be taken at initiation and then biweekly to the end. Caliper measurements will be taken biweekly to the end. Any adverse reactions will be reported immediately. Any individual animal with a single observation of > than 30% body weight loss or three consecutive measurements of >25% body weight loss will be euthanized. Any group with a mean body weight loss of >20 % or >10% mortality stopped dosing; the group will not be euthanized and recovery is allowed. Within a group with >20% weight loss, individuals hitting the individual body weight loss endpoint will be euthanized. If the group treatment related body weight loss is recovered to within 10% of the original weights, dosing will be resumed at a lower dose or less frequent dosing schedule. Exceptions to non-treatment body weight % recovery will be allowed on a case-by-case basis. Endpoint will be tumor growth delay (TGD). Animals will be monitored individually. The endpoint of the experiment will be a tumor volume of 1500 mm³ or 45 days, whichever comes first. Responders were followed longer. When the endpoint was reached, the animals will be euthanized.

Example 4. IFN in Combination with an Anti-PD1 Antibody

[0165] Inducible IFN prodrug will be tested in a B16F10 syngeneic tumor model with or without the addition of an anti-PD1 antibody (RMP1-14). 1×10^5 tumor cells will be implanted in the flank of animals and tumor growth will be monitored. Once tumors reached an average volume of $30\text{--}60 \text{ mm}^3$, the animals will be randomized and dosed as described in Table 8.

Table 8.

Group	N	Agent 1	Dose	Agent 2	Dose	Schedule
1	10	Vehicle	N/A	N/A	100 μL	Biwk x 2
2	10	Inducible IFN prodrug	100 $\mu\text{g}/\text{mouse}$	N/A	N/A	Biwk x 2
3	10	Inducible IFN prodrug	200 $\mu\text{g}/\text{mouse}$	N/A	N/A	Biwk x 2
4	10	N/A	N/A	Anti-PD-1	200 $\mu\text{g}/\text{mouse}$	Biwk x 2
5	10	Inducible IFN prodrug	100 $\mu\text{g}/\text{mouse}$	Anti-PD-1	200 $\mu\text{g}/\text{mouse}$	Biwk x 2
6	10	Inducible IFN prodrug	200 $\mu\text{g}/\text{mouse}$	Anti-PD-1	200 $\mu\text{g}/\text{mouse}$	Biwk x 2

[46] Tumor volumes and body weights will be recorded three times per week with a gap of 2-3 days in between two measurements. Treatment with inducible IFN prodrug will show dose dependent efficacy as a monotherapy. Monotherapy with anti-PD1 in this model will have no efficacy and tumor volumes in anti-PD1 treated mice will be similar to those in mice treated with vehicle only. But, it is expected that the combination therapy using inducible IFN prodrug and anti-PD1 will synergistically improve tumor control, and will be more effective than either inducible IFN prodrug or anti-PD-1.

Example 5. A20 Lymphoma Model

[47] The A20 cell line, a rapidly growing B cell lymphoma cell line, was used. Using this tumor model, the ability of inducible cytokine prodrugs to affect tumor growth was examined. Since human IL-12 and IFN α 2b are not cross reactive in mice, surrogate inducible cytokine prodrugs molecules were created, consisting of a mouse/human chimeric IL-12 (WW0757/636) or a mouse IFN α 1 (WW0610) to explore anti-tumor responses in syngeneic hematologic cancer models.

Table 9.

Group	N	Agent	Dose	Route	Schedule
1	10	Vehicle	-	ip	biwk x 2
2	10	WW0757/636	30 ug/animal	ip	biwk x 2
3	10	WW0757/636	100 ug/animal	ip	biwk x 2
4	10	WW0757/636	300 ug/animal	ip	biwk x 2
5	10	WW0610	45 ug/animal	ip	biwk x 2
6	10	WW0610	133 ug/animal	ip	biwk x 2

[48] 60 female BALB/c mice were set up with 5×10^5 A20 tumor cells in 0% Matrigel sc in flank. Cell injection volume was 0.1 mL/mouse. Mouse age at start date was 8 to 12 weeks. Pair matches were performed when tumors reach an average size of 90 - 130 mm³ and treatment began according to Table 9. This was Day 11 of study start. Caliper measurements were taken biweekly to the end. Any adverse reactions were reported immediately. Any individual animal with a single observation of > than 25% body weight loss or three consecutive measurements of >20% body weight loss was euthanized. If any group had a mean body weight loss of >20 % or >10% mortality then dosing was stopped; the group was not euthanized, and recovery was allowed. Within a group with >20% weight loss, individuals hitting the individual body weight loss endpoint were euthanized. If the group treatment related body weight loss recovered to within 10% of the original weights, dosing resumed at a lower dose or less frequent dosing schedule. Exceptions to non-treatment body weight % recovery were allowed on a case-by-case basis. Endpoint was tumor growth delay (TGD). Animals were monitored individually. The endpoint of the experiment was a tumor volume of 2000 mm³ or 40 days, whichever occurred first. When the endpoint was reached, the animals were euthanized. Results are shown in FIGs. 5A and 5B.

Example 6. EG7.OVA Lymphoma Model

[49] The EG7.OVA cell line, a rapidly growing T lymphoblast cell line, was used. Using this tumor model, the ability of fusion proteins to affect tumor growth was examined. Since human IL-12 and IFN α 2b are not cross reactive in mice, surrogate inducible cytokine prodrugs molecules were created, consisting of a mouse/human chimeric IL-12 (WW0757/636) or a mouse IFN α 1 (WW0610) to explore anti-tumor responses in syngeneic hematologic cancer models.

Table 10.

Group	N	Agent	Dose	Route	Schedule
1	10	Vehicle	-	ip	biwk x 2
2	10	WW0757/636	30 ug/animal	ip	biwk x 2
3	10	WW0757/636	100 ug/animal	ip	biwk x 2
4	10	WW0757/636	300 ug/animal	ip	biwk x 2
5	10	WW0610	45 ug/animal	ip	biwk x 2
6	10	WW0610	133 ug/animal	ip	biwk x 2

[50] 60 female C57Bl/6 mice were set up with 10x10⁵ EG7. OVA tumor cells in 0% Matrigel sc in flank. Cell injection volume was 0.1 mL/mouse. Mouse age at start date was 8 to 12 weeks. Pair matches were performed when tumors reach an average size of 63 - 135 mm³ and begin treatment according to Table 10. This was Day 5 of study start. Caliper measurements were taken biweekly to the end. Any adverse reactions were reported immediately. Any individual animal with a single observation of > than 25% body weight loss or three consecutive measurements of >20% body weight loss was euthanized. If any group had a mean body weight loss of >20 % or >10% mortality then dosing was stopped; the group was not euthanized, and recovery was allowed. Within a group with >20% weight loss, individuals hitting the individual body weight loss endpoint were euthanized. If the group treatment related body weight loss was recovered to within 10% of the original weights, dosing resumed at a lower dose or less frequent dosing schedule. Exceptions to non-treatment body weight % recovery were allowed on a case-by-case basis. Endpoint was tumor growth delay (TGD). Animals were monitored individually. The endpoint of the experiment was a tumor volume of 2000 mm³ or 40 days, whichever occurred first. When the endpoint was reached, the animals were euthanized. Results are shown in FIGs. 6A and 6B.

Example 7. CT26 Experiments – Treatment with Inducible IL-2 prodrug or anti-PD1 alone or in combination

[51] The CT26 cell line, a rapidly growing colon adenocarcinoma cell line that expresses MMP9 in vitro, was used. Using this tumor model, the ability of fusion proteins to affect tumor growth was examined.

Table. 11

Gr.	N	Treatment	Inducible IL-2 prodrug dose	Anti-PD1 Dose	Inducible IL-2 prodrug schedule	Anti-PD1 schedule
1 [#]	8	vehicle	na	na	na	na
2	8	inducible IL-2 prodrug	100 µg/animal	na	Days 1 and 8	na
3	8	Anti-PD-1	na	200 µg/animal	na	Days 1, 4, 8 and 11
4	8	inducible IL-2 prodrug + anti-PD-1	100 µg/animal	200 µg/animal	Days 1 and 8	Days 1, 4, 8 and 11

[52] Mice were anaesthetized with isoflurane for implant of cells to reduce the ulcerations. Female BALB/c mice were set up with 1.5×10^5 CT26 tumor cells sc in flank. Cell Injection Volume was 0.1 mL/mouse. Mouse age at start date was 8 to 12 weeks. Pair matches were performed when tumors reached an average size of 100 - 150 mm³ and treatment began according to Table 11. The inducible IL-2 prodrug was a two-chain polypeptide that contained WW0621 and WW0523, and the anti-PD-1 antibody was RMP1-14. Body weights were taken at initiation and then biweekly to the end of the study. Caliper measurements of tumor size were taken biweekly to the end of the study. Any adverse reactions were reported immediately. Any individual animal with a single observation of > than 30% body weight loss or three consecutive measurements of >25% body weight loss was euthanized. Any group with a mean body weight

loss of >20 % or >10% mortality resulted in stopped dosing; the group was not euthanized, and recovery was allowed. Animals were monitored individually. The endpoint of the study was a tumor volume of 1500 mm³ or 45 days, whichever comes first. Responders were followed longer. When the endpoint was reached, the animals were euthanized. Results are shown in FIGs. 7A-7F.

8. CONSTRUCTS

[53] The elements of the polypeptide constructs provided in Table 8 contain the abbreviations as follows: “X” refers to a linker. “X” refers to a cleavable linker. Linker 3 refers to a linker that comprises a CTSL-1 substrate motif sequence.

Table 11. Exemplary inducible cytokine prodrug constructs

Construct #	Construct Description
WW0706	anti-HSA-X-human_p40-L-mouse_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_R27E_T32D_IGLC2-01_X=Linker2)
WW0707	anti-HSA-X-human_p40-L-human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_R27E_T32D_IGLC2-01_X=Linker2)
WW0708	anti-HSA-X-human_p40-L-mouse_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_S30E_IGLC2-01_X=Linker2)
WW0709	anti-HSA-X-human_p40-L-human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_S30E_IGLC2-01_X=Linker2)
WW0710	anti-HSA-X-human_p40-L-mouse_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_S30E_N31E_IGLC2-01_X=Linker2)
WW0711	anti-HSA-X-human_p40-L-human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_S30E_N31E_IGLC2-01_X=Linker2)
WW0700	anti-HSA-X-human_p40-L-mouse_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_N31E_IGLC2-01_X=Linker2)
WW0701	anti-HSA-X-human_p40-L-human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_N31E_IGLC2-01_X=Linker2)
WW0712	anti-HSA-X-human_p40-L-mouse_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_IGLC2-01_X=Linker3)
WW0713	anti-HSA-X-human_p40-L-human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_IGLC2-01_X=Linker3)
WW0714	anti-HSA-X-human_p40-L-mouse_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_N31E_IGLC2-01_X=Linker3)
WW0715	anti-HSA-X-human_p40-L-human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_N31E_IGLC2-01_X=Linker3)

WW0805	human_p35-X-anti-HSA-L-Blocker_(Blocker=Opt1_Hv_D53E_D61E_Vl-Vh X=Linker3)
WW0754	anti-HSA-X-Human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_IGLC2-01 X=Linker2)
WW0756	anti-HSA-X-Human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_S30D_N31E_IGLC2-01 X=Linker2)
WW0762	anti-HSA-X-Human_p35-XL-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_IGLC2-01 X=Linker3)
WW0770	human_p40-L-human_p35-X-anti-HSA-L-Fab_Lambda_Blocker_(Blocker=Lambda_Fab_IGLC2-01 X=Linker2)
WW0636	Human_IL12B_(p40)
WW0727	Fab_Heavy_Blocker_(Blocker=IL-12_Heavy_Fab_D53E_D61E_IgG1_Fab)
WW0045	Blocker2-Linker-(cleav. link.)-IL2-(cleav. link.)-(anti-HSA)-6xHis
WW0046	(anti-HSA)-(cleav. link.)-Blocker2-Linker-(cleav. link.)-IL2-6xHis
WW0203	IL2-(cleav. link.)-(anti-HSA)-linker(cleav. link.)-blocker2
WW0204	IL2-X-anti-HSA-LX-blocker_(X=Linker4_Blocker=Vh-Vl)
WW0205	IL2-X-anti-HSA-LX-blocker_(X=Linker5_Blocker=Vh-Vl)
WW0234	IL2-X-HSA-LX-blocker_(X=Linker 1; Blocker=3TOW69)
WW0235	IL2-X-HSA-LX-blocker_(X=Linker 1; Blocker=3TOW85)
WW0236	IL2-X-HSA-LX-blocker_(X=Linker 1; Blocker=2TOW91)
WW0308	IL2-X-HSA-LX-blocker(QAPRL_FR2)_(X=Linker 1; Blocker=Vh/Vl)
WW0415	IL2-X-anti-HSA-LX-blocker_(Blocker=VHVL.F2.high. A02_Vh/Vl_A46S; X=Linker 2)
WW0621	IL2-X-anti-HSA-LX-Heavy_blocker_Fab_(Blocker= VH-CH1; X=Linker 3)
WW0520	IL2-X-anti-HSA-LX-Heavy_blocker_Fab_(Blocker=VH-CH1; X=Linker 2)
WW0735	IL2-X-anti-HSA-LL-Heavy_blocker_Fab_(Blocker=VH-CH1_X=Linker2)
WW0736	IL2-X-anti-HSA-LL-Heavy_blocker_Fab_(Blocker=VH-CH1_X=Linker3)
WW0523	Kappa_blocker_Fab_(Blocker=VHVL.F2.high. A02_A46S_Kappa)

9. SEQUENCE DISCLOSURE

SEQ ID NO:	Description	Sequence
1	WW0621 (Heterodimeric inducible IL-2 produg)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFK FYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPDLI SNINVIVLELKGETTFMCEYADETATIVEFLNRWITFCQSIIST LTSGGPALFKSSFPPGSEVQLVESGGGLVQPGNSLRRLSCAASG FTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGRF TISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTIV TVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGPALF KSSFPPGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYTLA WVRQAPGKGLEWVAIDSSSYTYSPDTRVGRFTISRDNAKNS LYLQMNSLRRAEDTAVYYCARDSDNWDALDYWGQGTIVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVKPKSC**

2	WW0520 (Heterodimeric inducible IL-2 prodrug)	APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFK FYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGETTFMCEYADETATIVEFLNRWITFCQSIIST LTSGGPGPAGLYAQPGEVQLVESGGGLVQPGNSLRRLSCAAS GFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGR FTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTL VTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGP AGLYAQPGEVQLVESGGGLVQPGSLRLSCAASGFTFSSYTL AWVRQAPGKGLEWVAIDSSSYTYSPDVRGRFTISRDNANKN SLYLQMNSLRAEDTAVYYCARDSDNWDALDYWGQGTITVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSC**
3	WW0735 (Heterodimeric inducible IL-2 prodrug)	APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFK FYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGETTFMCEYADETATIVEFLNRWITFCQSIIST LTSGGPGPAGLYAQPGEVQLVESGGGLVQPGNSLRRLSCAAS GFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGR FTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTL VTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGGSGGGGSEVQLVESGGGLVQPGSLRLSCAASGFTFSSYT LAWVRQAPGKGLEWVAIDSSSYTYSPDVRGRFTISRDNANK NSLYLQMNSLRAEDTAVYYCARDSDNWDALDYWGQGTITVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSC
4	WW0736 (Heterodimeric inducible IL-2 prodrug)	APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFK FYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGETTFMCEYADETATIVEFLNRWITFCQSIIST LTSGGPALFKSSFPPGEVQLVESGGGLVQPGNSLRRLSCAASG FTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGRF TISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLV TVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGLVQPGSLRLSCAASGFTFSSYTL AWVRQAPGKGLEWVAIDSSSYTYSPDVRGRFTISRDNANKN SLYLQMNSLRAEDTAVYYCARDSDNWDALDYWGQGTITVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSC
5	WW0523 Kappa_blocker_Fab (Blocker=VHVL.F 2.high. A02_A46S_Kappa)	DIQMTQSPSSLSASVGDRVTITCKAREKLWSAVAWYQQKPGK APKSLIYSASFYSGVPSRFSGSGSGTDFTLTISLQPEDFATYY CQQYYTYPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNIFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
6	WW0758 Heterodimeric IL- 12 polypeptide, anti-HSA sdAb,	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSggpALFK SSFpPgsmnlpvatpdpgmfpclhhsqnlrravsnmlqkarqtlfypctseidhedit kdkstveaclpleltknesclnsretsfingscslsrktsfmmlalclssiyedlkmyqvef

	scFv Blocker, 2 cleavage sites	ktmnakllmdpkrqifldqnm lavidelmqalnfns etvpqkssleepdfy ktkiklcill hafiravtidrvmsylnassggpALFKSSFPpgsgggsgggsgggsgggsgg ggsgggsgsQSVLTQPPSVSGAPGQRVTISCSGSRNIGSNTVK WYQQLPGTAPKLLIYYNDQRPSGVPDRFSGSKSGTSASLAI TGLQAEDEADYYCQSYDRYTHPALLFGTGTKVTVLggggsg ggsgggsgsQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYG MHWVRQAPGKGLEWVAFIRYcGSNKYYAeSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCKTHGSHDNWGQGTMTV TVSS**
7	WW0805 Heterodimeric IL-12 polypeptide, anti-HSA sdAb, scFv Blocker, 1 cleavage site	mlpvatpdp gmfpc lhhsqnllravsnmlqkarqt lefypctseeidhedi tkdktstvea clpleltknesclnsretsf itngsclsrktsfmmalclssiyedlkmyqvef ktmnakll mdpkrqifldqnm lavidelmqalnfns etvpqkssleepdfy ktkiklcillhafiravti drvmsylnassggpALFKSSFPpgsEVQLVESGGGLVQPGNSLRLS CAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYA ESVKGRFTISRDN AKTTLYLQMNSLRPEDTAVYYCTIGGSL SVSSQGT LVTVSSggggsgggsgggsgggsgggsgggsgggsgsQSVLTQ PPSVSGAPGQRVTISCSGSRNIGSNTVKWYQQLPGTAPKL LIYYNDQRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CQSYDRYTHPALLFGTGTKVTVLggggsgggsgggsgggsgsQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAFIRYcGSNKYYAeSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVYYCKTHGSHDNWGQGTMTVTVSS**
8	WW0754 Heterodimeric IL-12 polypeptide, anti-HSA sdAb, Fab Blocker, 2 cleavage sites	EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDN AKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSggpGPAG LYAQpgsrnlpvatpdp gmfpc lhhsqnllravsnmlqkarqt lefypctseeidhedi tkdktstveaclpleltknesclnsretsf itngsclsrktsfmmalclssiyedlkmyqvef ktmnakllmdpkrqifldqnm lavidelmqalnfns etvpqkssleepdfy ktkiklcill hafiravtidrvmsylnassggpGPAGLYAQpgsgggsgggsgggsgggsgggsgs ggsgggsgsQSVLTQPPSVSGAPGQRVTISCSGSRNIGSNTV KWYQQLPGTAPKLLIYYNDQRPSGVPDRFSGSKSGTSASL AITGLQAEDEADYYCQSYDRYTHPALLFGTGTKVTVLgqpk aapsvtlfppsseelqankatlvc lisd fypgavtvawkadsspvkagvett tpskqsnnk yaassylsltp eqwksrshrsyscq vthegstvektv aptecs**
9	WW0756 Heterodimeric IL-12 polypeptide, anti-HSA sdAb, Fab Blocker, 2 cleavage sites	EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDN AKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSggpGPAG LYAQpgsrnlpvatpdp gmfpc lhhsqnllravsnmlqkarqt lefypctseeidhedi tkdktstveaclpleltknesclnsretsf itngsclsrktsfmmalclssiyedlkmyqvef ktmnakllmdpkrqifldqnm lavidelmqalnfns etvpqkssleepdfy ktkiklcill hafiravtidrvmsylnassggpGPAGLYAQpgsgggsgggsgggsgggsgggsgs ggsgggsgsQSVLTQPPSVSGAPGQRVTISCSGSRNIGdeTVK WYQQLPGTAPKLLIYYNDQRPSGVPDRFSGSKSGTSASLAI TGLQAEDEADYYCQSYDRYTHPALLFGTGTKVTVLgqpkaa psvtlfppsseelqankatlvc lisd fypgavtvawkadsspvkagvett tpskqsnnkya assylsltp eqwksrshrsyscq vthegstvektv aptecs**
10	WW0762	EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDN AKTTLYL

[illegible]

		LRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSRD TLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTI GGSLSVSSQGT LVTVSS
15	WW0614	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSRD TLYAESVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSSGGPPLA QKLKSSPGSCDLPQTHSLGSRRTLMLLAQMRRISLFSLCKD RHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSA AWDETLLDKFYTEL YQQLNDLEACVIQGVGVTTETPLMKE DSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSL TNLQESLRSKESGGPPLAQKLKSSPGSEVQLVESGGGLVQ PNSLRRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISG SRD TLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTAVY YCTIGGSLSVSSQGT LVTVSS
16	WW0819	EAHKSEIAHRYNDLGEQHFGLVLIQAFSQYLQKCSYDEHA KLVQEVTDFAKTCVADESAANCDKSLHTLFGDKLCAIPNL RENYGELADCCTKQEPERNECF LQHKDDNPSPPPFERPEAE AMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAEQ YNEILTQCCAEADKESCLTPKLDGVKEKALVSSVRQRMKC SSMQKFGERAFAKAWAVARLSQTFPNADFAEITKLATDLTK VNKECCHGDLLECADDRAELAKYMCENQATISSKLQTC DKPLLKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKN YAEAKDVFLGTFLYEYSRRHPDYSVSLLLRLAKKYEATLE KCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNC DLYEKL GEYGFQNAILVRYTQKAPQVSTPTLVEAARNLGRVGT KCC TLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVT KCCSG SLVERRPCFSALTVD ETYVPKEFKAETFTFHS DICTLPEKEK QIKKQTAELV KHKPKATAEQLKTVMD DFAQFLDTCCK AADKDTCFSTEGPNLVTRCKDALASGGPALFKSSFPPGSCD LPQTHNLRNKRALTLLVQMRRLSPLSCLKDRKDFGFPQEK VDAQQIKKAQAIPVLS ETLQQLNIFTSKDSSAAWNTLLD SFCNDLHQQLNDLQGCLMQQVGVQEFPLTQEDALLAVRK YFHRITVYLREKKHSPCAWEVVRAEVWRALSSSANVLGR LREEKSGGPALFKSSFPPGSEVQLVESGGGLVQPGNSLR LS CAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSRD TLYA ESVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSL SVSSQGT LVTVSSHHHHHH
17	WW0821	DAHKSEVAHRFKDLGEENFKALVLIQAFQYLQQCPFEDHV KLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATL RETYGEMADCCAKQEPERNECF LQHKDDNP NLPRLVRPE VDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAK RYKAAFTECCQAADKAACLLPKLDEL RDEGKASSAKQRL KCA SLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDL TKVHTECCHGDLLECADDRAELAKYICENQDSISSKLKEC CEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKD VCKN YAEAKDVFLGMFLYEYARRHPDYSVLLLR LAKTYETTL KCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC E LFEQL GEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCC

		KHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCC TESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKC CKADDKETCFAEEGKKLVAASQAALGLSGGPALFKSSFPP GSCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFP QEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETL LDKFYTELYQQLNDEACVIQGVGVTTETPLMKEDSILAVR KYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKESGGPALFKSSFPPGSDAHKSEVAHRFKDLGEENFKAL VLIAFAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENC DKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECF LQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLKKYLYEI ARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQ RFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLA KYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADL PSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDY SVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLV EEPQNLIKQNCLEFEQLGEYKFQNALLVRYTKKVPQVSTPT LVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLC VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEF NAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQ LKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA ALGL
18	WW0834	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSSGGPALF KSSFPPGSCDLPQTHSLGNRRALILLAQMGRISHFSLKDR YDFGFPQEVFDGNQFQKAQAISAFHEMIQQTFNLFSTKDSS AAWDETL LDKFYIELFQQLNDEACVTQEVGVVEIALMNE DSILAVRKYFQRITLYLMGKKYSPCAWEVVRAEIMRSFSFS TNLQKGLRRKDSGGPALFKSSFPPGSEVQLVESGGGLVQP GNSLRRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGS GRDTLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTAVY YCTIGGSLSVSSQGT LVTVSS
19	WW0742	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSSGGPALF KSSFPPGSMYNLLGFLQRSSNFQCQKLLWQLNGRLEYCL KDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQD SSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRG KLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNF YFINRLTGYLNRSGGPALFKSSFPPGSEVQLVESGGGLVQP GNSLRRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGS GRDTLYAESVKGRFTISRDNAKTTLYLQMNSLRPEDTAVY YCTIGGSLSVSSQGT LVTVSS
20	WW0612	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAKTTLYL

		QMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSSGGPGPAGLYAQPGSCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTTETPLMKEDSLAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKESGGPGPAGLYAQPGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSS
21	WW0045 (Monomeric IL-2 inducible prodrug)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSSYTYSPDTRGRFTISRDNAAKNSLYLQMNSLRAEDTAVYYCARDNWDALDYWGQGTITVTVSSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCKASQNVGTNVGWYQQKPGKAPKALIYSASFYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYTYPYTFGGGTKEIKGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGPGPAGMKGLPGSAPTSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATVEFLNRWITFCQSIISTLTSGGPGPAGMKGLPGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSHHHHHH
22	WW0046 (Monomeric IL-2 inducible prodrug)	EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSSGGPGPAGMKGLPGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSSYTYSPDTRGRFTISRDNAAKNSLYLQMNSLRAEDTAVYYCARDNWDALDYWGQGTITVTVSSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCKASQNVGTNVGWYQQKPGKAPKALIYSASFYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYTYPYTFGGGTKEIKGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGPGPAGMKGLPGSAPTSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATVEFLNRWITFCQSIISTLTHHHHHH
23	WW0203 (Monomeric IL-2 inducible prodrug)	APTSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATVEFLNRWITFCQSIISTLTSGGPALFKSSFPPGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGPALFKSSFPPGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSSYTYSPDTRGRFTISRDNAAKNSLYLQMNSLRAEDTAVYYCARDNWDALDYWGQGTITVTVSSGGGGSGGGSGGGSGGGSDIQM

		TQSPSSLSASVGDRVTITCKASQNVGTNVGWYQQKPGKAP KALIYSASFRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQYYTYTPYTFGGGTKVEIKHHHHHHEPEA
24	WW0204 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPPLAQKLKSSPGSEVQLVESGGGLVQPGN SLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGR DTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYC TIGGSLSVSSQGTLLTVSSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSSGGPPLAQKLKSSPGSEVQLVESGGGLVQPG GSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSS YTYPDTPVRGRFTISRDNAAKNSLYLQMNSLRAEDTAVYYC ARDSNWDALDYWGQGTTLTVSSGGGGSGGGGSGGGGSD IQMTQSPSSLSASVGDRVTITCKASQNVGTNVGWYQQKPG KAPKALIYSASFRYSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQYYTYTPYTFGGGTKVEIKHHHHHHEPEA
25	WW0205 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPPGPGPAGIGALFKSSFPPLAQKLKSSPGSE VQLVESGGGLVQPGNSLRLSCAASGFTFSKFGMSWVRQAP GKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAAKTTLYLQ MNSLRPEDTAVYYCTIGGSLSVSSQGTLLTVSSGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSSGGPPGPGPAGIGALFKS SFPPLAQKLKSSPGSEVQLVESGGGLVQPGGSLRLSCAASG FTFSSYTLAWVRQAPGKGLEWVAIDSSSYTYPDTPVRGR FTISRDNAAKNSLYLQMNSLRAEDTAVYYCARDNWDALD YWGQGTTLTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSA SVGDRVTITCKASQNVGTNVGWYQQKPGKAPKALIYSASF RYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQYYTY PYTFGGGTKVEIKHHHHHHEPEA
26	WW0234 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPGPAGMKGLPGSEVQLVESGGGLVQPGN SLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGR DTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYC TIGGSLSVSSQGTLLTVSSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSSGGPAGMKGLPGQVQLQESGGGLVQTGG SLRLSCTTSGTIFSGYTMGWYRQAPGEQRELVAVISGGGD TNYADSVKGRFTISRDNATKDTMYLQMNSLKPEDTAVYYC YSREVTTPWKLYWGQGTQVTVSSAAAYPYDVPDYGSHHH HHH
27	WW0235 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPGPAGMKGLPGSEVQLVESGGGLVQPGN

		SLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGR DTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYC TIGGSLSVSSQGTLVTVSSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSSGGPGPAGMKGLPGQVQLQESGGGLVQEGG SLRLSCAASERIFSTDVMGWYRQAAEKQRELVAVVSARGT TNYLDAVKGRFTISRDNARNTLTLQMNDLKPEDTASYCY VRETTSPWRIYWGGGTQVTVSSAAAYPYDVPDYGSHHHH HH
28	WW0236 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPGPAGMKGLPGSEVQLVESGGGLVQPGN SLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGR DTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYC TIGGSLSVSSQGTLVTVSSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSSGGPGPAGMKGLPGQVQLQESGGGLVQAG GSLRLSCAASGSIFSANAMGWYRQAPGKQRELVAVISSGG STNYADSVKGRFTISRDNAAKNTVYLQMNSLKPEDTAVYY CMYSGSYYYTPNDYWGGGTQVTVSSAAAYPYDVPDYGS HHHHHH
29	WW0308 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPGPAGMKGLPGSEVQLVESGGGLVQPGN SLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGR DTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYC TIGGSLSVSSQGTLVTVSSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSSGGPGPAGMKGLPGSEVQLVESGGGLVQPG GSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSS YTYSPTVVRGRFTISRDNAAKNSLYLQMNSLRAEDTAVYYC ARDSNWDALDYWGQGTTVTVSSGGGGSGGGGSGGGGSD IQMTQSPSSLSASVGDRVITITCKASQNVGTNVGWYQQKPG QAPRLLIYSASFRYSGVPSRFSGSGSGTDFTLTISLQPEDFA TYYCQQYYTYPYTFGGGKTKVEIKHHHHHH
30	WW0415 (Monomeric IL-2 inducible prodrug)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTSGGPGPAGLYAQPGSEVQLVESGGGLVQPGN SLRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGR DTLYAESVKGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYC TIGGSLSVSSQGTLVTVSSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSSGGPGPAGLYAQPGSEVQLVESGGGLVQPG GSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSS YTYSPTVVRGRFTISRDNAAKNSLYLQMNSLRAEDTAVYYC ARDSNWDALDYWGQGTTVTVSSGGGGSGGGGSGGGGSD IQMTQSPSSLSASVGDRVITITCKAREKLWSAWYQQKPG KAPKSLIYSASFRYSGVPSRFSGSGSGTDFTLTISLQPEDFA TYYCQQYYTYPYTFGGGKTKVEIK

31	WW0706 Monomeric IL-12 (chimeric) polypeptide, anti-HSA sdAb, Fab Blocker, 2 cleavage sites	EVQLVESGGGLVQPGNSLR LSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDN AKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSsggpGPAG LYAQpgsiwelkkdvyyvvel dwypdaggemvvlctdtp eedgitwtldqssevlgs gklttiqvkefgdagqy tchkggevlshsllllh kkedgiwstdilkdqk epknkftlrcea knysgrftcwwlttistdl tfsvkssrgssdpqgv tcgaaatlsaervrgdn keyeysvecqe dsacpaaeeslpievmda vhlkkyenytsffirdi ikpdpknqlkplknsrqvevs wey pdtwtstphsyfsltfcvqv gqgkskrekkdrvftd ktsatvicrknasisvraqdryys sswsewasvpesggggsgggsgggsgggsv ipvsgparclsqsrnllktddm vktarekl khysctaedi dheditrdqtstlktclplelhknesclatretssttrgsc lppqktslmm tclcl gsiyedlkmyqtefqaina alqnhnhqqiildkgm lvaidelmqslnhn getlrqkppv geadpyrvkmkclcill hafstrvvtinrvmgyl ssasggpGPAGLYAQpgsggggs gggsgggsgggsgggsgggsggggsQSVLTQPPSVSGAPGQRVTISCS GSeSNIGSNdVKWYQQLPGTAPKLLIYYNDQRPSGV PDRFS GSKSGTSASLAITGLQAEDEADY YCQSYDRYTHPALLFGT GTKVTVLgqpkaapsv tlfppsseelqankatlvcl isdfypgavtawkadsspvk agvettptskqsnkyaassy lsltp eqwksrshyscqvthe gstvektv aptecs**
32	WW0707 Monomeric IL-12 polypeptide, anti-HSA sdAb, Fab Blocker, 2 cleavage sites	EVQLVESGGGLVQPGNSLR LSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDN AKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSsggpGPAG LYAQpgsiwelkkdvyyvvel dwypdaggemvvlctdtp eedgitwtldqssevlgs gklttiqvkefgdagqy tchkggevlshsllllh kkedgiwstdilkdqk epknkftlrcea knysgrftcwwlttistdl tfsvkssrgssdpqgv tcgaaatlsaervrgdn keyeysvecqe dsacpaaeeslpievmda vhlkkyenytsffirdi ikpdpknqlkplknsrqvevs wey pdtwtstphsyfsltfcvqv gqgkskrekkdrvftd ktsatvicrknasisvraqdryys sswsewasvpesggggsgggsgggsgggsggnlp vatpdp gmfpcllhhsqnllravsnml qkarqtlefypctseeid heditkdktstveaclple ltknesclnsretsfing sclasrktsf mmalclssiyedlkmyqve fktmnakllmdpkrqif ldqnm lavidelmqalnfnset vpqkssleepdfyktkiklc illhafiravtidrvmsy lnassggpGPAGLYAQpgs gggsgggsgggsgggsgggsgggsgggsggggsQSVLTQPPSVSGAPGQRVT ISCSGSeSNIGSNdVKWYQQLPGTAPKLLIYYNDQRPSGV PD RFSGSKSGTSASLAITGLQAEDEADY YCQSYDRYTHPALLF GTGTKVTVLgqpkaapsv tlfppsseelqankatlvcl isdfypgavtvawkadss pvkagvettptskqsnkyaassy lsltp eqwksrshyscqvthe gstvektv aptecs**
33	WW0708 Monomeric IL-12 (chimeric) polypeptide, anti-HSA sdAb, Fab Blocker, 2 cleavage sites	EVQLVESGGGLVQPGNSLR LSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDN AKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGT LVTVSSsggpGPAG LYAQpgsiwelkkdvyyvvel dwypdaggemvvlctdtp eedgitwtldqssevlgs gklttiqvkefgdagqy tchkggevlshsllllh kkedgiwstdilkdqk epknkftlrcea knysgrftcwwlttistdl tfsvkssrgssdpqgv tcgaaatlsaervrgdn keyeysvecqe dsacpaaeeslpievmda vhlkkyenytsffirdi ikpdpknqlkplknsrqvevs wey pdtwtstphsyfsltfcvqv gqgkskrekkdrvftd ktsatvicrknasisvraqdryys sswsewasvpesggggsgggsgggsgggsv ipvsgparclsqsrnllktddm vktarekl khysctaedi dheditrdqtstlktclplelhknesclatretssttrgsc lppqktslmm tclcl gsiyedlkmyqtefqaina alqnhnhqqiildkgm lvaidelmqslnhn getlrqkppv geadpyrvkmkclcill hafstrvvtinrvmgyl ssasggpGPAGLYAQpgsggggs gggsgggsgggsgggsgggsggggsQSVLTQPPSVSGAPGQRVTISCS GSRNIGeNTVKWYQQLPGTAPKLLIYYNDQRPSGV PDRFS

[illegible]

[illegible]

	Blocker, 2 cleavage sites	eypdwtstphsyfsltfcvqvqgkskrekkrdrvfdtktsatvicrknasisvraqdryyss wsewasvpesggsgsgsgsgsgsrvipvsghparclsqsnnllktddmvktareklk hysctaedidheditrdaqstlktclplelhknescelatretsstrgsclppqktslmmtlcgs iyedlkmyqtcfqainaalqnlnhqqiildkgmlvaidelmqlnlngsetlrqkppvge adpyrvkmklcillhafstrvtinrvmgylssasgppALFKSSFPpgsgsgsgsgg sgsgsgsgsgsgsgsgsgsgsgsgsQSVLTQPPSVSGAPGQRVTISCSGS RSNIGSeTVKWYQQLPGTAPKLLIYYNDQRPSGVDPDRFSGS KSGTSASLAITGLQAEDADYYCQSYDRTYTHPALLFGTGT KVTVLgqpkaapsvtlfppsseelqankatlvcisldfygpavtvawkadsspvkag vetttpsksqsnkyaaassyisltpewkwshrsyseqvthegstvektvaptcs**
40	WW0715 Monomeric IL-12 polypeptide, anti-HSA sdAb, Fab Blocker, 2 cleavage sites	EVQLVESGGGLVQPGENSLRLSCAASGFTFSKFGMSWVRQA PGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSsggpALFK SSFPpgsiwelkkdvyyveldwydpagemvvltcdtpeedgitwldqssevlgsg kltliqvkefgdagqytchkggevlshtlllhkkedgiwstdilkdqekpnkttflrceak nysgrftcwllttistdltsfvksrgssdpqgvctgaatlsaervrgdnkeyeysvecqed sacpaeeslpievmvdavhlklyenyttssffirdiikpdpknllqkplknsrqvevsw eypdwtstphsyfsltfcvqvqgkskrekkrdrvfdtktsatvicrknasisvraqdryyss wsewasvpesggsgsgsgsgsgsgsrnlpvatpdpgmfpclhhsqnlravsnmlqk arqtlefypctseeidhditkdktstveaclpleltknesclnsretsfingscslarktsfm malclssiyeidlmyqvfektmnakllmdpkqrqifldqnmvlavidelmqlnfnsetvp qkssleepdfytkiklclllafriravtidrvmsynassgppALFKSSFPpgsggg sgsgsgsgsgsgsgsgsgsgsgsgsQSVLTQPPSVSGAPGQRVTIS CSGSRSNIGSeTVKWYQQLPGTAPKLLIYYNDQRPSGVDPDR FSGSKSGTSASLAITGLQAEDADYYCQSYDRTYTHPALLFG TGTKVTVLgqpkaapsvtlfppsseelqankatlvcisldfygpavtvawkadssp vkagvetttpsksqsnkyaaassyisltpewkwshrsyseqvthegstvektvaptcs**
41-194		Place hold SEQ ID NOs.: 41-194
195	MMP14_1	GPAGLYAQ
196	MMP9_1	GPAGMKGL
197	FAPa_1	PGGPAGIG
198	CTSL1_1	ALFKSSFP
199	CTSL1_2	ALFFSSPP
200	ADAM17_1	LAQRLRSS
201	ADAM17_2	LAQKLKSS
202	ALU30-1	GALFKSSFPSGGGPAGLYAQGGSGKGGSGK
203	ALU30-2	RSGSGGPAGLYAQGGSGGPAGLYAQGGSGK
204	ALU30-3	KGGGPAGLYAQGPAGLYAQGPAGLYAQGSR
205	ALU30-4	RGGPAGLYAQGGPAGLYAQGGGPAGLYAQK
206	ALU30-5	KGGALFKSSFPGGPAGIGPLAQKLKSSGGS
207	ALU30-6	SGGPAGPAGIGALFKSSFPPLAQKLKSSGGG
208	ALU30-7	RGPLAQKLKSSALFKSSFPGGPAGIGGGGK
209	ALU30-8	GGGALFKSSFPPLAQKLKSSPGGPAGIGGGR
210	ALU30-9	RPGGPAGIGPLAQKLKSSALFKSSFPGGG
211	ALU30-10	RGGPLAQKLKSSPGGPAGIGALFKSSFP GK
212	ALU30-11	RSGGPAGLYAQALFKSSFPPLAQKLKSSGGG

213	ALU30-12	GGPLAQKLKSSALFKSSFPGPAGLYAQGGR
214	ALU30-13	GGALFKSSFPGPAGLYAQPLAQKLKSSGGK
215	ALU30-14	RGGALFKSSFPLAQKLKSSGPAGLYAQGGK
216	ALU30-15	RGGGPAGLYAQPLAQKLKSSALFKSSFPGG
217	ALU30-16	SGPLAQKLKSSGPAGLYAQALFKSSFPGSK
218	ALU30-17	KGGPGGPAGIGPLAQRLRSSALFKSSFPGR
219	ALU30-18	KSGPGGPAGIGALFFSSPPLAQKLKSSGGR
220	ALU30-19	SGGFPRSGGSFNPRTFGSKRKRRGSRGGGG
221	MMP14 substrate motif sequence	GPLGLKAQ
222	MMP14 substrate motif sequence	LPLGLKAQ
223	MMP14 substrate motif sequence	SPLGLKAQ
224	MMP14 substrate motif sequence	QPLGLKAQ
225	MMP14 substrate motif sequence	KPLGLKAQ
226	MMP14 substrate motif sequence	FPLGLKAQ
227	MMP14 substrate motif sequence	HPLGLKAQ
228	MMP14 substrate motif sequence	PPLGLKAQ
229	MMP14 substrate motif sequence	APLGLKAQ
230	MMP14 substrate motif sequence	DPLGLKAQ
231	MMP14 substrate motif sequence	GPHGLKAQ
232	MMP14 substrate motif sequence	GPSGLKAQ
233	MMP14 substrate motif sequence	GPQGLKAQ
234	MMP14 substrate motif sequence	GPPGLKAQ
235	MMP14 substrate motif sequence	GPEGLKAQ
236	MMP14 substrate motif sequence	GPFGLKAQ
237	MMP14 substrate motif sequence	GPRGLKAQ
238	MMP14 substrate motif sequence	GPGGLKAQ
239	MMP14 substrate motif sequence	GPAGLKAQ

240	MMP14 substrate motif sequence	LPAGLKGA
241	MMP14 substrate motif sequence	GPAGLYAQ
242	MMP14 substrate motif sequence	GPANLVAQ
243	MMP14 substrate motif sequence	GPAALVGA
244	MMP14 substrate motif sequence	GPANLRAQ
245	MMP14 substrate motif sequence	GPAGLRAQ
246	MMP14 substrate motif sequence	GPAGLVAQ
247	MMP14 substrate motif sequence	GPAGLRGA
248	MMP14 substrate motif sequence	LPAGLVGA
249	MMP14 substrate motif sequence	GPAGLKGA
250	MMP14 substrate motif sequence	GPLALKAQ
251	MMP14 substrate motif sequence	GPLNLKAQ
252	MMP14 substrate motif sequence	GPLHLKAQ
253	MMP14 substrate motif sequence	GPLYLKAQ
254	MMP14 substrate motif sequence	GPLPLKAQ
255	MMP14 substrate motif sequence	GPLELKAQ
256	MMP14 substrate motif sequence	GPLRLKAQ
257	MMP14 substrate motif sequence	GPLLLKAQ
258	MMP14 substrate motif sequence	GPLSLKAQ
259	MMP14 substrate motif sequence	GPLGLY AQ
260	MMP14 substrate motif sequence	GPLGLFAQ
261	MMP14 substrate motif sequence	GPLGLLAQ
262	MMP14 substrate motif sequence	GPLGLHAQ

263	MMP14 substrate motif sequence	GPLGLRAQ
264	MMP14 substrate motif sequence	GPLGLAAQ
265	MMP14 substrate motif sequence	GPLGLEAQ
266	MMP14 substrate motif sequence	GPLGLGAQ
267	MMP14 substrate motif sequence	GPLGLPAQ
268	MMP14 substrate motif sequence	GPLGLQAQ
269	MMP14 substrate motif sequence	GPLGLSAQ
270	MMP14 substrate motif sequence	GPLGLVAQ
271	MMP14 substrate motif sequence	GPLGLKLQ
272	MMP14 substrate motif sequence	GPLGLKFQ
273	MMP14 substrate motif sequence	GPLGLKEQ
274	MMP14 substrate motif sequence	GPLGLKKQ
275	MMP14 substrate motif sequence	GPLGLKQQ
276	MMP14 substrate motif sequence	GPLGLKSQ
277	MMP14 substrate motif sequence	GPLGLKGQ
278	MMP14 substrate motif sequence	GPLGLKHQ
279	MMP14 substrate motif sequence	GPLGLKPQ
280	MMP14 substrate motif sequence	GPLGLKAG
281	MMP14 substrate motif sequence	GPLGLKAF
282	MMP14 substrate motif sequence	GPLGLKAP
283	MMP14 substrate motif sequence	GPLGLKAL
284	MMP14 substrate motif sequence	GPLGLKAE
285	MMP14 substrate motif sequence	GPLGLKAA

286	MMP14 substrate motif sequence	GPLGLKAH
287	MMP14 substrate motif sequence	GPLGLKAK
288	MMP14 substrate motif sequence	GPLGLKAS
289	MMP14 substrate motif sequence	GPLGLFGA
290	MMP14 substrate motif sequence	GPLGLQGA
291	MMP14 substrate motif sequence	GPLGLVGA
292	MMP14 substrate motif sequence	GPLGLAGA
293	MMP14 substrate motif sequence	GPLGLLGA
294	MMP14 substrate motif sequence	GPLGLRGA
295	MMP14 substrate motif sequence	GPLGLYGA
296	CTSL1 substrate motif sequence	ALFKSSPP
297	CTSL1 substrate motif sequence	SPFRSSRQ
298	CTSL1 substrate motif sequence	KLFKSSPP
299	CTSL1 substrate motif sequence	HLFKSSPP
300	CTSL1 substrate motif sequence	SLFKSSPP
301	CTSL1 substrate motif sequence	QLFKSSPP
302	CTSL1 substrate motif sequence	LLFKSSPP
303	CTSL1 substrate motif sequence	PLFKSSPP
304	CTSL1 substrate motif sequence	FLFKSSPP
305	CTSL1 substrate motif sequence	GLFKSSPP
306	CTSL1 substrate motif sequence	VLFKSSPP
307	CTSL1 substrate motif sequence	ELFKSSPP
308	CTSL1 substrate motif sequence	AKFKSSPP

309	CTSL1 substrate motif sequence	AHFKSSPP
310	CTSL1 substrate motif sequence	AGFKSSPP
311	CTSL1 substrate motif sequence	APFKSSPP
312	CTSL1 substrate motif sequence	ANFKSSPP
313	CTSL1 substrate motif sequence	AFFKSSPP
314	CTSL1 substrate motif sequence	AAFKSSPP
315	CTSL1 substrate motif sequence	ASFKSSPP
316	CTSL1 substrate motif sequence	AEFKSSPP
317	CTSL1 substrate motif sequence	ALRKSSPP
318	CTSL1 substrate motif sequence	ALLKSSPP
319	CTSL1 substrate motif sequence	ALAKSSPP
320	CTSL1 substrate motif sequence	ALQKSSPP
321	CTSL1 substrate motif sequence	ALHKSSPP
322	CTSL1 substrate motif sequence	ALPKSSPP
323	CTSL1 substrate motif sequence	ALTKSSPP
324	CTSL1 substrate motif sequence	ALGKSSPP
325	CTSL1 substrate motif sequence	ALDKSSPP
326	CTSL1 substrate motif sequence	ALFFSSPP
327	CTSL1 substrate motif sequence	ALFHSSPP
328	CTSL1 substrate motif sequence	ALFTSSPP
329	CTSL1 substrate motif sequence	ALFASSPP
330	CTSL1 substrate motif sequence	ALFQSSPP
331	CTSL1 substrate motif sequence	ALFLSSPP

332	CTSL1 substrate motif sequence	ALFGSSPP
333	CTSL1 substrate motif sequence	ALFESSPP
334	CTSL1 substrate motif sequence	ALFPSSPP
335	CTSL1 substrate motif sequence	ALFKHSPP
336	CTSL1 substrate motif sequence	ALFKLSPP
337	CTSL1 substrate motif sequence	ALFKKSPP
338	CTSL1 substrate motif sequence	ALFKASPP
339	CTSL1 substrate motif sequence	ALFKISPP
340	CTSL1 substrate motif sequence	ALFKGSPP
341	CTSL1 substrate motif sequence	ALFKNSPP
342	CTSL1 substrate motif sequence	ALFKRSPP
343	CTSL1 substrate motif sequence	ALFKESPP
344	CTSL1 substrate motif sequence	ALFKFSPP
345	CTSL1 substrate motif sequence	ALFKPSPP
346	CTSL1 substrate motif sequence	ALFKSFPP
347	CTSL1 substrate motif sequence	ALFKSLPP
348	CTSL1 substrate motif sequence	ALFKSIPP
349	CTSL1 substrate motif sequence	ALFKSKPP
350	CTSL1 substrate motif sequence	ALFKSAPP
351	CTSL1 substrate motif sequence	ALFKSQPP
352	CTSL1 substrate motif sequence	ALFKSPPP
353	CTSL1 substrate motif sequence	ALFKSEPP
354	CTSL1 substrate motif sequence	ALFKSGPP

355	CTSL1 substrate motif sequence	ALFKSSFP
356	CTSL1 substrate motif sequence	ALFKSSLP
357	CTSL1 substrate motif sequence	ALFKSSGP
358	CTSL1 substrate motif sequence	ALFKSSSP
359	CTSL1 substrate motif sequence	ALFKSSVP
360	CTSL1 substrate motif sequence	ALFKSSHP
361	CTSL1 substrate motif sequence	ALFKSSAP
362	CTSL1 substrate motif sequence	ALFKSSNP
363	CTSL1 substrate motif sequence	ALFKSSKP
364	CTSL1 substrate motif sequence	ALFKSSEP
365	CTSL1 substrate motif sequence	ALFKSSPF
366	CTSL1 substrate motif sequence	ALFKSSPH
367	CTSL1 substrate motif sequence	ALFKSSPG
368	CTSL1 substrate motif sequence	ALFKSSPA
369	CTSL1 substrate motif sequence	ALFKSSPS
370	CTSL1 substrate motif sequence	ALFKSSPV
371	CTSL1 substrate motif sequence	ALFKSSPQ
372	CTSL1 substrate motif sequence	ALFKSSPK
373	CTSL1 substrate motif sequence	ALFKSSPL
374	CTSL1 substrate motif sequence	ALFKSSPD
375	MMP7	KRALGLPG
376	MMP7	(DE)8RPLALWRS(DR)8
	MMP9	PR(S/T)(L/I)(S/T)
378	MMP9	LEATA
379	MMP11	GGAANLVRGG
380	MMP14	SGRIGFLRTA
381	MMP	PLGLAG

382	MMP	PLGLAX
383	MMP	PLGC(me)AG
384	MMP	ESPAYYTA
385	MMP	RLQLKL
386	MMP	RLQLKAC
387	MMP2, MMP9, MMP14	EP(Cit)G(Hof)YL
388	Urokinase plasminogen activator (uPA)	SGRSA
389	Urokinase plasminogen activator (uPA)	DAFK
390	Urokinase plasminogen activator (uPA)	GGGRR
391	Lysosomal Enzyme	GFLG
392	Lysosomal Enzyme	ALAL
	Lysosomal Enzyme	FK
	Cathepsin B	NLL
395	Cathepsin D	PIC(Et)FF
396	Cathepsin K	GGPRGLPG
397	Prostate Specific Antigen	HSSKLQ
398	Prostate Specific Antigen	HSSKLQL
399	Prostate Specific Antigen	HSSKLQEDA
400	Herpes Simplex Virus Protease	LVLASSSFGY
401	HIV Protease	GVSQNYPIVG
402	CMV Protease	GVVQASCRLA
	Thrombin	F(Pip)RS
404	Thrombin	DPRSFL
405	Thrombin	PPRSFL
406	Caspase-3	DEVD
407	Caspase-3	DEVDP
408	Caspase-3	KGSGDVEG
409	Interleukin 1 β converting enzyme	GWEHDG
410	Enterokinase	EDDDDKA
411	FAP	KQEQNPGST

412	Kallikrein 2	GKAFRR
413	Plasmin	DAFK
414	Plasmin	DVLK
415	Plasmin	DAFK
416	TOP	ALLLALL
417		GPLGVRG
418		IPVSLRSG
419		VPLSLYSG
420		SGESPAYYTA
421	WW0757	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVR QAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNKTT LYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTTLTVSSsggp ALFKSSFPpgsrvipvsgparclsqsrnllkttddmvtareklkhsycta edidheditrddqstltklcpllelhknesclatretsstrgscldppqktsl mmtlclgsiyedlkmyqtefqainaalqnhnhqqjildkgmlvaidelmqsl nhngetlrqkppvgeadpyrvkmklcillhafstrvvtinrvmgylssasg gpALFKSSFPpgsgggsgsgggsgggsgggsgQSVLTQPPSVSGAPGQ RVTISCSGSRSNIGSNTVKWYQQLPGTAPKLLIYYNDQRPSGV PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDRYTHP ALLFGTGTKVTVLggggsgggsgggsgggsgQVQLVESGGGVVQP GRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFIR YeGSNKYYAeSVKGRFTISRDN SKNTLYLQMNSLRAEDTA VYYCKTHGSHDNWGQGTMTVSS
422	WW0610	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVR QAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNKTT LYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTTLTVSSSGG PALFKSSFPFGCDLPQTHNLRNKRALTLLVQMRRLSPLS CLKDRKDFGFPEQKVDAQQIKKAQAIPVLSELTQQILNIFT SKDSSAAWNTLLDSFCNDLHQQNLNDLQGCLMQQVG VQEFPLTQEDALLAVRKYFHRITVYLREKKHSPCAWEV VRAEVWRALSSSANVLGRLREEKSGGPALFKSSFPFGSE VQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVR QAPGKGLEWVSSISGSGRDTLYAESVKGRFTISRDNKTT LYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTTLTVSS

CLAIMS

1. A combination comprising a therapeutically effective amount of: (i) an inducible cytokine prodrug comprising a cytokine polypeptide [A], a blocking element [D], optionally a half-life extension element [H] and a protease-cleavable polypeptide linker; and (ii) an anti-PD-1 antibody or an anti-PD-L1 antibody for use in treating a cancer in a subject in need thereof;

wherein the cytokine polypeptide and the cytokine blocking element and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the inducible cytokine prodrug has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the inducible cytokine prodrug is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

2. A method of treating a cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of (i) an inducible cytokine prodrug comprising a cytokine polypeptide [A], a blocking element [D], optionally a half-life extension element [H] and a protease-cleavable polypeptide linker; and (ii) an anti-PD-1 antibody or an anti-PD-L1 antibody;

wherein the cytokine polypeptide and the cytokine blocking element and the optional half-life extension element when present are operably linked by the protease-cleavable polypeptide linker and the inducible cytokine prodrug has attenuated cytokine receptor activating activity, wherein the cytokine-receptor activating activity of the inducible cytokine prodrug is at least about 10X less than the cytokine receptor activating activity of the polypeptide that contains the cytokine polypeptide that is produced by cleavage of the protease cleavable linker.

3. The use of claim 1 or the method of claim 2, wherein the cytokine polypeptide is IL-2, IL-12, interferon alpha, interferon beta, interferon gamma, or a mutein, or an active fragment of any of the foregoing.

4. The use or method of claim 3, wherein the cytokine polypeptide is IL-2, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing.
5. The use or method of claim 3, wherein the cytokine polypeptide is IL-12, or a mutein, a variant, an active fragment, or a subunit of any of the foregoing.
6. The use or method of claim 3, wherein the cytokine polypeptide is interferon alpha, interferon beta, interferon gamma, or a mutein, or an active fragment of any of the foregoing.
7. The use or method of any one of the preceding claims, wherein the inducible cytokine prodrug has the formula:
[A]-[L1]-[H]-[L2]-[D]
[D]-[L2]-[H]-[L1]-[A]
[A]-[L1]-[D]-[L2]-[H]
[H]-[L2]-[D]-[L1]-[A]
[H]-[L1]-[A]-[L2']-[D]
[D]-[L1]-[A]-[L2']-[H]
wherein A is a cytokine polypeptide, D is a blocking moiety, H is a half-life extension moiety, L1 is a protease-cleavable polypeptide linker, L2 is an polypeptide linker that is optionally protease-cleavable, and L2' is a protease-cleavable polypeptide linker.
8. The use or method of any one of the preceding claims, wherein the inducible cytokine prodrug is a single polypeptide chain.
9. The use or method of any one of claims 1-8, wherein the inducible cytokine prodrug comprises at least two polypeptide chains.
10. The use or method of any one of claims 1-8, wherein the inducible cytokine prodrug comprises at least three polypeptide chains.

11. The use or method of any one of the preceding claims, wherein the cytokine polypeptide comprises Compound 1, Compound 2, Compound 3, Compound 4, or an amino acid sequence variant of the foregoing.

12. The use or method of claim 11, wherein a) Compound 1 comprises a first polypeptide chain of SEQ ID NO:1 and a second polypeptide chain of SEQ ID NO:5, and the amino acid sequence variant of Compound 1 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO:1 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5, b) Compound 2 comprises a first polypeptide chain of SEQ ID NO:2 and a second polypeptide chain of SEQ ID NO:5, and the amino acid sequence variant of Compound 2 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO:2 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5, c) Compound 3 comprises a first polypeptide chain of SEQ ID NO:3 and a second polypeptide chain of SEQ ID NO:5, and the amino acid sequence variant of Compound 3 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO:3 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5, and d) Compound 4 comprises a first polypeptide chain of SEQ ID NO:1 and a second polypeptide chain of SEQ ID NO:4, and the amino acid sequence variant of Compound 4 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO:4 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO:5.

12. The use or method of any one of claims 1-9, wherein the cytokine polypeptide is Compound 5, Compound 6, Compound 7, Compound 8, Compound 9, or Compound 10, or an amino acid sequence variant of the foregoing.

13. The use or method of claim 12, wherein a) Compound 5 comprises a first polypeptide chain of SEQ ID NO: 6 and a second polypeptide chain of SEQ ID NO:12, and the amino acid sequence variant of Compound 5 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 6 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 12, b) Compound 6 comprises a first polypeptide chain of SEQ ID NO: 7 and a second polypeptide chain of SEQ ID NO: 12, and the amino acid sequence variant of Compound

6 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO:7 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 12, C) Compound 7 comprises a first polypeptide chain of SEQ ID NO: 8 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 7 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 8 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 13, c) Compound 8 comprises a first polypeptide chain of SEQ ID NO: 9 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 8 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 9 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 13, d) Compound 9 comprises a first polypeptide chain of SEQ ID NO: 10 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 9 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 10 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 13, and e) Compound 10 comprises a first polypeptide chain of SEQ ID NO: 11 and a second polypeptide chain of SEQ ID NO: 13, and the amino acid sequence variant of Compound 10 comprises a first polypeptide chain that has at least about 80% identity to SEQ ID NO: 11 and a second polypeptide chain that has at least about 80% identity to SEQ ID NO: 1.

14. The use or method of any one of claims 1-9, wherein the inducible cytokine prodrug comprises the amino acid selected from 14-20, or an amino acid sequence that has at least about 80% identity to SEQ ID NOs 14-20.

15. The use or method of any one of the preceding claims, wherein an anti-PD-1 antibody is administered.

16. The use or method of claim 15, wherein the anti-PD-1 antibody is selected from the group consisting of AMP-224 (AstraZenica), 609A (3SBio), 704 (3SBio), 705 (3SBio), ABBV-181 (AbbVie), ADU-1503 / bion-004 (Chinook Therapeutics), AGEN2034 / balstilimab (Agenus), AK103 (Akeso), AK104 (Akeso), AK112 (Akeso), AK123 (Akeso), AMG 256 (Amgen), AMG 404 (Amgen), ANB030 (AnaptysBio), ANKEBIO Anti-PD1 product (Anhui Anke Biotechnology), Anti PD-1 / Anti-CD47 (DiNonA), ASKG915 (Ask Gene

Pharmaceuticals), AV-MEL-1 (Aivita Biomedical), BCD-100 (Biocad CJSC), BI 754091 (Boehringer Ingelheim), BiCKI-IL-7 (OSE Immunotherapeutics), Boehringer-PD-1-unknown (Boehringer Ingelheim), BSK-050K01 (Biosion), Camrelizumab (Jiangsu Hengrui Medicine), CB201 (Crescendo Biologics), CB213 (Crescendo Biologics), CC-90006 (AnaptsBio), cetrelimab (J&J), chPD1 (Kiromic Biopharma), CMAB819 (Mabpharm), CS1003 (CStone Pharmaceuticals), CS17938 (Shenzhen Chipscreen Biosciences), CTX-8371 (Compass Therapeutics), CX-072 (CytomX Therapeutics), CX-188 (CytomX Therapeutics), cypalizumab (Harbin Gloria Pharmaceuticals), DB004 (DotBio), EMB02 (EpimAb Biotherapeutics), Geptanblimab / genolimzumab (Apollomics), GS19 (Suzhou Zelgen Biopharmaceuticals), HLX10 (Shanghai Henlius Biotech), HX008 (Taizhou HanZhong Pharmaceuticals), HY003 (Juventas Cell Therapy), IBI315 / BH2950 (Innovent Biologics), IBI318 (Innovent Biologics), IBI319 (Innovent Biologics), IMM1802 (ImmuneOnco Biopharma), IMT200 (TrueBinding), Jemperli / dostarlimab (AnaptyBio), JTX-4014 (Jounce Therapeutics), Keytruda / pembrolizumab (Merck), LBL-006 (Nanjing Leads Biolabs), Libtayo / cemiplimab-rwlc (Regeneron Pharmaceuticals), LVGN3616 (Lyvgen Biopharma), LXF821 (Novartis), LY01015 (Luye Pharma Group), LY3462817 (Eli Lilly), MCLA-134 (Merus N.V.), MEDI5752 (AstraZenica), NIR178 (Novartis), ONCR-177 (Oncorus), ONO-4685 (Ono Pharmaceutical), Opdivo / nivolumab (Ono Pharmaceutical), MGD019 (MacroGenius), PD1-GDT CAR-T (Kiromic Biopharma), penpulimab (Akeso), PSB205 (Qilu Puget Sound Biotherapeutics), PT-001 (Merck), PT627 (Merck), RB-M1 (Refuge Biotechnologies), Retifanlimab (MacroGenics), RG6139 (Roche), RG6279 (Roche), RTX-002 (RubrYc Therapeutics), sasanlimab (Pfizer), Servier-PD1xLAG3-unknown (Servier), SL-279252 / TAK-252 (Shattuck Labs), Sofusa anti-PD1 (Sorrento Therapeutics), spartalizumab (Novartis), SSI-361 (Lyvgen Biopharma), Sym021 (Servier), Tebotelimab (MacroGenics), tislelizumab (BeiGene), TSR-075 (AnaptsBio), Tuhura-DO/PD-1-unknown (Tuhura Biopharma), toripalimab (Shanghai Junshi Biosciences), sintilimab (Innovent Biologics), Unicar-CAR-T&PD-1-unknown (Shanghai Unicar-Therapy Bio-Medicine Technology), Xdivane (Xbrane Biopharma), XmAb20717 (Xencor), XmAb23104 (Xencor), YBL-006 (Y-Biologics), zimberelimab (Arcus Biosciences)

17. The use or method of claim 16, wherein the anti-PD-1 antibody is pembrolizumab, dostarlimab, cemiplimab-rwlc, nivolumab, camrelizumab, tislelizumab, toripalimab, sintilimab or a biosimilar of any of the foregoing.

18. The use or method of any one of claims 1-17, wherein an anti-PD-L1 antibody is administered.

19. The use or method of claim 18, wherein the anti-PD-L1 antibody is chosen from the group consisting of A167 (Sichuan Kelun), ABL501 (ABL Bio), ABL503 (ABL Bio), ABSK041 (Abbisko Therapeutics), ACE1708 (Acepodia), ACE-NK-PDL1 (Acepodia), ADG104 (Adagene), AK106 (Akeso), ALPN-202 (Alpine Immune Sciences), AN4005 (Adlai Nortye Biopharma), BMS-936559 / MDX-1105 (BMS), APL-502 / TQB2450 (Apollomics), Arbutus-PD-L1-unknown (Arbutus Biopharma), ASC22 (Ascleitis Pharma), ATG-101 (Antengene), AVA-004 (Avacta Group), AVA021 (Avacta Group), AVA027 (Avacta Group), AVA-040-100 (Avacta Group), AVA04-Vbp (Avacta Group), Bavencio / avelumab (Merck), BCD-135 (Biocad CJSC), BGB-A333 (BeiGene), Bintrafusp alfa / GSK4045154 (Merck), CA-170 / aupm-170 (Dr. Reddy's Laboratories), CCX559 (ChemoCentryx), CDR101 (CDR-Life), cosibelimab (Checkpoint Therapeutics), CTX-8371 (Compass Therapeutics), DiNonA-Solid Tumors-unknown (DiNonA), DR30207 (Zhejiang Doer Biologics), DuoBody-PD-L1x4-1BB (Ligand Pharmaceuticals), envafolimab (Alphamab Oncology), EPIM-001 (Elpis Biopharmaceuticals), ES101 (Elpiscience Biopharma), INBRX-105 (Inhibrx), FAZ053 (Novartis), FS118 (F-star Therapeutics), GB262 (Genor Biopharma), GS-4224 (Gilead), GT900008 (Kintor Pharmaceuticals), GX-P2 (Genexine), Hamni-PS-L1/CD47-Unknown (Hanmi Pharmaceutical), HBM7015 (HBM Holdings), HBM9167 (HBM Holdings), HLX20 (Shanghai Henlius Biotech), HTI-1088 (Jiangsu Hengrui Medicine), IBI318 (Innovent Biologics), IBI322 (Innovent Biologics), IBI323 (Innovent Biologics), IGM-7354 (IGM Biosciences), IMC-001 (Sorrento Therapeutics), Imfinzi / durvalumab (AstraZenica), IMM25 (ImmuneOnco Biopharma), IMM2502 (ImmuneOnco Biopharma), IMM2503 (ImmuneOnco Biopharma), IMM2504 (ImmuneOnco Biopharma), INCB86550 (Incyte), IO103 (IO Biotech), JS003 (Shanghai Junshi Biosciences), Jubilant-PD-L1-unknown (Jubilant Therapeutics), KD033 (Kadmon Holdings), KN046 (Alphamab Oncology), KY1003 (Sanofi), KY1043 (Sanofi), LY3300054 (Eli Lilly),

LY3415244 (Eli Lilly), MRNA-6981 (Moderna), MSB2311 (Transcenta Holding), MT-6035 (Molecular Templates), ND021 / NM21-1480 (Numab Therapeutics), OX001R (Oxford BioTherapeutics), PD-L1 based BsAbs (I-Mab), PD-L1 Boltbody ISAC (Bolt Biotherapeutics), PDL-GEX (Glycotope GmbH), PMC-122 (PharmAbcine), PMI06 (D&D Pharmatech), Protheragen-RV-scFv-PDL1-unknown (Protheragen), PRS-344 (Pieris Pharmaceuticals), Q-1802 (Merck), RC98 (Yantai Rongchang Pharmaceutical), RV-scFv-PDL1 (Protheragen), SenI_TAAX22P (Hebei Senlang Biotechnology), SHC020 (Nanjing Sanhome Pharmaceutical), sugemalimab (Ligand Pharmaceuticals), atezolizumab (Roche), TST005 (Transcenta Holding), TT-01 (Topmunnity Therapeutics), TTX-siPDL1 (TransCode Therapeutics), UniCAR-T-PD-L1 (GEMoaB monoclonals), Vaximm (VXM10), and YBL-013 (Y-Biologics).

20. The use or method of claim 19, wherein the anti-PD-1 antibody is avelumab, durvalumab, atezolizumab or a biosimilar of any of the foregoing.

21. The use or method of any one of the preceding claims, wherein the half-life extension element is a human serum albumin, an antigen binding polypeptide that binds human serum albumin, or an immunoglobulin Fc or fragment thereof.

22. The use or method of any one of the preceding claims, wherein the protease cleavable linker comprises a sequence that is capable of being cleaved by a protease selected from kallikrein, thrombin, chymase, carboxypeptidase A, cathepsin, elastase, PR-3, granzyme M, a calpain, a matrix metalloproteinase (MMP), an ADAM, a FAP, a plasminogen activator, a caspase, a tryptase, or a tumor protease.

23. The use or method of any one of the preceding claims, wherein the protease is selected from cathepsin B, cathepsin C, cathepsin D, cathepsin E, cathepsin K, cathepsin L, or cathepsin G.

24. The use or method of any one of the preceding claims, wherein protease is selected from matrix metalloprotease (MMP) is MMP1, MMP2, MMP3, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, or MMP14.

25. The use or method of any one of the preceding claims, wherein the blocking element binds the cytokine polypeptide.

26. The use or method of any one of the preceding claims, wherein the blocking element comprises a ligand-binding domain or fragment of a cognate receptor for the IFN, an antibody or antigen-binding fragment of an antibody that binds to the IFN polypeptide.

27. The use or method of any one of the preceding claims, wherein the antibody or antigen-binding fragment is a single domain antibody, a Fab, or a scFv that binds the IFN polypeptide.

28. The use or method of any one of the preceding claims, wherein the inducible cytokine prodrug is administered before, concurrently with or after the anti-PD-1 antibody or the anti-PD-L1 antibody.

29. The use or method of any one of the preceding claims, wherein the cancer is adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, brain tumor, bile duct cancer, bladder cancer, bone cancer, breast cancer, bronchial tumor, carcinoma of unknown primary origin, cardiac tumor, cervical cancer, chordoma, colon cancer, colorectal cancer, craniopharyngioma, ductal carcinoma, embryonal tumor, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, fibrous histiocytoma, Ewing sarcoma, eye cancer, germ cell tumor, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gestational trophoblastic disease, glioma, head and neck cancer, hepatocellular cancer, histiocytosis, hypopharyngeal cancer, intraocular melanoma, islet cell tumor, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ, lung cancer, macroglobulinemia, malignant fibrous histiocytoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, midline tract carcinoma involving NUT gene, mouth cancer, multiple endocrine neoplasia syndrome, mycosis fungoides, myelodysplastic syndrome, myelodysplastic/myeloproliferative neoplasm, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oropharyngeal cancer,

osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytomas, pituitary tumor, pleuropulmonary blastoma, prostate cancer, rectal cancer, renal cell cancer, renal pelvis and ureter cancer, retinoblastoma, rhabdoid tumor, salivary gland cancer, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cord tumor, stomach cancer, T-cell lymphoma, teratoid tumor, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms tumor.

30. The use or method of claim 29, wherein the cancer is colon cancer, lung cancer, melanoma, renal cell carcinoma, or breast cancer.

31. The use or method of claim 29, wherein the cancer is melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell cancer (HNSCC), classical Hodgkin lymphoma (cHL), primary mediastinal large B cell lymphoma (PMBCL), urothelial carcinoma, microsatellite instability high or mismatch repair deficient cancer, microsatellite instability high or mismatch repair deficient colorectal cancer, gastric cancer, esophageal cancer, cervical cancer, hepatocellular carcinoma (HCC), merkel cell carcinoma (MCC), renal cell carcinoma (RCC), endometrial carcinoma, tumor mutational burden high cancer, cutaneous squamous cell carcinoma (cSCC), triple negative breast cancer (TNBC), colorectal cancer or oesophageal carcinoma.

32. The use or method of claim 29, wherein the cancer is metastatic renal clear cell carcinoma or metastatic cutaneous malignant melanoma.

33. A pharmaceutical composition comprising the inducible cytokine prodrug, an anti-PD-1 or an anti-PD-L1 antibody, and a suitable carrier.

34. The pharmaceutical composition of claim 33, wherein the composition is a liquid composition for intravenous administration.

35. The pharmaceutical composition of claim 33, wherein the composition is a lyophilized composition.

36. The pharmaceutical composition of claim 35, wherein the lyophilized composition is for reconstitution using water for formulation is suitable of intravenous administration.
37. The use, method or composition of any one of claims 1-32, wherein the cancer is a lymphoma.
38. The use, method or composition of claim 37, wherein the lymphoma is a B cell lymphoma or a T cell lymphoma.
39. The use, method or composition of claim 37, wherein the lymphoma is Non-Hodgkin lymphoma or Hodgkin lymphoma.
40. The use, method or composition of claim 37, wherein the lymphoma is diffuse large B-cell lymphoma, primary mediastinal B cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, marginal zone lymphoma, mantle cell lymphoma, Waldenstrom's macroglobulinemia, Burkitt lymphoma, peripheral T cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T cell lymphoma, central nervous system lymphoma, grey zone lymphoma, double hit lymphoma, triple hit lymphoma, high grade B cell lymphomas not otherwise specified, lymphoblastic lymphoma, lymphoplasmacytic lymphoma, MALT lymphoma, monocytoid B cell lymphoma, natural killer (NK) cell lymphoma, mycosis fungoides, Sezary syndrome, enteropathy-type T cell lymphoma, or hepatosplenic gamma/delta T cell lymphoma.
41. A method for treating lymphoma, comprising administering to a subject in need thereof an effective amount of an inducible cytokine prodrug.
42. An inducible cytokine prodrug for use in treating lymphoma.

43. The method or use of claim 41 or 42 wherein the inducible cytokine prodrug is an inducible IL-12 prodrug or an inducible IFN α prodrug.

44. The method of use of claim 41 or 42, wherein the lymphoma is a B cell lymphoma or a T cell lymphoma.

45. The method or use of claim 41 or 42, wherein the lymphoma is Non-Hodgkin lymphoma or Hodgkin lymphoma.

46. The method or use of claim 41 or 42, wherein the lymphoma is diffuse large B-cell lymphoma, primary mediastinal B cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, marginal zone lymphoma, mantle cell lymphoma, Waldenstrom's macroglobulinemia, Burkitt lymphoma, peripheral T cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T cell lymphoma, central nervous system lymphoma, grey zone lymphoma, double hit lymphoma, triple hit lymphoma, high grade B cell lymphomas not otherwise specified, lymphoblastic lymphoma, lymphoplasmacytic lymphoma, MALT lymphoma, monocytoid B cell lymphoma, natural killer (NK) cell lymphoma, mycosis fungoides, Sezary syndrome, enteropathy-type T cell lymphoma, or hepatosplenic gamma/delta T cell lymphoma.

FIG. 1A

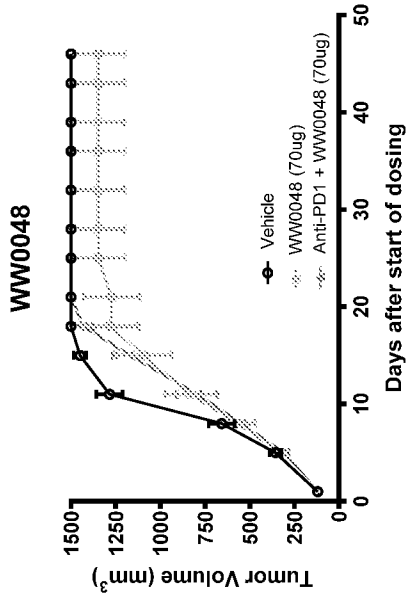


FIG. 1B

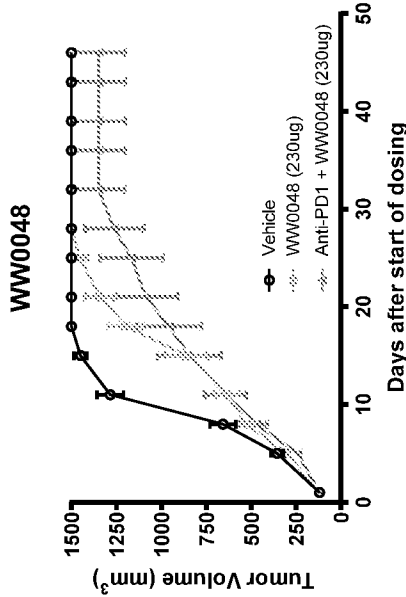


FIG. 1C

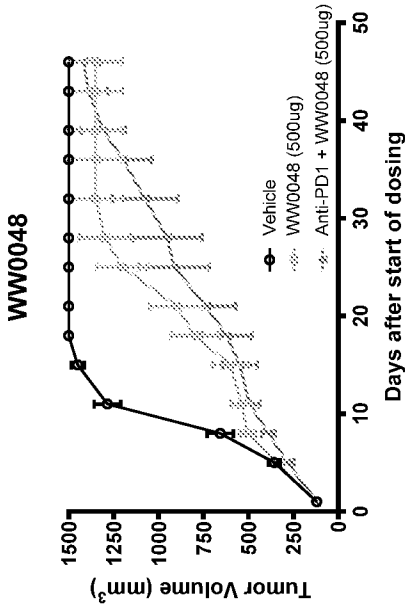


FIG. 1D

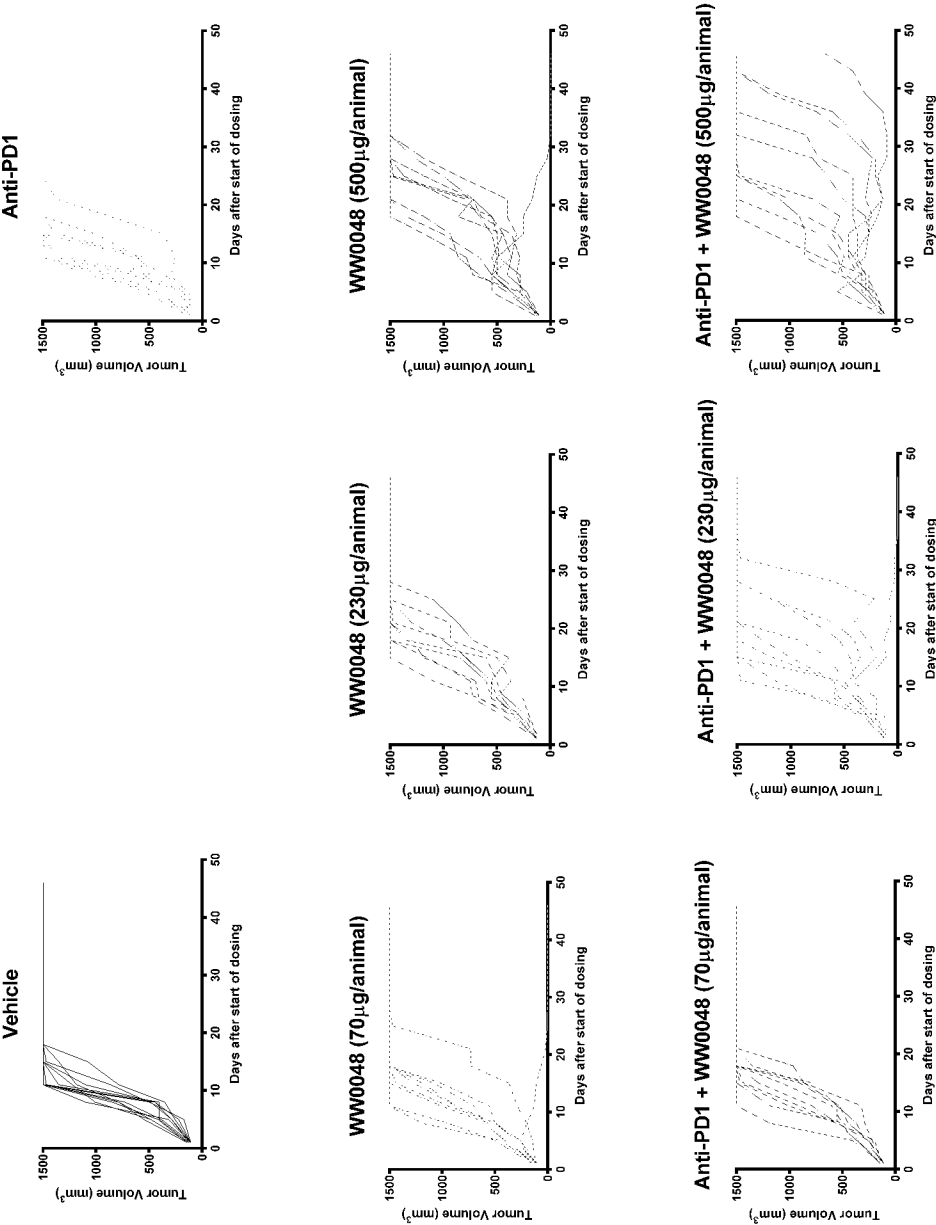


FIG. 1E

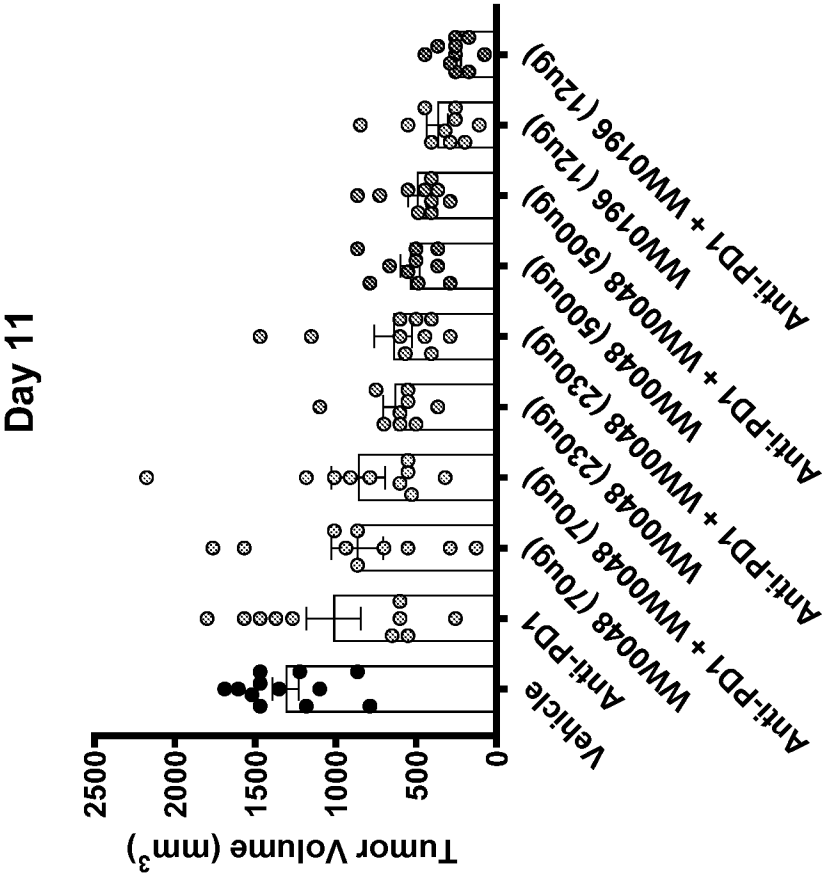


FIG. 1F

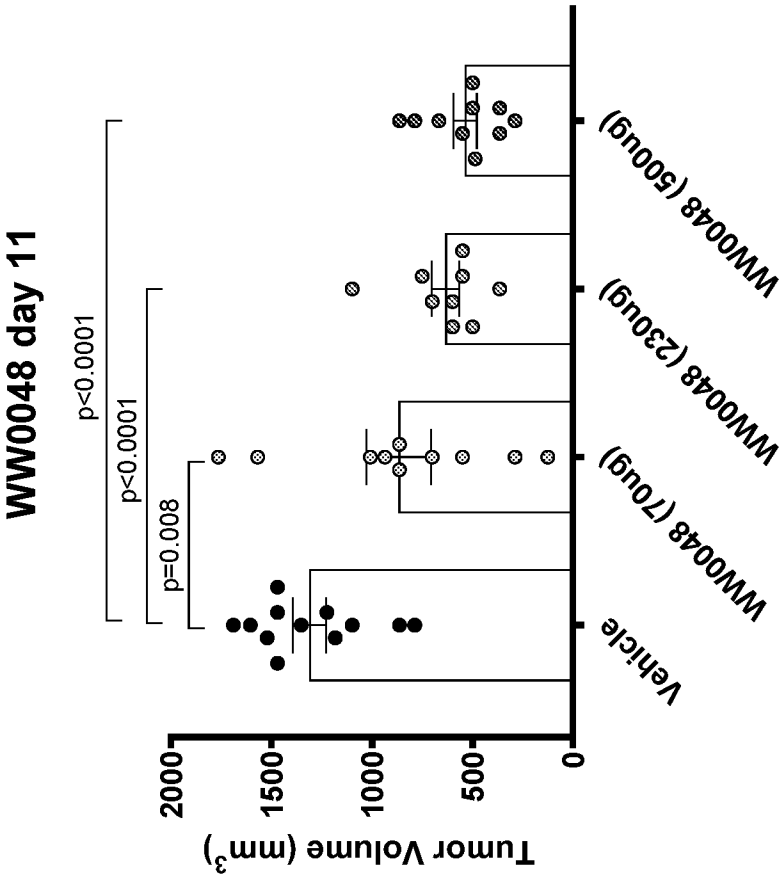


FIG. 2A

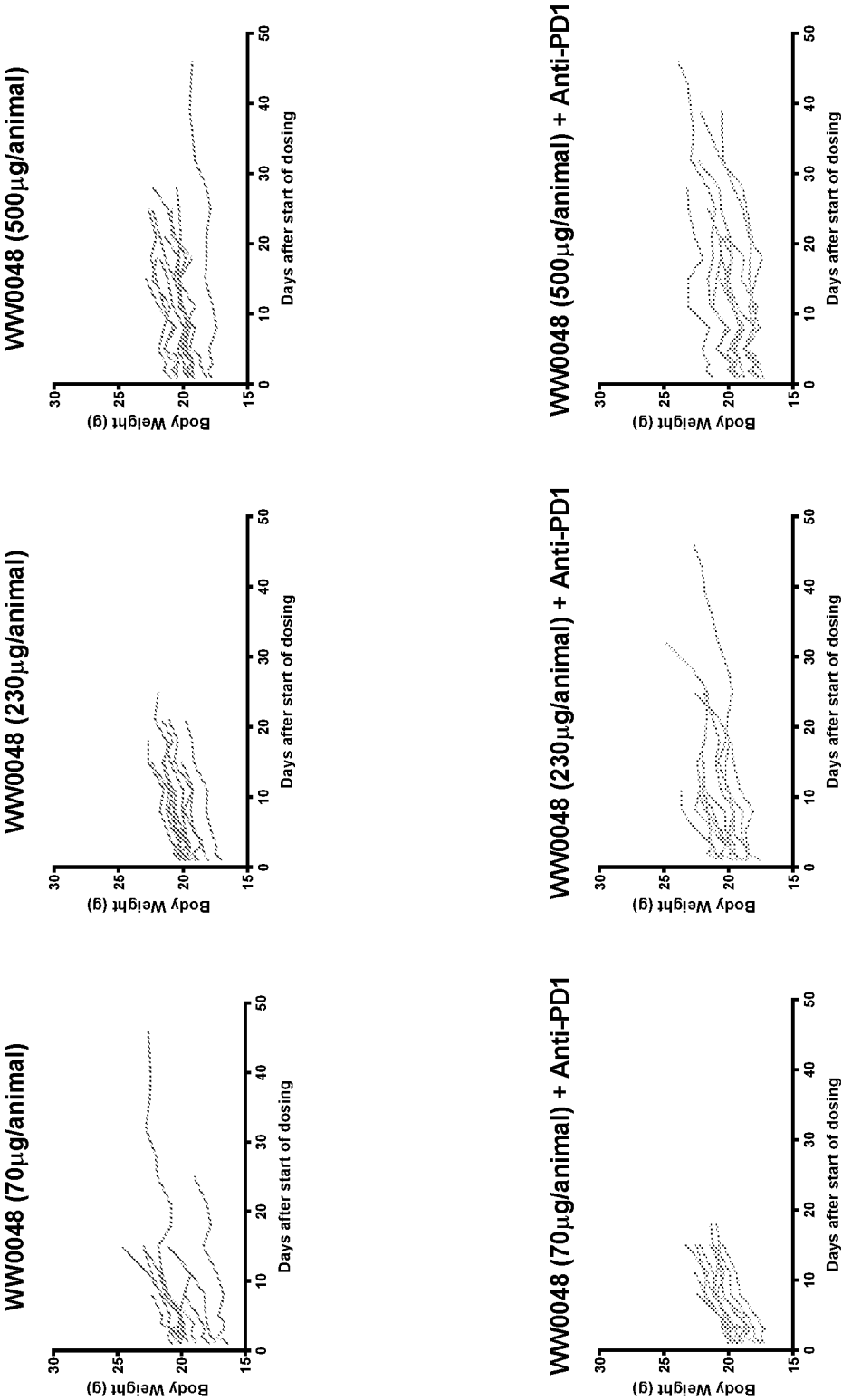


FIG. 2B

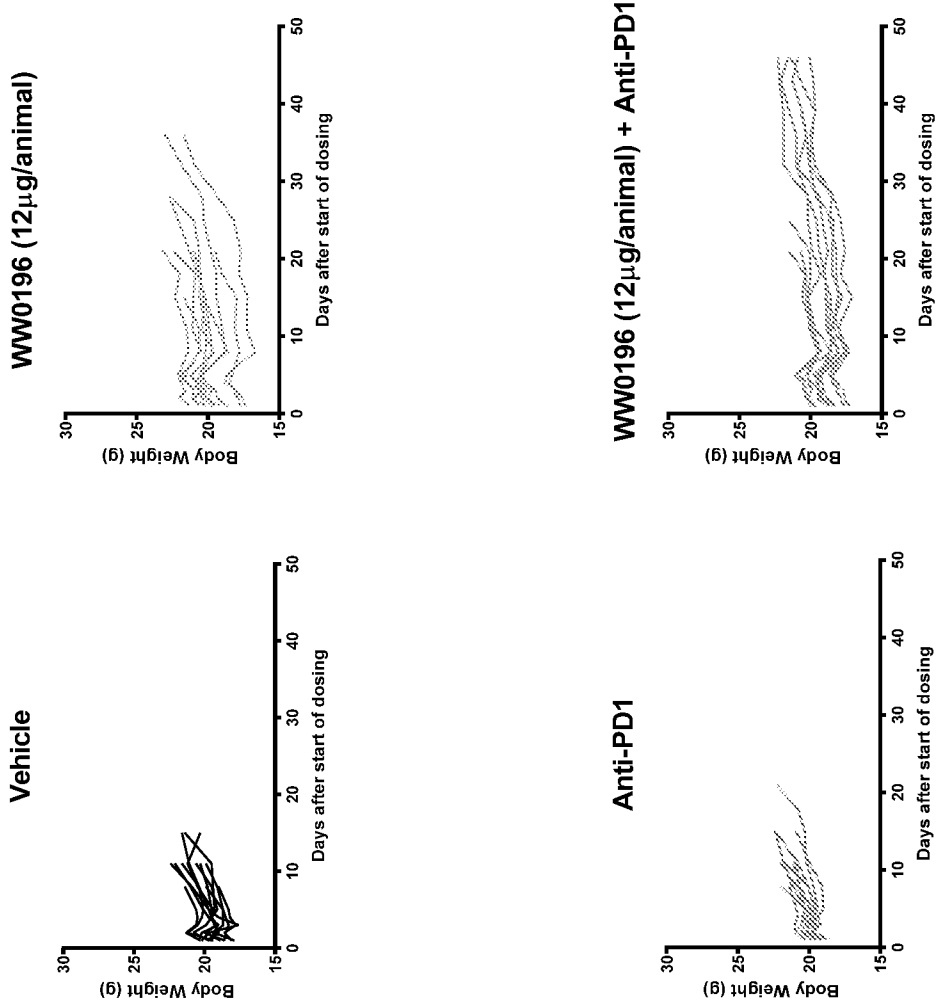


FIG. 3A

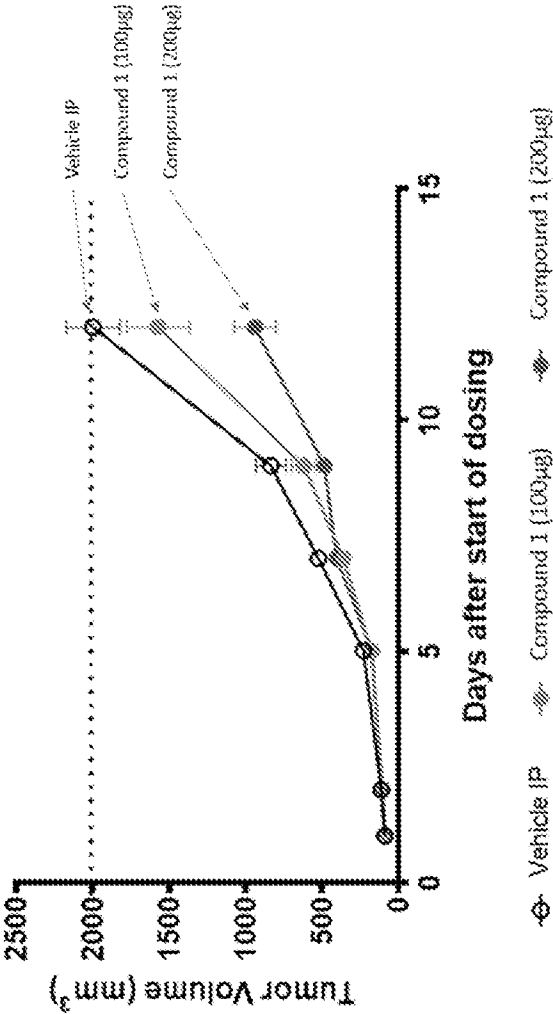


FIG. 3B

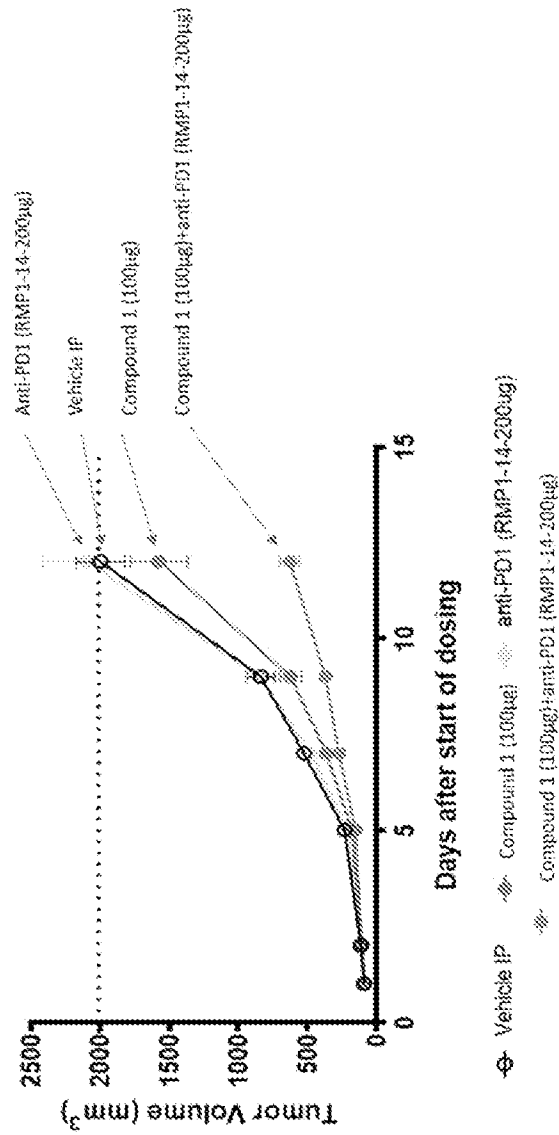
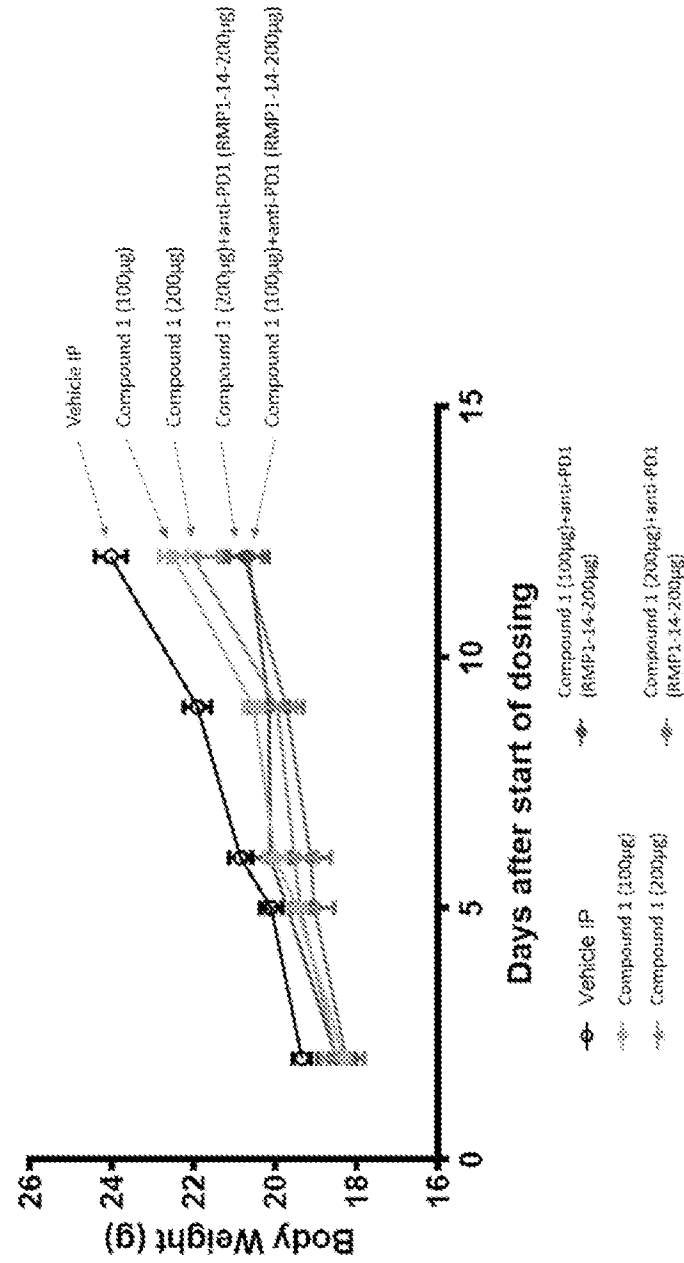


FIG. 4



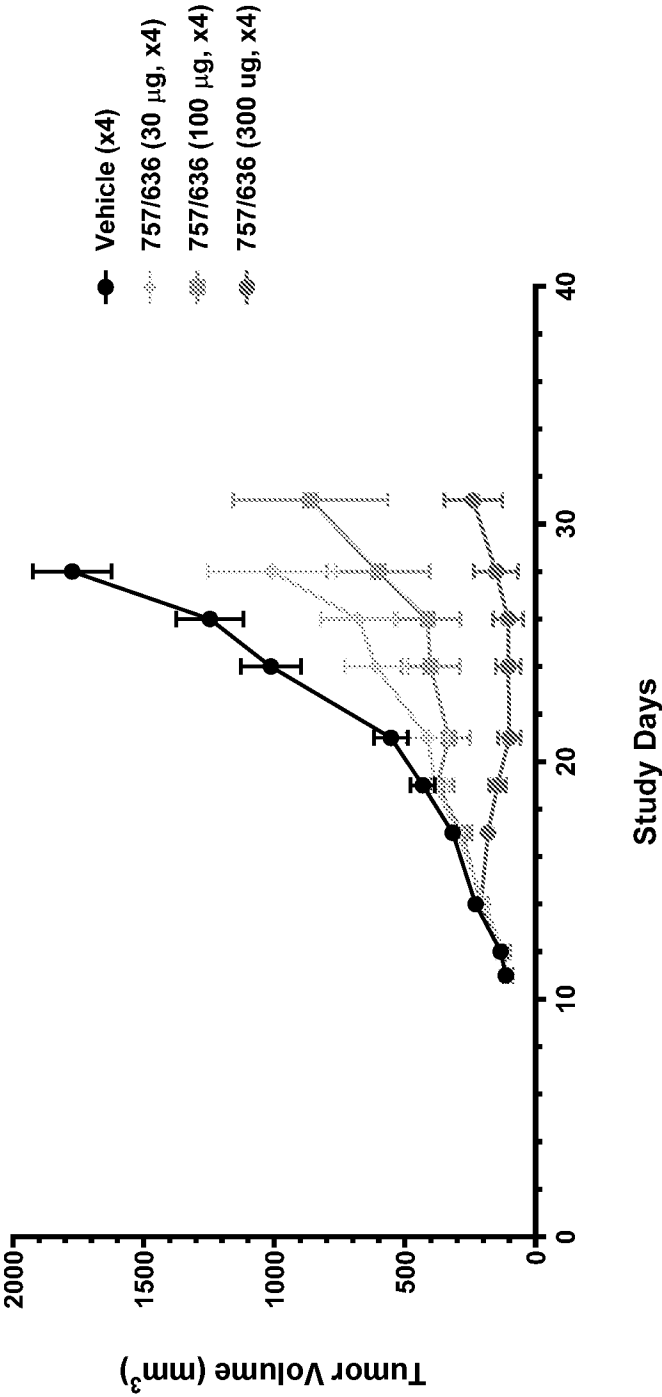


FIG. 5A

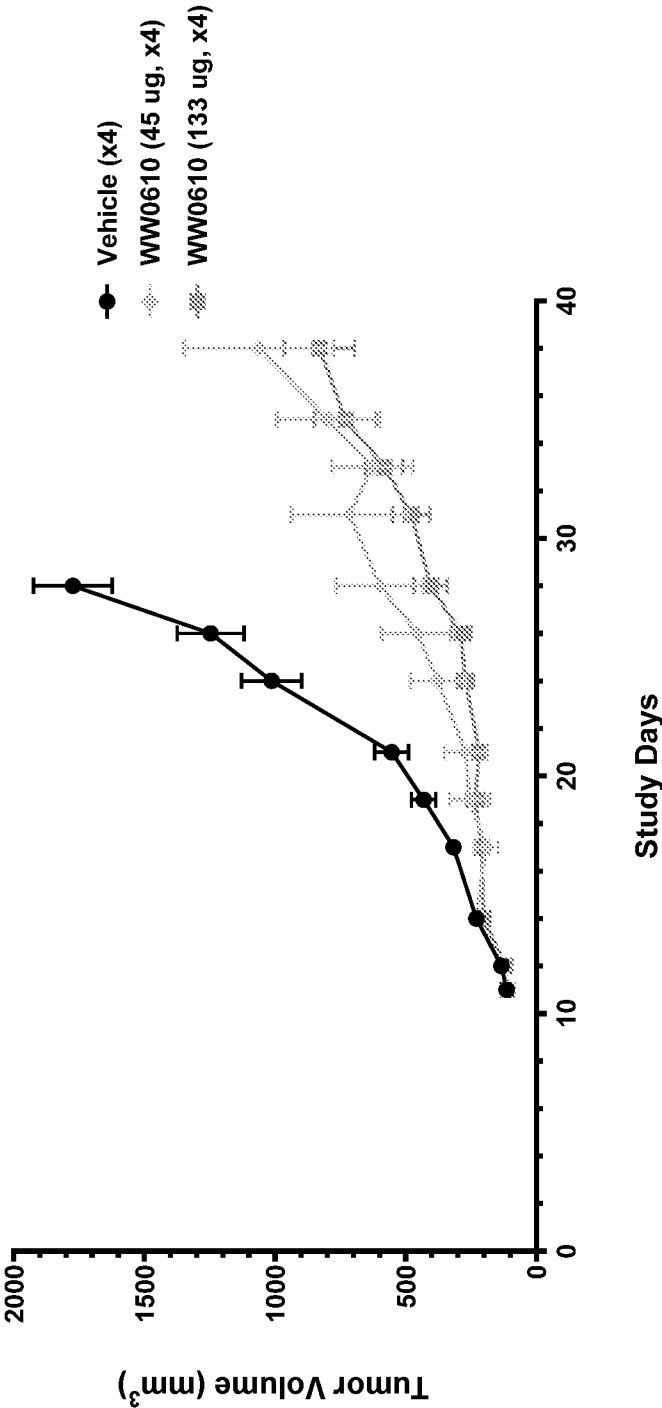


FIG. 5B

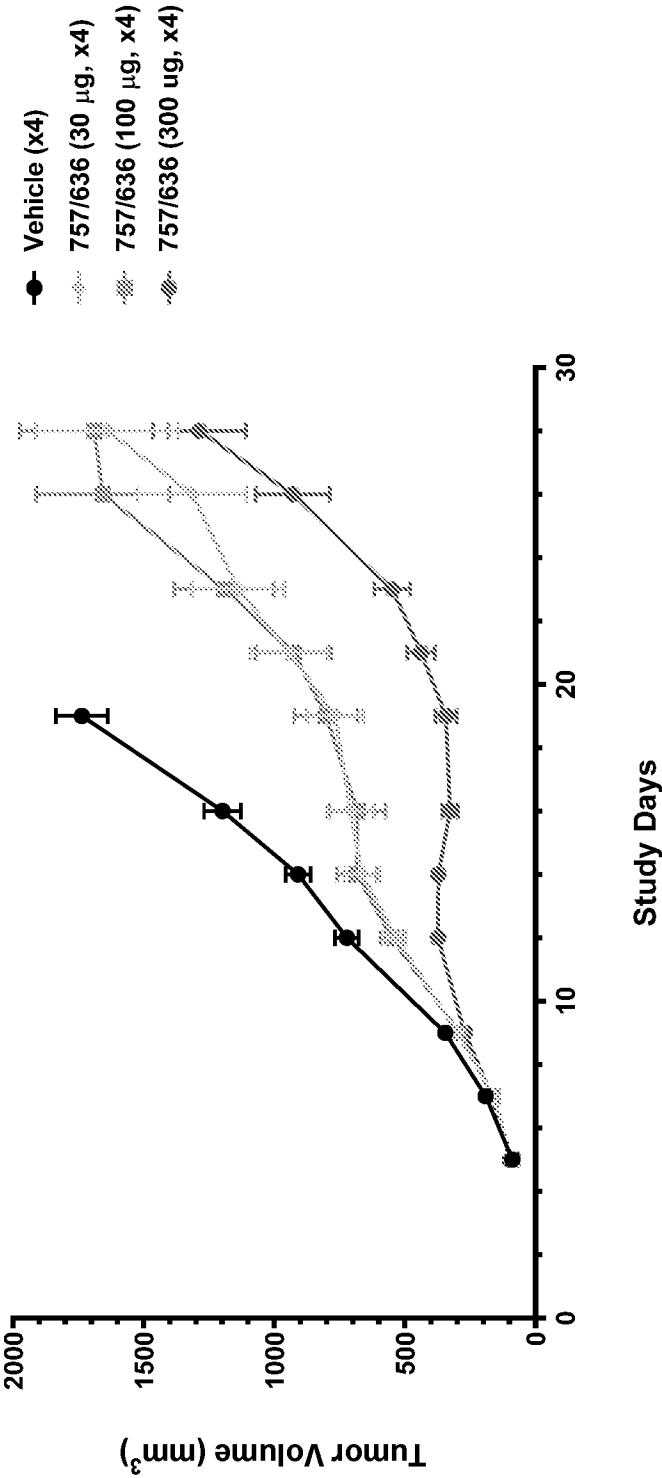


FIG. 6A

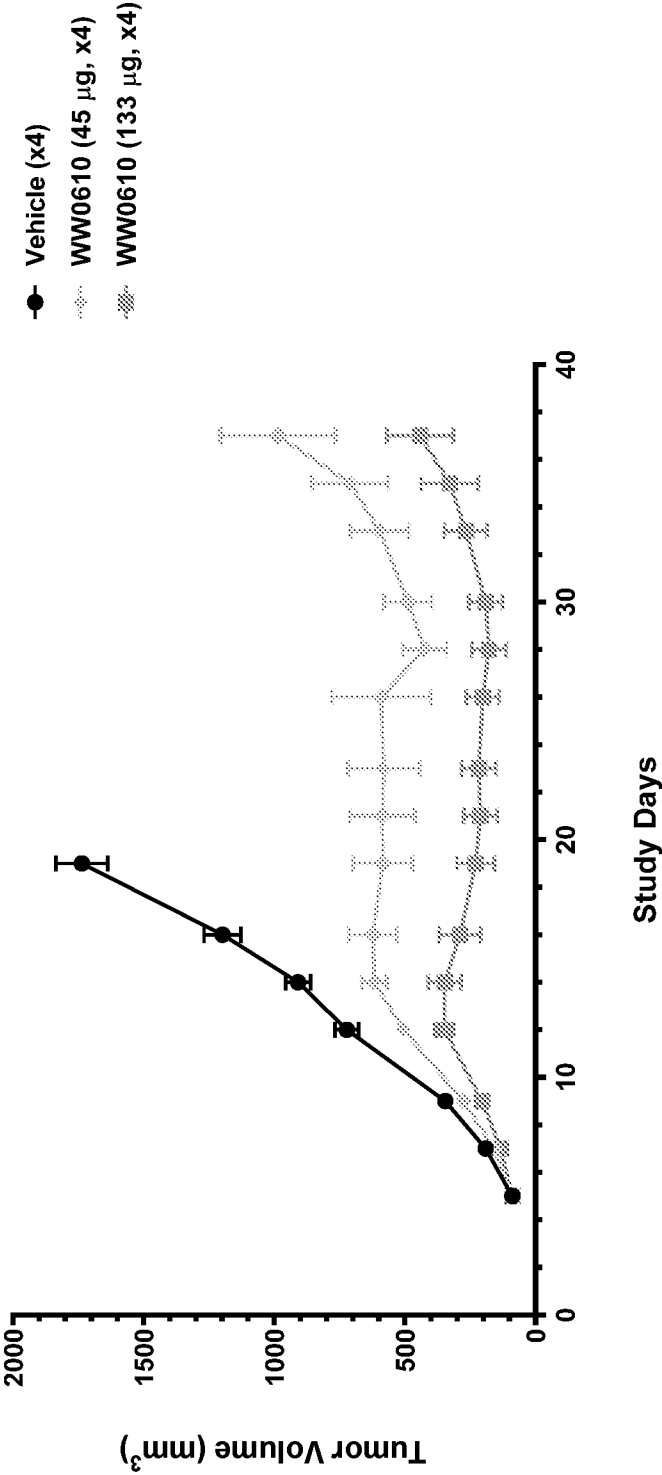


FIG. 6B

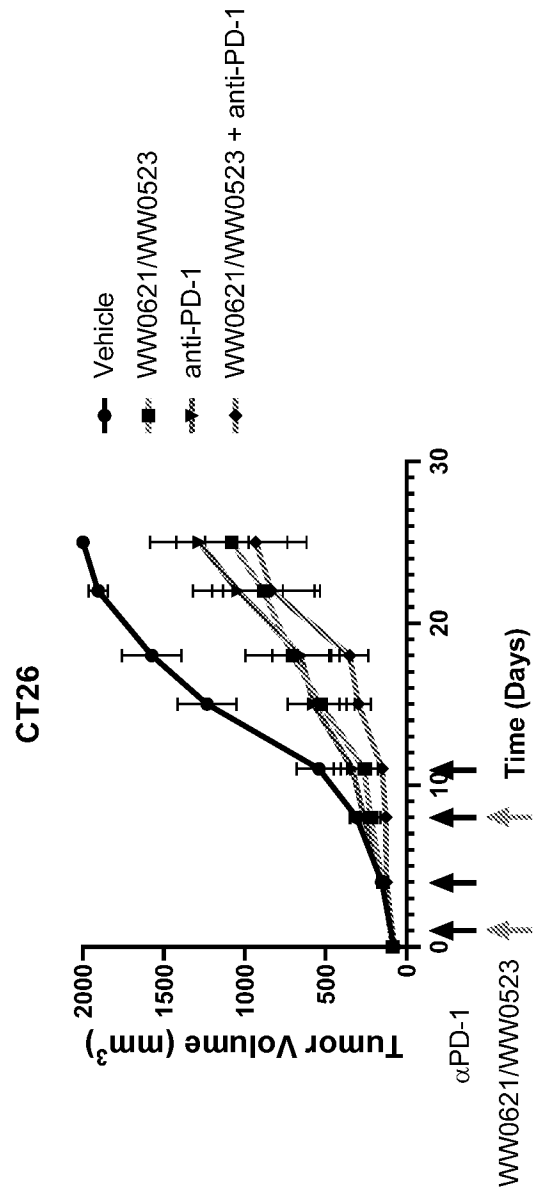


FIG. 7A

FIG. 7B

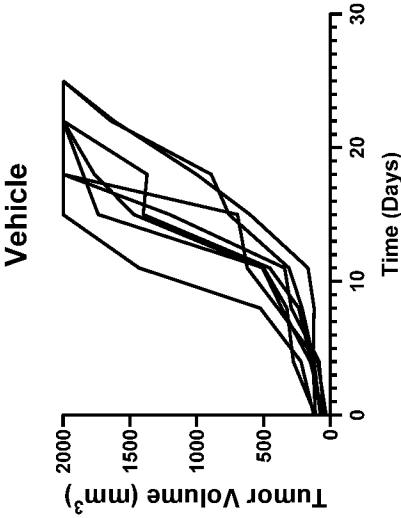


FIG. 7C

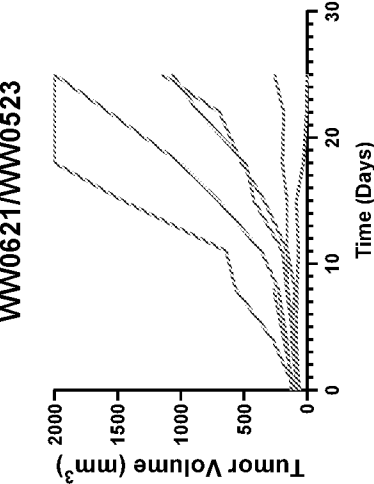


FIG. 7D

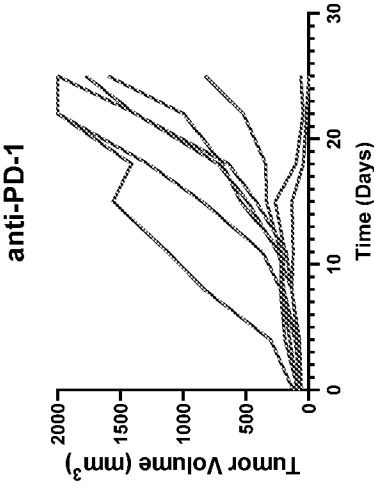


FIG. 7E

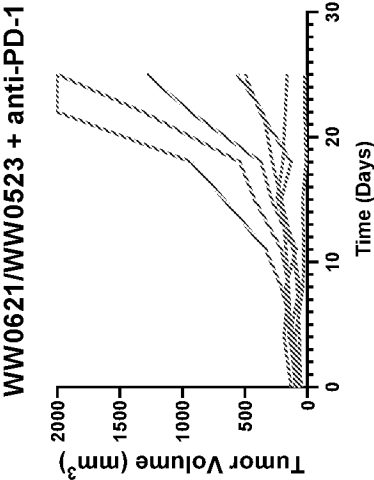
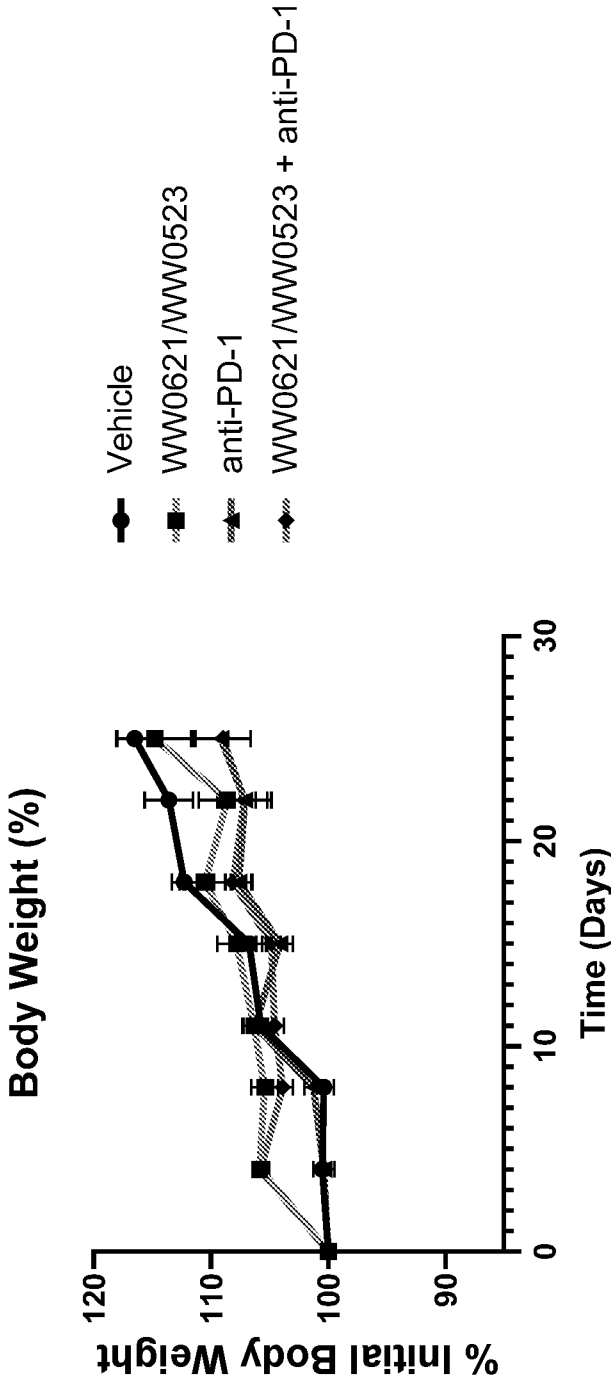


FIG. 7F



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/40478

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 38/20; A61K 38/21; A61K 39/395; A61P 35/00; C07K 14/54; C07K 14/555 (2022.01)

ADD.

CPC - INV. A61K 38/20; A61K 38/21; A61K 39/39558; A61P 35/00; C07K 14/54; C07K 14/555

ADD. C07K 2319/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2021/097376 A1 (WEREWOLF THERAPEUTICS INC.) 20 May 2021; paragraphs [6], [7], [11], [23], [40], [79], [81], [205], [206], [211], [222], [256], [257], [278], [279]	1-6, 33-34, 41-46 --- 35-36
Y	US 2,008,167,238 A1 (ROSEN, C. et al.) 10 July 2008; abstract; paragraphs [0005], [0540], [0546]	35-36

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

13 October 2022 (13.10.2022)

Date of mailing of the international search report

OCT 31 2022

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/40478

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/40478

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

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

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

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

3. ☒ Claims Nos.: 7-32, 37-40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.