PSEUDOTYPED ONCOLYTIC VIRAL DELIVERY OF THERAPEUTIC POLYPEPTIDES

Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Application No. 62/357,195, filed June 30, 2016, the content of which is incorporated herein by reference in its entirety.

Description of the Text Filed Submitted Electronically

[0002] The contents of the text filed submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (file name: ONCR_004_02WO_ST25.txt; date recorded: June 30, 2017; file size: 193 kilobytes).

Background of the Invention

[0003] Patients with certain hematologic and solid tumors remain in need of new therapies. The use of bispecific antibodies to direct cytotoxic T cells to tumor cells, and chimeric antigen receptors (CARs) to engineer antigen specificity onto an immune effector cell are being demonstrated to provide a therapeutic benefit. Also, oncolytic virus technologies are useful additions to the current standard of care of solid tumors, expected to have a safety profile and the ability to infect, replicate in, and lyse tumor cells. However, the antitumor efficacy of the bispecific antibodies, CARs and oncolytic virus are suboptimal, demonstrating the continued need for further advances of oncology, antibodies, and oncolytic virus therapy.

Summary of the Invention

[0004] In some embodiments, the present invention provides a pseudotyped oncolytic virus comprising a recombinant nucleic acid comprising (i) a first nucleic acid sequence encoding an engager polypeptide, wherein the engager polypeptide comprises an activation domain specific for an antigen expressed on an effector cell and an antigen recognition domain specific for a cell-surface antigen expressed on a target cell. In some embodiments, the antigen recognition domain specifically binds to a tumor antigen. In some embodiments, tumor antigen is selected from Table 2.

[0005] In some embodiments, the present invention provides a pseudotyped oncolytic virus comprising a recombinant nucleic acid comprising (i) a first nucleic acid sequence encoding an engager polypeptide, wherein the engager polypeptide comprises an activation domain specific for an antigen expressed on an effector cell and a therapeutic molecule domain that binds to an inhibitory antigen expressed on a cell surface. In some embodiments, the therapeutic molecule domain specifically binds to PD1, PDL1, or CD47. In some embodiments, the recombinant nucleic acid further comprises a second nucleic acid sequence encoding a therapeutic polypeptide. In some embodiments, the therapeutic polypeptide is an immune modulator polypeptide. In some embodiments, the immune modulator polypeptide is selected from a cytokine, a costimulatory molecule, an immune checkpoint polypeptide, an anti-angiogenesis factor, a matrix metalloprotease (MMP), or a nucleic acid.

[0006] In some embodiments, the immune checkpoint polypeptide comprises (i) an inhibitor of PD-1, PDL-1, CTLA-4, LAG3, TIM3, neuropilin, or CCR4; (ii) an agonist of GITR, OX-40, or CD28; or (iii) a combination of (i) and (ii). In some embodiments, the immune checkpoint polypeptide comprises an MMP, wherein the MMP is MMP9. In some embodiments, the immune checkpoint polypeptide comprises a cytokine, wherein the cytokine is selected from IL-15, IL-12, and CXCL10.

[0007] In some embodiments, the effector cell engaged by the engager molecules herein is a T cell, an NKT cell, an NK cell, or a macrophage. In some embodiments, the activation domain of the effector molecule specifically binds to CD3, CD4, CD5, CD8, CD16, CD28, CD40, CD134, CD137, or NKG2D.

[0008] In some embodiments, the recombinant nucleic acid provideds herein are multicistronic sequences. In some embodiments, the multicistronic sequence is a bicistronic sequence or a tricistronic sequence. In some embodiments, the multicistronic sequence comprises a picornavirus-2a-like sequence, and wherein the first and second nucleic acid sequences are expressed from a single promoter sequence present in the recombinant nucleic acid.

[0009] In some embodiments, the present invention provides a pseudotyped oncolytic virus comprising a recombinant nucleic acid sequence comprising (i) a first nucleic acid sequence encoding an engager polypeptide, wherein the engager polypeptide comprises an activation domain specific for an antigen expressed on an effector cell and an antigen recognition domain specific for a tumor cell antigen expressed on a target cell, wherein the antigen expressed on the effector cell is CD3, and wherein the tumor cell antigen is CD19. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 44. In some embodiments, the recombinant nucleic acid sequence

comprises SEQ ID NO: 43. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is IL-12. In such embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 54. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is IL-15. In such embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 53. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is CXCL10. In such embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 55. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is MMP9.

[0010] In some embodiments, the present invention provides a pseudotyped oncolytic virus comprising a recombinant nucleic acid sequence comprising (i) a first nucleic acid sequence encoding an engager polypeptide, wherein the engager polypeptide comprises an activation domain specific for an antigen expressed on an effector cell and an therapeutic molecule domain specific for an inhibitory antigen, wherein the antigen expressed on the effector cell is CD3, and wherein the inhibitory antigen is PDL1. In some embodiments, the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding a polypeptide sequence that is at least 90% identical to SEQ ID NO: 50. In some embodiments, the recombinant nucleic acid sequence comprises SEQ ID NO: 49. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is IL-12. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 63. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is IL-15. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 62. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is CXCL10. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 64. In some embodiments, the recombinant nucleic acid

sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is MMP9. In some embodiments, the engager molecule further comprises a third binding domain. In some embodiments, the third binding domain comprises an immunoglobulin Fc domain. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 52. In some embodiments, the recombinant nucleic acid sequence comprises SEQ ID NO: 51.

[0011] In some embodiments, the present invention provides a pseudotyped oncolytic virus comprising a recombinant nucleic acid sequence comprising (i) a first nucleic acid sequence encoding an engager polypeptide, wherein the engager polypeptide comprises an activation domain specific for an antigen expressed on an effector cell and an therapeutic molecule domain specific for an inhibitory antigen, wherein the antigen expressed on the effector cell is CD3, and wherein the inhibitory antigen is SIRP1α. In some embodiments, the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding a polypeptide sequence that is at least 90% identical to SEQ ID NO: 46 or 48. In some embodiments, the recombinant nucleic acid sequence comprises SEQ ID NO: 45 or 47. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is IL-12. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 58 or 59. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is IL-15. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 56 or 57. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is CXCL10. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 60 or 61. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is MMP9. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at least 90% identical to SEQ ID NO: 65 or 66. In some embodiments, the recombinant nucleic acid sequence further comprises (ii) a second nucleic acid sequence encoding a therapeutic molecule, wherein the therapeutic molecule is an anti-PDL1 scFv linked to an IgG1 Fc domain. In some embodiments, the recombinant nucleic acid sequence encodes a polypeptide sequence that is at

least 90% identical to SEQ ID NO: 68 or 70. In some embodiments, the recombinant nucleic acid sequence comprises SEQ ID NO: 67 or 69.

[0012] In some embodiments, the pseudotyped oncolytic viruses of the present invention are selected from adenovirus, herpes simplex virus 1 (HSV1), myxoma virus, reovirus, poliovirus, vesicular stomatitis virus (VSV), measles virus (MV), lassa virus (LASV), or Newcastle disease virus (NDV). In some embodiments, the pseudotyped oncolytic virus comprises a reduced neurotropism activity and/or neurotoxicity activity in a human subject as compared to a reference virus. In some embodiments, the reference virus is i) a non-pseudotyped oncolytic virus, or ii) a vaccinia virus. In some embodiments, the pseudotyped oncolytic virus is an attenuated oncolytic virus. In some embodiments, the virus is not a vaccinia virus.

[0013] In some embodiments, the pseudotyped oncolytic viruses of the present invention comprise a single recombinant nucleic acid. In some embodiments, the pseudotyped oncolytic viruses comprise a plurality of recombinant nucleic acids. In some embodiments, the oncolytic virus selectively infects a target cell. In some embodiments, the target cell is a tumor cell and wherein the oncolytic virus is capable of selectively replicating within the tumor cell.

[0014] In some embodiments, the engager polypeptide is a bipartite polypeptide and is comprised of an antibody, an antibody domain, a human immunoglobulin heavy chain variable domain, a dual-variable-domain antibody (DVD-Ig), a Tandab, a diabody, a flexibody, a dock-and-lock antibody, a Scorpion polypeptide, a single chain variable fragment (scFv), a BiTE, a DuoBody, an Fc-engineered IgG, an Fcab, a Mab2, or DART polypeptide.

[0015] In some embodiments, the present invention provides a pharmaceutical composition comprising any of the pseudotyped oncolytic viruses described herein. In some embodiments, the pseudotyped oncolytic virus induces an immune response. In some embodiments, immune response is selectively cytotoxic to a target cell. In some embodiments, the target cell is a solid tumor cell or a hematologic cancer cell. In some embodiments, the target cell expresses one or more tumor antigens. In some embodiments, the one or more tumor antigens are selected from Table 2.

[0016] In some embodiments, the present invention provides a method of treating a cancer in a subject in need thereof, comprising administering a therapeutically effective amount of an oncolytic virus described herein or a pharmaceutical composition described herein. In some embodiments, the method further comprises administering one or more additional therapies to the subject in need thereof. In some embodiments, the one or more additional therapies comprise surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof.

[0017] In some embodiments, the present invention provides a method of treating one or more tumors in a subject in need thereof comprising administering a therapeutically effective amount of an oncolytic virus described herein or a pharmaceutical composition described herein to a patient, wherein the one or more tumors express a tumor antigen.

[0018] In some embodiments, the present invention provides a method of selecting a patient for treatment comprising (a) determining the expression of a tumor antigen on one or more tumor cells derived from the patient; and (b) administering an oncolytic virus described herein or a pharmaceutical composition described herein if the tumor cells obtained from the patient express the one or more tumor antigens. In some embodiments, the one or more tumor antigens are selected from Table 2. In some embodiments, the present invention provides a method of delivering an engager polypeptide and a therapeutic polypeptide to a tumor site comprising administering to a patient in need thereof an oncolytic virus described herein or a pharmaceutical composition described herein.

Brief Description of the Drawings

[0019] FIG. 1 illustrates an amino acid sequence of a CD19-CD3 bipartite polypeptide comprising a first single chain variable fragment (scFv) directed against CD19 linked to a second scFv directed against CD3.

[0020] FIG. 2 illustrates an amino acid sequence of a CD19-CD3-IL15 construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising a first scFv directed against CD19 linked to a second scFv directed against CD3. A second gene encoding IL-15 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0021] FIG. 3 illustrates an amino acid sequence of a CD19-CD3-IL12 construct encoded by a multicistronic gene. The first gene encodes a bipartite polypeptide comprising a first scFv directed against CD19 linked to a second scFv directed against CD3. A second gene encoding the p35 subunit of IL-12 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker and a third gene encoding the p40 subunit of IL-12 is linked by a T2A self-cleaving polypeptide linker.

[0022] FIG. 4 illustrates an amino acid sequence of a CD19-CD3-CXCL10 construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising a first scFv directed against CD19 linked to a second scFv directed against CD3. A second gene encoding CXCL10 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0023] FIG. 5 illustrates an amino acid sequence of a SIRP1α-CD3 bipartite polypeptide comprising a first protein comprising the first 120 amino acids of SIRP1α linked by a single amino acid linker to an scFv directed against CD3.

[0024] FIG. 6 illustrates an amino acid sequence of a SIRP1α-CD3-LL bipartite polypeptide comprising a first protein comprising the first 120 amino acids of SIRP1α linked by a G4S motif linker to an scFv directed against CD3.

[0025] FIG. 7 illustrates an amino acid sequence of a SIRP1α-CD3-IL15 construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a single amino acid linker to an scFv directed against CD3. A second gene encoding IL-15 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0026] FIG. 8 illustrates an amino acid sequence of a SIRP1α-CD3-IL15-LL construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a G4S motif linker to an scFv directed against CD3. A second gene encoding IL-15 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0027] FIG. 9 illustrates an amino acid sequence of a SIRP1α-CD3-IL12 construct encoded by a multicistronic gene. The first gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a single amino acid linker to an scFv directed against CD3. A second gene encoding the p35 subunit of IL-12 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker and a third gene encoding the p40 subunit of IL-12 is linked by a T2A self-cleaving polypeptide linker.

[0028] FIG. 10 illustrates an amino acid sequence of a SIRP1α-CD3-IL12-LL construct encoded by a multicistronic gene. The first gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a G4S motif linker to an scFv directed against CD3. A second gene encoding the p35 subunit of IL-12 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker and a third gene encoding the p40 subunit of IL-12 is linked by a T2A self-cleaving polypeptide linker.

[0029] FIG. 11 illustrates an amino acid sequence of a SIRP1α-CD3-CXCL10 construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a single amino acid linker to an scFv directed against CD3. A second gene encoding CXCL10 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0030] FIG. 12 illustrates an amino acid sequence of a SIRP1α-CD3-CXCL10-LL construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a G4S motif linker to an scFv directed against CD3. A second gene encoding CXCL10 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0031] FIG. 13 illustrates an amino acid sequence of a PDL1-CD3 bipartite polypeptide comprising a first scFv directed against PDL1 linked to a second scFv directed against CD3.

[0032] FIG. 14 illustrates an amino acid sequence of a PDL1-CD3-IL15 construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising a first scFv directed against PDL1 linked to a second scFv directed against CD3. A second gene encoding IL-15 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0033] FIG. 15 illustrates an amino acid sequence of a PDL1-CD3-IL12 construct encoded by a multicistronic gene. The first gene encodes a bipartite polypeptide comprising a first scFv directed against PDL1 linked to a second scFv directed against CD3. A second gene encoding the p35 subunit of IL-12 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker and a third gene encoding the p40 subunit of IL-12 is linked by a T2A self-cleaving polypeptide linker.

[0034] FIG. 16 illustrates an amino acid sequence of a PDL1-CD3-CXCL10 construct encoded by a bicistronic gene. The first gene encodes a bipartite polypeptide comprising a first scFv directed against PDL1 linked to a second scFv directed against CD3. A second gene encoding CXCL10 is linked to the bipartite gene sequence by a T2A self-cleaving polypeptide linker.

[0035] FIG. 17 illustrates an amino acid sequence of a PDL1-CD3-Fc tripartite polypeptide comprising a first scFv directed against CD3, linked by a G4S motif linker to a second scFv directed against PDL1, which is in turn linked to the CH2-CH3 domain of human IgG1 by an IgG1 hinge.

[0036] FIG. 18 illustrates an amino acid sequence of a SIRP1α-CD3-MMP9-SL construct encoded by a bicistronic gene (FIG. 18A) and an amino acid sequence of a SIRP1α-CD3-MMP9-LL construct encoded by a bicistronic gene (FIG. 18B).

[0037] FIG. 19A – 19C illustrate the binding of CD19-CD3 BiTE constructs (FIG. 19A), SIRP1α-CD3 BiTE constructs (FIG. 19B), and PDL1-CD3-Fc tripartite T cell engagers (FIG. 19C) $CD3^+$ T cells.

[0038] FIG. 20 illustrates the quatification of the T cell engager construct binding shown in FIG. 19.

[0039] FIG. 21 illustrates the CD3-specific binding of CD19-CD3 BiTE constructs (FIG. 21A), SIRP1α-CD3 BiTE constructs (FIG. 21B), and PDL1-CD3-Fc tripartite T cell engagers (FIG. 21C) through the use of an anti-CD3 antibody, OKT3.

[0040] FIG. 22 illustrates the specificity of the CD47-binding SIRP1α arm of a SIRP1α-CD3 BiTE construct.

[0041] FIG. 23A – FIG. 23B illustrate the binding of CD19-CD3 and SIRP1α-CD3 BiTE constructs (FIG. 23A) to Raji cells ($CD19^+CD47^+$). % binding is quantified in FIG. 23B.

[0042] FIG. 24A – FIG 24B illustrate the binding of CD19-CD3 and SIRP1α-CD3 BiTE constructs (FIG. 24A) to U2OS cells ($CD19^-CD47^+$). % binding is quantified in FIG. 24B.

[0043] FIG. 25A – FIG. 25B illustrate the binding of CD19-CD3 and SIRP1α-CD3 BiTE constructs (FIG. 25A) to GBM30-luc cells ($CD19^-CD47^+$). % binding is quantified in FIG. 25B.

[0044] FIG. 26A – FIG. 26B illustrate the binding of CD19-CD3 and SIRP1α-CD3 BiTE constructs (FIG. 26A) to U251 cells ($CD19^-CD47^+$). % binding is quantified in FIG. 26B.

[0045] FIG. 27A – FIG. 27C illustrate the binding of PDL1-Fc-CD3 tripartite T cell engagers to U251 cells. The binding of the PDL1-Fc-CD3 constructs (FIG. 27B) is compared to the binding of an anti-PDL1 antibody (FIG. 27A). Binding was not mediated by FcγRs, as U251 cells do not express FcγRI, FcγRII, or FcγRIII (FIG. 27C).

[0046] FIG. 28 illustrates CD19-CD3 BiTE, SIRP1α-CD3 BiTE, and PDL1-CD3-Fc tripartite T cell engager-mediated T cell-dependent cytotoxicity (TDCC) of Raji cells.

[0047] FIG. 29 illustrates CD19-CD3 BiTE and PDL1-CD3-Fc tripartite T cell engager-mediated TDCC of THP1 cells.

[0048] FIG. 30 illustrates CD19-CD3 BiTE and PDL1-CD3-Fc tripartite T cell engager-mediated TDCC of U251 cells.

[0049] FIG. 31 illustrates SIRP1α-CD3 BiTE-mediated TDCC of 293F cells compared to an osteopontin-fusion control construct.

[0050] FIG. 32 illustrates expression of SIRP1α-CD3 BiTE constructs from oncolytic-HSV vectors. Expression of SIRP1α-CD3 BiTE constructs with short linkers (Lanes 1-

4 and ONCR085 in lanes 5-6, shown in FIG. 5) and SIRP1α-CD3 BiTE constructs with long linkers (ONCR087 in lanes 7-8, shown in FIG. 6) are shown.

[0051] FIG. 33 illustrates expression of PDL1-CD3-Fc BiTE constructs from oncolytic-HSV vectors. Purifed PDL1-CD3-Fc BiTE protein is shown in lanes 1-4. Concentrated viral supernatants are shown in lanes 5-6.

[0052] FIG. 34A – FIG. 34B illustrates TDCC of U251 cells by virally produced SIRP1α-CD3, SIRP1α-CD3-LL, and PDL1-CD3-Fc BiTE constructs. Photographs of U251 cell cultures after incubation with the indicated BiTE constructs and CD8+ T cells are shown in FIG. 34A. Activity of virally produced BiTE constructs, measured by % of cell killing and quantified by flow cytometry, is shown in FIG. 34B.

[0053] FIG. 35 illustrates that Amicon ultrafiltration effectively removes virus from samples, as determined by Western blotting with polyclonal anti-HSV antibody, and indicated that BiTE-killing is due to the BiTE and not viral infection.

[0054] FIG. 36 illustrates a cartoon representation of the production of a pseudotyped oncolytic virus and a recombinant oncolytic virus and infection of a target cell by the respective pseudotyped oncolytic virus and the recombinant oncolytic virus.

[0055] FIG. 37 illustrates an amino acid sequence of a SIRP1α-CD3-PDL1-Fc (SL) construct encoded by a bicistronic gene wherein the first gene encodes an anti-PDL1 scFv linked to an IgG1 Fc domain and the second gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a single amino acid linker to an scFv directed against CD3.

[0056] FIG. 38 illustrates an amino acid sequence of a SIRP1α-CD3-PDL1-Fc (LL) construct encoded by a bicistronic gene wherein the first gene encodes an anti-PDL1 scFv linked to an IgG1 Fc domain and the second gene encodes a bipartite polypeptide comprising the first 120 amino acids of SIRP1α linked by a G4S motif linker to an scFv directed against CD3.

[0057] FIG. 39 illustrates a schematic of a SIRP1α-CD3-PDL1-Fc expression plasmid. Two plasmid constructs, one for SIRP1α-CD3-PDL1-Fc (SL) and one for SIRP1α-CD3-PDL1-Fc (LL) were generated.

[0058] FIG. 40 illustrates purification of the SIRP1α-CD3 BiTE (SL), SIRP1α-CD3 BiTE (LL), and the anti-PDL1-Fc compounds from supernatants of transfected 293 T cells. FIG. 40A shows purification of anti-PDL1-Fc compounds assessed by Coomassie. FIG. 40B illustrates purification of SIRP1α-CD3 BiTE compounds as assessed by Western Blot using an anti-His detection antibody.

[0059] FIG. 41 shows results of a PD1/PDL1 blockade assay. A schematic of the assay is shown in FIG. 41A – FIG. 41B. The results of the PD1/PDL1 blockade assay using the anti-PDL1-Fc compound produced from 293 cells transfected are shown in FIG. 41C

Detailed Description of the Invention

[0060] The present disclosure provides novel engineered oncolytic viruses, in particular pseudotyped oncolytic viruses that produce multipartite polypeptides and/or other therapeutic polypeptides for the treatment of cancer including solid tumors (*e.g.*, advanced solid tumors) and hematologic malignancies. In some embodiments, the oncolytic virus is engineered by pseudotyping or other recombinant technology in order to modulate the tropism of the virus to result in a viral infection specific for tumor cells and/or surrounding tumor stroma and/or for other beneficial purposes as provided herein. In some embodiments, the multipartite and/or therapeutic polypeptides produced by the oncolytic viruses described herein mediate or enhance the anti-tumor effects of the oncolytic viruses, such as by effector-cell mediated lysis of target cells (*e.g.*, tumor cells). The oncolytic viruses described herein may have multiple (*e.g.* dual) modes of action, including effector cell-mediated cytolysis of target cells as a result of the expression of multipartite polypeptides, and viral-mediated destruction of target cells. The present disclosure further provides therapeutic compositions comprising the engineered oncolytic viruses and methods of use in the treatment of solid tumors and hematologic malignancies.

Overview

[0061] In some embodiments, the present invention provides pseudotyped oncolytic viruses, compositions thereof, and methods of use for the treatment of cancer. The pseudotyped oncolytic viruses provided herein comprise recombinant nucleic acids that encode engager polypeptides and/or other therapeutic molecules (*e.g.*, therapeutic polypeptides). Typically, the engager polypeptides function as effector cell engagers and generally comprise a first domain directed against an activation molecule expressed on an effector cell (*e.g.*, an activation domain or an engager domain) and a second domain directed against a target cell antigen (*e.g.*, an antigen recognition domain) or other cell-surface molecule (*e.g.*, a therapeutic molecule domain). Also provided are bipartite, tripartite or multipartite polypeptides (*e.g.*, comprising one or multiple engager domains, one or multiple antigen recognition domains, or one or multiple therapeutic molecule domains, and optionally one or multiple other functional domains).

[0062] Also provided are methods of treating cancer, comprising the step of delivering to human subject in need thereof a therapeutically effective amount of the oncolytic viruses or pharmaceutical compositions thereof provided herein. Such methods optionally include the step of delivering to the human subject an additional cancer therapy, such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof.

Definitions

[0063] As used herein, the singular forms "a," "an," or "the" include plural references unless the context clearly dictates otherwise.

[0064] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0065] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 30%, 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0066] As used herein the specification, "subject" or "subjects" or "individuals" include, but are not limited to, mammals such as humans or non-human mammals, including domesticated, agricultural or wild, animals, as well as birds, and aquatic animals. In some embodiments, subjects are livestock such as cattle, sheep, goats, cows, swine, and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals such as dogs and cats. In some embodiments (*e.g.*, particularly in research contexts) subjects are rodents (*e.g.*, mice, rats, hamsters), rabbits, primates, or swine such as inbred pigs and the like. In particular embodiments, the subject is a human. "Patients" are subjects suffering from or at risk of developing a disease, disorder, or condition or otherwise in need of the compositions and methods provided herein. None of the terms require or are limited to situations characterized by the supervision (*e.g.* constant or intermittent) of a health care worker (*e.g.* a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly or a hospice worker).

[0067] As used herein, "treating" or "treatment" refers to any indicia of success in the treatment or amelioration of a disease or condition, particularly cancer. Treating or treatment may be performed *in vitro* and/or *in vivo*, and may comprise delivering an oncolytic virus, or composition thereof, described herein to a patient or subject in need thereof. In some embodiments, treating includes, for example, reducing, delaying or alleviating the severity of one or more symptoms of the disease or condition, and/or reducing the frequency with which symptoms of a disease, defect, disorder, or adverse condition are experienced by a subject or patient. Herein, "treat or prevent" is used herein to refer to a method that results in some level of treatment or amelioration of the disease or condition, and contemplates a range of results directed to that end, including but not restricted to prevention of the condition entirely.

[0068] As used herein, "preventing" refers to the prevention of a disease or condition, *e.g.*, tumor formation, in a patient or subjet and may also be refered to as "prophylactic treatment." Prevention of disease development can refer to complete prevention of the symptoms of disease, a delay in disease onset, or a lessening of the severity of the symptoms in a subsequently developed disease. As a non-limiting illustrative example, if an individual at risk of developing a tumor or other form of cancer is treated with the methods of the present invention and does not later develop the tumor or other form of cancer, then the disease has been prevented, at least over a period of time, in that individual.

[0069] The terms "therapeutically effective amount" and "therapeutically effective dose" are used interchangeably herein and refer to the amount of an oncolytic viruse or composition thereof that is sufficient to provide a beneficial effect or to otherwise reduce a detrimental non-beneficial event (*e.g.* an amount or dose sufficient to treat a disease). The exact amount or dose of an oncolytic virus comprised within a therapeutically effective amount or therapeutically effective dose will depend on variety of factors including: the purpose of the treatment; the weight, sex, age, and general health of the subject or patient; the route of administration; the timing of administrations; and the nature of the disease to be treated. The therapeutically effective amount for a given subject or patient is ascertainable by one skilled in the art using known techniques (see, *e.g.* Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); and Pickar, *Dosage Calculations* (1999)).

[0070] "Pseudotype" refers to a virus particle, wherein a portion of the virus particle (*e.g.*, the envelope or capsid) comprises heterologous proteins, such as viral proteins derived from a heterologous virus or non-viral proteins. Non-viral proteins may include antibodies and antigen-binding fragments thereof. Preferably, a pseudotyped virus is capable of i)

altered tropism relative to non-pseudotyped virus, and/or ii) reduction or elimination of a non-beneficial effect. For example, in some embodiments a pseudotyped virus demonstrates reduced toxicity or reduced infection of non-tumor cells or non-tumor tissue as compared to a non-pseudotyped virus.

[0071] The term "targeting moiety" refers herein to a heterologous protein linked to a virus particle that is capable of binding to a protein on the cell surface of a selected cell type in order to direct interaction between the virus particle and the selected cell type. The targeting moiety may be covalently or non-covalently linked and is generally linked to an envelope protein, *e.g.*, E1, E2, or E3. Representative targeting moieties include antibodies, antigen binding fragments thereof, and receptor ligands. A viral "envelope" protein, or "Env" protein, refers to any polypeptide sequence that resides on the surface lipid bilayer of a virion and whose function is to mediate the adsorption to and the penetration of host cells susceptible to infection.

[0072] The term "vector" is used herein to refer to a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. In some embodiments, the vector is a virus (*i.e.*, a viral vector or oncolytic viral vector) and the transferred nucleic acid sequence is a recombinant nucleic acid sequence encoding an engager molecule and/or a therapeutic molecule. A viral vector may sometimes be referred to as a "recombinant virus" or a "virus." The terms "oncolytic virus" and "oncolytic vector" are used interchangeably herein.

[0073] "Nucleic acid genome" or "viral genome" refers to the nucleic acid component of a virus particle, which encodes the genome of the virus particle including any proteins required for replication and/or integration of the genome. In some embodiments, a viral genome acts as a viral vector and may comprise a heterologous gene operably linked to a promoter. The promoter may be either native or heterologous to the gene and may be viral or non-viral in origin. The viral genomes described herein may be based on any virus, may be an RNA or DNA genome, and may be either single stranded or double stranded. Preferably, the nucleic acid genome is from the family Rhabdoviridae.

[0074] "Retroviral vectors," as used herein, refer to viral vectors based on viruses of the Retroviridae family. In their wild-type (WT) form, retroviral vectors typically contain a nucleic acid genome. Provided herein are pseudotyped retroviral vectors that also comprise a heterologous gene, such as a recombinant nucleic acid sequence described herein.

[0075] The term "antibody fragment or derivative thereof" includes polypeptide sequences containing at least one CDR and capable of specifically binding to a target antigen. The term further relates to single chain antibodies, or fragments thereof, synthetic antibodies, antibody fragments, such as a Camel Ig, Ig NAR, Fab fragments, Fab' fragments, F(ab)'2 fragments, F(ab)'3 fragments, Fv, single chain Fv antibody ("scFv"), bis-scFv, (scFv)2, minibody, diabody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), and single-domain antibody (sdAb, nanobody), etc., or a chemically modified derivative of any of these. In some embodiments, antibodies or their corresponding immunoglobulin chain(s) are further modified by using, for example, amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other modification(s) (*e.g.* posttranslational and chemical modifications, such as glycosylation and phosphorylation), either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art.

[0076] The term "single-chain" as used in accordance with the present disclosure refers to the covalent linkage of two or more polypeptide sequences, preferably in the form of a co-linear amino acid sequence encoded by a single nucleic acid molecule.

[0077] The terms "binding to" and "interacting with" are used interchangeably herein and refer to the interaction of at least two "antigen-interaction-sites" with each other. An "antigen-interaction-site" refers to a motif of a polypeptide (*e.g.*, an antibody or antigen binding fragment thereof) capable of specific interaction with an antigen or a group of antigens. The binding/interaction is also understood to define a "specific interaction" or "specific binding."

[0078] The terms "specific binding" or "specific interaction" refer to an antigen-interaction-site that is capable of specifically interacting with and/or binding to at least two amino acids of a target molecule as defined herein. The term relates to the ability of the antigen-interaction-site to discriminate between the specific regions (*e.g.* epitopes) of the target molecules defined herein such that it does not, or essentially does not, cross-react with polypeptides of similar structures. In some embodiments, the epitopes are linear. In some embodiments, the epitopes are conformational epitopes, a structural epitope, or a discontinuous epitope consisting of two regions of the human target molecules or parts thereof. In context of this disclosure, a conformational epitope is defined by two or more discrete amino acid sequences separated in the primary sequence which come together on the surface of the folded protein. Specificity and/or cross-reactivity of a panel of antigen bindings construct under investigation can be tested, for example, by assessing binding of the panel of the constructs to the polypeptide of interest as well as to a number of more or less (structurally and/or functionally)

closely related polypeptides under conventional conditions (see, *e.g.*, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988 and Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999). Only those constructs that bind to the polypeptide/protein of interest and do not, or essentially do not, bind to any of the other polypeptides are considered specific for the polypeptide/protein of interest. Examples of specific interactions of an antigen-interaction-site with a specific antigen include the interaction of ligands which induce a signal upon binding to its specific receptor, the specificity of a ligand for its receptor, such as cytokines that bind to specific cytokine receptors, and the binding of an antigen binding site of an antibody to an antigenic epitope, among others.

[0079] In some instances, the specific interaction of the antigen-interaction-site with a specific antigen results in the initiation of a signal, *e.g.* due to the induction of a change of the conformation of the antigen, oligomerization of the antigen, etc. In some embodiments, specific binding encompasses a "key-lock-principle." Therefore in some embodiments, specific motifs in the amino acid sequence of the antigen-interaction-site interact with specific motifs in the antigen and bind to each other as a result of their primary, secondary or tertiary structure, or as the result of secondary modifications of said structure. In some embodiments, the specific interaction of the antigen-interaction-site with its specific antigen results in a simple binding of the site to the antigen.

Oncolytic Viruses

[0080] Oncolytic viruses are able to infect, replicate in, and lyse tumor cells, and are further capable of spreading to other tumor cells in successive rounds of replication. While past oncolytic virus therapy has shown promise in preclinical models and clinical studies, anti-tumor efficacy of these oncolytic virus, such as vaccinia, has been suboptimal. For example, these viruses demonstrated limited viral spread throughout the tumor and/or limited activation of anti-tumor T cell responses within the tumor. Therefore, the present disclosure provides an oncolytic virus that 1) facilitates tumor infiltration and activation of effector cells (*e.g.*, T cells), and 2) effectively lyses tumor cells that are not infected the virus (also known as by-stander killing).

[0081] In some embodiments, provided are viral vectors which have advantages including one or more of the following properties:

(i) the vectors are oncolytic and have a particularly high oncolytic activity compared to other previously described oncolytic viral vectors;

(ii) the vectors replicate preferentially in tumor cells and have a particularly high replication capability compared to other oncolytic viral vectors;

(iii) the vectors infect actively dividing cells as well as resting cells;

(iv) the vectors induce a strong innate, humoral, and cellular immune response;

(v) the vectors replicate purely cytoplasmatically, i.e., as RNA viruses they cannot integrate into the host cell genome or recombine into replication-competent viruses;

(vi) the vectors are easy to package; and/or

(vii) the native viral glycoprotein is interchangeable with a foreign envelope protein.

[0082] Some embodiments of the invention relate to recombinant vesicular stomatitis viruses (VSV) and VSV vectors. The VSV genome includes five genes, l, m, n, p and g, which encode the proteins L, M, N, P and G and are essential for the reproduction of the virus. N is a nucleoprotein which packages the VSV genomic RNA. The VSV genome is replicated as RNA-protein complex and L and P together form a polymerase complex which replicates the VSV genome and transcribes the VSV mRNA. M is a matrix protein which provides structural support between the lipid envelope and nucleocapsid and is important for particle sprouting at the cell membrane. G is the envelope protein which is incorporated in the viral envelope and is essential for the infectivity and tropism of the virus.

Pseudotyped oncolytic viruses

[0083] In some embodiments, the present invention provides oncolytic viruses that are capable of being pseudotyped or otherwise engineered. "Pseudotyped viruses" refer to viruses in which one or more of the viral coat proteins (*e.g.*, envelope proteins) have been replaced or modified. In some embodiments, a pseudotyped virus is capable of infecting a cell or tissue type that the corresponding non-pseudotyped virus is not capable of infecting. In some embodiments, a pseudotyped virus is capable of perferentially infecting a cell or tissue type compared to a non-pseudotyped virus.

[0084] In general, viruses have natural host cell populations that they infect most efficiently. For example, retroviruses have limited natural host cell ranges, while adenoviruses and adeno-associated viruses are able to efficiently infect a relatively broader range of host cells, although some cell types are refractory to infection by these viruses. The proteins on the surface of a virus (*e.g.*, envelope proteins or capsid proteins) meditate attachment to and entry into a susceptible host cell and thereby determine the tropism of the virus, *i.e.*, the ability of a particular virus to infect a particular cell or tissue type. In some embodiments, the oncolytic viruses described herein comprise a single types of protein on the surface of the virus. For example,

retroviruses and adeno-associated viruses have a single protein coating their membrane. In some embodiments, the oncolytic viruses described herein comprise more than one type of protein on the surface of the virus. For example, adenoviruses are coated with both an envelope protein and fibers that extend away from the surface of the virus.

[0085] The proteins on the surface of the virus can bind to cell-surface molecules such as heparin sulfate, thereby localizing the virus to the surface of the potential host cell. The proteins on the surface of the virus can also mediate interactions between the virus and specific protein receptors expressed on a host cell that induce structural changes in the viral protein in order to mediate viral entry. Alternatively, interactions between the proteins on the surface of the virus and cell receptors can facilitate viral internalization into endosomes, wherein acidification of the endosomal lumen induces refolding of the viral coat. In either case, viral entry into potential host cells requires a favorable interaction between at least one molecule on the surface of the virus and at least one molecule on the surface of the cell.

[0086] In some embodiments, the oncolytic viruses described herein comprise a viral coat (*e.g.*, a viral envelop or viral capsid), wherein the proteins present on the surface of the viral coat (*e.g.*, viral envelop proteins or viral capsid proteins) modulate recognition of a potential target cell for viral entry. In some instances, this process of determining a potential target cell for entry by a virus is referred to as host tropism. In some embodiments, the host tropism is cellular tropism, wherein viral recognition of a receptor occurs at a cellular level, or tissue tropism, wherein viral recognition of cellular receptors occurs at a tissue level. In some instances, the viral coat of a virus recognizes receptors present on a single type of cell. In other instances, the viral coat of a virus recognizes receptors present on multiple cell types (*e.g.*, 2, 3, 4, 5, 6 or more different cell types). In some instances, the viral coat of a virus recognizes cellular receptors present on a single type of tissue. In other instances, the viral coat of a virus recognizes cellular receptors present on multiple tissue types (*e.g.*, 2, 3, 4, 5, 6 or more different tissue types).

[0087] In some embodiments, the oncolytic viruses described herein comprise a viral coat that has been modified to incorporate surface proteins from a different virus in order to facilitate viral entry to a particular cell or tissue type. Such oncolytic viruses are refered to herein as pseudotyped oncolytic viruses. In some embodiments, a pseudotyped oncolytic viruses comprises a viral coat wherein the viral coat of a first virus is exchanged with a viral coat of second, wherein the viral coat of the second virus is allows the pseudotyped oncolytic virus to infect a particular cell or tissue type. In some embodiments, the viral coat comprises a viral envelope. In some instances, the viral envelope comprises a phospholipid bilayer and proteins such as proteins obtained from a host membrane. In some embodiments, the viral envelope

further comprises glycoproteins for recognition and attachment to a receptor expressed by a host cell. In some embodiments, the viral coat comprises a capsid. In some instances, the capsid is assembled from oligomeric protein subunits termed protomers. In some embodiments, the capsid is assembled from one type of protomer or protein, or is assembled from two, three, four, or more types of protomers or proteins.

[0088] In some embodiments, it is advantageous to limit or expand the range of cells susceptible to transduction by an oncolytic virus for the purpose of oncolytic therapy. To this end, many viruses have been developed in which the endogenous viral coat proteins (*e.g.*, viral envelope or capsid proteins) proteins have been replaced by viral coat proteins from other viruses or by chimeric proteins. In some embodiments, the chimeric proteins are comprised of parts of a viral protein necessary for incorporation into the virion, as well proteins or nucleic acids designed to interact with specific host cell proteins, such as a targeting moiety.

[0089] In some embodiments, the pseudotyped oncolytic viruses described herein are pseudotyped in order to limit or control the viral tropism (*i.e.*, to reduce the number of cell or tissue types that the pseudotyped oncolytic virus is capable of infecting). Most strategies adopted to limit tropism have used chimeric viral coat proteins (*e.g.*, envelope proteins) linked antibody fragments. These viruses show great promise for the development of oncolytic therapies. In some embodiments, the pseudotyped oncolytic viruses described herein are pseudotyped in order to expand the viral tropism (*i.e.*, to increase the number of cell or tissue types that the pseudotyped oncolytic virus is capable of infecting). One mechanism for expanding the cellular tropism of viruses (*e.g.*, enveloped viruses) is through the formation of phenotypically mixed particles or pseudotypes, a process that commonly occurs during viral assembly in cells infected with two or more viruses. For example, human immunodeficiency virus type 1 (HIV-1). HIV1 infects cells that express CCR4 with an appropriate co-receptor. However, HIV1 forms pseudotypes by the incorporation of heterologous glycoproteins (GPs) through phenotypic mixing, such that the virus can infect cells that do not express the CD4 receptor and/or an appropriate co-receptor, thereby expanding the tropism of the virus. Several studies have demonstrated that wild type HIV-1 produced in cells infected with xenotropic murine leukemia virus (MLV), amphotropic MLV, or herpes simplex virus gives rise to phenotypically mixed virions with an expanded host range, indicating that pseudotyped virions had been produced. Phenotypic mixing of viral GPs has also been shown to occur between HIV-1 and VSV in coinfected cell cultures. These early observations were key to the subsequent design of HIV-1-based lentiviral vectors bearing heterologous GPs.

[0090] There is an ever-growing list of alternative GPs for pseudotyping lentiviruses, each with specific advantages and disadvantages. The widespread use of VSV G-proteins (VSV-G) to pseudotype lentiviruses has made this GP in effect the standard against which the usefulness of other viral GPs to form pseudotypes are compared. Addtional non-limiting examples of lentivirus pseudotypes include pseudotypes bearing lyssavirus-derived GPs, pseudotyped lentiviruses bearing lymphocytic choriomeningitis virus GPs, lentivirus pseudotypes bearing alphavirus GPs (*e.g.*, lentiviral vectors pseudotyped with the RRV and SFV GPs, lentiviral vectors pseudotyped with sindbis virus GPs), pseudotypes bearing filovirus GPs, and lentiviral vector pseudotypes containing the baculovirus GP64.

[0091] In some embodiments, the engineered (*e.g.*, pseudotyped) viruses are capable of binding to a tumor and/or tumor cell, typically by binding to a protein, lipid, or carbohydrate expressed on a tumor cell. In such embodiments, the engineered viruses described herein may comprise a targeting moiety that directs the virus to a particular host cell. In some instances, any cell surface biological material known in the art or yet to be identified that is differentially expressed or otherwise present on a particular cell or tissue type (*e.g.*, a tumor or tumor cell, or tumor associated stroma or stromal cell) may be used as a potential target for the oncolytic viruses the present invention. In particular embodiments, the cell surface material is a protein. In some embodiments, the targeting moiety binds cell surface antigens indicative of a disease, such as a cancer (*e.g.*, breast, lung, ovarian, prostate, colon, lymphoma, leukemia, melanoma, and others); an autoimmune disease (*e.g.* myasthenia gravis, multiple sclerosis, systemic lupus erythymatosis, rheumatoid arthritis, diabetes mellitus, and others); an infectious disease, including infection by HIV, HCV, HBV, CMV, and HPV; and a genetic disease including sickle cell anemia, cystic fibrosis, Tay-Sachs, J3-thalassemia, neurofibromatosis, polycystic kidney disease, hemophilia, etc. In certain embodiments, the targeting moiety targets a cell surface antigen specific to a particular cell or tissue type, *e.g.*, cell-surface antigens present in neural, lung, kidney, muscle, vascular, thyroid, ocular, breast, ovarian, testis, or prostate tissue.

[0092] Exemplary antigens and cell surface molecules for targeting include, *e.g.* P-glycoprotein, Her2/Neu, erythropoietin (EPO), epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGF-R), cadherin, carcinoembryonic antigen (CEA), CD4. CD8, CD19. CD20, CD33, CD34, CD45, CD117 (c-kit), CD133, HLA-A. HLA-B, HLA-C, chemokine receptor 5 (CCR5), stem cell marker ABCG2 transporter, ovarian cancer antigen CA125, immunoglobulins, integrins, prostate specific antigen (PSA), prostate stem cell antigen (PSCA), dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), thyroglobulin, granulocyte-macrophage colony stimulating factor (GM-CSF),

myogenic differentiation promoting factor-1 (MyoD-1), Leu-7 (CD57), LeuM-1, cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67 (Ki-67), viral envelope proteins, HIV gp120, transferrin receptor, etc. Additional antigens and cell surface molecules for targeting are shown in Table 2.

[0093] In some embodiments, the pseudotyped oncolytic viruses provided herein are capable of selectively entering, replicating in, and/or lysing tumor cells. Such an embodiment is illustrated in FIG. 36, wherein the pseudotyped oncolytic virus gains entry to the target cell due to the incorporation of viral glycoproteins derived from a different (*i.e.*, heterologous) virus that allow for entry of the pseudotyped oncolytic virus into the target cell. In contrast, the non-pseudotyped oncolytic virus is unable to gain entry into the target cell due to the non-permissive nature of the envelope proteins. In some instances, the ability of a pseudotyped oncolytic virus to selectively enter, replicate in, and/or lyse a tumor cells is due to a reduced or otherwise ineffective cellular interferon (IFN) response. In some embodiments, the pseudotyped oncolytic viruses produce an engager molecule and/or a therapeutic molecule, such as an immune modulating polypeptide, that interferes or impairs the cellular IFN response, thereby enhancing the replication of the pseudotyped or engineered virus.

[0094] The pseudotyped oncolytic viruses described herein may be derived from a variety of viruses, non-limiting examples of which include vaccinia virus, adenovirus, herpes simplex virus 1 (HSV1), myxoma virus, reovirus, poliovirus, vesicular stomatitis virus (VSV), measles virus (MV), lassa virus (LASV) and Newcastle disease virus (NDV). In some embodiments, the pseudotyped oncolytic viruses described herein can infect substantially any cell type. An exemplary lentivirus for use in oncolytic therapy is Simian immunodeficiency virus coated with the envelope proteins, G-protein (GP), from VSV. In some instances, this virus is referred to as VSV G-pseudotyped lentivirus, and is known to infect an almost universal set of cells.

[0095] In some embodiments, the pseudotyped oncolytic viruses of the present invention are VSV viruses pseudotyped against healthy brain cells, i.e., neurons and exhibit considerably reduced toxicity. Since neurotropism is a dose-limiting factor in all applications of oncolytic VSV, the use of the vector according to some embodiments of the present invention is that they are used for all tumors types of solid tumors.

[0096] In some embodiments, the pseudotyped VSV vectors have one or more key attributes including: (i) the VSV is not cell-toxic; (ii) the vectors are concentrated by ultracentrifugation without loss of infectivity; and (iii) the vectors show a tropism for tumor cells, whereas neurons and other non-tumor cells are infected inefficiently. To increase the safety

during the use of replicable viruses in therapeutic uses, some embodiments of the present invention provide a vector system which ensures that replication, oncolysis and the production of VSV viruses takes place only in cells which are infected by at least two replication-deficient, mutually complementing vectors.

[0097] In some embodiments, the genetic material (*e.g.*, the viral coat protein or the core genetic material) for generating a pseudotyped oncolytic virus is obtained from a DNA virus, an RNA virus, or from both virus types. In some embodiments, a DNA virus is a single-stranded (ss) DNA virus, a double-stranded (ds) DNA virus, or a DNA virus that contains both ss and ds DNA regions. In some embodiments, an RNA virus is a single-stranded (ss) RNA virus or a double-stranded (ds) RNA virus. In some embodiments, an ssRNA virus is further classified into a positive-sense RNA virus or a negative-sense RNA virus.

[0098] In some instances, the genetic material for generating a pseudotyped oncolytic virus is obtained from a dsDNA virus of any one of the following families: Myoviridae, Podoviridae, Siphoviridae, Alloherpesviridae, Herpesviridae, Malacoherpesviridae, Lipothrixviridae, Rudiviridae, Adenoviridae, Ampullaviridae, Ascoviridae, Asfaviridae, Baculoviridae, Bicaudaviridae, Clavaviridae, Corticoviridae, Fuselloviridae, Globuloviridae, Guttaviridae, Hytrosaviridae, Iridoviridae, Marseilleviridae, Mimiviridae, Nimaviridae, Pandoraviridae, Papillomaviridae, Phycodnaviridae, Plasmaviridae, Polydnaviruses, Polyomaviridae, Poxviridae, Sphaerolipoviridae, or Tectiviridae.

[0099] In some cases, the genetic material for generating a pseudotyped oncolytic virus is obtained from a ssDNA virus of any one of the following families: Anelloviridae, Bacillariodnaviridae, Bidnaviridae, Circoviridae, Geminiviridae, Inoviridae, Microviridae, Nanoviridae, Parvoviridae, or Spiraviridae.

[00100] In some embodiments, the genetic material for generating a pseudotyped oncolytic virus is obtained from a DNA virus that contains both ssDNA and dsDNA regions. In some cases, the DNA virus is from the group pleolipoviruses. In some cases, the pleolipoviruses include Haloarcula hispanica pleomorphic virus 1, Halogeometricum pleomorphic virus 1, Halorubrum pleomorphic virus 1, Halorubrum pleomorphic virus 2, Halorubrum pleomorphic virus 3, or Halorubrum pleomorphic virus 6.

[00101] In some cases, the genetic material for generating a pseudotyped oncolytic virus is obtained from a dsRNA virus of any one of the following families: Birnaviridae, Chrysoviridae, Cystoviridae, Endornaviridae, Hypoviridae, Megavirnaviridae, Partitiviridae, Picobirnaviridae, Reoviridae, Rotavirus or Totiviridae.

[00102] In some instances, the genetic material for generating a pseudotyped oncolytic virus is obtained from a positive-sense ssRNA virus of any one of the following families: Alphaflexiviridae, Alphatetraviridae, Alvernaviridae, Arteriviridae, Astroviridae, Barnaviridae, Betaflexiviridae, Bromoviridae, Caliciviridae, Carmotetraviridae, Closteroviridae, Coronaviridae, Dicistroviridae, Flaviviridae, Gammaflexiviridae, Iflaviridae, Leviviridae, Luteoviridae, Marnaviridae, Mesoniviridae, Narnaviridae, Nodaviridae, Permutotetraviridae, Picornaviridae, Potyviridae, Roniviridae, Secoviridae, Togaviridae, Tombusviridae, Tymoviridae, or Virgaviridae.

[00103] In some cases, the genetic material for generating a pseudotyped oncolytic virus is obtained from a negative-sense ssRNA virus of any one of the following families: Bornaviridae, Filoviridae, Paramyxoviridae, Rhabdoviridae, Nyamiviridae, Arenaviridae, Bunyaviridae, Ophioviridae, or Orthomyxoviridae.

[00104] In some instances, the genetic material for generating a pseudotyped oncolytic virus is obtained from oncolytic DNA viruses that comprise capsid symmetry that is isocahedral or complex. In some cases, isosahedral oncolytic DNA viruses are naked or comprise an envelope. Exemplary families of oncolytic DNA viruses include the Adenoviridae (for example, Adenovirus, having a genome size of 36-38kb), Herpesviridae (for example, HSV1, having a genome size of 120-200 kb), and Poxviridae (for example, Vaccinia virus and myxoma virus, having a genome size of 130-280 kb).

[00105] In some cases, the genetic material for generating a pseudotyped oncolytic virus is obtained from oncolytic RNA viruses include those having icosahedral or helical capsid symmetry. In some cases, icosahedral oncolytic viruses are naked without envelope and include Reoviridae (for example, Reovirus, having a genome of 22-27 kb) and Picornaviridae (for example, Poliovirus, having a genome size of 7.2-8.4 kb). In other cases, helical oncolytic RNA viruses are enveloped and include Rhabdoviridae (for example, VSV, having genome size of 13-16 kb) and Paramyxoviridae (for example MV and NDV, having genome sizes of 16-20 kb).

[00106] In some instances, the genetic material for generating a pseudotyped oncolytic virus is obtained from a virus such as Abelson leukemia virus, Abelson murine leukemia virus, Abelson's virus, Acute laryngotracheobronchitis virus, Adelaide River virus, Adeno associated virus group, Adenovirus, African horse sickness virus, African swine fever virus, AIDS virus, Aleutian mink disease parvovirus, Alpharetrovirus, Alphavirus, ALV related virus, Amapari virus, Aphthovirus, Aquareovirus, Arbovirus, Arbovirus C, arbovirus group A, arbovirus group B, Arenavirus group, Argentine hemorrhagic fever virus, Argentine hemorrhagic fever virus, Arterivirus, Astrovirus, Ateline herpesvirus group, Aujezky's disease virus, Aura

virus, Ausduk disease virus, Australian bat lyssavirus, Aviadenovirus, avian erythroblastosis virus, avian infectious bronchitis virus, avian leukemia virus, avian leukosis virus, avian lymphomatosis virus, avian myeloblastosis virus, avian paramyxovirus, avian pneumoencephalitis virus, avian reticuloendotheliosis virus, avian sarcoma virus, avian type C retrovirus group, Avihepadnavirus, Avipoxvirus, B virus, B19 virus, Babanki virus, baboon herpesvirus, baculovirus, Barmah Forest virus, Bebaru virus, Berrimah virus, Betaretrovirus, Birnavirus, Bittner virus, BK virus, Black Creek Canal virus, bluetongue virus, Bolivian hemorrhagic fever virus, Boma disease virus, border disease of sheep virus, boma virus, bovine alphaherpesvirus 1, bovine alphaherpesvirus 2, bovine coronavirus, bovine ephemeral fever virus, bovine immunodeficiency virus, bovine leukemia virus, bovine leukosis virus, bovine mammillitis virus, bovine papillomavirus, bovine papular stomatitis virus, bovine parvovirus, bovine syncytial virus, bovine type C oncovirus, bovine viral diarrhea virus, Buggy Creek virus, bullet shaped virus group, Bunyamwera virus supergroup, Bunyavirus, Burkitt's lymphoma virus, Bwamba Fever, CA virus, Calicivirus, California encephalitis virus, camelpox virus, canarypox virus, canid herpesvirus, canine coronavirus, canine distemper virus, canine herpesvirus, canine minute virus, canine parvovirus, Cano Delgadito virus, caprine arthritis virus, caprine encephalitis virus, Caprine Herpes Virus, Capripox virus, Cardiovirus, caviid herpesvirus 1, Cercopithecid herpesvirus 1, cercopithecine herpesvirus 1, Cercopithecine herpesvirus 2, Chandipura virus, Changuinola virus, channel catfish virus, Charleville virus, chickenpox virus, Chikungunya virus, chimpanzee herpesvirus, chub reovirus, chum salmon virus, Cocal virus, Coho salmon reovirus, coital exanthema virus, Colorado tick fever virus, Coltivirus, Columbia SK virus, common cold virus, contagious ecthyma virus, contagious pustular dermatitis virus, Coronavirus, Corriparta virus, coryza virus, cowpox virus, coxsackie virus, CPV (cytoplasmic polyhedrosis virus), cricket paralysis virus, Crimean-Congo hemorrhagic fever virus, croup associated virus, Cryptovirus, Cypovirus, Cytomegalovirus, cytomegalovirus group, cytoplasmic polyhedrosis virus, deer papillomavirus, deltaretrovirus, dengue virus, Densovirus, Dependovirus, Dhori virus, diploma virus, *Drosophila* C virus, duck hepatitis B virus, duck hepatitis virus 1, duck hepatitis virus 2, duovirus, Duvenhage virus, Deformed wing virus DWV, eastern equine encephalitis virus, eastern equine encephalomyelitis virus, EB virus, Ebola virus, Ebola-like virus, echo virus, echovirus, echovirus 10, echovirus 28, echovirus 9, ectromelia virus, EEE virus, EIA virus, EIA virus, encephalitis virus, encephalomyocarditis group virus, encephalomyocarditis virus, Enterovirus, enzyme elevating virus, enzyme elevating virus (LDH), epidemic hemorrhagic fever virus, epizootic hemorrhagic disease virus, Epstein-Barr virus, equid alphaherpesvirus 1, equid alphaherpesvirus 4, equid herpesvirus 2, equine abortion virus, equine

arteritis virus, equine encephalosis virus, equine infectious anemia virus, equine morbillivirus, equine rhinopneumonitis virus, equine rhinovirus, Eubenangu virus, European elk papillomavirus, European swine fever virus, Everglades virus, Eyach virus, felid herpesvirus 1, feline calicivirus, feline fibrosarcoma virus, feline herpesvirus, feline immunodeficiency virus, feline infectious peritonitis virus, feline leukemia/sarcoma virus, feline leukemia virus, feline panleukopenia virus, feline parvovirus, feline sarcoma virus, feline syncytial virus, Filovirus, Flanders virus, Flavivirus, foot and mouth disease virus, Fort Morgan virus, Four Corners hantavirus, fowl adenovirus 1, fowlpox virus, Friend virus, Gammaretrovirus, GB hepatitis virus, GB virus, German measles virus, Getah virus, gibbon ape leukemia virus, glandular fever virus, goatpox virus, golden shinner virus, Gonometa virus, goose parvovirus, granulosis virus, Gross' virus, ground squirrel hepatitis B virus, group A arbovirus, Guanarito virus, guinea pig cytomegalovirus, guinea pig type C virus, Hantaan virus, Hantavirus, hard clam reovirus, hare fibroma virus, HCMV (human cytomegalovirus), hemadsorption virus 2, hemagglutinating virus of Japan, hemorrhagic fever virus, hendra virus, Henipaviruses, Hepadnavirus, hepatitis A virus, hepatitis B virus group, hepatitis C virus, hepatitis D virus, hepatitis delta virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, hepatitis nonA nonB virus, hepatitis virus, hepatitis virus (nonhuman), hepatoencephalomyelitis reovirus 3, Hepatovirus, heron hepatitis B virus, herpes B virus, herpes simplex virus, herpes simplex virus 1, herpes simplex virus 2, herpesvirus, herpesvirus 7, Herpesvirus ateles, Herpesvirus hominis, Herpesvirus infection, Herpesvirus saimiri, Herpesvirus suis, Herpesvirus varicellae, Highlands J virus, Hirame rhabdovirus, hog cholera virus, human adenovirus 2, human alphaherpesvirus 1, human alphaherpesvirus 2, human alphaherpesvirus 3, human B lymphotropic virus, human betaherpesvirus 5, human coronavirus, human cytomegalovirus group, human foamy virus, human gammaherpesvirus 4, human gammaherpesvirus 6, human hepatitis A virus, human herpesvirus 1 group, human herpesvirus 2 group, human herpesvirus 3 group, human herpesvirus 4 group, human herpesvirus 6, human herpesvirus 8, human immunodeficiency virus, human immunodeficiency virus 1, human immunodeficiency virus 2, human papillomavirus, human T cell leukemia virus, human T cell leukemia virus I, human T cell leukemia virus II, human T cell leukemia virus III, human T cell lymphoma virus I, human T cell lymphoma virus II, human T cell lymphotropic virus type 1, human T cell lymphotropic virus type 2, human T lymphotropic virus I, human T lymphotropic virus II, human T lymphotropic virus III, Ichnovirus, infantile gastroenteritis virus, infectious bovine rhinotracheitis virus, infectious haematopoietic necrosis virus, infectious pancreatic necrosis virus, influenza virus A, influenza virus B, influenza virus C, influenza virus D, influenza virus pr8, insect iridescent virus, insect virus, iridovirus, Japanese B virus, Japanese

encephalitis virus, JC virus, Junin virus, Kaposi's sarcoma-associated herpesvirus, Kemerovo virus, Kilham's rat virus, Klamath virus, Kolongo virus, Korean hemorrhagic fever virus, kumba virus, Kysanur forest disease virus, Kyzylagach virus, La Crosse virus, lactic dehydrogenase elevating virus, lactic dehydrogenase virus, Lagos bat virus, Langur virus, lapine parvovirus, Lassa fever virus, Lassa virus, latent rat virus, LCM virus, Leaky virus, Lentivirus, Leporipoxvirus, leukemia virus, leukovirus, lumpy skin disease virus, lymphadenopathy associated virus, Lymphocryptovirus, lymphocytic choriomeningitis virus, lymphoproliferative virus group, Machupo virus, mad itch virus, mammalian type B oncovirus group, mammalian type B retroviruses, mammalian type C retrovirus group, mammalian type D retroviruses, mammary tumor virus, Mapuera virus, Marburg virus, Marburg-like virus, Mason Pfizer monkey virus, Mastadenovirus, Mayaro virus, ME virus, measles virus, Menangle virus, Mengo virus, Mengovirus, Middelburg virus, milkers nodule virus, mink enteritis virus, minute virus of mice, MLV related virus, MM virus, Mokola virus, Molluscipoxvirus, Molluscum contagiosum virus, monkey B virus, monkeypox virus, Mononegavirales, Morbillivirus, Mount Elgon bat virus, mouse cytomegalovirus, mouse encephalomyelitis virus, mouse hepatitis virus, mouse K virus, mouse leukemia virus, mouse mammary tumor virus, mouse minute virus, mouse pneumonia virus, mouse poliomyelitis virus, mouse polyomavirus, mouse sarcoma virus, mousepox virus, Mozambique virus, Mucambo virus, mucosal disease virus, mumps virus, murid betaherpesvirus 1, murid cytomegalovirus 2, murine cytomegalovirus group, murine encephalomyelitis virus, murine hepatitis virus, murine leukemia virus, murine nodule inducing virus, murine polyomavirus, murine sarcoma virus, Muromegalovirus, Murray Valley encephalitis virus, myxoma virus, Myxovirus, Myxovirus multiforme, Myxovirus parotitidis, Nairobi sheep disease virus, Nairovirus, Nanirnavirus, Nariva virus, Ndumo virus, Neethling virus, Nelson Bay virus, neurotropic virus, New World Arenavirus, newborn pneumonitis virus, Newcastle disease virus, Nipah virus, noncytopathogenic virus, Norwalk virus, nuclear polyhedrosis virus (NPV), nipple neck virus, O'nyong'nyong virus, Ockelbo virus, oncogenic virus, oncogenic viruslike particle, oncornavirus, Orbivirus, Orf virus, Oropouche virus, Orthohepadnavirus, Orthomyxovirus, Orthopoxvirus, Orthoreovirus, Orungo, ovine papillomavirus, ovine catarrhal fever virus, owl monkey herpesvirus, Palyam virus, Papillomavirus, Papillomavirus sylvilagi, Papovavirus, parainfluenza virus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3, parainfluenza virus type 4, Paramyxovirus, Parapoxvirus, paravaccinia virus, Parvovirus, Parvovirus B19, parvovirus group, Pestivirus, Phlebovirus, phocine distemper virus, Picodnavirus, Picornavirus, pig cytomegalovirus-pigeonpox virus, Piry virus, Pixuna virus, pneumonia virus of mice, Pneumovirus, poliomyelitis virus, poliovirus, Polydnavirus, polyhedral

virus, polyoma virus, Polyomavirus, Polyomavirus bovis, Polyomavirus cercopitheci, Polyomavirus hominis 2, Polyomavirus maccacae 1, Polyomavirus muris 1, Polyomavirus muris 2, Polyomavirus papionis 1, Polyomavirus papionis 2, Polyomavirus sylvilagi, Pongine herpesvirus 1, porcine epidemic diarrhea virus, porcine hemagglutinating encephalomyelitis virus, porcine parvovirus, porcine transmissible gastroenteritis virus, porcine type C virus, pox virus, poxvirus, poxvirus variolae, Prospect Hill virus, Provirus, pseudocowpox virus, pseudorabies virus, psittacinepox virus, quailpox virus, rabbit fibroma virus, rabbit kidney vaculolating virus, rabbit papillomavirus, rabies virus, raccoon parvovirus, raccoonpox virus, Ranikhet virus, rat cytomegalovirus, rat parvovirus, rat virus, Rauscher's virus, recombinant vaccinia virus, recombinant virus, reovirus, reovirus 1, reovirus 2, reovirus 3, reptilian type C virus, respiratory infection virus, respiratory syncytial virus, respiratory virus, reticuloendotheliosis virus, Rhabdovirus, Rhabdovirus carpia, Rhadinovirus, Rhinovirus, Rhizidiovirus, Rift Valley fever virus, Riley's virus, rinderpest virus, RNA tumor virus, Ross River virus, Rotavirus, rougeole virus, Rous sarcoma virus, rubella virus, rubeola virus, Rubivirus, Russian autumn encephalitis virus, SA 11 simian virus, SA2 virus, Sabia virus, Sagiyama virus, Saimirine herpesvirus 1, salivary gland virus, sandfly fever virus group, Sandjimba virus, SARS virus, SDAV (sialodacryoadenitis virus), sealpox virus, Semliki Forest Virus, Seoul virus, sheeppox virus, Shope fibroma virus, Shope papilloma virus, simian foamy virus, simian hepatitis A virus, simian human immunodeficiency virus, simian immunodeficiency virus, simian parainfluenza virus, simian T cell lymphotrophic virus, simian virus, simian virus 40, Simplexvirus, Sin Nombre virus, Sindbis virus, smallpox virus, South American hemorrhagic fever viruses, sparrowpox virus, Spumavirus, squirrel fibroma virus, squirrel monkey retrovirus, SSV 1 virus group, STLV (simian T lymphotropic virus) type I, STLV (simian T lymphotropic virus) type II, STLV (simian T lymphotropic virus) type III, stomatitis papulosa virus, submaxillary virus, suid alphaherpesvirus 1, suid herpesvirus 2, Suipoxvirus, swamp fever virus, swinepox virus, Swiss mouse leukemia virus, TAC virus, Tacaribe complex virus, Tacaribe virus, Tanapox virus, Taterapox virus, Tench reovirus, Theiler's encephalomyelitis virus, Theiler's virus, Thogoto virus, Thottapalayam virus, Tick borne encephalitis virus, Tioman virus, Togavirus, Torovirus, tumor virus, Tupaia virus, turkey rhinotracheitis virus, turkeypox virus, type C retroviruses, type D oncovirus, type D retrovirus group, ulcerative disease rhabdovirus, Una virus, Uukuniemi virus group, vaccinia virus, vacuolating virus, varicella zoster virus, Varicellovirus, Varicola virus, variola major virus, variola virus, Vasin Gishu disease virus, VEE virus, Venezuelan equine encephalitis virus, Venezuelan equine encephalomyelitis virus, Venezuelan hemorrhagic fever virus, vesicular stomatitis virus, Vesiculovirus, Vilyuisk virus,

viper retrovirus, viral haemorrhagic septicemia virus, Visna Maedi virus, Visna virus, volepox virus, VSV (vesicular stomatitis virus), Wallal virus, Warrego virus, wart virus, WEE virus, West Nile virus, western equine encephalitis virus, western equine encephalomyelitis virus, Whataroa virus, Winter Vomiting Virus, woodchuck hepatitis B virus, woolly monkey sarcoma virus, wound tumor virus, WRSV virus, Yaba monkey tumor virus, Yaba virus, Yatapoxvirus, yellow fever virus, and the Yug Bogdanovac virus.

Methods of producing pseudotyped oncolytic viruses

[00107] In some instances, a pseudotyped oncolytic virus described herein is generated using methods well known in the art. In some instances, the methods involve one or more transfection steps and one or more infection steps. In some instances, a cell line such as a mammalian cell line, an insect cell line, or a plant cell line is infected with a pseudotyped oncolytic virus described herein to produce one or more viruses. Exemplary mammalian cell lines include: 293A cell line, 293FT cell line, 293F cells, 293 H cells, CHO DG44 cells, CHO-S cells, CHO-K1 cells, Expi293F™ cells, Flp-In™ T-REx™ 293 cell line, Flp-In™-293 cell line, Flp-In™-3T3 cell line, Flp-In™-BHK cell line, Flp-In™-CHO cell line, Flp-In™-CV-1 cell line, Flp-In™-Jurkat cell line, FreeStyle™ 293-F cells, FreeStyle™ CHO-S cells, GripTite™ 293 MSR cell line, GS-CHO cell line, HepaRG™ cells, T-REx™ Jurkat cell line, Per.C6 cells, T-REx™-293 cell line, T-REx™-CHO cell line, T-REx™-HeLa cell line, 3T6, A549, A9, AtT-20, BALB/3T3, BHK-21, BHL-100, BT, Caco-2, Chang, Clone 9, Clone M-3, COS-1, COS-3, COS-7, CRFK, CV-1, D-17, Daudi, GH1, GH3, H9, HaK, HCT-15, HEp-2, HL-60, HT-1080, HT-29, HUVEC, I-10, IM-9, JEG-2, Jensen, K-562, KB, KG-1, L2, LLC-WRC 256, McCoy, MCF7, VERO, WI-38, WISH, XC, or Y-1. Exemplary insect cell lines include *Drosophila* S2 cells, Sf9 cells, Sf21 cells, High Five™ cells, or expresSF+® cells. Exemplary plant cell lines include algae cells such as for example *Phaeocystis pouchetii*.

[00108] Any method known to one skilled in the art is used for large scale production of recombinant oncolytic vectors and vector constructs, such as pseudotyped oncolytic vectors. For example, master and working seed stocks can be prepared under GMP conditions in qualified primary CEFs or by other methods. In some instances, cells are plated on large surface area flasks, grown to near confluency, and infected at selected MOI. The produced virus can then be purified. In some cases, cells are harvested and intracellular virus is released by mechanical disruption. In some embodiments, cell debris is removed by large-pore depth filtration and/or host cell DNA is digested with an endonuclease. In some cases, virus particles are subsequently purified and concentrated by tangential-flow filtration, followed by diafiltration.

The resulting concentrated virus can formulated by dilution with a buffer containing one or more stabilizers, filled into vials, and lyophilized. Compositions and formulations can be stored for later use. In some embodiments, a lyophilized virus is reconstituted by addition of one or more diluents.

Engager Molecules

[00109] In some embodiments, the oncolytic viral vectors provided herein are pseudotyped oncolytic viruses that are further engineered to include a polynucleotide sequence that encodes an engager molecule, *e.g.*, an engager polypeptide. The engager molecules of the present invention comprise at least two domains each capable of binding to a different cell surface molecule. In some embodiments, engager polypeptides comprise an antigen recognition domain and an activation domain that recognize particular cell surface proteins (*e.g.*, cell-surface receptors or ligands) expressed by target and effector cells, respectively. As used herein, an "antigen recognition domain" is a polypeptide that binds one or more molecules present on the cell surface of a target cell (*e.g.*, a tumor antigen), and an "activation domain" is a polypeptide that binds to one or more molecules present on the cell surface of an effector cell (*e.g.*, an activation molecule). An activation domain may also be referred to as an "engager domain."

[00110] In some embodiments, engager polypeptides comprise a therapeutic molecule domain and an activation domain. A therapeutic molecule domain is a polypeptide that binds to a particular cell surface protein expressed on an effector cell (*e.g.*, cell-surface receptors or ligands) and that is distinct from the cell surface protein recognized by the activation domain. In particular embodiments, the therapeutic molecule domain binds to a cell surface protein that is a negative regulator of effector cell function (*e.g.*, an immune checkpoint molecule or other inhibitory molecule). Exemplary cell-surface antigen for targeting by a therapeutic domain include CD47, PD1, PDL1, CTLA4, TIM2, LAG3, BTLA, KIR, TIGIT, OX40, FITR, CD27, SLAMF7, and CD200.

[00111] In some embodiments, binding of an activation domain to a molecule present on the surface of the effector cell results in activation of the effector cell. In certain embodiments, binding of an activation domain to a molecule on an effector cell and binding of an antigen recognition domain to a molecule present on a target cell brings the effector cell in close proximity to the target cell and thereby facilitates the destruction of the target cell by the effector cell. In certain embodiments, binding of an activation domain to an activation molecule on an effector cell and binding of a therapeutic molecule domain to an inhibitory molecule present on

an effector cell enhances the activation of the effector cell and thereby facilitates the destruction of one or more bystander target cells by the effector cell.

[00112] In certain embodiments, the engager molecule is a protein, *e.g.*, an engineered protein. In some embodiments, the engager molecule is a bipartite polypeptide. In some embodiments, the engager molecule is a tripartite or multipartite polypeptide. In such embodiments, the engager molecule may comprise one or more activation domains and/or antigen recognition domains, or other domains, including one or more co-stimulatory domains, one or more dimerization or trimerization domains, or other domain capable of binding a molecule expressed on the cell surface. Alternatively, the one or more additional domains are optionally present on a separate polypeptide. In some embodiments, the engager molecule comprises an antibody or antibody fragment. In some embodiments, the engager molecule is a is a trifunctional antibody, an Fab_2, a bi-specific scFv such as a bi-specific T-cell engager (BiTE), a bivalent minibody, a bispecific diabody, a DuoBody, or an Mab2. In certain embodiments, the engager molecule is a bipartite T cell engager (BiTE) or a tripartite T cell engager (TiTE).

[00113] In some embodiments, the activation domain, the antigen recognition domain, and/or the therapeutic molecule domain of the engager molecule comprises an antibody or an antigen-binding fragment thereof, *e.g.*, a single chain variable fragment (scFv), a monoclonal antibody, Fv, Fab, minibody, diabody. In some embodiments, the activation domain, the antigen recognition domain, and/or the therapeutic molecule domain of the engager molecule comprises a ligand, a peptide, a peptide that recognize and interacts with a soluble TCR, or combinations thereof. In some embodiments, these antibody-derived fragments or derivatives may be modified by chemical, biochemical, or molecular biological methods. Corresponding methods are known in the art and described, *inter alia*, in laboratory manuals (see Sambrook et al.; Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, 2nd edition 1989 and 3rd edition 2001; Gerhardt et al.; Methods for General and Molecular Bacteriology; ASM Press, 1994; Lefkovits; Immunology Methods Manual: The Comprehensive Sourcebook of Techniques; Academic Press, 1997; Golemis; Protein-Protein Interactions: A Molecular Cloning Manual; Cold Spring Harbor Laboratory Press, 2002). In some instances, the polypeptides, antibodies, or antigen-binding fragments thereof used in the construction of the engager molecules described herein are humanized or deimmunized constructs. Methods for the humanization and/or deimmunization of polypeptides and, in particular, antibody constructs are known to the person skilled in the art.

[00114] In some embodiments, for any of the engagers described herein, the respective domains are in any order from N-terminus to C-terminus. For example, in some

embodiments, the engager molecule may comprise an N-terminal activation domain and a C-terminal antigen recognition domain. In some embodiments, the engager molecule may comprise an N-terminal antigen recognition domain and a C-terminal activation domain. In some embodiments, the engager molecule may comprise an N-terminal activation domain and a C-terminal therapeutic molecule domain. In some embodiments, the engager molecule may comprise an N-terminal therapeutic molecule domain and a C-terminal activation domain. In certain embodiments, T-cells are modified to secrete engager molecules that have an antigen recognition domain or therapeutic molecule domain N-terminal to an activation domain.

[00115] In particular embodiments, two or more of the domains of an engager molecule are linked by a linker. In some instances, the linker is of any suitable length, and such a parameter is routinely optimized in the art. For example, linkers are of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities. The term "peptide linker" refers to an amino acid sequence by which the amino acid sequences of a first domain (*e.g.*, an activation domain) and a second domain (*e.g.*, an antigen recognition domain or therapeutic molecule domain) of a defined construct are linked together. In some instance, one technical feature of such peptide linker is that said peptide linker does not comprise any polymerization activity and/or does not promote formation of secondary structures. Such peptide linkers are known in the art and described, for example, in Dall'Acqua et al. (Biochem. (1998) 37, 9266-9273); Cheadle et al. (Mol Immunol (1992) 29, 21-30); and Raag and Whitlow (FASEB (1995) 9(1), 73-80). In some embodiments, the peptide linkers of the present invention comprise less than 5 amino acids, less than 4 amino acids, less than 3 amino acids, less than 2 amino acids, or 1 amino acid. In some embodiments, the peptide linker is a single amino acid linker. In such embodiments, the single amino acid is typically a glycine (Gly). In some embodiments, peptide linkers that also do not promote any secondary structures are preferred. Methods for preparing fused, operatively-linked constructs and their expression in mammalian or bacterial cells are well-known in the art (*See e.g.*, International PCT Publication No. WO 99/54440; Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. 1989 and 1994; and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

[00116] In some embodiments, the engager molecule is a single chain bi-specific antibody construct. The term "single chain bispecific antibody construct" refers to a construct comprising two antibody-derived binding domains. One of the binding domains comprises variable regions (or parts thereof) of both heavy chain (VH) and light chain (VL) of an antibody

or antigen binding fragments or derivatives thereof, capable of specifically binding to/interacting with an activation molecule expressed on an effector cell (*e.g.*, CD3). The second binding domain comprises variable regions (or parts thereof) of both heavy chain (VH) and light chain (VL) of an antibody or antigen binding fragments or derivatives thereof, capable of specifically binding to/interacting with a target antigen expressed on a target cell (*e.g.*, CD19) or an antigen expressed by and effector cell (*e.g.*, an inhibitor molecule). In particular embodiments, each of the two antibody or antigen binding fragments or derivatives comprise at least one complementary determining region (CDR), particularly a CDR3. In some embodiments, the single chain bi-specific antibody construct is a bispecific scFv or diabody.

[00117] In specific embodiments, the single chain bispecific antibody construct is a single chain bispecific scFv. An scFv in general contains a VH and VL domain connected by a linker peptide. In some embodiments, a single chain bispecific scFv is comprised of a signal peptide to allow for secretion from cells, followed by two scFvs connected by one or more linker peptides (Lx, Ly, Lz). Bispecific single chain molecules are known in the art and are described in International PCT Publication No. WO 99/54440; Mack, J. Immunol. (1997), 158, 3965-3970; Mack, PNAS, (1995), 92, 7021-7025; Kufer, Cancer Immunol. Immunother., (1997), 45, 193-197; Loftier, Blood, (2000), 95, 6, 2098-2103; and Bruhl, J. Immunol., (2001), 166, 2420-2426.

[00118] In some embodiments, the molecular format of the polynucleotide encoding a single chain bi-specific scFv polypeptide comprises nucleic acid sequence encoding a signal peptide (such as the signal sequences of SEQ ID NO: 2 and 4) followed by two or more antibody-derived regions (*e.g.*, a first scFv and a second scFv). Each antibody-derived region (*e.g.*, scFv) comprises one VH and one VL chain. In specific embodiments, the two or more antibody-derived regions are scFvs and are linked by a peptide linker to form a single chain bi-specific scFv construct. In some embodiments, the bi-specific scFv is a tandem bi-scFv or a diabody. Bispecific scFvs can be arranged in different formats including the following: VHO -Lx-V_{La}-Ly-V_H -Lz-ViJ3, V_{La}-Lx-V_{Ha}-Ly-VH -Lz-ViJ3, V_{La}-Lx-V_H -Ly-VL -Lz-VH , V_H -Lx-V_{La}-Ly-VL -Lz-VH , V_H -Lx-VL -Ly-VH -Lz-V_{La}, V_{La}-Lx-VL -Ly-VH -Lz-V_H , VH -Lx-VH -Ly-VL -Lz-VLa,VLa-Lx-VH -Ly-VL -Lz-V_H , VH -Lx-V_{La}-Ly-V_H -Lz-VL , VL -Lx-V_{La}-Ly-V_H -Lz-VH , V_H -Lx-VH -Ly-VLa-Lz-VL ,VL -Lx-VH -Ly-VLa-Lz-V_H.

[00119] In some embodiments, the engager molecule comprises multiple (*e.g.*, 2, 3, 4, 5 or more) antigen binding domains to allow targeting of multiple antigens. In some embodiments, the engager molecule comprises multiple (*e.g.*, 2, 3, 4, 5 or more) activation domains to activate effector cells. In some embodiments, the engager molecule comprises multiple (*e.g.*, 2, 3, 4, 5 or more) therapeutic molecule domains to activate effector cells.

[00120] In specific embodiments of the disclosure, the engager molecule comprises additional domains for the isolation and/or preparation of recombinantly produced constructs, such as a tag or a label. The tag or label may be a short peptide sequence, such as a histidine tag (SEQ ID NO: 12), or may be a tag or label that is capable of being imaged, such as fluorescent or radioactive label.

[00121] In particular embodiments, the engager molecules of the present invention specifically bind to/interact with a particular conformational/structural epitope(s) of a target antigen expressed on a target cell and an activation molecule expressed on an effector cell (*e.g.*, an activation domain that specifically binds to one of the two regions of the human CD3 complex, or parts thereof). In particular embodiments, the engager molecules of the present invention specifically bind to/interact with a particular conformational/structural epitope(s) of an activation molecule expressed on an effector cell and a different cell-surface protein expressed on an effector cell. Accordingly, specificity in some instances is determined experimentally by methods known in the art and methods as disclosed and described herein. Such methods comprise, but are not limited to Western blots, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), radioimmunoprecipitation (RIP), electrochemiluminescence (ECL), immunoradiometric assay (IRMA), enzyme immunoassay (EIA), and peptide scans.

Activation Molecules and Target Cell Antigens

[00122] In some embodiments, binding of the activation domain of an engager molecule to an activation molecule on the cell surface of an effector cell results in activation of the effector cell. As used herein, the term "effector cell" refers to any mammalian cell type that is capable of facilitating the death of a target cell. In particular embodiments, the effector cells of the present invention are immune cells, such as a T cell, a B cell, an innate lymphocyte, a natural killer (NK) cell, a natural killer T cell (NKT), a granulocyte (*e.g.*, a neutrophil, basophil, mast cell, or eosinophil), a macrophage, a monocyte, or a dendritic cell. Exemplary effector cell types include T cells, NK cells, NKT cells, and macrophages.

[00123] In some embodiments, activation of an effector cell may result in one or more of the following: (i) increased proliferation of the effector cell; (ii) changes in the expression or activity of one or more cell surface proteins of the effector cell; (iii) change in expression or activity of one or more intracellular proteins expressed by the effector cell; (iv) changes in the amount or nature of factors produced and/or secreted by the effector cell, such as cytokines, chemokines or reactive oxygen species; (v) changes in the morphology of the effector cell; (vi) changes in the chemotactic potential of the effector cell, such as through increased or

decreased expression of one or more chemokine receptors; (vii) changes in the functional activity of the effector cell, such as increased cytolytic activity and/or increased phagocytic activity. Activation of an effector cell, or population of effector cells, can be determined by any means known in the art. For example, changes in proliferation, protein expression, production, or secretion can be determined by flow cytometry, Western blot, ELISA, immunohistochemistry, immunoprecipitation, or immunofluorescence and changes in cell morphology can be determined by numerous types of microscopy known in the art.

[00124] The skilled artisan will recognize that the nature of the activating molecule may vary according to the nature of the effector cell, although different groups of effector cells may share expression of certain types of activation molecules. For example, T cells express different surface receptors, i.e. different activating receptors, than NK cells or macrophages. As an illustrative example, CD3 is an activating receptor expressed by T-cells that is not expressed by NK cells or macrophages, whereas CD1, CD16, NKG2D, and/or NKp30 are activating receptors expressed by NK cells that are not expressed by T cells. Therefore, in some instances, engager molecules that activate T-cells have a different activation domain than engager molecules that activate NK cells, macrophages, NKT cells, or other types of effector cells. Exemplary activation molecules are described below and shown in Table 1.

[00125] In some embodiments, the effector cell is a T cell and the activation domain of the engager molecule binds to an activation molecule expressed by the T cell. The T-cell repertoire is comprised of numerous sub-types of T cell, including NKT cells, cytotoxic T cells (Tc or CTL), memory T cells, helper T cells (*e.g.*, Th1, Th2, Th17, Th9, and/or Th22 cells), suppressor T cells (*e.g.*, regulator T cells (Tregs)), mucosal-associated invariant T cells, and γδ T cells. In some instances, one or more surface receptors expressed by one T cell subtype are not expressed by another T cell subtype. In some instances, one or more surface receptors expressed by one T cell subtype are expressed by at least one other T cell subtype. In some instances, one or more surface receptors expressed by one T cell subtype are generally expressed by all, or most, T cell subtypes. For example, CD3 is a signaling component of the T cell receptor (TCR) complex and is expressed in multiple T cell subtypes. Exemplary activation molecules expressed by T cells (*e.g.*, NKT, Tc, memory T cells, or helper T cell), include, but are not limited to one or more components of CD3, (*e.g.*, CD3γ, CD3δ, CD3ε or CD3ξ), CD2, CD4, CD5, CD6, CD7, CD8, CD25, CD27, CD28, CD30, CD38, CD40, CD57, CD69, CD70, CD73, CD81, CD82, CD134, CD137, CD152, or CD278. In some embodiments, the effector cell is an NKT-cell. In such embodiments, the activation molecule includes, but is not limited to, CD3 or an invariant TCR.

[00126] In some embodiments, the effector cell is an NK cell and the activation domain of the engager molecule binds to an activation molecule expressed by the NK cell. Exemplary activation molecules expressed by NK cells include, but are not limited to, CD16, CD94/NKG2 (*e.g.*, NKG2D), NKp30, NKp44, NKp46, or killer activation receptors (KARs).

Table 1: Exemplary Activation Molecules

T cell Activation Molecules	NKT cell Activation Molecules
CD3 or components thereof (*e.g.*, CD3γ, CD3δ, CD3ε or CD3ξ)	CD3
CD2	invariant TCR
CD4	**NK Cell Activation Molecules**
CD5	CD16
CD6	CD94/NKG2 (*e.g.*, NKG2D)
CD7	NKp30
CD8	NKp44
CD16	NKp46
CD25	KARs
CD27	
CD28	
CD30	
CD38	
CD40	
CD57	
CD69	
CD70	
CD73	
CD81	
CD82	
CD134	
CD137	
CD152	
CD278	

[00127] In some embodiments, binding of an engager molecule to a target cell and an effector cell (*e.g.*, binding of an activation domain to a molecule on an effector cell and binding of an antigen recognition domain to a molecule present on a target cell) brings the effector cell in close proximity to the target cell and thereby facilitates the destruction of the target cell by the effector cell. As used herein, the term "target cell" refers to a mammalian cell

that should be killed, attacked, destroyed, and/or controlled. In particular, target cells are cells that are in some way altered compared to a normal cell of the same cell type, such as a cancerous cell, a bacterially-infected cell, a virally-infected cell, a fungally-infected cell, and/or an autoimmune cell. In particular embodiments, the target cells of the present invention are cancerous cells (*e.g.*, tumor cells). Destruction (*i.e.*, death) of a target cell can be determined by any means known in the art, such as flow cytometry (*e.g.*, by AnnexinV, propidium iodide, or other means), cell counts, and/or microscopy to determine the cellular morphology of the target cells.

[00128] In some embodiments, the antigen recognition domain of an engager molecule brings a target cell (*e.g.*, tumor cell) into the vicinity of an effector cell via interaction between the antigen recognition domain and surface antigens expressed by the target cell (*e.g.*, target cell antigens). In some embodiments, the target-cell antigen is a tumor antigen. In some embodiments, a tumor antigen is a tumor-specific antigen (TSA), and is expressed only by tumor cells. In some embodiments, the target cell angien is a tumor-associated antigen (TAA), and is expressed by tumor cells and one or more types of normal cells or non-tumor cells. In some cases, TSA is also present in one or more types of normal cells or non-tumor cells, but is predominantly expressed by tumor cells. In some instances, a tumor antigen (*e.g.*, TSA or TAA) is present in one cancer type. In some instances, a tumor antigen is present in multiple cancer types. In one embodiment, a tumor antigen is expressed on a blood cancer cell. In another embodiment, a tumor antigen is expressed on a cell of a solid tumor. In some embodiments, the solid tumor is a glioblastoma, a non-small cell lung cancer, a lung cancer other than a non-small cell lung cancer, breast cancer, prostate cancer, pancreatic cancer, liver cancer, colon cancer, stomach cancer, a cancer of the spleen, skin cancer, a brain cancer other than a glioblastoma, a kidney cancer, a thyroid cancer, or the like. In more specific embodiments, a tumor antigen is expressed by a tumor cell in an individual.

[00129] Exemplary tumor antigens (*e.g.*, TSAs or TAAs) include, but are not limited to, alphafetoprotein (AFP), carcinoembryonic antigen (CEA), CA-125, epithelial tumor antigen (ETA), tyrosinase, CD10 (also known as neprilysin, membrane metallo-endopeptidase (MME), neutral endopeptidase (NEP), or common acute lymphoblastic leukemia antigen (CALLA)), CD15, CD19, CD20, CD21, CD22, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD70, CD123, CD138, CD171, ras, p53, v-raf murine sarcoma viral oncogene homolog B1 (BRAF), calcium binding tyrosine-(Y)-phosphorylation regulated (CABYR), cysteine-rich secretory protein 3 (CRISP3), CSAG family, member 2 (CSAG2), cancer/testis antigen 2 (CTAG2), dihydrofolate reductase (DHFR), ferritin, heavy polypeptide 1; testis-

specific expression (FTHL17), G antigen 1 (GAGE1), lactate dehydrogenase C (LDHC), melanoma antigen family A (MAGEA) 1, MAGEA3, MAGEA4, (melanoma antigen family B, 6) MAGEB6, mitogen-activated protein kinase 1 (MAPK1), MHC Class I polypeptide-related sequence A (MICA), mucin (MUC) 1, cell surface associated (MUC1), MUC16, NLR family, pyrin domain containing 4 (NLRP4), New York esophageal squamous cell carcinoma 1 (NY-ESO-1), PDZ binding kinase (PB), preferentially expressed antigen in melanoma (PRAME), sex determining region Y-box (SOX)-2, SOX10, SOX11, sperm protein associated with the nucleus , X-linked, family member A1 (SPANXA1), synovial sarcoma, X (SSX) breakpoint 2 (SSX2), SSX4, SSX5, testis specific, 10 (TSGA10), testis-specific serine kinase 6 (TSSK6), tubby like protein (TULP2), X antigen family, member 2 (XAGE2), zinc finger protein 165 (ZNF165), absent in melanoma 2 (AIM2), BMI1 polycomb ring finger oncogene (BMI1), cyclooxygenase-2 (COX-2), tyrosine related protein (TRP)-1, TRP-2, glycoprotein 100 (GP100), epidermal growth factor receptor variant III (EGFRvIII), enhancer of zeste homolog 2 (EZH2), human LI cell adhesion molecule (LICAM), Livin, multidrug resistance protein 3 (MRP-3), Nestin, oligodendrocyte transcription factor (OLIG2), antigen recognized by T cells (ART)-1, ART4, squamous cell carcinoma antigen recognized by T cells (SART)-1, SART2, SART3, B-cyclin, β-catenin, glioma-associated oncogene homlog 1 (Glil), caveolin-1 (Cav-1), cathepsin B, cluster of differentiation (CD)-74, epithelial calcium-dependent adhesion (E-cadherin), EPH receptor A2 (EphA2), EphA2/epithelial kinase (EphA2/Eck), fos-related antigen 1 (Fra-l/Fosl 1), Ganglioside/GD2, GD3, acetylglucosaminyltransferase-V (GnT-V, β1,6-N), human epidermal growth factor receptor 2 (Her2/Neu), nuclear proliferation-associated antigen of antibody Ki67 (Ki67), human Ku heterodimer proteins subunits (u70/80), interleukin-13 receptor subunit alpha-2 (IL-13Rα2), melanoma antigen recognized by T cells (MART-1), prospero homeobox protein 1 (PROXl), prostate stem cell antigen (PSCA), Survivin, urokinase-type plasminogen activator receptor (UPAR), Wilms' tumor protein 1 (WT-1), Folate receptor a, Glypican-3, 5T4, 8H9, $\alpha_v\beta_6$ integrin, B7-H3, B7-H6, CAIX, CA9, CSPG4, EGP2, EGP40, EpCAM, ERBB3, ERBB4, ErbB3/4, FAP, FAR, FBP, fetal AchR, HLA-AI, HLA-A2, IL-1Rα, KDR, Lambda, Lewis-Y, MCSP, Mesothelin, NCAM, NKG2D ligands, PSC1, PSMA, ROR1, TAG72, TEM1, TEM8, VEGRR2, HMW-MAA, VEGF, VEGF receptors, P-glycoprotein, erythropoietin (EPO), cadherin, CD4, CD8, CD45, CD117 (c-kit), CD133, HLA-A. HLA-B, HLA-C, chemokine receptor 5 (CCR5), stem cell marker ABCG2 transporter, immunoglobulins, integrins, prostate specific antigen (PSA), prostate stem cell antigen (PSCA), dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), thyroglobulin, granulocyte-macrophage colony stimulating factor (GM-CSF), myogenic differentiation promoting factor-1 (MyoD-1),

Leu-7 (CD57), LeuM-1, cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67 (Ki-67), viral envelope proteins, HIV gp120, and transferrin receptor. Other exemplary tumor antigens are antigens that are present in the extracelluar matrix of tumors, such as oncofetal variants of fibronectin, tenascin, or necrotic regions of tumors.

Table 2: Exemplary Target Cell Antigens

Antigen
5T4
8H9
ABCG2 transporter
AFP
AIM2
ART1
ART4
B7-H3
B7-H6
B-cyclin
BMI1
BRAF
CA9
CABYR
CAIX
cathepsin B
Cav-1
CCR5
CD10
CD117
CD123
CD133
CD138
CD15
CD171
CD19
CD20
CD21
CD22
CD30
CD33
CD38
CD4
CD44
CD44v6
CD44v7/8
CD45
CD70
CD74

CD8
CEA
COX-2
CRISP3
CSAG2
CSPG4
CTAG2
DC-SIGN
DHFR
E-cadherin
EGFR
EGFRvIII
EGP2
EGP40
EPCAM
EphA2
EphA2/Eck
ERBB3
ErbB3/4
ERBB4
erythropoietin (EPO)
ETA
EZH2
FAP
FAR
FBP
fetal AchR
Folate Receptor a
Fra-l/Fosl 1
FTHL17
GAGE1
GD2
GD3
Glil
Glypican-3
GnT-V, β1,6-N
GP100
Her2/Neu
HIV sp120
HLA A
HLA B
HLA C
HLA-A2
HLA-AI
HMW-MAA
IL-13Rα2
IL-1Rα
kappa light chain

KDR
Ki67
Lambda
LDHC
Leu-7 (CD57)
LeuM-1
Lewis-Y
LICAM
Livin
MAGEA1
MAGEA3
MAGEA4
MAGEB6
MAPK1
MART-1
MCSP
Mesothelin
MICA
MRP-3
MUC1
MUC16 or CA125
MyoD1
NCAM
necrotic regions of tumors
Nestin
NKG2D ligands
NLRP4
NY-ESO-1
OLIG2
oncofetal variants of fibronectin
p53
PB
P-glycoprotein
PRAME
PROX1
PSA
PSC1
PSCA
PSCA
PSMA
Ras
ROR1
SART1
SART2
SART3
SOX10
SOX11
SOX2

SPANXA1
SSX2
SSX4
SSX5
Survivin
TAG72
TEM1
TEM8
tenascin
thyroglobulin
transferrin receptor
TRP-1
TRP-2
TSGA10
TSSK6
TULP2
tyrosinase
u70/80
UPAR
VEGF
VEGF Receptors
VEGRR2
WT-1
XAGE2
ZNF165
$\alpha_v\beta_6$ integrin
β-catenin

[00130] In certain embodiments, the antigen recognition domain of an engager molecule specifically binds a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA). In certain embodiments, the antigen recognition domain comprises an antibody or an antibody fragment or an antigen-binding fragment or portion thereof, such as for example, a monoclonal antibody, Fv, a scFv, Fab, minibody, or diabody that is specific for a TAA or TSA. In certain embodiments, the antigen recognition domain of the engager is an scFv that is specific for a TAA or TSA. In a specific embodiment, the TAA or TSA is expressed on a cancer cell. In one embodiment, the TAA or TSA is expressed on a blood cancer cell. In another embodiment, the TAA or TSA is expressed on a cell of a solid tumor. In more specific embodiments, the solid tumor is a glioblastoma, a non-small cell lung cancer, a lung cancer other than a non-small cell lung cancer, breast cancer, prostate cancer, pancreatic cancer, liver cancer, colon cancer, stomach cancer, a cancer of the spleen, skin cancer, a brain cancer other than a glioblastoma, a kidney cancer, a thyroid cancer, or the like. In more specific embodiments, the TAA or TSA is expressed

by a tumor cell in an individual. In some embodiments, the antigen-recognition domain of the engager molecule is specific for one or more target cell antigens shown in Table 2.

EphA2

[00131] In some embodiments, EphA2 is referred to as EPH receptor A2 (ephrin type-A receptor 2; EPHA2; ARCC2; CTPA; CTPP1; or ECK), which is a protein that in humans is encoded by the EPHA2 gene in the ephrin receptor subfamily of the protein-tyrosine kinase family. Receptors in this subfamily generally comprise a single kinase domain and an extracellular region comprising a Cys-rich domain and 2 fibronectin type III repeats; embodiments of the antibodies of the disclosure target any of these domains. An exemplary human EphA2 nucleic sequence is in GenBank® Accession No. NM_004431, and an exemplary human EphA2 polypeptide sequence is in GenBank® Accession No. NP_004422, both of which sequences are incorporated herein in their entirety. An exemplary human EphA2 nucleic sequence is in GenBank® Accession No. NM_004448.2, and an exemplary human EphA2 polypeptide sequence is in GenBank® Accession No. NP_004439, both of which sequences are incorporated herein in their entirety.

[00132] The Eph family, the largest group among tyrosine kinase receptor families, is comprised of the EphA (EphA1–10) or EphB (EphB1–6) subclasses of receptors classified as per their sequence homologies and their binding affinity for their ligands, Ephrins (Eph receptor interacting protein). The human EphA2 gene is located on chromosome 1, encodes a receptor tyrosine kinase of 976 amino acids with an apparent molecular weight of 130 kDa and has a 90% amino acid sequence homology to the mouse EphA2. The Eph family contains an extracellular conserved N-terminal ligand-binding domain followed by a cysteine-rich domain with an epidermal growth factor-like motif and two fibronectin type-III repeats. The extracellular motif is followed by a membrane spanning region and a cytoplasmic region that encompasses a juxtamembrane region, a tyrosine kinase domain, a sterile alpha motif (SAM), and a post synaptic domain (disc large and zona occludens protein (PDZ) domain-binding motif). EphA2 shows 25–35% sequence homologies with other Eph receptors, and the tyrosine residues are conserved within the juxtamembrane and kinase domain.

[00133] EphA2 mRNA expression is observed in the skin, bone marrow, thymus, uterus, testis, prostate, urinary bladder, kidney, small intestine, colon, spleen, liver, lung and brain. EphA2 expression in the colon, skin, kidney and lung was over ten-fold relative to the bone marrow. EphA2 is also expressed during gastrulation in the ectodermal cells and early embryogenesis in the developing hind brain. In the skin, EphA2 is present in keratinocytes of

epidermis and hair follicles but not in dermal cells (fibroblasts, vascular cells and inflammatory cells). EphA2 is also expressed in proliferating mammary glands in female mice at puberty and differentially expressed during the estrous cycle. Besides its expression in embryo and in normal adult tissues, EphA2 is overexpressed in several cancers, such as breast cancer, gastric cancer, melanoma, ovarian cancer, lunch cancer, gliomas, urinary bladder cancer, prostate cancer, esophageal, renal, colon and vulvar cancers. In particular, a high level of EphA2 is detected in malignant cancer-derived cell lines and advanced forms of cancer. In light of the EphA2 overexpression in pre-clinical models and clinical specimens of many different types of cancer, the increased level of EphA2 expression is informative in both the prediction of cancer outcomes and in the clinical management of cancer. The differential expression of EphA2 in normal cells compared to cancer cells also signifies its importance as a therapeutic target.

HER2

[00134] In some embodiments, HER2 is referred to as human Epidermal Growth Factor Receptor 2 (Neu, ErbB-2, CD340, or pi 85), which is a protein that in humans is encoded by the ERBB2 gene in the epidermal growth factor receptor (EFR/ErbB) family. HER2 contains an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain that interacts with a multitude of signaling molecules. HER2 is a member of the epidermal growth factor receptor family having tyrosine kinase activity. Dimerization of the receptor results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors and initiates a variety of signaling pathways leading to cell proliferation and tumorigenesis. Amplification or overexpression of HER2 occurs in approximately 15–30% of breast cancers and 10–30% of gastric/gastroesophageal cancers and serves as a prognostic and predictive biomarker. HER2 overexpression has also been seen in other cancers like ovary, endometrium, bladder, lung, colon, and head and neck. HER2 is overexpressed in 15–30% of invasive breast cancers, which has both prognostic and predictive implications. Overexpression of HER2 protein, determined using IHC was found in 23% and gene amplification determined using FISH in 27% of 200 resected tumors in a gastric cancer study. HER2 overexpression is directly correlated with poorer outcome in gastric cancer. In a study of 260 gastric cancers, HER2 overexpression was an independent negative prognostic factor and HER2 staining intensity was correlated with tumor size, serosal invasion, and lymph node metastases. Other studies also confirmed the negative impact of HER2 overexpression in gastric cancer. HER2 overexpression is reported in 0–83% of esophageal cancers, with a tendency towards higher rates of positivity in adenocarcinoma (10–83%) compared to squamous cell carcinomas (0–56%). Overexpression of HER2 is seen in 20–

30% patients with ovarian cancer. In endometrial serous carcinoma, the reported rates of HER2 overexpression range between 14% and 80% with HER2 amplification (by fluorescence in situ hybridization [FISH]) ranging from 21% to 47%. Embodiments of the antibodies of the disclosure target the extracellular ligand binding domain.

Disialoganglioside GD2

[00135] Disialoganglioside GD2 is a sialic acid-containing glycosphingolipid expressed primarily on the cell surface. The function of this carbohydrate antigen is not completely understood; however, it is thought to play an important role in the attachment of tumor cells to extracellular matrix proteins. GD2 expression in normal fetal and adult tissues is primarily restricted to the central nervous system, peripheral nerves, and skin melanocytes, although GD2 expression has been described in the stromal component of some normal tissues and white pulp of the spleen. In malignant cells, GD2 is uniformly expressed in neuroblastomas and most melanomas and to a variable degree in a variety of other tumors, including bone and soft-tissue sarcomas, small cell lung cancer, and brain tumors. GD2 is present and concentrated on cell surfaces, with the two hydrocarbon chains of the ceramide moiety embedded in the plasma membrane and the oligosaccharides located on the extracellular surface, where they present points of recognition for extracellular molecules or surfaces of neighboring cells. Because of the relatively tumor-selective expression combined with its presence on the cell surface, GD2 is an attractive target for tumor-specific antibody therapy. Embodiments of the antibodies of the disclosure target the extracellular domain.

Therapeutic Molecules

[00136] In some embodiments, the pseudotyped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and one or more additional nucleic acid sequences that encode one or more therapeutic molecules. As used herein, a "therapeutic molecule" refers to a molecule that enhances the therapeutic efficacy of an oncolytic virus described herein. In general, the therapeutic molecules described herein are proteins, nucleic acids, or a combination thereof. Exemplary therapeutic molecules include cytokines, chemokines, antibodies or antigen binding fragments thereof, proteases, RNA polynucleotides, and DNA polynucleotides.

[00137] In some embodiments, the therapeutic molecule is capable of increasing or enhancing the therapeutic efficacy of an oncolytic virus described herein by stimulating, or activating, a cellular immune response. In some embodiments, the therapeutic molecule is

capable of increasing or enhancing the therapeutic efficacy of an oncolytic virus described herein by antagonizing a suppressive or regulatory immune response. In some embodiments, reduction of a suppressive immune response occurs in a tumor microenvironment. In some instances, reduction of a suppressive immune response by the therapeutic molecule enhances the oncolytic effects of a pseudotyped oncolytic virus described herein. In some embodiments, the therapeutic molecule further reduces immunoregulatory T cell activity in a subject treated with a pseudotyped oncolytic virus described herein. In some embodiments, the therapeutic molecule modulates or impairs the production level of a protein at a nucleic acid level or at a protein level, or disrupts a protein function.

[00138] In some embodiments, a nucleic acid sequence encoding an engager molecule and a nucleic acid sequence encoding one or more therapeutic molecules are comprised within the same vector. In some embodiments, a nucleic acid sequence encoding an engager molecule and a nucleic acid sequence encoding one or more therapeutic molecules are comprised in different vectors. In some embodiments, the vector is a viral vector. In some instances, a therapeutic molecule comprises a polypeptide or a nucleic acid polymer. In some embodiments, the additional nucleic acid sequence is inserted into a viral vector which allows higher expression levels and production of the therapeutic molecule.

[00139] In some embodiments, the therapeutic molecule is a polypeptide. In some instances, the polypeptide is an immune modulator polypeptide. In some cases, the immune modulator polypeptide is a cytokine, a co-stimulatory domain, a domain that inhibits negative regulatory molecules of T-cell activation (*e.g.*, an immune checkpoint inhibitor), or a combination thereof.

[00140] In some embodiments, the immune modulator polypeptide modulates the activity of one or more cell types, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), dendritic cells, and/or T cells. Exemplary Treg modulatory polypeptides include CCR4, Helios, TIGIT, GITR, neuropilin, neuritin, CD103, CTLA-4, ICOS, and Swap70. Exemplary MDSC modulatory polypeptides include TGF-βR1, GM-CSF, INFγ, interleukins such as IL-β, IL-1F2, IL-6, IL-10, IL-12, IL-13, IL-6, IL-6Rα, IL-6/IL-6R complex, TGF-β1, M-CSF, Prostaglandin E2/PGE2, Prostaglandin E Synthase 2, S100A8, and VEGF. Exemplary dendritic-cell directed modulatory polypeptides include GM-CSF and/or IL-13. Exemplary T cell-directed modulatory polypeptides include IL-12, OX-40, GITR, CD28, or IL-28, or an antibody that agonizes a pathway comprising IL-12, OX-40, GITR, CD28, or IL-28.

[00141] In other embodiments, the therapeutic polypeptides modulate the fibrotic stroma. Exemplary fibrotic stromal polypeptides include fibroblast activation protein-alpha

(FAP). In some embodiments, the therapeutic polypeptide is a protease. In particular embodiments, the protease is capable of altering the extracellular matrix, particularly the extracellular matrix within a tumor microenvironment. Exemplary proteases include matrixmetalloproteases (MMP), such as MMP9, collagenases, and elastases.

Cytokines as Therapeutic molecules

[00142] In some cases, the immune modulator polypeptide is a cytokine. Cytokines are a category of small proteins between about 5-20 kDa that are involved in cell signaling and include chemokines, interferons (INF), interleukins (IL), and tumor necrosis factors (TNF), among others. Chemokines play a role as a chemoattractant to guide the migration of cells and are classified into four subfamilies: CXC, CC, CX3C, and XC. Exemplary chemokines include chemokines from the CC subfamily, such as CCL1, CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL6, CCL7, CCL8, CCL9 (or CCL10), CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, and CCL28; the CXC subfamily, such as CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, and CXCL17; the XC subfamily, such as XCL1 and XCL2; and the CX3C subfamily, such as CX3CL1.

[00143] Interferons (IFNs) comprise Type I IFNs (*e.g.* IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω), Type II IFNs (*e.g.* IFN-γ), and Type III IFNs. In some embodiments, IFN-α is further classified into about 13 subtypes including IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, and IFNA21.

[00144] Interleukins are a broad class of cytokine that promote the development and differentiation of immune cells, including T and B cells, and other hematopoietic cells. Exemplary interleukins include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35, and IL-36.

[00145] Tumor necrosis factors (TNFs) are a group of cytokines that modulate apoptosis. In some instances, there are about 19 members within the TNF family, including, not limited to, TNFα, lymphotoxin-alpha (LT-α), lymphotoxin-beta (LT-β), T cell antigen gp39 (CD40L), CD27L, CD30L, FASL, 4-1BBL, OX40L, and TNF-related apoptosis inducing ligand (TRAIL).

[00146] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes a

cytokine selected from chemokine, interferon, interleukin, or tumor necrosis factor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and an additional nucleic acid sequence that encodesa chemokine, an interferon, an interleukin, and/or a tumor necrosis factor.

Co-stimulatory domains as Therapeutic molecules

[00147] In some embodiments, the immune modulator polypeptide is a co-stimulatory domain. In some cases, the co-stimulatory domain enhances antigen-specific cytotoxicity. In some cases, the co-stimulatory domain further enhances cytokine production. In some embodiments, the co-stimulatory domain comprises CD27, CD28, CD70, CD80, CD83, CD86, CD134 (OX-40), CD134L (OK-40L), CD137 (41BB), CD137L (41BBL), or CD224.

[00148] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes a co-stimulatory domain. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes a co-stimulatory domain selected from CD27, CD28, CD80, CD83, CD86, CD134, CD134L, CD137, CD137L, or CD224.

Immune checkpoint inhibitors as Therapeutic molecules

[00149] In some embodiments, the immune modulator polypeptide is an immune checkpoint inhibitor polypeptide that inhibits a negative regulatory molecule of T-cell activation. Immune checkpoint inhibitor bind to immune checkpoint molecules, which are a group of molecules on the cell surface of CD4 and CD8 T cells. In some instances, these molecules effectively serve as "brakes" to down-modulate or inhibit an anti-tumor immune response. An immune checkpoint inhibitor refers to any molecule that modulates or inhibits the activity of an immune checkpoint molecule. In some instances, immune checkpoint inhibitors include antibodies, antibody-derivatives (*e.g.*, Fab fragments, scFvs, minobodies, diabodies), antisense oligonucleotides, siRNA, aptamers, or peptides.

[00150] Exemplary immune checkpoint molecules include, but are not limited to, programmed death-ligand 1 (PDL1, also known as B7-H1, CD274), programmed death 1 (PD-1), PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD16, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, inducible T cell costimulatory (ICOS), KIR, LAIR1, LIGHT, macrophage receptor with collageneous structure (MARCO), OX-40, phosphatidylserine

(PS), SLAM, TIGHT, VISTA, and VTCN1. In some embodiments, an immune checkpoint inhibitor inhibits on or more of PDL1, PD-1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, OX-40, PS, SLAM, TIGHT, VISTA, and VTCN1.

[00151] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and an additional nucleic acid sequence that encodes an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor reduces the expression or activity of one or more immune checkpoint molecules. In some embodiments, the immune checkpoint inhibitor reduces the interaction between an immune checkpoint molecule and its ligand (*e.g.*, reduced the interaction between PD-1 and PDL1). In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes an immune checkpoint inhibitor that inhibits one or more of PDL1, PD-1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, OX-40, PS, SLAM, TIGHT, VISTA, and VTCN1.

[00152] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain, wherein the therapeutic molecule domain is an immune checkpoint inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain, wherein the therapeutic molecule domain is an immune checkpoint inhibitor that inhibits one or more of PDL1, PD-1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, OX-40, PS, SLAM, TIGHT, VISTA, and VTCN1.

a) *PDL1 Inhibitors*

[00153] In some embodiments, the immune checkpoint inhibitor is an inhibitor of PDL1. In some embodiments, the immune checkpoint inhibitor is an antibody (*e.g.*, a monoclonal antibody or antigen-binding fragments thereof, or a humanized or chimeric antibody or antigen-binding fragments thereof) against PDL1. In some embodiments, the inhibitor of PDL1 reduces the expression or activity of PDL1. In some embodiments, the inhibitor of PDL1 reduces the

interaction between PD-1 and PDL1. Exemplary inhibitors of PDL1 include anti-PDL1 antibodies, RNAi molecules (*e.g.*, anti-PDL1 RNAi), antisense molecules (*e.g.*, an anti-PDL1 antisense RNA), or dominant negative proteins (*e.g.*, a dominant negative PDL1 protein). Exemplary anti-PDL1 antibodies includes clone EH12; MPDL3280A (Genentech, RG7446); anti-mouse PDL1 antibody Clone 10F.9G2 (BioXcell, Cat # BE0101); anti-PDL1 monoclonal antibody MDX-1105 (BMS-936559 and BMS-935559 from Bristol-Meyers Squibb; MSB0010718C; mouse anti-PDL1 Clone 29E.2A3; and AstraZeneca's MEDI4736.

[00154] In some embodiments, the anti-PDL1 antibody is an anti-PDL1 antibody disclosed in International PCT Publication Nos. WO 2013/079174; WO 2010/036959; WO 2013/056716; WO 2007/005874; WO 2010/089411; WO 2010/077634; WO 2004/004771; WO 2006/133396; WO 2013/09906; WO 2012/145493; WO 2013/181634; U.S. Patent Application Publication No. 20140294898; or Chinese Patent Application Publication No. CN 101104640.

[00155] In some embodiments, the PDL1 inhibitor is a nucleic acid inhibitor of PDL1 expression. In some embodiments, the PDL1 inhibitor is one disclosed in International PCT Publication Nos. WO 2011/127180 or WO 2011/000841. In some embodiments, the PDL1 inhibitor is rapamycin.

[00156] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to PDL1 (*e.g.*, an anti-PDL1 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

[00157] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to PDL1. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes a PDL1 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes PDL1 inhibitor selected from EH12, Genentech's MPDL3280A (RG7446); Anti-mouse PDL1 antibody Clone 10F.9G2 (Cat # BE0101) from BioXcell; anti-PDL1 monoclonal antibody MDX-1105 (BMS-936559) and BMS-935559 from Bristol-Meyer's Squibb; MSB0010718C; mouse anti-PDL1 Clone 29E.2A3; and AstraZeneca's MEDI4736.

b) PD-L2 Inhibitors

[00158] In some embodiments, the immune checkpoint inhibitor is an inhibitor of PD-L2. In some embodiments, the inhibitor of PD-L2 is an antibody (*e.g.*, a monoclonal antibody or fragments, or a humanized or chimeric antibody or fragments thereof) against PD-L2. In some embodiments, the inhibitor of PD-L2 reduces the expression or activity of PD-L2. In other embodiments, the inhibitor of PD-L2 reduces the interaction between PD-1 and PD-L2. Exemplary inhibitors of PD-L2 include antibodies (*e.g.*, an anti-PD-L2 antibody), RNAi molecules (*e.g.*, an anti-PD-L2 RNAi), antisense molecules (*e.g.*, an anti-PD-L2 antisense RNA), or dominant negative proteins (*e.g.*, a dominant negative PD-L2 protein).

[00159] In some embodiments, the PD-L2 inhibitor is GlaxoSmithKline's AMP-224 (Amplimmune). In some embodiments, the PD-L2 inhibitor is rHIgM12B7.

[00160] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes a PD-L2 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes PD-L2 inhibitor selected from AMP-224 (Amplimmune) or rHIgM12B7.

[00161] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to PDL2. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to PD-L2 (*e.g.*, an anti-PDL2 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

c) PD-1 Inhibitors

[00162] In some embodiments, the immune checkpoint inhibitor is an inhibitor of PD1. In some embodiments, the inhibitor of PDL1 is an antibody (*e.g.*, a monoclonal antibody or fragments, or a humanized or chimeric antibody or fragments thereof) against PD-1. Exemplary antibodies against PD-1 include: anti-mouse PD-1 antibody Clone J43 (Cat # BE0033-2) from BioXcell; anti-mouse PD-1 antibody Clone RMP1-14 (Cat # BE0146) from BioXcell; mouse anti-PD-1 antibody Clone EH12; Merck's MK-3475 anti-mouse PD-1 antibody (Keytruda, pembrolizumab, lambrolizumab); and AnaptysBio's anti-PD-1 antibody, known as ANB011; antibody MDX-1 106 (ONO-4538); Bristol-Myers Squibb's human IgG4 monoclonal antibody

nivolumab (Opdivo®, BMS-936558, MDX1106); AstraZeneca's AMP-514, and AMP-224; and Pidilizumab (CT-011), CureTech Ltd.

[00163] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes a PD1 inhibitor selected from ANB011; antibody MDX-1 106 (ONO-4538); Bristol-Myers Squibb's human IgG4 monoclonal antibody nivolumab (Opdivo®, BMS-936558, MDX1106); AstraZeneca's AMP-514, and AMP-224; and Pidilizumab (CT-011). In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes PD-1 inhibitor selected from ANB011; antibody MDX-1 106 (ONO-4538); Bristol-Myers Squibb's human IgG4 monoclonal antibody nivolumab (Opdivo®, BMS-936558, MDX1106); AstraZeneca's AMP-514, and AMP-224; and Pidilizumab (CT-011).

[00164] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes a PD-L2 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and an additional nucleic acid sequence that encodes PD-L2 inhibitor selected from AMP-224 (Amplimmune) or rHIgM12B7.

[00165] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to PD1. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to PD1 (*e.g.*, an anti-PD1 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

d) *CTLA-4 Inhibitors*

[00166] In some embodiments, the immune checkpoint inhibitor is an inhibitor of CTLA-4. In some embodiments, the an inhibitor of CTLA-4 is an antibody (*e.g.*, a monoclonal antibody or fragments, or a humanized or chimeric antibody or fragments thereof) against CTLA-4. In one embodiment, the anti-CTLA-4 antibody blocks the binding of CTLA-4 to CD80 (B7-1) and/or CD86 (B7-2) expressed on antigen presenting cells. Exemplary antibodies against CTLA-4 include ipilimumab (also known as Yervoy®, MDX-010, BMS-734016 and MDX-101, Bristol

Meyers Squibb); anti-CTLA4 antibody clone 9H10 from Millipore; tremelimumab (CP-675,206, ticilimumab, Pfizer); and anti-CTLA4 antibody clone BNI3 from Abcam.

[00167] In some embodiments, the anti-CTLA-4 antibody is one disclosed in any of International PCT Publication Nos. WO 2001/014424; WO 2004/035607; WO 2003/086459; WO 2012/120125; WO 2000/037504; WO 2009/100140; WO 2006/09649; WO 2005/092380; WO 2007/123737; WO 2006/029219; WO 2010/0979597; WO 2006/12168; WO 1997/020574 U.S. Patent Application Publication No. 2005/0201994; or European Patent Application Publication No. EP 1212422. Additional CTLA-4 antibodies are described in U.S. Patent Nos. 5,811,097; 5,855,887; 5,977,318; 6,051,227; 6,682,736; 6,984,720; 7,109,003; 7,132,281; International PCT Publication Nos. WO 01/14424 and WO 00/37504; and in U.S. Patent Application Publication Nos. 2002/0039581 and 2002/086014. In some embodiments, the anti-CTLA-4 antibody is one disclosed in any of International PCT Publication Nos. WO 1998/42752; U.S. Patent Nos. 6,682,736 and 6,207, 156; Hurwitz et al, Proc. Natl. Acad. Sci. USA, 95(17): 10067-10071 (1998); Camacho et al, J. Clin. Oncol., 22(145): Abstract No. 2505 (2004) (antibody CP- 675206); Mokyr et al, Cancer Res., 58:5301-5304 (1998).

[00168] In some embodiments, the CTLA-4 inhibitor is a CTLA-4 ligand as disclosed in International PCT Publication No. WO 1996/040915.

[00169] In some embodiments, the CTLA-4 inhibitor is a nucleic acid inhibitor of CTLA-4 expression, such as an RNAi molecule. In some embodiments, anti-CTLA4 RNAi molecules take the form of those described in any of International PCT Publication Nos. WO 1999/032619 and WO 2001/029058; U.S. Patent Application Publication Nos. 2003/0051263, 2003/0055020, 2003/0056235, 2004/265839, 2005/0100913, 2006/0024798, 2008/0050342, 2008/0081373, 2008/0248576, and 2008/055443; and/or U.S. Patent Nos. 6,506,559; 7,282,564; 7,538,095; and 7,560,438. In some instances, the anti-CTLA4 RNAi molecules are double stranded RNAi molecules, such as those disclosed in European Patent No. EP 1309726. In some instances, the anti-CTLA4 RNAi molecules are double stranded RNAi molecules, such as those described in U.S. Patent Nos. 7,056,704 and 7,078, 196. In some embodiments, the CTLA4 inhibitor is an aptamer, such as those described in International PCT Publication No. WO 2004/081021, such as Del 60 or M9-14 del 55. Additionally, in some embodiments, the anti-CTLA4 RNAi molecules of the present invention are RNA molecules, such as those described in U.S. Patent Nos. 5,898,031, 6,107,094, 7,432,249, and 7,432,250, and European Application No. EP 0928290.

[00170] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen

recognition domain, and an additional nucleic acid sequence that encodes a CTLA-4 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and an additional nucleic acid sequence that encodes a CTLA-4 inhibitor selected from ipilimumab (also known as Yervoy®, MDX-010, BMS-734016 and MDX-101); anti-CTLA4 Antibody, clone 9H10 from Millipore; Pfizer's tremelimumab (CP-675,206, ticilimumab); and anti-CTLA4 antibody clone BNI3 from Abcam.

[00171] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to CTLA-4. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to CTLA-4 (*e.g.*, an anti-CTLA-4 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

e) *LAG3 Inhibitors*

[00172] In some embodiments, the immune checkpoint inhibitor is an inhibitor of LAG3 (CD223). In some embodiments, the inhibitor of LAG3 is an antibody (*e.g.*, a monoclonal antibody or fragments, or a humanized or chimeric antibody or fragments thereof) against LAG3. In additional embodiments, an antibody against LAG3 blocks the interaction of LAG3 with major histocompatibility complex (MHC) class II molecules. Exemplary antibodies against LAG3 include: anti-Lag-3 antibody clone eBioC9B7W (C9B7W) from eBioscience; anti-Lag3 antibody LS-B2237 from LifeSpan Biosciences; IMP321 (ImmuFact) from Immutep; anti-Lag3 antibody BMS-986016; and the LAG-3 chimeric antibody A9H12. In some embodiments, the anti-LAG3 antibody is an anti-LAG3 antibody disclosed in International PCT Publication Nos. WO 2010/019570; WO 2008/132601; or WO 2004/078928.

[00173] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes LAG3 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and an additional nucleic acid sequence that encodes LAG3 inhibitor selected from anti-Lag-3 antibody clone eBioC9B7W (C9B7W) from eBioscience; anti-Lag3 antibody LS-B2237 from LifeSpan Biosciences; IMP321 (ImmuFact) from Immutep; anti-Lag3 antibody BMS-986016; and the LAG-3 chimeric antibody A9H12.

[00174] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to LAG3. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to LAG3 (*e.g.*, an anti-LAG3 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

f) *TIM3 Inhibitors*

[00175] In some embodiments, the immune checkpoint inhibitor is an inhibitor of TIM3. In some embodiments, the inhibitor of TIM3 is an antibody (*e.g.*, a monoclonal antibody or fragments, or a humanized or chimeric antibody or fragments thereof) against TIM3 (also known as HAVCR2). In additional embodiments, an antibody against TIM3 blocks the interaction of TIM3 with galectin-9 (Gal9). In some embodiments, the anti-TIM3 antibody is an anti-TIM3 antibody disclosed in Interantional PCT Publication Nos. WO 2013/006490; WO 2011/55607; WO 2011/159877; or WO 2001/17057. In another embodiment, a TIM3 inhibitor is a TIM3 inhibitor disclosed in International PCT Publication No. WO 2009/052623.

[00176] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes TIM3 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule and an additional nucleic acid sequence that encodes TIM3 inhibitor such as an antibody against TIM3 blocks the interaction of TIM3 with galectin-9 (Gal9).

[00177] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to TIM3. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to LAG3 (*e.g.*, an anti-TIM3 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

g) *B7-H3 Inhibitors*

[00178] In some embodiments, the immune checkpoint inhibitor is an inhibitor of B7-H3. In some embodiments, the inhibitor of B7-H3 is an antibody (*e.g.*, a monoclonal antibody or fragments, or a humanized or chimeric antibody or fragments thereof) against B7-H3. In some embodiments, the inhibitor of B7-H3 is MGA271 (MacroGenics).

[00179] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and an antigen recognition domain, and an additional nucleic acid sequence that encodes a B7-H3 inhibitor. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager and an additional nucleic acid sequence that encodes a B7-H3 inhibitor such as MGA271.

[00180] In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain and a therapeutic molecule domain that binds to B7-H3. In some embodiments, a pseudytoped oncolytic virus comprises a nucleic acid sequence that encodes an engager molecule comprising an activation domain that binds to CD3 (*e.g.*, an anti-CD3 scFv) and a therapeutic molecule domain that binds to B7-H3 (*e.g.*, an anti-B7-H3 scFv). In such embodiments, the pseudytoped oncolytic virus may further comprise an additional nucleic acid sequence that encodes an additional therapeutic molecule.

[00181] In certain other embodiments, the engager molecule additionally comprises one or more other domains, *e.g.*, one or more of a cytokine, a co-stimulatory domain, a domain that inhibits negative regulatory molecules of T-cell activation, or a combination thereof. In alternative embodiments, the engager is a first polypeptide provided within the pseudotyped oncolytic virus with a second polypeptide having one or more other domains, *e.g.*, one or more of a cytokine, a co-stimulatory domain, a domain that inhibits negative regulatory molecules of T-cell activation, or a combination thereof. In some embodiments, the first polypeptide and the second polypeptide are encoded in the same vector (*e.g.*, viral vector). In some embodiments, the first polypeptide and the second polypeptide are encoded in different vectors (*e.g.*, viral vectors). In specific embodiments, the cytokine is IL-15, IL-2, and/or IL-7. In other specific embodiments, the co-stimulatory domain is CD27, CD80, CD83, CD86, CD134, or CD137. In other specific embodiments, the domain that inhibits negative regulatory molecules of T-cell activation is PD-1, PDL1, CTLA4, or B7-H4.

[00182] In some embodiments, the therapeutic molecule is a polypeptide such as an anti-angiogenic factor. Angiogenesis or neovascularization is the formation of new microvessels from an established vascular network. In some instances, the angiogenic process involves communications from multiple cell types such as endothelial cells (EC) and circulating endothelial progenitor cells, pericytes, vascular smooth muscle cells, stromal cells, including stem cells, and parenchymal cells. These communications or interactions occur through secreted factors such as VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), or angiopoietins. In some instances, an anti-angiogenic factor is a polypeptide that disrupts one or more of the interactions of the cell types: endothelial cells (EC) and circulating endothelial progenitor cells, pericytes, vascular smooth muscle cells, stromal cells, including stem cells, and parenchymal cells. In some instances, an anti-angiogenic factor is a polypeptide that disrupts one or more of the interactions of secreted factors such as VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) or angiopoietins.

[00183] In other embodiments, provided are pseudotyped oncolytic viruses comprising nucleic acids that encode therapeutic polypeptides that modulate regulatory T cells. In some instances, regulatory T cells maintain the tolerance to self-antigens and in some instances abrogate autoimmune. In some cases, Treg supresses or downregulates induction and proliferation of effector T cells. Exemplary Treg modulatory polypeptides include CCR4, Helios, TIGIT, GITR, neuropilin, neuritin, CD103, CTLA-4, ICOS, and Swap70.

[00184] In other embodiments, provided are pseudotyped oncolytic viruses comprising nucleic acids that encode therapeutic polypeptides that modulate myeloid-derived suppressor cells (MDSCs). MDSCs are a heterogenous population of immune cells from the myeloid lineage (a cluster of different cell types that originate from bone marrow stem cells), to which also includes dendritic cells, macrophages and neutrophils. In some instances, myeloid cells interact with T cells to regulate the T cell's function. Exemplary MDSC modulatory polypeptides include TGF-βR1, GM-CSF, IFN-γ, Interleukins (*e.g.*, IL-β, IL-1F2, IL-6, IL-10, IL-12, IL-13, IL-6, IL-6Rα, IL-6/IL-6R complex, TGF-β1, M-CSF, Prostaglandin E2/PGE2, Prostaglandin E Synthase 2, S100A8, and VEGF.

[00185] In other embodiments, provided are pseudotyped oncolytic viruses comprising nucleic acids that encode therapeutic polypeptides that modulate the fibrotic stroma. In some embodiments, fibrosis occurs in response to inflammation, either chronic or recurrent. Over time, the repeated bouts of inflammation irritate and scar the tissue, causing buildups of fibrous tissue. In some intances, if enough fibrous material develops, it turns into stromal

fibrosis. Exemplary fibrotic stromal polypeptides include fibroblast activation protein-alpha (FAP).

Nucleic acid polymers as Therapeutic Molecules

[00186] In some embodiments, the therapeutic molecule is a nucleic acid polymer. In some instances, the nucleic acid polymer is a RNA polymer. In some instances, the RNA polymer is an antisense polymer those sequence is complementary to a microRNA (miRNA or miR) target sequence. In some instances, the RNA polymer is a microRNA polymer. In some embodiments, the RNA polymer comprises a DNA-directed RNAi (ddRNAi) sequence, which enables in vivo production of short hairpin RNAs (shRNAs).

[00187] In some embodiments, a microRNA polymer is a short non-coding RNA that is expressed in different tissue and cell types which suppresses the expression of a target gene. For example, miRNAs are transcribed by RNA polymerase II as part of the capped and polyadenylated primary transcripts (pri-miRNAs). In some instances, the primary transcript is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA star (miRNA*) products. In some instances, the mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA and in some instances results in translational inhibition or destabilization of the target mRNA.

[00188] In some instances, dysregulated microRNA expression is correlated with one or more types of cancer. In some embodiments, the microRNA is referred to as an oncomiR. In some instances, the dysregulated microRNA expression is an elevated expression. In some instances, the elevated expression level of microRNA correlates to one or more types of cancer. For example, overexpression of microRNA-155 (miR-155) has been observed in cancers such as Burkitt lymphoma, or laryngeal squamous cell carcinoma (LSCC) and overexpression of microRNA-21 (miR-21) has been observed in breast cancer.

[00189] In some embodiments, exemplary microRNAs with an elevated expression level include, but are not limited to, miR-10 family (*e.g.*, miR-10b), miR-17, miR-21, miR-106 family (*e.g.*, miR-106a), miR-125 family (*e.g.*, miR-125b), miR-145, miR-146 family (*e.g.*, miR-146a, miR-146b), miR-155, miR-96, miR-182, miR-183, miR-221, miR-222, and miR-1247-5p.

[00190] In some instances, the nucleic acid polymer is an antisense polymer those sequence complements an oncomiR. In some instances, the nucleic acid polymer is an antisense polymer those sequence complements an oncomiR that is characterized with an overexpression.

In some instances, the nucleic acid polymer is an antisense polymer those sequence complements a microRNA target sequence. In some instances, the nucleic acid polymer is an antisense polymer those sequence complements a microRNA target sequence that is characterized with an overexpression. In some instances, the therapeutic molecule is an antisense polymer those sequence complements a microRNA target sequence. In some instances, the therapeutic molecule is an antisense polymer those sequence complements a microRNA target sequence that is characterized with an overexpression. In some instances, the overexpression level is relative to the endogenous expression level of the microRNA.

[00191] In some instances, the dysregulated microRNA expression is a reduced expression. In some instances, the reduced expression level of microRNA correlates to one or more types of cancer. For example, a depleted level of miR-31 has been observed in both human and mouse metastatic breast cancer cell lines.

[00192] In some embodiments, exemplary microRNAs with reduced expression levels include, but are not limited to, miR-31, miR-34 family (*e.g.*, miR34a, miR-34b, and miR-34c), miR-101, miR-126, miR-145, miR-196a, and the miR-200 family.

[00193] In some instances, the nucleic acid polymer is an oncomiR. In some instances, the oncomiR is equivalent to an endogeous oncomiR wherein the endogeous oncomiR is characterized with a reduced expression level. In some instances, the nucleic acid polymer is a microRNA polymer. In some instances, the therapeutic molecule is a microRNA polymer. In some instances, the microRNA is equivalent to an endogeous microRNA polymer wherein the endogenous microRNA is characterized with a reduced expression level.

[00194] As described above, in some instances the RNA polymer comprises a DNA-directed RNAi (ddRNAi) sequence. In some instances, a ddRNAi construct encoding a shRNA is packaged into a viral vector such as a viral vector of a pseudotyped oncolytic virus described herein. In some instances upon entry into the target cell (*e.g.*, a tumor cell), the viral genome is processed to produce the encoded shRNAs. The shRNAs are then processed by endogenous host systems and enter the RNAi pathway to modulate or silence the desired gene target. In some instances, the gene target is a gene that is overexpressed in a cancer type. In some instances, the gene target is a gene that is overexpressed in a solid tumor. In some instances, the gene target is a gene that is overexpressed in a hematologic cancer. Exemplary genes that are overexpressed in cancer include, but are not limited to, *TP53*, human epidermal growth factor receptor 2 (*HER2*), mucin 1-cell surface associated (*MUC1*), human pituitary tumour-transforming gene 1 (*hPPTG1*), prostate and breast cancer overexpressed gene 1 protein (*PBOV1*), and the like.

[00195] In some instances, the nucleic acid polymer comprises a ddRNAi sequence. In some instances, the nucleic acid polymer is comprises a ddRNAi sequence which targets a gene that is overexpressed in a cancer. In some instances, the therapeutic molecule comprises a ddRNAi sequence. In some instances, the therapeutic molecule comprises a ddRNAi sequence which targets a gene that is overexpressed in a cancer.

Exemplarly Engager Molecules

[00196] In some embodiments, the engager molecules described herein comprise a bi-specific antibody construct comprising an activation domain and an antigen recognition domain, in which the activation domain interacts or binds to an effector cell surface receptor shown in Table 1; and the antigen recognition domain interacts or binds to a target-cell antigen shown in Table 2. In some embodiments, the engager molecules described herein comprise a bi-specific antibody construct comprising an activation domain and a therapeutic molecule domain, in which the activation domain interacts or binds to an effector cell surface receptor shown in Table 1; and the therapeutic molecule domain interacts or binds to a cell surface antigen shown in Table 2.

[00197] In some embodiments, the engager molecules provided herein comprise an activation domain, wherein the activation domain comprises an anti-CD3 scFv. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 20 and a heavy chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment comprising an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 20 and a heavy chain variable fragment that is 100% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment comprising the amino acid sequence of SEQ ID NO: 20 and a heavy chain variable fragment comprising the amino acid sequence of of SEQ ID NO: 22. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment consisting of the amino acid sequence of SEQ ID NO: 20 and a heavy chain variable fragment consisting of the amino acid sequence of of SEQ ID NO: 22.

[00198] In some embodiments, the engager molecules provided herein comprise an activation domain, wherein the activation domain comprises an anti-CD3 scFv, wherein the anti-

CD3 scFv comprises a light chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 19 and a heavy chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 21. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment nucleic acid sequence that is 100% identical to the nucleic acid sequence of SEQ ID NO: 19 and a heavy chain variable fragment nucleic acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment nucleic acid sequence comprising SEQ ID NO: 19 and a heavy chain variable fragment nucleic acid sequence comprising SEQ ID NO: 21. In some embodiments, the anti-CD3 scFv comprises a light chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 19 and a heavy chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 21.

[00199] In some embodiments, the engager molecules provided herein comprise an antigen recognition domain, wherein the antigen recognition domain comprises an anti-CD19 scFv. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 16 and a heavy chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 18. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment comprising an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 16 and a heavy chain variable fragment that is 100% identical to the amino acid sequence of SEQ ID NO: 18. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment comprising the amino acid sequence of SEQ ID NO: 16 and a heavy chain variable fragment comprising the amino acid sequence of of SEQ ID NO: 18. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment consisting of the amino acid sequence of SEQ ID NO: 16 and a heavy chain variable fragment consisting of the amino acid sequence of of SEQ ID NO: 18.

[00200] In some embodiments, the engager molecules provided herein comprise an antigen recognition domain, wherein the antigen recognition domain comprises an anti-CD19 scFv, wherein the anti-CD19 scFv comprises a light chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%,

at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 15 and a heavy chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 17. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment nucleic acid sequence that is 100% identical to the nucleic acid sequence of SEQ ID NO: 15 and a heavy chain variable fragment nucleic acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment nucleic acid sequence comprising SEQ ID NO: 15 and a heavy chain variable fragment nucleic acid sequence comprising SEQ ID NO: 17. In some embodiments, the anti-CD19 scFv comprises a light chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 15 and a heavy chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 17.

[00201] In some embodiments, the engager molecules provided herein comprise a therapeutic molecule domain, wherein the therapeutic molecule domain comprises an anti-PDL1 scFv. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 38. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment comprising an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment that is 100% identical to the amino acid sequence of SEQ ID NO: 38. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment comprising the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment comprising the amino acid sequence of of SEQ ID NO: 38. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment consisting of the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment consisting of the amino acid sequence of of SEQ ID NO: 38.

[00202] In some embodiments, the engager molecules provided herein comprise a therapeutic molecule domain, wherein the therapeutic molecule domain comprises an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 35 and a

heavy chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 37. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence that is 100% identical to the nucleic acid sequence of SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 37. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence comprising SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence comprising SEQ ID NO: 37. In some embodiments, the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 37.

[00203] In some embodiments, the engager molecules provided herein comprise a therapeutic molecule domain, wherein the therapeutic molecule domain comprises a SIRP1α polypeptide fragement. In some embodiments, the SIRP1α polypeptide fragement comprises an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 32. In some embodiments, the SIRP1α polypeptide fragement comprises an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 32. In some embodiments, the SIRP1α polypeptide fragement comprises the amino acid sequence of SEQ ID NO: 32. In some embodiments, the SIRP1α polypeptide fragement consists of the amino acid sequence of SEQ ID NO: 32.

[00204] In some embodiments, the engager molecules provided herein comprise a therapeutic molecule domain, wherein the therapeutic molecule domain comprises a SIRP1α polypeptide fragement, wherein the SIRP1α polypeptide fragement comprises a nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 31. In some embodiments, the SIRP1α polypeptide fragement comprises a nucleic acid sequence that is 100% identical to the nucleic acid sequence of SEQ ID NO: 31. In some embodiments, the SIRP1α polypeptide fragement comprises the nucleic acid sequence of SEQ ID NO: 31. In some embodiments, the SIRP1α polypeptide fragement consists of the nucleic acid sequence of SEQ ID NO: 31.

[00205] In some embodiments, the engager molecules comprise an activation domain comprising an scFv that binds to CD3 and an antigen recognition domain comprising an scFv that binds to CD19, referred to herein as a CD19-CD3 BiTE, or a CD19 BiTE. A schematic

of an exemplary CD19-CD3 BiTE is shown in FIG. 1 (SEQ ID NO: 44). In such embodiments, the anti-CD3 scFv and the anti-CD19 scFv are linked together by a G4S linker (SEQ ID NO: 6). In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes a CD19-CD3 BiTE and a second nucleic acid sequence encodes a therapeutic molecule such as IL-15 (FIG. 2, SEQ ID NO: 53), IL-12 (FIG. 3, SEQ ID NO: 54), or CXCL10 (FIG. 4, SEQ ID NO: 55). In such embodiments, the CD19-CD3 BiTE (*e.g.*, SEQ ID NO: 44) is linked to the therapeutic molecule, *e.g.*, IL-15 (SEQ ID NO: 24), IL-12 p35 (SEQ ID NO: 28), IL-12 p40 (SEQ ID NO: 26), and/or CXCL10 (SEQ ID NO: 30), by a T2A self-cleaving peptide linker (SEQ ID NO: 14).

[00206] In some embodiments, the engager molecules comprise an activation domain comprising an scFv that binds to CD3 and a therapeutic molecule domain comprising a SIRP1α polypeptide fragement that binds to CD47 (SEQ ID NO: 32), referred to herein as an SIRP1α-CD3 BiTE or a SIRP1α BiTE. A schematic of an exemplary SIRP1α-CD3 BiTE is shown in FIG. 5 (SIRP1α-CD3 (SL), SEQ ID NO: 46) and FIG. 6 (SIRP1α-CD3 (LL), SEQ ID NO: 48). In some embodiments, the anti-CD3 scFv and the SIRP1α peptide fragment are linked together by a single amino acid linker, or a "short linker" (SL) (*e.g.*, SIRP1α-CD3 (SL) as shown in FIG. 5). In some embodiments, the anti-CD3 scFv and the SIRP1α peptide fragment are linked together by G4S linker, or a "long linker" (LL) (*e.g.*, SIRP1α-CD3 (LL) as shown in FIG. 6). In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes a SIRP1α-CD3 BiTE and a second nucleic acid sequence encodes a therapeutic molecule such as IL-15 (FIG. 7, SEQ ID NO: 56 and FIG. 8, SEQ ID NO: 57), IL-12 (FIG. 9, SEQ ID NO: 58 and FIG. 10, SEQ ID NO: 59), or CXCL10 (FIG. 11, SEQ ID NO: 60 and FIG. 12, SEQ ID NO: 61). In such embodiments, the SIRP1α-CD3 BiTE (*e.g.*, SEQ ID NO: 46 or SEQ ID NO: 48) is linked to the therapeutic molecule, *e.g.*, IL-15 (SEQ ID NO: 24), IL-12 p35 (SEQ ID NO: 28), IL-12 p40 (SEQ ID NO: 26), and/or CXCL10 (SEQ ID NO: 30), by a T2A self-cleaving peptide linker (SEQ ID NO: 14).

[00207] In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes a SIRP1α-CD3 BiTE and a second nucleic acid sequence encodes a therapeutic molecule such as MMP9 (FIG. 18A, SEQ ID NO: 65 and FIG. 18B, SEQ ID NO: 66). In such embodiments, the SIRP1α-CD3 BiTE (*e.g.*, SEQ ID NO: 65 or 66) is linked to the MMP9 polypeptide (SEQ ID NO: 34) by a T2A self-cleaving peptide linker (SEQ ID NO: 14).

[00208] In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes a SIRP1α-CD3 BiTE and a second nucleic acid sequence encodes a therapeutic molecule comprising an anti-PDL1 scFv linked to an IgG1 Fc domain (*e.g.*, comprises an IgG1 CH2-CH3-Hinge, SEQ ID NO: 40), such as the SIRP1α-CD3-PDL1-Fc (SL) construct shown in FIG. 37 (SEQ ID NO: 68) or the SIRP1α-CD3-PDL1-Fc (LL) construct show in FIG. 38 (SEQ ID NO: 70).

[00209] In some embodiments, the engager molecules comprise an activation domain comprising an scFv that binds to CD3 and a therapeutic molecule domain comprising an scFv that binds to PDL1, referred to herein as an PDL1-CD3 BiTE or a PDL1 BiTE. Exemplary PDL1-CD3 BiTEs are shown in FIG. 13 (SEQ ID NO: 50). In some embodiments, the anti-CD3 scFv and the anti-PDL1 scFv are linked together by G4S linker (SEQ ID NO: 6). In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes a PDL1-CD3 BiTE and a second nucleic acid sequence encodes a therapeutic molecule such as IL-15 (FIG. 14, SEQ ID NO: 62), IL-12 (FIG. 15, SEQ ID NO: 63), or CXCL10 (FIG. 16, SEQ ID NO: 64). In such embodiments, the SIRP1α-CD3 BiTE (*e.g.*, SEQ ID NO: 50) is linked to the therapeutic molecule, *e.g.*, IL-15 (SEQ ID NO: 24), IL-12 p35 (SEQ ID NO: 28), IL-12 p40 (SEQ ID NO: 26), and/or CXCL10 (SEQ ID NO: 30), by a T2A self-cleaving peptide linker (SEQ ID NO: 14).

[00210] In some embodiments, the engager molecule is a tripartite engager molecule and comprises an activation domain comprising an scFv that binds to CD3, a therapeutic molecule domain comprising an scFv that binds to PDL1, and a third domain comprising an IgG1 Fc domain (*e.g.*, comprises an IgG1 CH2-CH3-Hinge, SEQ ID NO: 40) and capable of binding to one or more FcγRs, referred to herein as an PDL1-CD3-Fc tripartite T cell engager, or TiTE, or a PDL1 TiTE. A schematic of an exemplary PDL1-CD3-Fc TiTE is shown in FIG. 17 (SEQ ID NO: 52).

[00211] The amino acid sequences of exemplary engager molecules and therapeutic molecules are shown in Table 3.

Table 3: Amino acid sequences of exemplary engager molecules and therapeutic molecules

BiTE	Amino Acid Sequence	SEQ ID NO:
CD19-CD3	MEFGLSWVFLVALFRGVQCDIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNW YQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTE	44

	DPWTFGGGTKLEIKGGGGSGGGGSGGGGSQVQLQQSGAELVRPGSSVKISCKASGYAF SSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLA SEDSAVYFCARRETTTVGRYYYAMDYWGQGTTVTVSSGGGGSDIKLQQSGAELARPGA SVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGG SGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKV ASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHH H-	
SIRP1α-CD3-SL	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASDIKLQQSGAELARPGASVKMSCKTSGYTFTRY TMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIM SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTS YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHH-	46
SIRP1α-CD3-LL	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASGGGGSDIKLQQSGAELARPGASVKMSCKTSGY TFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQ SPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGS GSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHH-	48
PDL1-CD3	MEFGLSWVFLVALFRGVQCDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQ RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCA RYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEK VTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISS MEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSDIQMTQSPSSLSASVGDRVTITC RASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPGG SLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAHHHHHH-	50
PDL1-CD3-Fc	MEFGLSWVFLVALFRGVQCDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQ RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCA RYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEK VTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISS MEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSDIQMTQSPSSLSASVGDRVTITC RASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPGG SLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAVDEAKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGHHHHHH-	52
CD19-CD3-IL15	MEFGLSWVFLVALFRGVQCDIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNW YQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTE DPWTFGGGTKLEIKGGGGSGGGGSGGGGSQVQLQQSGAELVRPGSSVKISCKASGYAF SSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLA SEDSAVYFCARRETTTVGRYYYAMDYWGQGTTVTVSSGGGGSDIKLQQSGAELARPGA SVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGG SGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKV ASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHH HRRKREGRGSLLTCGDVEENPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFI LGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLL ELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF VHIVQMFINTS-	53
CD19-CD3-IL12	MEFGLSWVFLVALFRGVQCDIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNW YQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTE DPWTFGGGTKLEIKGGGGSGGGGSGGGGSQVQLQQSGAELVRPGSSVKISCKASGYAF SSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLA	54

	SEDSAVYFCARRETTTVGRYYYAMDYWGQGTTVTVSSGGGGSDIKLQQSGAELARPGA SVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGG SGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKV ASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHH HRRKREGRGSLLTCGDVEENPGPMWPPGSASQPPPSPAAATGLHPAARPVSLQCRLSM CPARSLLLVATLVLLDHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTL EFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTS FMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASRRKREGRGSLLTCG DVEENPGPPMCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLT CDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKK EDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDP QGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENY TSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKS KREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS-	
CD19-CD3-CXCL10	MEFGLSWVFLVALFRGVQCDIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNW YQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTE DPWTFGGGTKLEIKGGGGSGGGGSGGGGSQVQLQQSGAELVRPGSSVKISCKASGYAF SSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLA SEDSAVYFCARRETTTVGRYYYAMDYWGQGTTVTVSSGGGGSDIKLQQSGAELARPGA SVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGG SGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKV ASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHH HRRKREGRGSLLTCGDVEENPGPMNQTAILICCLIFLTLSGIQGVPLSRTVRCTCISI SNQPVNPRSLEKLEIIPASQFCPRVEIIATMKKKGEKRCLNPESKAIKNLLKAVSKER SKRSP-	55
SIRP1α-CD3-IL15 (SL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASDIKLQQSGAELARPGASVKMSCKTSGYTFTRY TMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIM SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTS YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSLLTCGD VEENPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWV NVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHD TVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS-	56
SIRP1α-CD3-IL15 (LL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASGGGGSDIKLQQSGAELARPGASVKMSCKTSGY TFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQ SPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGS GSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSL LTCGDVEENPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKT EANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS -	57
SIRP1α-C3-IL12 (SL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASDIKLQQSGAELARPGASVKMSCKTSGYTFTRY TMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIM SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTS YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSLLTCGD VEENPGPMWPPGSASQPPPSPAAATGLHPAARPVSLQCRLSMCPARSLLLVATLVLLD HLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDIT KDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKM YQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKT KIKLCILLHAFRIRAVTIDRVMSYLNASRRKREGRGSLLTCGDVEENPGPPMCHQQLV	58

	ISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQS SEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEP KNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVR GDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPK NLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSAT VICRKNASISVRAQDRYYSSSWSEWASVPCS-	
SIRP1α-CD3-IL12 (LL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASGGGGSDIKLQQSGAELARPGASVKMSCKTSGY TFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQ SPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGS GSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSL LTCGDVEENPGPMWPPGSASQPPPSPAAATGLHPAARPVSLQCRLSMCPARSLLLVAT LVLLDHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEID HEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIY EDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEP DFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASRRKREGRGSLLTCGDVEENPGPPMC HQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITW TLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILK DQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLS AERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIK PDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTD KTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS-	59
SIRP1α-CD3-CXCL10 (SL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASDIKLQQSGAELARPGASVKMSCKTSGYTFTRY TMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIM SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTS YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSLLTCGD VEENPGPMNQTAILICCLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRSLEKLEII PASQFCPRVEIIATMKKKGEKRCLNPESKAIKNLLKAVSKERSKRSP-	60
SIRP1α-CD3-CXCL10 (LL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASGGGGSDIKLQQSGAELARPGASVKMSCKTSGY TFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQ SPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGS GSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSL LTCGDVEENPGPMNQTAILICCLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRSLE KLEIIPASQFCPRVEIIATMKKKGEKRCLNPESKAIKNLLKAVSKERSKRSP-	61
PDL1-CD3-IL15	MEFGLSWVFLVALFRGVQCDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQ RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCA RYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEK VTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISS MEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSDIQMTQSPSSLSASVGDRVTITC RASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPGG SLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAHHHHHHRRKREGRG SLLTCGDVEENPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLP KTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS-	62
PDL1-CD3-IL12	MEFGLSWVFLVALFRGVQCDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQ RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCA RYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEK VTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISS MEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSDIQMTQSPSSLSASVGDRVTITC RASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPE	63

	DFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPGG SLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAHHHHHHRRKREGRG SLLTCGDVEENPGPMWPPGSASQPPPSPAAATGLHPAARPVSLQCRLSMCPARSLLLV ATLVLLDHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEE IDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLE EPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASRRKREGRGSLLTCGDVEENPGPP MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGI TWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDI LKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAAT LSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDI IKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS-	
PDL1-CD3-CXCL10	MEFGLSWVFLVALFRGVQCDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQ RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCA RYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEK VTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISS MEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSDIQMTQSPSSLSASVGDRVTITC RASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPGG SLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAHHHHHHRRKREGRG SLLTCGDVEENPGPMNQTAILICCLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRS LEKLEIIPASQFCPRVEIIATMKKKGEKRCLNPESKAIKNLLKAVSKERSKRSP-	64
SIRP1α-CD3-MMP9 (SL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASDIKLQQSGAELARPGASVKMSCKTSGYTFTRY TMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIM SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTS YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHHRRKREGRGSLLTCGD VEENPGPMSLWQPLVLVLLVLGCCFAAPRQRQSTLVLFPGDLRTNLTDRQLAEEYLYR YGYTRVAEMRGESKSLGPALLLLQKQLSLPETGELDSATLKAMRTPRCGVPDLGRFQT FEGDLKWHHHNITYWIQNYSEDLPRAVIDDAFARAFALWSAVTPLTFTRVYSRDADIV IQFGVAEHGDGYPFDGKDGLLAHAFPPGPGIQGDAHFDDDELWSLGKGVVVPTRFGNA DGAACHFPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTDDRFGFCPSERLYTRDGNA DGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATTANYDRDKLFGFCPTRADSTVMGGN SAGELCVFPFTFLGKEYSTCTSEGRGDGRLWCATTSNFDSDKKWGFCPDQGYSLFLVA AHEFGHALGLDHSSVPEALMYPMYRFTEGPPLHKDDVNGIRHLYGPRPEPEPRPPTTT TPQPTAPPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLSPVDDA CNVNIFDAIAEIGNQLYLFKDGKYWRFSEGRGSRPQGPFLIADKWPALPRKLDSVFEE PLSKKLFFFSGRQVWVYTGASVLGPRRLDKLGLGADVAQVTGALRSGRGKMLLFSGRR LWRFDVKAQMVDPRSASEVDRMFPGVPLDTHDVFQYREKAYFCQDRFYWRVSSRSELN QVDQVGYVTYDILQCPED-	65
SIRP1α-CD3-MMP9 (LL)	METDTLLLWVLLLWVPGSTGDEEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQ WFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFR KGSPDDVEFKSGAGTELSVRAKPSASGGGGSDIKLQQSGAELARPGASVKMSCKTSGY TFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQ SPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGS GSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHHRRKREGRGSL LTCGDVEENPGPMSLWQPLVLVLLVLGCCFAAPRQRQSTLVLFPGDLRTNLTDRQLAE EYLYRYGYTRVAEMRGESKSLGPALLLLQKQLSLPETGELDSATLKAMRTPRCGVPDL GRFQTFEGDLKWHHHNITYWIQNYSEDLPRAVIDDAFARAFALWSAVTPLTFTRVYSR DADIVIQFGVAEHGDGYPFDGKDGLLAHAFPPGPGIQGDAHFDDDELWSLGKGVVVPT RFGNADGAACHFPFIFEGRSYSACTTDGRSDGLPWCSTTANYDTDDRFGFCPSERLYT RDGNADGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATTANYDRDKLFGFCPTRADST VMGGNSAGELCVFPFTFLGKEYSTCTSEGRGDGRLWCATTSNFDSDKKWGFCPDQGYS LFLVAAHEFGHALGLDHSSVPEALMYPMYRFTEGPPLHKDDVNGIRHLYGPRPEPEPR PPTTTTPQPTAPPTVCPTGPPTVHPSERPTAGPTGPPSAGPTGPPTAGPSTATTVPLS	66

	PVDDACNVNIFDAIAEIGNQLYLFKDGKYWRFSEGRGSRPQGPFLIADKWPALPRKLD SVFEEPLSKKLFFFSGRQVWVYTGASVLGPRRLDKLGLGADVAQVTGALRSGRGKMLL FSGRRLWRFDVKAQMVDPRSASEVDRMFPGVPLDTHDVFQYREKAYFCQDRFYWRVSS RSELNQVDQVGYVTYDILQCPED-	
SIRP1α-CD3-PDL1-Fc (SL)	METDRLLLWVLLLWVPGSTGDYPYDVPDYAGAQPADDIQMTQSPSSLSASVGDRVTIT CRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPG GSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISAD TSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAVDEAKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKVDEQKLISEED LNRRKREGRGSLLTCGDVEENPGPMETDRLLLWVLLLWVPGSTGDEEELQIIQPDKSV LVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNN MDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPSASDIKLQQSG AELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKD KATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGS GGSGGSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKR WIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKL ELKHHHHHH-	68
SIRP1α-CD3-PDL1-Fc (LL)	METDRLLLWVLLLWVPGSTGDYPYDVPDYAGAQPADDIQMTQSPSSLSASVGDRVTIT CRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYLYHPATFGQGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVQPG GSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISAD TSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSAVDEAKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKVDEQKLISEED LNRRKREGRGSLLTCGDVEENPGPMETDRLLLWVLLLWVPGSTGDEEELQIIQPDKSV LVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVTTVSDTTKRNN MDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPSASGGGGSDIK LQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYN QKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS VEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSG TSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFG AGTKLELKHHHHHH-	70

[00212] In some embodiments, the present invention provides recombinant nucleic acid sequences encoding an engager molecule and/or a therapeutic molecule. Exemplary recombinant nucleic acid sequences are shown in Table 4.

[00213] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule, wherein the therapeutic molecule is IL-15. In some embodiments, the nucleic acid sequences provided herein encode an IL-15 therapeutic molecule comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 24. In some embodiments, the nucleic acid sequences provided herein encode an IL-15 therapeutic molecule that is 100% identical to the amino acid sequence of SEQ ID NO: 24. In some embodiments, the nucleic acid sequences provided herein encode an IL-15 therapeutic molecule comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, the

nucleic acid sequences provided herein encode an IL-15 therapeutic molecule consisting of the amino acid sequence of SEQ ID NO: 24. In some embodiments, the nucleic acid sequences provided herein encode an IL-15 therapeutic molecule and comprise a sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 23. In some embodiments, the nucleic acid sequences provided herein encode an IL-15 therapeutic molecule and comprise the nucleic acid sequence of SEQ ID NO: 23. In some embodiments, the nucleic acid sequences provided herein encode an IL-15 therapeutic molecule and consist of the nucleic acid sequence of SEQ ID NO: 23.

[00214] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule, wherein the therapeutic molecule is IL-12 (*i.e.*, IL-12 p35 and/or IL-12 p40). In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 26. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule that is 100% identical to the amino acid sequence of SEQ ID NO: 26. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule consisting of the amino acid sequence of SEQ ID NO: 26. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and comprise a sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 25. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and comprise the nucleic acid sequence of SEQ ID NO: 25. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and consist of the nucleic acid sequence of SEQ ID NO: 25.

[00215] In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule that is 100% identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule comprising the amino acid sequence of SEQ ID NO: 28. In

some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule consisting of the amino acid sequence of SEQ ID NO: 28. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and comprise a sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 27. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and comprise the nucleic acid sequence of SEQ ID NO: 27. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and consist of the nucleic acid sequence of SEQ ID NO: 27.

[00216] In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule comprising an amino acid sequence of SEQ ID NO: 26 and 28. In some embodiments, the nucleic acid sequences provided herein encode an IL-12 therapeutic molecule and comprise the nucleic acid sequences of SEQ ID NO: 25 and 27.

[00217] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule, wherein the therapeutic molecule is CXCL10. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 30. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule that is 100% identical to the amino acid sequence of SEQ ID NO: 30. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule consisting of the amino acid sequence of SEQ ID NO: 30. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule and comprise a sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 29. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule and comprise the nucleic acid sequence of SEQ ID NO: 29. In some embodiments, the nucleic acid sequences provided herein encode a CXCL10 therapeutic molecule and consist of the nucleic acid sequence of SEQ ID NO: 29.

[00218] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule, wherein the therapeutic molecule is MMP9. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule comprising an

amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 34. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule that is 100% identical to the amino acid sequence of SEQ ID NO: 34. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule comprising the amino acid sequence of SEQ ID NO: 34. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule consisting of the amino acid sequence of SEQ ID NO: 34. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule and comprise a sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 33. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule and comprise the nucleic acid sequence of SEQ ID NO: 33. In some embodiments, the nucleic acid sequences provided herein encode an MMP9 therapeutic molecule and consist of the nucleic acid sequence of SEQ ID NO: 33.

[00219] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule, wherein the therapeutic molecule comprises an anti-PDL1 scFv. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment comprising an amino acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 38. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment comprising an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment that is 100% identical to the amino acid sequence of SEQ ID NO: 38. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment comprising the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment comprising the amino acid sequence of of SEQ ID NO: 38. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain

variable fragment consisting of the amino acid sequence of SEQ ID NO: 36 and a heavy chain variable fragment consisting of the amino acid sequence of of SEQ ID NO: 38.

[00220] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 37. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence that is 100% identical to the nucleic acid sequence of SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO: 37. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence comprising SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence comprising SEQ ID NO: 37. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv, wherein the anti-PDL1 scFv comprises a light chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 35 and a heavy chain variable fragment nucleic acid sequence consisting of SEQ ID NO: 37.

[00221] In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain, wherein the IgG1 Fc domain comprises an amino acid sequence that is that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 40. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain, wherein the IgG1 Fc domain is 100% identical to the amino acid sequence of SEQ ID NO: 40. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain, wherein the IgG1 Fc domain comprises the amino acid sequence of SEQ ID NO: 40. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc

domain, wherein the IgG1 Fc domain consists of the amino acid sequence of SEQ ID NO: 40. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain, wherein the IgG1 Fc domain nucleic acid sequence is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the nucleic acid sequence of SEQ ID NO: 39. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain, wherein the IgG1 Fc domain nucleic acid sequence comprises SEQ ID NO: 39. In some embodiments, the nucleic acid sequences provided herein encode a therapeutic molecule comprising an anti-PDL1 scFv and an IgG1 Fc domain, wherein the IgG1 Fc domain nucleic acid sequence comprises SEQ ID NO: 39.

[00222] In some embodiments, the nucleic acid sequences provided herein comprise a nucleic acid sequence selected from SEQ ID NOs: 43, 45, 47, 49, 51, 67, and 69. In some embodiments, the nucleic acid sequences provided herein are at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a nucleic acid sequence selected from SEQ ID NOs: 43, 45, 47, 49, 51, 67, and 69. In some embodiments, the nucleic acid sequences provided herein are 100% identical to a nucleic acid sequence selected from SEQ ID NOs: 43, 45, 47, 49, 51, 67, and 69. In some embodiments, the nucleic acid sequences provided herein consist of a nucleic acid sequence selected from SEQ ID NOs: 43, 45, 47, 49, 51, 67, and 69.

[00223] In some embodiments, the nucleic acid sequences provided herein encode an engager molecule and/or therapeutic molecule that is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, and 52. In some embodiments, the nucleic acid sequences provided herein encode an engager molecule protein that is 100% identical to an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, and 52. In some embodiments, the nucleic acid sequences provided herein encode an engager molecule protein comprising an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, and 52. In some embodiments, the nucleic acid sequences provided herein encode an engager molecule protein consisting of an amino acid sequence selected from SEQ ID NOs: 44, 46, 48, 50, and 52.

[00224] In some embodiments, the recombinant nucleic acid sequences provided herein encode an engager molecule and a therapeutic molecule. In some embodiments, the recombinant nucleic acid sequences encode an amino acid sequence comprising an engager molecule and a therapeutic molecule, wherein the amino acid sequence is at least 80%, at least, 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical

to an amino acid sequence selected from SEQ ID NOs: 53 – 66, 68 and 70. In some embodiments, the nucleic acid sequences encode an amino acid sequence comprising an engager molecule and a therapeutic molecule, wherein the amino acid sequence is 100% identical to an amino acid sequences selected from SEQ ID NOs: 53 – 66, 68 and 70. In some embodiments, the nucleic acid sequences encode an amino acid sequence comprising an engager molecule and a therapeutic molecule, wherein the amino acid sequence consists of an amino acid sequence selected from SEQ ID NOs: 53 – 66, 68 and 70.

Table 4: Nucleic sequences of exemplary engager molecules

BiTE	Nucleic Acid Sequence	SEQ ID NO:
CD19-CD3	ATGGAGTTCGGCCTGAGCTGGGTGTTCCTGGTGGCCCTGTTCAGGGGCGTGCAGTGCG ACATCCAGCTGACCCAGAGCCCCGCCAGCCTGGCCGTGAGCCTGGGCCAGAGGGCCAC CATCAGCTGCAAGGCCAGCCAGAGCGTGGACTACGACGGCGACAGCTACCTGAACTGG TACCAGCAGATCCCCGGCCAGCCCCCCAAGCTGCTGATCTACGACGCCAGCAACCTGG TGAGCGGCATCCCCCCCAGGTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGAA CATCCACCCCGTGGAGAAGGTGGACGCCGCCACCTACCACTGCCAGCAGAGCACCGAG GACCCCTGGACCTTCGGCGGCGGCACCAAGCTGGAGATCAAGGGCGGCGGCGGCAGCG GCGGCGGCGGCAGCGGCGGCGGCGGCAGCCAGGTGCAGCTGCAGCAGAGCGGCGCCGA GCTGGTGAGGCCCGGCAGCAGCGTGAAGATCAGCTGCAAGGCCAGCGGCTACGCCTTC AGCAGCTACTGGATGAACTGGGTGAAGCAGAGGCCCGGCCAGGGCCTGGAGTGGATCG GCCAGATCTGGCCCGGCGACGGCGACACCAACTACAACGGCAAGTTCAAGGGCAAGGC CACCCTGACCGCCGACGAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGCCTGGCC AGCGAGGACAGCGCCGTGTACTTCTGCGCCAGGAGGGAGACCACCACCGTGGGCAGGT ACTACTACGCCATGGACTACTGGGGCCAGGGCACCACCGTGACCGTGAGCAGCGGCGG CGGCGGCAGCGACATCAAGCTGCAGCAGAGCGGCGCCGAGCTGGCCAGGCCCGGCGCC AGCGTGAAGATGAGCTGCAAGACCAGCGGCTACACCTTCACCAGGTACACCATGCACT GGGTGAAGCAGAGGCCCGGCCAGGGCCTGGAGTGGATCGGCTACATCAACCCCAGCAG GGGCTACACCAACTACAACCAGAAGTTCAAGGACAAGGCCACCCTGACCACCGACAAG AGCAGCAGCACCGCCTACATGCAGCTGAGCAGCCTGACCAGCGAGGACAGCGCCGTGT ACTACTGCGCCAGGTACTACGACGACCACTACTGCCTGGACTACTGGGGCCAGGGCAC CACCCTGACCGTGAGCAGCGTGGAGGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGC AGCGGCGGCGTGGACGACATCCAGCTGACCCAGAGCCCCGCCATCATGAGCGCCAGCC CCGGCGAGAAGGTGACCATGACCTGCAGGGCCAGCAGCAGCGTGAGCTACATGAACTG GTACCAGCAGAAGAGCGGCACCAGCCCCAAGAGGTGGATCTACGACACCAGCAAGGTG GCCAGCGGCGTGCCCTACAGGTTCAGCGGCAGCGGCAGCGGCACCAGCTACAGCCTGA CCATCAGCAGCATGGAGGCCGAGGACGCCGCCACCTACTACTGCCAGCAGTGGAGCAG CAACCCCCTGACCTTCGGCGCCGGCACCAAGCTGGAGCTGAAGCACCACCACCACCAC CACTAG	43
SIRP1α-CD3 (SL)	ATGGAGACCGATACCCTGCTCTTGTGGGTTTTGCTTCTTTGGGTGCCAGGATCTACAG GTGATGAAGAAGAATTGCAGATCATCCAACCAGACAAATCCGTACTCGTGGCCGCAGG AGAGACCGCTACCCTCAGATGTACCATCACTTCTCTCTTCCCCGTTGGCCCCATCCAG TGGTTTCGAGGCGCAGGACCAGGACGAGTGCTTATTTACAATCAACGACAGGGCCCAT TCCCAAGAGTGACAACAGTATCCGATACCACCAAGCGCAATAATATGGACTTTAGCAT TAGAATCGGCAACATAACACCCGCTGACGCCGGTACATACTATTGTATTAAATTTCGA AAGGGCTCACCAGACGACGTGGAATTTAAGTCAGGGGCCGGAACCGAACTCTCAGTTA GAGCAAAACCTTCTGCTAGCGACATCAAGCTGCAGCAGAGCGGCGCCGAGCTGGCCAG GCCCGGCGCCAGCGTGAAGATGAGCTGCAAGACCAGCGGCTACACCTTCACCAGGTAC ACCATGCACTGGGTGAAGCAGAGGCCCGGCCAGGGCCTGGAGTGGATCGGCTACATCA ACCCCAGCAGGGGCTACACCAACTACAACCAGAAGTTCAAGGACAAGGCCACCCTGAC CACCGACAAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGCCTGACCAGCGAGGAC AGCGCCGTGTACTACTGCGCCAGGTACTACGACGACCACTACTGCCTGGACTACTGGG	45

	GCCAGGGCACCACCCTGACCGTGAGCAGCGTGGAGGGCGGCAGCGGCGGCAGCGGCGG CAGCGGCGGCAGCGGCGGCGTGGACGACATCCAGCTGACCCAGAGCCCCGCCATCATG AGCGCCAGCCCCGGCGAGAAGGTGACCATGACCTGCAGGGCCAGCAGCAGCGTGAGCT ACATGAACTGGTACCAGCAGAAGAGCGGCACCAGCCCCAAGAGGTGGATCTACGACAC CAGCAAGGTGGCCAGCGGCGTGCCCTACAGGTTCAGCGGCAGCGGCAGCGGCACCAGC TACAGCCTGACCATCAGCAGCATGGAGGCCGAGGACGCCGCCACCTACTACTGCCAGC AGTGGAGCAGCAACCCCCTGACCTTCGGCGCCGGCACCAAGCTGGAGCTGAAGCACCA CCATCATCACCACTGAG	
SIRP1α-CD3 (LL)	ATGGAGACCGATACCCTGCTCTTGTGGGTTTTGCTTCTTTGGGTGCCAGGATCTACAG GTGATGAAGAAGAATTGCAGATCATCCAACCAGACAAATCCGTACTCGTGGCCGCAGG AGAGACCGCTACCCTCAGATGTACCATCACTTCTCTCTTCCCCGTTGGCCCCATCCAG TGGTTTCGAGGCGCAGGACCAGGACGAGTGCTTATTTACAATCAACGACAGGGCCCAT TCCCAAGAGTGACAACAGTATCCGATACCACCAAGCGCAATAATATGGACTTTAGCAT TAGAATCGGCAACATAACACCCGCTGACGCCGGTACATACTATTGTATTAAATTTCGA AAGGGCTCACCAGACGACGTGGAATTTAAGTCAGGGGCCGGAACCGAACTCTCAGTTA GAGCAAAACCTTCTGCTAGCGGCGGCGGCGGCAGCGACATCAAGCTGCAGCAGAGCGG CGCCGAGCTGGCCAGGCCCGGCGCCAGCGTGAAGATGAGCTGCAAGACCAGCGGCTAC ACCTTCACCAGGTACACCATGCACTGGGTGAAGCAGAGGCCCGGCCAGGGCCTGGAGT GGATCGGCTACATCAACCCCAGCAGGGGCTACACCAACTACAACCAGAAGTTCAAGGA CAAGGCCACCCTGACCACCGACAAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGC CTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGGTACTACGACGACCACTACT GCCTGGACTACTGGGGCCAGGGCACCACCCTGACCGTGAGCAGCGTGGAGGGCGGCAG CGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCGGCGTGGACGACATCCAGCTGACCCAG AGCCCCGCCATCATGAGCGCCAGCCCCGGCGAGAAGGTGACCATGACCTGCAGGGCCA GCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGAAGAGCGGCACCAGCCCCAAGAG GTGGATCTACGACACCAGCAAGGTGGCCAGCGGCGTGCCCTACAGGTTCAGCGGCAGC GGCAGCGGCACCAGCTACAGCCTGACCATCAGCAGCATGGAGGCCGAGGACGCCGCCA CCTACTACTGCCAGCAGTGGAGCAGCAACCCCCTGACCTTCGGCGCCGGCACCAAGCT GGAGCTGAAGCACCACCACCACCACCACTAG	47
PDL1-CD3	ATGGAGTTCGGCCTGAGCTGGGTGTTCCTGGTGGCCCTGTTCAGGGGCGTGCAGTGCG ACATCAAGCTGCAGCAGAGCGGCGCCGAGCTGGCCAGGCCCGGCGCCAGCGTGAAGAT GAGCTGCAAGACCAGCGGCTACACCTTCACCAGGTACACCATGCACTGGGTGAAGCAG AGGCCCGGCCAGGGCCTGGAGTGGATCGGCTACATCAACCCCAGCAGGGGCTACACCA ACTACAACCAGAAGTTCAAGGACAAGGCCACCCTGACCACCGACAAGAGCAGCAGCAC CGCCTACATGCAGCTGAGCAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCC AGGTACTACGACGACCACTACTGCCTGGACTACTGGGGCCAGGGCACCACCCTGACCG TGAGCAGCGTGGAGGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCGT GGACGACATCCAGCTGACCCAGAGCCCCGCCATCATGAGCGCCAGCCCCGGCGAGAAG GTGACCATGACCTGCAGGGCCAGCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGA AGAGCGGCACCAGCCCCAAGAGGTGGATCTACGACACCAGCAAGGTGGCCAGCGGCGT GCCCTACAGGTTCAGCGGCAGCGGCAGCGGCACCAGCTACAGCCTGACCATCAGCAGC ATGGAGGCCGAGGACGCCGCCACCTACTACTGCCAGCAGTGGAGCAGCAACCCCCTGA CCTTCGGCGCCGGCACCAAGCTGGAGCTGAAGGGCGGCGGCGGCAGCGATATCCAGAT GACACAGAGCCCATCATCTCTGTCTGCAAGCGTAGGAGACCGAGTCACCATTACATGC AGAGCCTCCCAAGACGTTTCCACAGCAGTGGCCTGGTATCAGCAAAAACCTGGTAAGG CGCCCAAGCTTCTCATCTATTCAGCCAGTTTTCTGTATAGCGGCGTTCCCAGCCGATT CTCTGGCTCTGGATCCGGCACGGACTTTACTTTGACAATTTCCTCTCTTCAGCCCGAA GATTTTGCAACCTACTACTGTCAGCAATATCTCTACCATCCAGCCACATTCGGACAGG GCACCAAAGTCGAAATCAAAAGAGGCGGCGGCGGCAGTGGCGGCGGGGGTTCAGGAGG CGGGGGTTCTGAAGTGCAACTCGTTGAAAGCGGAGGAGGGCTTGTCCAACCTGGCGGG TCACTGCGGTTGAGCTGCGCCGCAAGCGGATTCACCTTCTCAGACTCTTGGATCCATT GGGTGCGCCAGGCTCCCGGAAAAGGCTTGGAATGGGTTGCTTGGATTTCACCGTATGG CGGTTCCACATACTACGCTGACAGCGTTAAGGGTCGATTCACCATCTCTGCAGATACT TCAAAAAACACAGCCTACCTTCAGATGAATAGTTTGCGCGCCGAGGACACAGCGGTTT ATTATTGTGCCCGAAGACATTGGCCCGGCGGTTTCGACTACTGGGGGCAAGGTACGTT GGTGACTGTGAGCGCCCACCACCATCATCACCACTGA	49
PDL1-CD3-Fc	ATGGAGTTCGGCCTGAGCTGGGTGTTCCTGGTGGCCCTGTTCAGGGGCGTGCAGTGCG ACATCAAGCTGCAGCAGAGCGGCGCCGAGCTGGCCAGGCCCGGCGCCAGCGTGAAGAT GAGCTGCAAGACCAGCGGCTACACCTTCACCAGGTACACCATGCACTGGGTGAAGCAG AGGCCCGGCCAGGGCCTGGAGTGGATCGGCTACATCAACCCCAGCAGGGGCTACACCA ACTACAACCAGAAGTTCAAGGACAAGGCCACCCTGACCACCGACAAGAGCAGCAGCAC	51

	CGCCTACATGCAGCTGAGCAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCC AGGTACTACGACGACCACTACTGCCTGGACTACTGGGGCCAGGGCACCACCCTGACCG TGAGCAGCGTGGAGGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCGT GGACGACATCCAGCTGACCCAGAGCCCCGCCATCATGAGCGCCAGCCCCGGCGAGAAG GTGACCATGACCTGCAGGGCCAGCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGA AGAGCGGCACCAGCCCCAAGAGGTGGATCTACGACACCAGCAAGGTGGCCAGCGGCGT GCCCTACAGGTTCAGCGGCAGCGGCAGCGGCACCAGCTACAGCCTGACCATCAGCAGC ATGGAGGCCGAGGACGCCGCCACCTACTACTGCCAGCAGTGGAGCAGCAACCCCCTGA CCTTCGGCGCCGGCACCAAGCTGGAGCTGAAGGGCGGCGGCGGCAGCGATATCCAGAT GACACAGAGCCCATCATCTCTGTCTGCAAGCGTAGGAGACCGAGTCACCATTACATGC AGAGCCTCCCAAGACGTTTCCACAGCAGTGGCCTGGTATCAGCAAAAACCTGGTAAGG CGCCCAAGCTTCTCATCTATTCAGCCAGTTTTCTGTATAGCGGCGTTCCCAGCCGATT CTCTGGCTCTGGATCCGGCACGGACTTTACTTTGACAATTTCCTCTCTTCAGCCCGAA GATTTTGCAACCTACTACTGTCAGCAATATCTCTACCATCCAGCCACATTCGGACAGG GCACCAAAGTCGAAATCAAAAGAGGCGGCGGCGGCAGTGGCGGCGGGGGTTCAGGAGG CGGGGGTTCTGAAGTGCAACTCGTTGAAAGCGGAGGAGGGCTTGTCCAACCTGGCGGG TCACTGCGGTTGAGCTGCGCCGCAAGCGGATTCACCTTCTCAGACTCTTGGATCCATT GGGTGCGCCAGGCTCCCGGAAAAGGCTTGGAATGGGTTGCTTGGATTTCACCGTATGG CGGTTCCACATACTACGCTGACAGCGTTAAGGGTCGATTCACCATCTCTGCAGATACT TCAAAAAACACAGCCTACCTTCAGATGAATAGTTTGCGCGCCGAGGACACAGCGGTTT ATTATTGTGCCCGAAGACATTGGCCCGGCGGTTTCGACTACTGGGGGCAAGGTACGTT GGTGACTGTGAGCGCCGTAGATGAAGCAAAATCTTGTGACAAAACCCATACCTGCCCA CCATGCCCAGCCCCAGAACTTCTTGGCGGACCCTCTGTCTTCCTTTTCCCTCCGAAGC CCAAGGATACCCTGATGATCAGCCGAACCCCGGAGGTAACATGTGTGGTGGTCGATGT TAGCCATGAGGATCCTGAAGTCAAATTTAACTGGTATGTAGACGGTGTTGAGGTGCAC AACGCTAAAACTAAGCCCAGGGAGGAGCAGTACAACTCAACCTATCGCGTCGTATCTG TGCTTACCGTCCTGCATCAAGACTGGCTCAATGGTAAGGAATATAAATGTAAAGTGAG TAACAAGGCACTGCCAGCACCTATCGAAAAAACCATCTCAAAGGCGAAGGGACAGCCC AGGGAACCCCAGGTCTATACTCTGCCACCTTCTCGGGATGAATTGACCAAGAACCAAG TTAGCCTGACATGTCTGGTGAAAGGTTTCTATCCAAGCGATATAGCTGTCGAGTGGGA GTCCAATGGCCAACCTGAGAACAATTATAAGACCACCCCACCCGTTCTGGACAGCGAC GGATCCTTTTTCCTGTACTCAAAACTCACTGTCGATAAATCAAGATGGCAACAAGGCA ACGTTTTTAGCTGTAGCGTGATGCACGAAGCACTTCATAATCACTATACACAGAAGTC ACTCTCTCTTTCTCCAGGACACCACCATCATCACCACTGA	
SIRP1α-CD3-PDL1-Fc (SL)	ATGGAAACCGATACACTTCTGTTGTGGGTGCTGCTGCTGTGGGTCCCTGGTTCAACAG GCGATTATCCCTACGATGTGCCCGACTACGCAGGCGCTCAGCCAGCTGATGATATCCA GATGACACAGAGCCCATCATCTCTGTCTGCAAGCGTAGGAGACCGAGTCACCATTACA TGCAGAGCCTCCCAAGACGTTTCCACAGCAGTGGCCTGGTATCAGCAAAAACCTGGTA AGGCGCCCAAGCTTCTCATCTATTCAGCCAGTTTTCTGTATAGCGGCGTTCCCAGCCG ATTCTCTGGCTCTGGATCCGGCACGGACTTTACTTTGACAATTTCCTCTCTTCAGCCC GAAGATTTTGCAACCTACTACTGTCAGCAATATCTCTACCATCCAGCCACATTCGGAC AGGGCACCAAAGTCGAAATCAAAAGAGGCGGCGGCGGCAGTGGCGGCGGGGGTTCAGG AGGCGGGGGTTCTGAAGTGCAACTCGTTGAAAGCGTAGGAGGGCTTGTCCAACCTGGC GGGTCACTGCGGTTGAGCTGCGCCGCAAGCGGATTCACCTTCTCAGACTCTTGGATCC ATTGGGTGCGCCAGGCTCCCGGAAAAGGCTTGGAATGGGTTGCTTGGATTTCACCGTA TGGCGGTTCCACATACTACGCTGACAGCGTTAAGGGTCGATTCACCATCTCTGCAGAT ACTTCAAAAAACACAGCCTACCTTCAGATGAATAGTTTGCGCGCCGAGGACACAGCGG TTTATTATTGTGCCCTAAGACATTGGCCCGGCGGTTTCGACTACTGGGGGCAAGGTAC GTTGGTGACTGTGAGCGCCGTAGATGAAGCAAAATCTTGTGACAAAACCCATACCTGC CCACCATGCCCAGCCCCAGAACTTCTTGGCGTACCCTCTGTCTTCCTTTTCCCTCCGA AGCCCAAGGATACCCTGATGATCAGCCGAACCCCGGAGGTAACATGTGTGGTGGTCGA TGTTAGCCATGAGGATCCTGAAGTCAAATTTAACTGGTATGTAGACGGTGTTGAGGTG CACAACGCTAAAACTAAGCCCAGGGAGGAGCAGTACAACTCAACCTATCGCGTCGTAT CTGTGCTTACCGTCCTGCATCAAGACTGGCTCAATGGTAAGGAATATAAATGTAAAGT GAGTAACAAGGCACTGCCAGCACCTATCGAAAAAACCATCTCAAAGGCGAAGGGACAG CCCAGGGAACCCCAGGTCTATACTCTGCAACCTTCTCGGGATGAATTGACCAAGAACC AAGTTAGCCTGACATGTCTGGTGAAAGGTTTCTATCCAAGCGATATAGCTGTCGAGTG GGAGTCCAATGGCCAACCTGAGAACAATTATAAGACCACCCCACCCGTTCTGGACAGC GACGGATCCTTTTTCCTGTACTCAAAACTCACTGTCGATAAATCAAGATGGCAACAAG GCAACGTTTTTAGCTGTAGCGTGATGCACGAAGCACTTCATAATCACTATACACAGAA GTCACTCTCTCTTTCTCCAGGAAAGGTTGACGAACAGAAATTGATATCCGAGGAAGAT	67

	CTCAATAGGAGGAAGAGAGAAGGCAGGGGGAGCCTTCTCACTTGCGGCGATGTCGAGG AAAATCCGGGGCCTATGGAGACCGATACCCTGCTCTTGTGGGTTTTGCTTCTTTGGGT GCCAGGATCTACAGGTGATGAAGAAGAATTGCAGATCATCCAACCAGACAAATCCGTA CTCGTGGCCGCAGGAGAGACCGCTACCCTCAGATGTACCATCACTTCTCTCTTCCCCG TTGGCCCCATCCAGTGGTTTCGAGGCGCAGGACCAGGACGAGTGCTTATTTACAATCA ACGACAGGGCCCATTCCCAAGAGTGACAACAGTATCCGATACCACCAAGCGCAATAAT ATGGACTTTAGCATTAGAATCGGCAACATAACACCCGCTGACGCCGGTACATACTATT GTATTAAATTTCGAAAGGGCTCACCAGACGACGTGGAATTTAAGTCAGGGGCCGGAAC CGAACTCTCAGTTAGAGCAAAACCTTCTGCTAGCGACATCAAGCTGCAGCAGAGCGGC GCCGAGCTGGCCAGGCCCGGCGCCAGCGTGAAGATGAGCTGCAAGACCAGCGGCTACA CCTTCACCAGGTACACCATGCACTGGGTGAAGCAGAGGCCCGGCCAGGGCCTGGAGTG GATCGGCTACATCAACCCCAGCAGGGGCTACACCAACTACAACCAGAAGTTCAAGGAC AAGGCCACCCTGACCACCGACAAGAGCAGCAGCACCGCCTACATGCAGCTGAGCAGCC TGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGGTACTACGACGACCACTACTG CCTGGACTACTGGGGCCAGGGCACCACCCTGACCGTGAGCAGCGTGGAGGGCGGCAGC GGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCGTGGACGACATCCAGCTGACCCAGA GCCCCGCCATCATGAGCGCCAGCCCCGGCGAGAAGGTGACCATGACCTGCAGGGCCAG CAGCAGCGTGAGCTACATGAACTGGTACCAGCAGAAGAGCGGCACCAGCCCCAAGAGG TGGATCTACGACACCAGCAAGGTGGCCAGCGGCGTGCCCTACAGGTTCAGCGGCAGCG GCAGCGGCACCAGCTACAGCCTGACCATCAGCAGCATGGAGGCCGAGGACGCCGCCAC CTACTACTGCCAGCAGTGGAGCAGCAACCCCCTGACCTCCGGCGCCGGCACCAAGCTG GAGCTGAAGCACCACCATCATCACCACTGA	
SIRP1α-CD3-PDL1-Fc (LL)	ATGGAAACCGATACACTTCTGTTGTGGGTGCTGCTGCTGTGGGTCCCTGGTTCAACAG GCGATTATCCCTACGATGTGCCCGACTACGCAGGCGCTCAGCCAGCTGATGATATCCA GATGACACAGAGCCCATCATCTCTGTCTGCAAGCGTAGGAGACCGAGTCACCATTACA TGCAGAGCCTCCCAAGACGTTTCCACAGCAGTGGCCTGGTATCAGCAAAAACCTGGTA AGGCGCCCAAGCTTCTCATCTATTCAGCCAGTTTTCTGTATAGCGGCGTTCCCAGCCG ATTCTCTGGCTCTGGATCCGGCACGGACTTTACTTTGACAATTTCCTCTCTTCAGCCC GAAGATTTTGCAACCTACTACTGTCAGCAATATCTCTACCATCCAGCCACATTCGGAC AGGGCACCAAAGTCGAAATCAAAAGAGGCGGCGGCGGCAGTGGCGGCGGGGGTTCAGG AGGCGGGGGTTCTGAAGTGCAACTCGTTGAAAGCGTAGGAGGGCTTGTCCAACCTGGC GGGTCACTGCGGTTGAGCTGCGCCGCAAGCGGATTCACCTTCTCAGACTCTTGGATCC ATTGGGTGCGCCAGGCTCCCGGAAAAGGCTTGGAATGGGTTGCTTGGATTTCACCGTA TGGCGGTTCCACATACTACGCTGACAGCGTTAAGGGTCGATTCACCATCTCTGCAGAT ACTTCAAAAAACACAGCCTACCTTCAGATGAATAGTTTGCGCGCCGAGGACACAGCGG TTTATTATTGTGCCCTAAGACATTGGCCCGGCGGTTTCGACTACTGGGGGCAAGGTAC GTTGGTGACTGTGAGCGCCGTAGATGAAGCAAAATCTTGTGACAAAACCCATACCTGC CCACCATGCCCAGCCCCAGAACTTCTTGGCGTACCCTCTGTCTTCCTTTTCCCTCCGA AGCCCAAGGATACCCTGATGATCAGCCGAACCCCGGAGGTAACATGTGTGGTGGTCGA TGTTAGCCATGAGGATCCTGAAGTCAAATTTAACTGGTATGTAGACGGTGTTGAGGTG CACAACGCTAAAACTAAGCCCAGGGAGGAGCAGTACAACTCAACCTATCGCGTCGTAT CTGTGCTTACCGTCCTGCATCAAGACTGGCTCAATGGTAAGGAATATAAATGTAAAGT GAGTAACAAGGCACTGCCAGCACCTATCGAAAAAACCATCTCAAAGGCGAAGGGACAG CCCAGGGAACCCCAGGTCTATACTCTGCAACCTTCTCGGGATGAATTGACCAAGAACC AAGTTAGCCTGACATGTCTGGTGAAAGGTTTCTATCCAAGCGATATAGCTGTCGAGTG GGAGTCCAATGGCCAACCTGAGAACAATTATAAGACCACCCCACCCGTTCTGGACAGC GACGGATCCTTTTTCCTGTACTCAAAACTCACTGTCGATAAATCAAGATGGCAACAAG GCAACGTTTTTAGCTGTAGCGTGATGCACGAAGCACTTCATAATCACTATACACAGAA GTCACTCTCTCTTTCTCCAGGAAAGGTTGACGAACAGAAATTGATATCCGAGGAAGAT CTCAATAGGAGGAAGAGAGAAGGCAGGGGGAGCCTTCTCACTTGCGGCGATGTCGAGG AAAATCCGGGGCCTATGGAGACCGATACCCTGCTCTTGTGGGTTTTGCTTCTTTGGGT GCCAGGATCTACAGGTGATGAAGAAGAATTGCAGATCATCCAACCAGACAAATCCGTA CTCGTGGCCGCAGGAGAGACCGCTACCCTCAGATGTACCATCACTTCTCTCTTCCCCG TTGGCCCCATCCAGTGGTTTCGAGGCGCAGGACCAGGACGAGTGCTTATTTACAATCA ACGACAGGGCCCATTCCCAAGAGTGACAACAGTATCCGATACCACCAAGCGCAATAAT ATGGACTTTAGCATTAGAATCGGCAACATAACACCCGCTGACGCCGGTACATACTATT GTATTAAATTTCGAAAGGGCTCACCAGACGACGTGGAATTTAAGTCAGGGGCCGGAAC CGAACTCTCAGTTAGAGCAAAACCTTCTGCTAGCGGCGGCGGCGGCAGCGACATCAAG CTGCAGCAGAGCGGCGCCGAGCTGGCCAGGCCCGGCGCCAGCGTGAAGATGAGCTGCA AGACCAGCGGCTACACCTTCACCAGGTACACCATGCACTGGGTGAAGCAGAGGCCCGG CCAGGGCCTGGAGTGGATCGGCTACATCAACCCCAGCAGGGGCTACACCAACTACAAC	69

	CAGAAGTTCAAGGACAAGGCCACCCTGACCACCGACAAGAGCAGCAGCACCGCCTACA TGCAGCTGAGCAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGGTACTA CGACGACCACTACTGCCTGGACTACTGGGGCCAGGGCACCACCCTGACCGTGAGCAGC GTGGAGGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCGTGGACGACA TCCAGCTGACCCAGAGCCCCGCCATCATGAGCGCCAGCCCCGGCGAGAAGGTGACCAT GACCTGCAGGGCCAGCAGCAGCGTGAGCTACATGAACTGGTACCAGCAGAAGAGCGGC ACCAGCCCCAAGAGGTGGATCTACGACACCAGCAAGGTGGCCAGCGGCGTGCCCTACA GGTTCAGCGGCAGCGGCAGCGGCACCAGCTACAGCCTGACCATCAGCAGCATGGAGGC CGAGGACGCCGCCACCTACTACTGCCAGCAGTGGAGCAGCAACCCCCTGACCTTCGGC GCCGGCACCAAGCTGGAGCTGAAGCACCACCACCACCACCACTAG	

[00225] Additional exemplarly embodiments of engager molecules include engager molecules comprising an activation domain comprising an anti-CD3 scFv (*e.g.*, comprised of SEQ ID NOs: 20 and 22) and a therapeutic domain comprising an scFv that binds to a cell surface protein such as CTLA4, TIM3, LAG3, BTLA, KIR, TIGIT, OX40, or GITR. In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes an engager molecules comprising an activation domain comprising an anti-CD3 scFv (*e.g.*, comprised of SEQ ID NOs: 20 and 22) and a therapeutic domain comprising an scFv that binds to a cell surface protein such as CTLA4, TIM3, LAG3, BTLA, KIR, TIGIT, OX40, CD47, or GITR, and a second nucleic acid sequence encoding a therapeutic molecule such as IL-15 (SEQ ID NO: 24), IL-12 (SEQ ID NOs: 26 and 28), CXCL10 (SEQ ID NO: 30), or MMP9 (SEQ ID NO: 34). In such embodiments, the engager molecule is linked to the therapeutic molecule polypeptide by a T2A self-cleaving peptide linker (SEQ ID NO: 14).

[00226] Additional exemplarly embodiments of engager molecules include engager molecules comprising an activation domain comprising an anti-CD3 scFv (*e.g.*, comprised of SEQ ID NOs: 20 and 22) and an antigen recognition domain comprising an scFv that binds to SLAMF7 (also known as CD319) or CD27 (either the membrane bound form of CD27 or the soluble form of CD27). In some embodiments, the oncolytic viruses described herein comprise a bicistronic or multicistronic nucleic acid sequence, wherein a first nucleic acid sequence encodes an engager molecules comprising an activation domain comprising an anti-CD3 scFv (*e.g.*, comprised of SEQ ID NOs: 20 and 22) and an antigen-recognition domain comprising an scFv that binds to a target cell antigen such as SLAMF7 or CD27, and a second nucleic acid sequence encoding a therapeutic molecule such as IL-15 (SEQ ID NO: 24), IL-12 (SEQ ID NOs: 26 and 28), CXCL10 (SEQ ID NO: 30), or MMP9 (SEQ ID NO: 34). In such embodiments, the engager molecule is linked to the therapeutic molecule polypeptide by a T2A self-cleaving peptide linker (SEQ ID NO: 14).

[00227] Additional cell surface proteins that are suitable for target by the engager molecules described herein are shown below in Table 5. Additional proteins that are suitable for use as therapeutic molecules are show below in Table 6.

Table 5: Cell-surface proteins suitable for targeting by engager molecules

Cell-surface protein	NCBI Reference Sequence (RefSeq) Identifier
human SLAMF7	NP_067004.3
human NKGD2L	NP_079494.1
human CTLA4	NP_005205.2
human TIM3	NP_116171.3
human LAG3	NP_002277.4
human BTLA (isoform 1 and 2, respectively)	NP_001078826.1; NP_861445.3
human KIR	
human TIGIT	NP_776160.2
human OX40	NP_003318.1
human GITR (isoform 1, 2, 3 respectively)	NP_004186.1; NP_683699.1; NP_683700.1
human CD27	NP_001233.1
human CD40 (isoforms 1-5, respectively)	NP_001241.1; NP_690593.1; NP_001289682.1; NP_001309350.1; NP_001309351.1
human NKGD2L	NP_079494.1
human CD200	NP_005935.4

Table 6: Proteins suitable for use as therapeutic molecules

Molecule	NCBI Reference Sequence (RefSeq) Identifier
human TNFα	NP_000585.2
human CX3CL1	NP_002987.1
human CCR4	NP_005499.1
human CSF-1	NP_000748.3
human TGFβ	NP_000651.3
human IL-7	NP_000871.1
human GM-CSF	NP_000749.2

Therapeutic Uses of Oncolytic Viruses

[00228] In some embodiments, the present invention provides compositions and methods of use for the prevention, treatment, and/or amelioration of a cancerous disease. In some embodiments, the methods described herein comprise administering an effective amount (*e.g.*, a therapeutically effective amount) of an oncolytic virus described herein to a subject in need thereof, wherein the virus expresses an engager molecule or an engager molecule and a therapeutic molecule.

[00229] In some embodiemnts, compositions and methods of the present invention are useful for all stages and types of cancer, including for minimal residual disease, early solid tumor, advanced solid tumor and/or metastatic solid tumor. In some embodiments, compositions and methods of the present invention are used to treat a variety of solid tumors associated with a number of different cancers. The term "solid tumors" refers to relapsed or refractory tumors as well as metastases (wherever located), other than metastatses observed in lymphatic cancer.

[00230] Exemplarly solid tumors include, but are not limited to, brain and other central nervous system tumors (*e.g.* tumors of the meninges, brain, spinal cord, cranial nerves and other parts of central nervous system, *e.g.* glioblastomas or medulla blastomas); head and/or neck cancer; breast tumors; circulatory system tumors (*e.g.* heart, mediastinum and pleura, and other intrathoracic organs, vascular tumors and tumor-associated vascular tissue); excretory system tumors (*e.g.* kidney, renal pelvis, ureter, bladder, other and unspecified urinary organs); gastrointestinal tract tumors (*e.g.* oesophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus and anal canal), tumors involving the liver and intrahepatic bile ducts, gall bladder, other and unspecified parts of biliary tract, pancreas, other and digestive organs); head and neck; oral cavity (lip, tongue, gum, floor of mouth, palate, and other parts of mouth, parotid gland, and other parts of the salivary glands, tonsil, oropharynx, nasopharynx, pyriform sinus, hypopharynx, and other sites in the lip, oral cavity and pharynx); reproductive system tumors (*e.g.* vulva, vagina, Cervix uteri, Corpus uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); respiratory tract tumors (*e.g.* nasal cavity and middle ear, accessory sinuses, larynx, trachea, bronchus and lung, *e.g.* small cell lung cancer or non-small cell lung cancer); skeletal system tumors (*e.g.* bone and articular cartilage of limbs, bone articular cartilage and other sites); skin tumors (*e.g.* malignant melanoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues including peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneum and peritoneum, eye and adnexa, thyroid, adrenal gland and other endocrine glands and related structures, secondary and unspecified malignant neoplasm of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasm of other sites, oligodendroglioma, oligoastrocytoma, astrocytoma, glioblastoma or medulloblastoma or other solid tumor.

[00231] In particular embodiments, the solid tumor is a brain tumor. In some instances, the brain tumor includes, but is not limited to, a glioma, in particular ependymoma, oligodendroglioma, oligoastrocytoma, astrocytoma, glioblastoma, or a medulloblastoma.

[00232] In some embodiments, compositions and methods of the present invention are used to treat a hematologic cancer. The term "hematologic cancer" refers herein to a cancer of the blood system and includes relapsed or refractory hematologic cancer as well as a metastasized hematologic cancer (wherever located). In some instances, the hematologic cancer is a T-cell malignancy or a B-cell malignancy. Exemplary T-cell malignancies include, but are not limited to, peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma (ATLL), blastic NK-cell lymphoma, enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphomas, or treatment-related T-cell lymphomas.

[00233] Exemplary B-cell malignancies include, but are not limited to, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, a non-CLL/SLL lymphoma, prolymphocytic leukemia (PLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis. In some cases, the hematologic cancer is a relapsed or refractory hematologic cancer. In some cases, the hematologic cancer is a metastasized hematologic cancer.

[00234] In some embodiments, the oncolytic virus is engineered to produce a high level of expression of the engager molecule and/or the therapeutic polypeptide prior to the death of the virally-infected cell, *e.g.*, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours of infection, or within 2, 3, 4, 5, or 6 days of infection. Expression of the engager molecule and/or the therapeutic polypeptide can be determined by methods known in the art, including Western blot, ELISA, immunoprecipitation, or electrophoresis, among others. In general, a "high level of expression" in reference to a therapeutic molecule refers to a level of expression that is greater than the basal level of expression of a corresponding polypeptide in a cell that is not infected with the oncolytic virus

Compositions and routes of administration

[00235] In some embodiments, a therapeutically effective amount of an oncolytic virus or compositions thereof are administered to a subject.In accordance with this disclosure, the term "pharmaceutical composition" relates to a composition for administration to an individual. Administration of the compositions described herein can be local or systemic and can be effected by different ways, *e.g.*, by intravenous, subcutaneous, intraperitoneal, intramuscular, topical or intradermal administration. In some embodiments, compositions disclosed herein are administered by any means known in the art. For example, the compositions described herein may be administered to a subject intravenously, intratumorally, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intrathecally, subcutaneously, subconjunctival, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion, via a catheter, via a lavage, in a cream, or in a lipid composition. In particular embodiments, the composition is administered to the individual via infusion or injection. In some embodiments, administration is parenteral, *e.g.*, intravenous. In some embodiments, the oncolytic virus or composition thereof is administered directly to the target site, *e.g.*, by biolistic delivery to an internal or external target site or by catheter to a site in an artery. In particular embodiments, the compositions described herein are administered subcutaneously or intravenously. In some embodiments, the oncolytic viruses or compositions thereof described herein are administered intravenously or intraarterially.

[00236] In a preferred embodiment, the compositions described herein are formulated for a particular route of administration, for parenteral, transdermal, intraluminal, intra-arterial, intrathecal, intravenous administration, or for direct injection into a cancer. In some embodiments, the compositions further comprise a pharmaceutically acceptable carrier. "Pharmaceutically or pharmacologically acceptable" refer herein to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate. In some embodiments, the pharmaceutical compositions of the present disclosure further comprise a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, buffer, stabilizing formulation, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc. Compositions comprising such carriers are formulated by well-known conventional methods. In some embodiments,

supplementary active ingredients are also incorporated into the compositions. For human administration, the compositions described herein are met with sterility, pyrogenicity, and general safety and purity standards as required by FDA Office of Biologics standards.

[00237] In some embodiments, the compositions described herein comprise a carrier such as a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity is maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms is brought about by various antibacterial and antifungal agents known in the art. In many cases, it is preferable to include isotonic agents, for example, sugars or sodium chloride. In some embodiments, prolonged absorption of the injectable compositions is brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00238] In some embodiments, the oncolytic viruses described herein are formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups are derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[00239] Pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In some cases, the form is sterile and is fluid. In some cases, it is stable under the conditions of manufacture and certain storage parameters (*e.g.* refrigeration and freezing) and is preserved against the contaminating action of microorganisms, such as bacteria and fungi. Aqueous compositions of some embodiments herein include an effective amount of a virus, nucleic acid, therapeutic protein, peptide, construct, stimulator, inhibitor, and the like, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Aqueous compositions of vectors expressing any of the foregoing are also contemplated.

[00240] In certain embodiments, biological material is extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready

formulation into a desired vehicle, where appropriate. In some embodiments, the active compounds or constructs are formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, intralesional, intranasal or intraperitoneal routes. Any route used for vaccination or boost of a subject is used. The preparation of an aqueous composition that contains an active component or ingredient is known to those of skill in the art in light of the present disclosure. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for use in preparing solutions or suspensions upon the addition of a liquid prior to injection is also prepared; and the preparations are also emulsified.

[00241] In some instances, the oncolytic virus is dispersed in a pharmaceutically acceptable formulation for injection. In some embodiments, sterile injectable solutions are prepared by incorporating the active compounds or constructs in the required amount in the appropriate solvent with any of the other ingredients enumerated above, as required, followed by filtered sterilization.

[00242] Upon formulation, the compositions described herein are administered in a manner compatible with disease to be treated and the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but also as slow release capsules or microparticles and microspheres and the like.

[00243] For parenteral administration in an aqueous solution, for example, the solution is suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intratumorally, intramuscular, subcutaneous and intraperitoneal administration. In this context, sterile aqueous media that is employed is known to those of skill in the art in light of the present disclosure. For example, one dosage is dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermolysis fluid or injected at the proposed site of infusion.

[00244] In addition to the compounds formulated for parenteral administration, such as intravenous, intratumorally, intradermal or intramuscular injection, other pharmaceutically acceptable forms include, *e.g.*, tablets or other solids for oral administration; liposomal formulations; time release capsules; biodegradable and any other form currently used.

[00245] In some embodiments, the viruses are encapsulated to inhibit immune recognition and placed at the site of a tumor.

[00246] In some instances, 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 replenishes, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives are also present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In addition, the pharmaceutical composition of the present disclosure might comprise proteinaceous carriers, like, *e.g.*, serum albumin or immunoglobulin, preferably of human origin. It is envisaged that the pharmaceutical composition of the disclosure might comprise, in addition to the proteinaceous bispecific single chain antibody constructs or nucleic acid molecules or vectors encoding the same (as described in this disclosure), further biologically active agents, depending on the intended use of the pharmaceutical composition.

[00247] In some embodiments, tumor-infiltrating virus-producing cells which continuously release vectors are formulated for direct implantation into a tumor in order to increase the viral oncolysis and the transfer efficiency of the therapeutic genes.

[00248] Intranasal formulations are known in the art and are described in, for example, U.S. Patent Nos. 4,476,116; 5,116,817; and 6,391,452. Formulations which are prepared according to these and other techniques well-known in the art are prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, for example, Ansel, H. C. et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, Sixth Ed. (1995). Preferably these compositions and formulations are prepared with suitable nontoxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of nasal dosage forms and some of these are found in Remington: The Science and Practice of Pharmacy, 21st edition, 2005, a standard reference in the field. The choice of suitable carriers is highly dependent upon the exact nature of the nasal dosage form desired, *e.g.*, solutions, suspensions, ointments, or gels. Nasal dosage forms generally contain large amounts of water in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, gelling agents, or buffering and other stabilizing and solubilizing agents are also present. The nasal dosage form is isotonic with nasal secretions.

[00249] For administration by inhalation described herein is in a form as an aerosol, a mist or a powder. Pharmaceutical compositions described herein are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with

the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit is determined by providing a valve to deliver a metered amount. Capsules and cartridges of, such as, by way of example only, gelatin for use in an inhaler or insufflator is formulated containing a powder mix of the compound described herein and a suitable powder base such as lactose or starch.

Therapeutically Effective Amounts and Therapeutic Regimens

[00250] In some embodiments, the oncolytic viruses and compositions thereof described herein are administered to a subject at therapeutically effective amount. The therapeutically effective amount will depend on the subject to be treated, the state (*e.g.*, general health) of the subject, the protection desired, the disease to be treated, the route of administration, and/or the nature of the virus. In some embodiments, the person responsible for administration (*e.g.*, an attending physician) will determine the appropriate dose for an individual. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, weight, body surface area, age, sex, and general health, the particular compound to be administered, the particular disease to be treated, timing and route of administration, and other drugs being administered concurrently. Therefore, it is expected that for each individual patient, even if the viruses that are administered to the population at large, each patient is monitored for the proper dosage for the individual, and such practices of monitoring a patient are routine in the art.

[00251] In some embodiments, the therapeutically effective amount of an oncolytic virus described herein is administered in a single dose. In some embodiments of the present invention, the pseudotyped oncolytic viruses or compositions thereof are administered to a subject at a dose ranging from about $1x10^{+5}$ pfu to about $1x10^{+15}$ pfu (plaque forming units), about $1x10^{+8}$ pfu to about $1x10^{+15}$ pfu, about $1x10^{+10}$ pfu to about $1x10^{+15}$ pfu, or about $1x10^{+8}$ pfu to about $1x10^{+12}$ pfu. For example, in some embodiments, the pseudotyped oncolytic viruses or compositions thereof are administered to a subject at a dose of about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} pfu of virus. In some embodiments, the dose depends, on the age of the subject to which a composition is being administered. For example, a lower dose may be required if the subject is juvenile, and a higher dose may be required if the subject is an adult human subject. In certain embodiments, for example, a juvenile subject receives about $1x10^{+8}$ pfu and about $1x10^{+10}$ pfu, while an adult human subject receives a dose between about $1x10^{+10}$ pfu and about $1x10^{+12}$ pfu. In some embodiments, the therapeutically effective amount of an

oncolytic virus described herein is administered over the course of two or more doses. In some embodiments, the two or more doses are administered simultaneously (*e.g.*, on the same day or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[00252] In some embodiments, the oncolytic viruses or compositions thereof described herein are administered to a subject once. In some embodiments, the oncolytic viruses or compositions thereof described herein are administered to a subject more than once. For example, a composition disclosed herein may be administered multiple times, including 1, 2, 3, 4, 5, 6, or more times. In some embodiments, a composition disclosed herein may be administered to a subject on a daily or weekly basis for a time period or on a monthly, bi-yearly, or yearly basis depending on need or exposure to a pathogenic organism or to a condition in the subject (*e.g.* cancer). In particular embodiments, the oncolytic viruses and compositions thereof are formulated in such a way, and administered in such and amount and/or frequency, that they are retained by the subject for extended periods of time.

[00253] In some embodiments, the pseudotyped oncolytic viruses or compositions thereof are administered for therapeutic applications or is administered as a maintenance therapy, such as for example, for a patient in remission. In some embodiments, the pseudotyped oncolytic viruses or compositions thereof are administered once every month, once every 2 months, once every 6 months, once a year, twice a year, three times a year, once every two years, once every three years, or once every five years.

[00254] Im some embodiments wherein a patient's status does improve, the pseudotyped oncolytic viruses or compositions thereof may be administered continuously upon the doctor's discretion. In some embodiments, the dose composition is temporarily reduced and/or administration of the composition is temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some embodiments, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, or 100 %.

[00255] In some embodiments, once improvement of a patient's conditions has occurred, a maintenance dose may be administered if necessary. In some embodiments, the dosage and/or the frequency of administration of the composition is reduced, as a function of the

symptoms, to a level at which the improved disease, disorder or condition is retained. In some embodiments, patients may require intermittent treatment on a long-term basis upon any recurrence of symptoms.

[00256] In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD_{50} (the dose lethal to 50 % of the population) and the ED_{50} (the dose therapeutically effective in 50 % of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it is expressed as the ratio between LD_{50} and ED_{50}. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with minimal toxicity. The dosage varies within this range depending upon the dosage form employed and the route of administration utilized.

[00257] In some instances, tumor antigen expression levels are evaluated to assess the progress of treatment in a patient, to stratify a patient, and/or to modulate a therapeutic regimen. In some instances, assessment of antigen expression levels include the use of immunohistochemistry (IHC) (including semi-quantitative or quantitative IHC) or other antibody-based assays (Western blot, fluorescent immunoassay (FIA), fluorescence in situ hybridization (FISH), radioimmunoassay (RIA), radioimmunoprecipitation (RIP), enzyme-linked immunosorbent assay (ELISA), immunoassay, immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, gel electrophoresis), or indirectly by quantitating the transcripts for these genes (*e.g.* by *in situ* hybridization, nuclease protection, Northern blot, polymerase chain reaction (PCR) including reverse transcriptase PCR (RT-PCR)). In some instances, cells, for example, lymphocytes, are analyzed using FACs technology or paraffin embedded tumor sections using antibodies.

[00258] In some instances, antibodies are used to characterize the protein content of target cells through techniques such as immunohistochemistry, ELISAs and Western blotting. In some cases, this provides a screen *e.g.* for the presence or absence of a subject likely to respond favorably to oncolytic virus therapy and/or a need for co-administering an immune stimulating agent with an oncolytic virus.

[00259] In some embodiments, immunohistochemistry is performed on a sample of tissue from a biopsy. In some cases, the sample is examined fresh or frozen. In some instances, antibodies against antigens presented in the cell are added to the sample on a slide and the antibodies bind wherever the antigens are present. In some embodiments, excess antibody is then

washed away. In some cases, the antibodies that remain bound to the cell are further labeled by a secondary antibody for visualization under a microscope.

[00260] In some embodiments, test samples are obtained from a subject such as for example, from tissue (*e.g.* tumor biopsy), cerebrospinal fluid (CSF), lymph, blood, plasma, serum, peripheral blood mononuclear cells (PBMCs), lymph fluid, lymphocytes, synovial fluid and urine. In particular embodiments, the test sample is obtained from CSF or tumor tissue. In other particular embodiments, the test sample is obtained from tumor tissue and *e.g.* the relative number of $CD4^+$ and/or $CD8^+$ cells in the sample is determined and/or the level of one or more Thl and/or Th2 cytokines in the sample is measured *e.g.* by immunofluorescent staining of fixed and permeabilized cells from the sample with antibodies against the Thl and/or Th2 cytokines. In other particular embodiments, the test sample is obtained from blood and *e.g.* the level of one or more Thl and/or Th2 cytokines in the sample is measured by ELISA.

Combination Therapy

[00261] In some embodiments, the viruses, expression constructs, nucleic acid molecules and/or vectors described herein are administered in combination with another therapeutic agent. In some embodiments, the oncolytic viruses and an adddtional thereapeutic agent are formulated in the same compositions. In such embodiments, the composition may further comprise a pharmaceutically acceptable carrier or excipient. In some embodiments, the oncolytic viruses and an additional thereapeutic agent are formulated in separate compositions (*e.g.*, two or more compositions suitable for administration to patient or subject). The disclosure further encompasses co-administration protocols with other cancer therapies, *e.g.* bispecific antibody constructs, targeted toxins or other compounds, including those which act via immune cells, including T-cell therapy. The clinical regimen for co-administration of the inventive composition(s) encompass(es) co-administration at the same time, before and/or after the administration of the other component. Particular combination therapies include chemotherapy, radiation, surgery, hormone therapy, and/or other types of immunotherapy.In some embodiments, a therapeutically effective amount of a pseudotyped oncolytic virus is administered to a subject in need thereof in combination with an additional therapeutic agent. In some instances, the additional therapeutic agent is a chemotherapeutic agent, a steroid, an immunotherapeutic agent, a targeted therapy, or a combination thereof.

[00262] In some embodiments, pharmaceutical compositions are administered in conjuction with an adjuvant therapy. For examples, activating adjuvant treatments are administered prior to, contemporaneous with, or after one or more administrations (e.g.,

intratumoral injection of the pseudotyped virus). For example, adjuvant therapy includes modulation of Toll-like receptor (TLR) ligands, such as TLR9 activation by DNA molecules comprising CpG sequences, or TLR9 activation (e.g., by RNA ligands). Other adjuvant treatments include agonizing antibodies or other polypeptides (e.g., activation of CD40 or GITR by CD40 Ligand (CD40L) or GITR Ligand (GITRL), respectively). Further, provided are cyclic dinucleotides (e.g., c-di-GMP) that modulate STING. Another activating adjuvant includes interleukins such as IL-33.

[00263] In some embodiments, the additional therapeutic agent comprises an agent selected from: bendamustine, bortezomib, lenalidomide, idelalisib (GS-1101), vorinostat, everolimus, panobinostat, temsirolimus, romidepsin, vorinostat, fludarabine, cyclophosphamide, mitoxantrone, pentostatine, prednisone, etopside, procarbazine, and thalidomide.

[00264] In some embodiments, the additional therapeutic agent is a multi-agent therapeutic regimen. In some embodiments the additional therapeutic agent comprises the HyperCVAD regimen (cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with methotrexate and cytarabine). In some embodiments, the HyperCVAD regimen is administered in combination with rituximab.

[00265] In some embodiments the additional therapeutic agent comprises the R-CHOP regiment (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).

[00266] In some embodiments the additional therapeutic agent comprises the FCR regimen (FCR (fludarabine, cyclophosphamide, rituximab).

[00267] In some embodiments the additional therapeutic agent comprises the FCMR regimen (fludarabine, cyclophosphamide, mitoxantrone, rituximab).

[00268] In some embodiments the additional therapeutic agent comprises the FMR regimen (fludarabine, mitoxantrone, rituximab).

[00269] In some embodiments the additional therapeutic agent comprises the PCR regimen (pentostatin, cyclophosphamide, rituximab).

[00270] In some embodiments the additional therapeutic agent comprises the PEPC regimen (prednisone, etoposide, procarbazine, cyclophosphamide).

[00271] In some embodiments the additional therapeutic agent comprises radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan or ^{131}I-tositumomab.

[00272] In some embodiments, the additional therapeutic agent is an autologous stem cell transplant.

[00273] In some embodiments, the additional therapeutic agent is selected from: nitrogen mustards such as for example, bendamustine, chlorambucil, chlormethine,

cyclophosphamide, ifosfamide, melphalan, prednimustine, trofosfamide; alkyl sulfonates like busulfan, mannosulfan, treosulfan; ethylene imines like carboquone, thiotepa, triaziquone; nitrosoureas like carmustine, fotemustine, lomustine, nimustine, ranimustine, semustine, streptozocin; epoxides such as for example, etoglucid; other alkylating agents such as for example dacarbazine, mitobronitol, pipobroman, temozolomide; folic acid analogues such as for example methotrexate, permetrexed, pralatrexate, raltitrexed; purine analogs such as for example cladribine, clofarabine, fludarabine, mercaptopurine, nelarabine, tioguanine; pyrimidine analogs such as for example azacitidine, capecitabine, carmofur, cytarabine, decitabine, fluorouracil, gemcitabine, tegafur; vinca alkaloids such as for example vinblastine, vincristine, vindesine, vinflunine, vinorelbine; podophyllotoxin derivatives such as for example etoposide, teniposide; colchicine derivatives such as for example demecolcine; taxanes such as for example docetaxel, paclitaxel, paclitaxel poliglumex; other plant alkaloids and natural products such as for example trabectedin; actinomycines such as for example dactinomycin; antracyclines such as for example aclarubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, pirarubicin, valrubicin, zorubincin; other cytotoxic antibiotics such as for example bleomycin, ixabepilone, mitomycin, plicamycin; platinum compounds such as for example carboplatin, cisplatin, oxaliplatin, satraplatin; methylhydrazines such as for example procarbazine; sensitizers such as for example aminolevulinic acid, efaproxiral, methyl aminolevulinate, porfimer sodium, temoporfin; protein kinase inhibitors such as for example dasatinib, erlotinib, everolimus, gefitinib, imatinib, lapatinib, nilotinib, pazonanib, sorafenib, sunitinib, temsirolimus; other antineoplastic agents such as for example alitretinoin, altretamine, amzacrine, anagrelide, arsenic trioxide, asparaginase, bexarotene, bortezomib, celecoxib, denileukin diftitox, estramustine, hydroxycarbamide, irinotecan, lonidamine, masoprocol, miltefosein, mitoguazone, mitotane, oblimersen, pegaspargase, pentostatin, romidepsin, sitimagene ceradenovec, tiazofurine, topotecan, tretinoin, vorinostat; estrogens such as for example diethylstilbenol, ethinylestradiol, fosfestrol, polyestradiol phosphate; progestogens such as for example gestonorone, medroxyprogesterone, megestrol; gonadotropin releasing hormone analogs such as for example buserelin, goserelin, leuprorelin, triptorelin; anti-estrogens such as for example fulvestrant, tamoxifen, toremifene; anti-androgens such as for example bicalutamide, flutamide, nilutamide, enzyme inhibitors, aminoglutethimide, anastrozole, exemestane, formestane, letrozole, vorozole; other hormone antagonists such as for example abarelix, degarelix; Immunostimulants such as for example histamine dihydrochloride, mifamurtide, pidotimod, plerixafor, roquinimex, thymopentin; immunosuppressants such as for example everolimus, gusperimus, leflunomide, mycophenolic acid, sirolimus; calcineurin inhibitors such as for example ciclosporin, tacrolimus;

other immunosuppressants such as for example azathioprine, lenalidomide, methotrexate, thalidomide; and Radiopharmaceuticals such as for example, iobenguane.

[00274] In some embodiments, the additional therapeutic agent is selected from: interferons, interleukins, tumor necrosis factors, growth factors, or the like.

[00275] In some embodiments, the additional therapeutic agent is selected from: ancestim, filgrastim, lenograstim, molgramostim, pegfilgrastim, sargramostim; Interferons such as for example IFNα natural, IFN α-2a, IFN α-2b, IFN alfacon-1, IFN α-n1, IFN βnatural, IFN β-1a, IFN β-1b, IFN γ, peginterferon α-2a, peginterferon α-2b; interleukins such as for example aldesleukin, oprelvekin; other immunostimulants such as for example BCG vaccine, glatiramer acetate, histamine dihydrochloride, immunocyanin, lentinan, melanoma vaccine, mifamurtide, pegademase, pidotimod, plerixafor, poly I:C, poly ICLC, roquinimex, tasonermin, thymopentin; Immunosuppressants such as for example abatacept, abetimus, alefacept, antilymphocyte immunoglobulin (horse), antithymocyte immunoglobulin (rabbit), eculizumab, efalizumab, everolimus, gusperimus, leflunomide, muromab-CD3, mycophenolic acid, natalizumab, sirolimus; TNFα inhibitors such as for example adalimumab, afelimomab, certolizumab pegol, etanercept, golimumab, infliximab; Interleukin Inhibitors such as for example anakinra, basiliximab, canakinumab, daclizumab, mepolizumab, rilonacept, tocilizumab, ustekinumab; calcineurin inhibitors such as for example ciclosporin, tacrolimus; other immunosuppressants such as for example azathioprine, lenalidomide, methotrexate, thalidomide.

[00276] In some embodiments, the additional therapeutic agent is selected from: Adalimumab, Alemtuzumab, Basiliximab, Bevacizumab, Cetuximab, Certolizumab pegol, Daclizumab, Eculizumab, Efalizumab, Gemtuzumab, Ibritumomab tiuxetan, Infliximab, Muromonab-CD3, Natalizumab, Panitumumab, Ranibizumab, Rituximab, Tositumomab, Trastuzumab, or the like, or a combination thereof.

[00277] In some embodiments, the additional therapeutic agent is selected from: monoclonal antibodies such as for example alemtuzumab, bevacizumab, catumaxomab, cetuximab, edrecolomab, gemtuzumab, panitumumab, rituximab, trastuzumab; Immunosuppressants, eculizumab, efalizumab, muromab-CD3, natalizumab; TNF alpha Inhibitors such as for example adalimumab, afelimomab, certolizumab pegol, golimumab, infliximab; Interleukin Inhibitors, basiliximab, canakinumab, daclizumab, mepolizumab, tocilizumab, ustekinumab; Radiopharmaceuticals, ibritumomab tiuxetan, tositumomab; additional monoclonal antibodies such as for example abagovomab, adecatumumab, alemtuzumab, anti-CD30 monoclonal antibody Xmab2513, anti-MET monoclonal antibody MetMab, apolizumab, apomab, arcitumomab, basiliximab, bispecific antibody 2B1,

blinatumomab, brentuximab vedotin, capromab pendetide, cixutumumab, claudiximab, conatumumab, dacetuzumab, denosumab, eculizumab, epratuzumab, epratuzumab, ertumaxomab, etaracizumab, figitumumab, fresolimumab, galiximab, ganitumab, gemtuzumab ozogamicin, glembatumumab, ibritumomab, inotuzumab ozogamicin, ipilimumab, lexatumumab, lintuzumab, lintuzumab, lucatumumab, mapatumumab, matuzumab, milatuzumab, monoclonal antibody CC49, necitumumab, nimotuzumab, oregovomab, pertuzumab, ramacurimab, ranibizumab, siplizumab, sonepcizumab, tanezumab, tositumomab, trastuzumab, tremelimumab, tucotuzumab celmoleukin, veltuzumab, visilizumab, volociximab, zalutumumab.

[00278] In some embodiments, the additional therapeutic agent is selected from: agents that affect the tumor micro-enviroment such as cellular signaling network (*e.g.* phosphatidylinositol 3-kinase (PI3K) signaling pathway, signaling from the B-cell receptor and the IgE receptor). In some embodiments, the additional therapeutic agent is a PI3K signaling inhibitor or a syc kinase inhibitor. In one embodiment, the syk inhibitor is R788. In another embodiment is a PKCγ inhibitor such as by way of example only, enzastaurin.

[00279] Examples of agents that affect the tumor micro-environment include PI3K signaling inhibitor, syc kinase inhibitor, protein kinase inhibitors such as for example dasatinib, erlotinib, everolimus, gefitinib, imatinib, lapatinib, nilotinib, pazonanib, sorafenib, sunitinib, temsirolimus; other angiogenesis inhibitors such as for example GT-111, JI-101, R1530; other kinase inhibitors such as for example AC220, AC480, ACE-041, AMG 900, AP24534, Arry-614, AT7519, AT9283, AV-951, axitinib, AZD1152, AZD7762, AZD8055, AZD8931, bafetinib, BAY 73-4506, BGJ398, BGT226, BI 811283, BI6727, BIBF 1120, BIBW 2992, BMS-690154, BMS-777607, BMS-863233, BSK-461364, CAL-101, CEP-11981, CYC116, DCC-2036, dinaciclib, dovitinib lactate, E7050, EMD 1214063, ENMD-2076, fostamatinib disodium, GSK2256098, GSK690693, INCB18424, INNO-406, JNJ-26483327, JX-594, KX2-391, linifanib, LY2603618, MGCD265, MK-0457, MK1496, MLN8054, MLN8237, MP470, NMS-1116354, NMS-1286937, ON 01919.Na, OSI-027, OSI-930, Btk inhibitor, PF-00562271, PF-02341066, PF-03814735, PF-04217903, PF-04554878, PF-04691502, PF-3758309, PHA-739358, PLC3397, progenipoietin, R547, R763, ramucirumab, regorafenib, RO5185426, SAR103168, SCH 727965, SGI-1176, SGX523, SNS-314, TAK-593, TAK-901, TKI258, TLN-232, TTP607, XL147, XL228, XL281RO5126766, XL418, XL765.

[00280] In some embodiments, the additional therapeutic agent is selected from: inhibitors of mitogen-activated protein kinase signaling, *e.g.*, U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002; Syk inhibitors; mTOR inhibitors; and antibodies (*e.g.*, rituxan).

[00281] In some embodiments, the additional therapeutic agent is selected from: 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9- dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-such as for example growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin;

lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1 -based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D;

spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[00282] In some embodiments, the additional therapeutic agent is selected from: alkylating agents, antimetabolites, natural products, or hormones, *e.g.*, nitrogen mustards (*e.g.*, mechloroethamine, cyclophosphamide, chlorambucil, etc.), alkyl sulfonates (*e.g.*, busulfan), nitrosoureas (*e.g.*, carmustine, lomusitne, ete.), or triazenes (decarbazine, etc.). Examples of antimetabolites include but are not limited to folic acid analog (*e.g.*, methotrexate), or pyrimidine analogs (*e.g.*, Cytarabine), purine analogs (*e.g.*, mercaptopurine, thioguanine, pentostatin).

[00283] In some embodiments, pharmaceutical compositions are administered in conjuction with an adjuvant therapy. For examples, activating adjuvant treatments are administered prior to, contemporaneous with, or after one or more administrations (*e.g.*, intratumoral injection of the pseudotyped virus). For example, adjuvant therapy includes modulation of Toll-like receptor (TLR) ligands, such as TLR9 activation by DNA molecules comprising CpG sequences, or TLR9 activation (*e.g.*, by RNA ligands). Other adjuvant treatments include agonizing antibodies or other polypeptides (*e.g.*, activation of CD40 or GITR by CD40 Ligand (CD40L) or GITR Ligand (GITRL), respectively). Further, provided are cyclic dinucleotides (*e.g.*, c-di-GMP) that modulate STING. Another activating adjuvant includes interleukins such as IL-33. In some instances, the pharmaceutical compositions described herein are administered in conjuction with an adjuvant therapy.

Kits

[00284] In some embodiments, the present invention provides kits comprising one or more oncolytic viruses as described herein, a nucleic acid sequence as described herein, a

vector as described herein, and/or a host cell as described herein. In some embodiments, the kits comprise a pharmaceutical composition as described herein above, either alone or in combination with further therapeutic agents to be administered to an individual in need thereof.

[00285] In some embodiments, the present invention provides kits for the use of vectors and virus-producing cells according to the invention as drugs in therapeutic methods. In particular, the vectors and virus producing cells according to some embodiments of the invention are used for the therapy or treatment of solid tumors in a subject. In some embodiments, the therapeutic effect is caused by the oncolytic properties of the recombinant vectors and viruses as well as by the use of therapeutic genes.

[00286] In some embodiments, the present invention provides kits for use with methods and compositions. Some embodiments concern kits having vaccine compositions of use to reduce onset of or treat subjects having one or more solid tumors. Other embodiments concern kits for making and using molecular constructs described herein. In some instances, kits also include a suitable container, for example, vials, tubes, mini- or microfuge tubes, test tube, flask, bottle, syringe or other container. Where an additional component or agent is provided, the kit contains one or more additional containers into which this agent or component is placed. Kits herein also include a means for containing the constructs, vaccine compositions and any other reagent containers in close confinement for commercial sale. Such containers include injection or blow-molded plastic containers into which the desired vials are retained. Optionally, one or more additional agents such as other anti-viral agents, anti-fungal or anti-bacterial agents are needed for compositions described, for example, for compositions of use as a vaccine.

[00287] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

EXAMPLES

[00288] The examples below further illustrate the described embodiments without limiting the scope of the invention.

Example 1: Preparation of pseudotyped VSV-G

[00289] The following protocol was adopted to prepare an exemplary pseudotyped VSV-G, by combining VSV-Glycoprotein (VSV-GP) with HIV1-gag and rev proteins.

[00290] *Cell culture and transfection:* DNA of the following packaging plasmids was mixed and prepared for transfection into 293T cells: pMDLg/pRRE expressing HIV-1 GAG/POL; pRSV/REV expressing HIV-1 REV; and pMD2.G 5 60 5.8 VSV glycoprotein. The DNA mix was added to 500 µL of pre-warmed Optimem II medium. A working stock of polyethyleneimine transfection reagent (PEI) was prepared at 1 µg/µL in 1xPBS, pH 4.5, and 88 µL of the working stock was added to the mixture, maintaining a 4:1 v/w ratio of PEI:DNA. The mixture was vortexed briefly and left for 5-10 min at room temperature to form a PEI:DNA transfection complex. A total of 2.5 x 10^6 low passage (less than P20) 293T cells were seeded per 15 cm dish in 15 mL DMEM supplemented with 10% serum and 1% Pen/Strep. 2 hours prior to transfection, the cell culture medium was aspirated and replaced with 15 mL of fresh pre-warmed growth medium (GM). The transfection complex was then added drop-wise to each 15 cm plate, swirled briefly to mix and incubated for 8 hrs in 10% CO_2, 35° C. After 8 hours, the medium was replaced with 10 mL of fresh growth medium containing 25 mM HEPES and 10% serum. The mixture was then incubated for 48 hrs post-transfection.

[00291] *Virus collection:* The medium from each dish was removed, pooled, and filtered through a 0.22 µm low protein binding/fast flow filter unit and stored at 4° C. A 5 mL volume of fresh growth medium was added to each dish and incubated overnight at 4° C (60-72 hours post transfection). The second lot of medium from each dish was collected, as in the previous step, and pooled with previous media harvest. The plasmid carry-over is removed by digestion with DNASE-I (1 mg/mL stock). A 1 µg/mL solution the viral supernatant, supplemented with 1 µL of 1M $MgCl_2$, was incubated at room temperature for 30 min followed by 2-4 hrs at 4° C. The filtered supernatants can be used directly on cultured cells, or aliquoted and stored at -80° C. The pseudotyped VSV-G viral supernatant can be optionally concentrated and purified.

Example 2: Construction of pseudotyped VSV-G expressing a CD28-CA125 bispecific antibody engager molecule

[00292] Pseudotyped VSV-G is prepared as described in Example 1 and further processed to express a nucleic acid encoding an engager polypeptide comprising an activation domain comprising an anti-CD28 molecule and an antigen recognition domain comprising an anti-CA125 molecule, and a nucleic acid encoding an anti-PD1 immune modulatory peptide. The resulting oncolytic virus is a pseudotyped onlcolytic VSV-G virus encoding a CD28-CA125 engager molecule and an anti-PD1 therapeutic molecule (CD28-CA125-PD1 VSV-G).

Example 3: CD28-CA125-PD1 VSV-G activates human T cells and exhibits anti-tumor activity

[00293] Human T cells are infected with the pseudotyped CD28-CA125-PD1 VSV-G virus. 24 hrs to 48 hrs post viral infection, the T cell culture medium is collected and checked for the presence of proinflammatory cytokines. These results will show that T cells are activated by CD28-CA125-PD1 VSV-G, as evidenced by presence of proinflammatory cytokines such as IFN-β and IL-2 in the cell culture supernatant of CD28-CA125-PD1 VSV-G infected human T cells.

[00294] EphA2-overexpressing gastric cancer cells, from KATO3 cell line, are infected with pseudotyped CD28-CA125-PD1 VSV-G or non-pseudotyped CD28-CA125-PD1 VSV virus and the cell proliferation is assessed. These results will show that cell proliferation is significantly reduced in cells KATO3 cells infected with pseudotyped CD28-CA125-PD1 VSV-G compared to KATO3 cells infected with non-pseudotyped CD28-CA125-PD1 VSV virus.

Example 4: CD19-CD3, SIRP1α-CD3, and PDL1-CD3-Fc engager molecules specifically bind to T-cells via CD3

[00295] The binding of bipartite (CD19-CD3 and SIRP1α-CD3) and triparte (PDL1-CD3-Fc) engager molecules to T cells was assessed. Briefly, 25,000 T cells were stimulated with 200 U/mL IL-2 for 12 days. After 12 days, T cell were incubated with varying concentrations of engager molecules (500, 1000, or 2000 ng/mL for CD19-CD3 and SIRP1α-CD3; neat supernatant for PDL1-CD3-Fc) for 20 minutes at room temperature in triplicate. Cells were then washed twice, followed by staining with an anti-6XHis APC antibody at 500 ng/mL for an additional 20 minutes. Cells were washed again and treated with propidium iodide (PI) to exclude dead cells from further analysis. Stained cells were analyzed by flow cytometry on a BD LSR Fortesa cytometer and the percentage of the cell population positive for staining was set at 2% of the secondary only control.

[00296] Results for CD19-CD3 (FIG. 19A), SIRP1α-CD3 (FIG. 19B), and PDL1-CD3-Fc (FIG. 19C) show that the CD3 binding moiety of each of these molecules functional binds to CD3-expressing 293F T cells, as indicated by an increase in the percentage of cells that are positive for the engager molecules compared to the secondary antibody alone. In particular, a dose dependent increase in the % positive cells is observed for CD19-CD3 (FIG. 19A), while the SIRP1α-CD3 construct demonstrated maximal binding at all concentrations. The amount of the neat PDL1-CD3-Fc supernatant used resulted in binding of the construct to the majority of T cells (FIG. 19C).

[00297] The results of this experiment are quantified in FIG. 20. In particular, all of the constructs demonstrated a significant increase in the % positive T cells compared to samples where no engager molecule was added.

[00298] Additional experiments demonstrated that the binding of the CD19-CD3, SIRP1α-CD3, and PDL1-CD3-Fc was mediated by interactions of the anti-CD3 domain of the engager molecules with CD3 expressed by the T cells. Prior to exposure of T cells to the engager molecules, the T cells were incubated with an anti-CD3 monoclonal antibody (OKT3). Preincubation with the OKT3 inhibited binding of the CD19-CD3 engager, and substantially reduced binding of the PDL1-CD3-Fc engager. The lack of inhibition of binding of the SIRP1α-CD3 engager by preincubation with OKT3 (FIG. 21C) is likely due to an incomplete inhibition of CD3 by OKT3 in these samples.

Example 5: SIRP1α-CD3 constructs specifically bind to CD47

[00299] Experiments were performed to determine the binding specificity of the SIRP1α-CD3 engager constructs. Raji cells were preincubated with SIRP1α-CD3 engagers for 20 min at RT. Cells were then washed and incubated with a fluorescently labelled anti-CD47 monoclonal antibody for 20 min at RT, after which cells were washed and analyzed by flow cytometry. Raji cells that were not preincubated with the SIRP1α-CD3 engager showed significant binding of the anti-CD47 monoclonal antibody (FIG. 22, IgG control histogram vs. the anti-CD47 histogram). Preincubation of Raji cells with the SIRP1α-CD3 engager blocked binding of the anti-CD47 monoclonal antibody (FIG. 22, anti-CD47 histogram vs. anti-CD47 +SIRP1α-CD3 histograph).

Example 6: Binding of SIRP1α-CD3 and CD19-CD3 engager molecules to target cells

[00300] Experiments were performed to determine the ability of SIRP1α-CD3 and CD19-CD3 BiTEs to bind to Raji ($CD19^+CD47^+$, FIG. 23), U2OS ($CD19^-CD47^+$, FIG. 24), GBM30-luc ($CD19^-CD47^+$, FIG. 25), and U251 ($CD19^-CD47^+$, FIG. 26) target cell types. For each target cell type, cells were treated with 500 or 1000 ng/mL of either (i) His-tagged soluble SIRP1α; (ii) SIRP1α-CD3 BiTE; or (iii) or CD19-CD3 BiTE. Cells were then stained with a fluorescently labelled anti-His antibody and analyzed by flow cytometry.

[00301] The results of SIRP1α-CD3 and CD19-CD3 binding to $CD19^+CD47^+$ Raji cells are shown in FIG. 23. Relative to the negative control Ig (2^o only), soluble SIRP1α, SIRP1α-CD3 BiTE, and CD19-CD3 BiTE were able to bind to Raji cells, as indicated by a shift

towards the right of the engager histograms compared to the IgG control histogram (FIG. 23A). Quantitation of the binding data showing percentage of BiTE positive cells is show in FIG. 23B.

[00302] The results of SIRP1α-CD3 and CD19-CD3 binding to $CD19^{-}CD47^{+}$ U2OS cells are shown in FIG. 24. Relative to the negative control Ig (2° only), soluble SIRP1α, SIRP1α-CD3 BiTE were able to bind to U2OS cells at all concentrations used, as indicated by a shift towards the right of the engager histograms compared to the IgG control histogram (FIG. 24A). CD19-CD3 BiTEs were unable to bind to U2OS cells, which was expected based on the lack of CD19 expression by U2OS cells. Quantitation of these binding data showing percentage of BiTE positive cells is show in FIG. 24B.

[00303] The results of SIRP1α-CD3 and CD19-CD3 binding to $CD19^{-}CD47^{+}$ GBM30-luc cells are shown in FIG. 25. Relative to the negative control Ig (2° only), SIRP1α-CD3 BiTE were able to bind to GBM30-luc cells at all concentrations used, as indicated by a shift towards the right of the engager histograms compared to the IgG control histogram (FIG. 25A). In constast, CD19-CD3 BiTEs were unable to bind to GBM30-luc cells, which was expected based on the lack of CD19 expression by GBM30-luc cells. Quantitation of these binding data showing percentage of BiTE positive cells is show in FIG. 25B.

[00304] The results of SIRP1α-CD3 and CD19-CD3 binding to $CD19^{-}CD47^{+}$ U251 cells are shown in FIG. 26. Relative to the negative control Ig (2° only), SIRP1α-CD3 BiTE were able to bind to U251 cells at all concentrations used, as indicated by a shift towards the right of the engager histograms compared to the IgG control histogram (FIG. 26A). In constast, CD19-CD3 BiTEs were unable to bind to U251 cells, which was expected based on the lack of CD19 expression by U251 cells. Quantitation of these binding data showing percentage of BiTE positive cells is show in FIG. 26B.

Example 7: Binding of PDL1-CD3-Fc TiTEs to U251 cells is mediated by CD47, not FcγRs

[00305] As the PDL1-CD3-Fc TiTE construct comprises 2 domains that are capable of binding to target cells (the anti-PDL1 and the Fc domain) experiments were performed to assess the binding specificity of these constructs. $CD19^{-}CD47^{+}$ U251 cells were treated with 2 μg/mL of a fluorescently labled anti-PDL1 antibody, an isotype control, or PDL1-CD3-Fc transfection supernant. Relative to negative control Ig, the PDL1-CD3-Fc TiTE bound to U251 cells (FIG. 27B). To assess whether this observed binding was due to interactions with CD47 or FcγRs expressed by U251 cells, the FcγR expression on U251 cells was determined. Cells were incubated with 2 μg/mL of fluorophore-conjugated anti-CD16/32 (recognizing FcγRIII/FcγRII) or anti-CD64 (recognizing FcγRI) mAbs for 20 min at RT. Cells were then washed and analyzed

by flow cytometry using a BD LSR Fortessa cytometer. As shown in FIG. 27C, U251 cells do not express FcγRI, FcγRII, or FcγRIII, indicating the binding of the PDL1-CD3-Fc construct was mediated by interactions with CD47 and not FcγRs.

Example 8: CD19-CD3, SIRP1α-CD3, and PDL1-CD3-Fc constructs stimulate $CD8^+$ T cell-mediated killing of target cells

[00306] Experiments were performed to determine the ablity of CD19-CD3, SIRP1α-CD3, and PDL1-CD3-Fc constructs to mediate killing of target cells. Briefly, $CD8^+$ T cells were stimulated for 8-12 days in the presence of 200 U/mL IL-2 and Dynabeads. Prior to co-culture with target cells, all Dynabeads were removed by magnet and cells were washed to remove IL-2. Raji (FIG. 28), THP1 (FIG. 29), U251 (FIG. 30), and 293F (FIG. 31) target cells were labeled with the fluorescent membrane dye PKH67 green before plating. $CD8^+$ effector T cells were then co-cultured with target cells at an effector to target ratio of 1:1 along with 1000 ng/mL CD19-CD3 BiTE, SIRP1α-CD3 BiTEs, or a 1:3 dilution of PDL1-CD3-Fc transfection supernatant. Co-cultures of target and effector cells were incubated for 18 hours, after which they were stained with 7-AAD andlive/dead analysis was performed by flow cytometry on a BD LSR Fortesa cytometer.

[00307] The results of these experiments indicate that the CD19-CD3, SIRP1α-CD3 and PDL1-CD3-Fc engager constructs were all capable of inducing effector cell-mediated death of Raji target cells (FIG. 28), The EC_{50} for each of the CD19-CD3, SIRP1α-CD3 and PDL1-CD3-Fc engager molecules on Raji cells are shown below in Table 7.

Table 7: EC_{50} of engager molecules on Raji cells

Engager Molecule	EC_{50} (ng/mL)
CD19-CD3	0.6997
SIRP1α-CD3	0.0137
PDL1-CD3-Fc	0.8907

[00308] The results of these experiments further indicate that the PDL1-CD3-Fc engager constructs, but not the CD19-CD3 constructs, were capable of inducing effector cell-mediated death of THP1 target cells (FIG. 29). This is likely due to the lack of/relatively low expression of CD19 by THP1 cells.

[00309] Further, the PDL1-CD3-Fc engager constructs were capable of inducing effector cell-mediated death of U251 target cells (FIG. 30), while the CD19-CD3 constructs did not induce effector cell-mediated death of U251 cells due to a lack of CD19 expression by U251 cells. The EC_{50} for each of the CD19-CD3 and PDL1-CD3-Fc constructs on U251 cells are shown below in Table 8.

Table 8: EC_{50} of engager molecules on U251 cells

Engager Molecule	EC_{50} (ng/mL)
CD19-CD3	2.247
PDL1-CD3-Fc	2.611

[00310] Further, the SIRP1α-CD3 engager constructs were capable of inducing effector cell-mediated death of 293F target cells (FIG. 31), indicated by the increase in cell death in SIRP1α-CD3 containing cultures compared to a control osteopontin-fusion protein (OPN 1). The EC_{50} for SIRP1α-CD3 engager molecules on 293F cells is shown below in Table 9.

Table 9: EC_{50} of SIRP1α-CD3 on 293F cells

Engager Molecule	EC_{50} (ng/mL)
SIRP1α-CD3	0.0184

Example 9: PDL1-CD3-Fc BiTE enhances primary NK cell killing of U251 cells

[00311] Experiments are performed to assess the ability of PDL1-CD3-Fc constructs to induce NK cell-mediated killing of target cells. Briefly, U251 cells are lableled with cell membrane dye PKH67 green, and then seeded and allowed to adhere to wells over night (FIG. 32). Primary NK cells (StemCell Technologies, Inc.) are then added to each well at an effector to target ratio of 1:1, along with varying amounts of virally produced PDL1-CD3-Fc protein. Effector/target cell co-culture are incubated at 37° C for 6 hours prior to live/dead analysis by 7-AAD staining. Stained cells are analyzed by flow cytometry on a BD LSR Fortesa cytometer.

[00312] These results will demonstrate that virally produced PDL1-CD3-Fc compounds are able to stimulate NK cell-mediated death of target cells such as U251.

Example 10: oHSV-infected Vero cells express SIRP-1α-CD3 BiTEs

[00313] To demonstrate that the oncolytic viruses described here are capable or producing the engager molecules, Vero cells were infected with oHSV expressing SIRP1α-CD3 BiTEs (FIG. 32) with either a short linker (SL) (ONCR-085; 2A5B SIRP1α-CD3 (SL) BiTE) or long linker (LL) (ONCR-087; 2A5B SIRP1α-CD3 (LL) BiTE), or with oHSV expressing PDL1-CD3-Fc TiTEs (ONCR-089, FIG. 33). Cells were infected for 3 days, after whichsupernatants from infected cells were passed through a 100K MWCO ultrafiltration membrane to remove any viral particles. The flowthrough was concentrated with a 10K MWCO ultrafiltration membrane. Concentrated viral supernatants and 100 ng, 50 ng, 25 ng, or 12.5 ng of purified SIRP1α-CD3 or PDL1-CD3-Fc protein were then analyzed by PAGE followed by Western blotting with an anti-6xHis detection antibody in order to determine the amount of engager protein present in the viral supernatants.

[00314] The results demonstrate that cells infected with either ONCR-085 or ONCR-087 produced the SIRP1α-CD3 (SL) and SIRP1α-CD3 (LL) protein, respectively (FIG. 32). Further, cells infected with ONCR-089 produced the PDL1-CD3-Fc protein (FIG. 33). The ability of the 100K and 10K Amicon filtration and concentration steps to remove remaining virus was assessed by Western blot. The workflow for clarifying viral supernatants comprises low-speed centrifugation of the supernatants followed by filtration through a 0.8 μm filter membrane. Supernatant filtrates are then passaged through an Amicon 100 kDa filter to entrain the virus, followed by passage of the filtrate through an Amicon 10 kDa filter to entrain remaining protein. Aliquots of supernatants from virally-infected cells were taken before and after processing with the Amicon filters and the presence of HSV was determined by blotting with an anti-HSV polyclonal antibody. These results show that the ultrafiltration steps used to purify the engager constructs effectively removed virus (FIG. 35). Therefore, any target cell killing observed in the presence of these engager constructs is due to the engager construct itself, and not a result of viral infection of the target cells.

Example 11: Virally-produced SIRP1α-CD3 and PDL1-CD3-Fc engager constructs induced effector-cell mediated killing of target cells

[00315] Experiments were performed to assess the ability of virally-produced engager molecules (SIRP1α-CD3 and PDL1-CD3-Fc constructs) to mediate target cell killing. Briefly, SIRP1α-CD3 (SL), SIRP1α-CD3 (LL) and PDL1-CD3-Fc proteins were prepared from Vero cellsas described in Example 10. 50 μL of the resulting SIRP1α-CD3 (SL), SIRP1α-CD3 (LL), and PDL1-CD3-Fc engager proteins protein samples were diluted 1:1 in tissue culture

media containing 20% FBS. The diluted engager proteins were then incubated with activated $CD8^+$ effector T cells co-cultured with fluorescently labelled U251 target cells at a target to effector ratio of 1:1 for 18 hours. Cell death of U251 cells was assessed by flow cytometry on a BD LSR Fortesa cytometer.

[00316] The results of this experiment demonstrate that virally-produced engager constructs direct T-cell mediated killing of U251 target cells (FIG. 34A). These results are quantified in FIG. 34B.

Example 12: Expression of SIRP1α-CD3/PDL1-Fc compounds from 293 T cells

[00317] Two expression plasmids encoding a SIRP1α-CD3 engager molecule and a PDL1-Fc therapeutic molecule were generated. One construct comprised a first gene encoding an HA-tagged PDL1-Fc linked to a second gene encoding a His-tagged SIRP1α-CD3 BiTE. The SIRP1α amino acid sequence was linked to the anti-CD3 scFv by a single amino acid linker (*i.e.*, a short linker) (SIRP1α-CD3/PDL1-Fc (SL), FIG. 37). The other construct comprised a first gene encoding a PDL1-Fc linked to a second gene encoding a SIRP1α-CD3 BiTE. The SIRP1α amino acid sequence was linked to the anti-CD3 scFv by a G4S linker (*i.e.*, a long linker) (SIRP1α-CD3/PDL1-Fc (LL), FIG. 38). The constructs were inserted into a plasmid (FIG. 39) and the resultant SIRP1α-CD3/PDL1-Fc expression plasmids were transfected into 293 Free Style T cells. Four days after plasmid transfection, culture supernatants were collected.

[00318] Anti-PDL1-Fc compounds were purified from the culture supernatants using a HiTrap MabSelect SuRe Protein A column HiTrap column (GE Healthcare). Briefly, supernatants from 293 T cells transfected with either the SIRP1α-CD3/PDL1-Fc (LL) or the SIRP1α-CD3/PDL1-Fc (LL) expression plasmids were loaded onto the column to purify the anti-PDL1-Fc compounds by binding of the HA-tag to the column. Flow through was collected for SIRP1α-CD3 BiTE detection by Western Blot using an anti-His antibody (FIG. 40B). Columns were washed with wash buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.4). Bound anti-PDL1-Fc protein was eluted with IgG elution buffer (pH 2.8, Pierce) and was immediately neutralized with a 1 M Tris-HCl buffer, pH 8.

[00319] The anti-PDL1-Fc protein content of different elution fractions then were visualized by Coomassie staning. Briefly, elution fractions were run on a 4% - 12% Bis-Tris NuPAGE gel in MOPS buffer at 180 volts for 1 hour. Gels were stained for 1 hour in Simply Blue SafeStain followed by destaining with water. Anti-PDL1-Fc protein content for each elution fraction is show in FIG. 40A. After Coomassie analysis, elution fractions were combined and

dialyzed against PBS at 4° C. Total anti-PDL1-Fc protein concentration was then determined by a BCA assay.

Example 13: Isolated PDL1-Fc proteins stimulate T cell-mediated death of target cells

[00320] The ability of the anti-PDL1-Fc proteins to induce effector cell-mediated death of target cells was assessed by a PD1/PDL1 blockade assay. A general schematic of the assay is show in FIG. 41A – 41B. Briefly, $CD8^+$ T cells were co-cultured with PDL1-expressing target cells (CHO-K1 cells). Varying concentrations of the anti-PDL1-Fc protein isolated as described in Example 12 were then added to the culture. The highest concentration of anti-PDL1-Fc used was 50 μg/mL. 8, 2.5 fold serial dilutions were then performed to generate the remainder of the anti-PDL1-Fc concentrations. Cell death was analyzed by a CytoTox-Glo™ cytotoxicity assay in the presence (FIG. 41B) and absence (FIG. 41A) of the anti-PDL1-Fc. Results are quantified in FIG. 41C. The EC_{50} of the anti-PDL1-Fc is shown in Table 10. These results demonstrate that the anti-PDL1-Fc therapeutic molecules produced from the expression constructs described herein are capable of mediating effector cell-mediated death of target cells.

Table 10. EC_{50} of anti-PDL1-Fc compounds

Compound	EC_{50}
anti-PDL1-Fc	0.45 μg/mL

Example 13: oHSV-infected Vero cells express MMP9 and anti-PDL1-Fc therapeutic molecules

[00321] In addition to producing the engager molecules as described in Example 10, experiments are performed to demonstrate that the oncolytic viruses described here are capable or producing the MMP9 and anti-PDL1-Fc therapeutic molecules. Vero cells are infected with oHSV expressing SIRP1α-CD3/PDL1-Fc constructs BiTEs (FIG. 37 and FIG. 38) or with oHSV expressing SIRP1α-CD3/MMP9 constructs (FIG. 18A and FIG. 18B). Cells are infected for 3 days, after which supernatants from infected cells are passed through a 100K MWCO ultrafiltration membrane to remove any viral particles. The flowthrough is concentrated with a 10K MWCO ultrafiltration membrane. MMP9 and anti-PDL1-Fc are purified from filtered, concentrated supernatants according to the protocol outlined in Example 11. Protein A-isolated MMP9 and anti-PDL1 fractions are analyzed by PAGE followed by Coomassie staining.

SIRP1α-CD3 BiTEs present in the Protein A flowthrough are analyzed by Western blotting with an anti-6x His detection antibody.

[00322] The results will demonstrate that cells infected with oHSV vectors encoding either SIRP1α-CD3/PDL1-Fc constructs or SIRP1α-CD3/MMP9 constructs produce the SIRP1α-CD3 (SL) and SIRP1α-CD3 (LL) BiTE protein, MMP9, and anti-PDL1-Fc.

Example 14: Virally-produced SIRP1α-CD3/MMP9 and SIRP1α-CD3/PDL1-Fc engager constructs induce effector-cell mediated killing of target cells

[00323] Experiments are performed to assess the ability of virally-produced engager molecules (SIRP1α-CD3) and therapeutic molecules (MMP9 and anti-PDL1-Fc) to mediate target cell killing. Briefly, SIRP1α-CD3 (SL), SIRP1α-CD3 (LL), MMP9, and anti-PDL1-Fc proteins are prepared from Vero cells as described in Example 13. 50 µL of the resulting protein samples are diluted in tissue culture media containing 20% FBS. The diluted proteins are then incubated with activated $CD8^+$ effector T cells or NK effector cells and are co-cultured with fluorescently labelled target cells at a target to effector ratio of 1:1 for 18 hours. Cell death of target cells is assessed by flow cytometry on a BD LSR Fortesa cytometer.

[00324] The results of this experiment will demonstrate that virally-produced SIRP1α-CD3 engager constructs and therapeutic molecules MMP9 and anti-PDL1-Fc are able to direct T-cell and/or NK cell mediated killing of target cells.

[00325] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

1. A oncolytic herpes simplex virus (HSV) comprising a recombinant nucleic acid comprising:

i) a first nucleic acid sequence encoding a polypeptide comprising a first domain specific for an antigen expressed on an effector cell and a second domain that binds to an antigen expressed on a target cell, wherein the antigen expressed on the effector cell is CD3 and wherein the antigen expressed on the target cell is PDL1 or CD47; and

ii) a second nucleic acid sequence encoding an immune modulator polypeptide selected from the group consisting of a cytokine, a costimulatory molecule, an immune checkpoint polypeptide, an anti-angiogenesis factor, and a matrix metalloprotease (MMP).

2. The oncolytic HSV of claim 1, wherein the first and second nucleic acid sequences are expressed from a single promoter sequence present in the recombinant nucleic acid.

3. The oncolytic HSV of claim 1, wherein the first and second nucleic acids have a size of 7.2-38kb.

4. The oncolytic HSV of claim 1, wherein the HSV is HSV-1.

5. The oncolytic HSV of claim 1, wherein the immune modulator polypeptide is a cytokine selected from IL-15, IL-12, and CXCL10.

6. The oncolytic HSV of claim 1, wherein one or more of the HSV glycoproteins are modified to alter the trophism of the oncolytic HSV.