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(54) Titre : POLYTHERAPIE UTILISANT UN RECEPTEUR ANTIGENIQUE CHIMERIQUE
(54) Title: COMBINATION THERAPY USING A CHIMERIC ANTIGEN RECEPTOR

(57) Abrégé/Abstract:
The invention provides compositions and methods for treating diseases such as cancer. The invention also relates to a method of administering a chimeric antigen receptor (CAR) therapy and an additional therapeutic agent, e.g., one or more cytokine molecules, e.g., a virus comprising a nucleic acid molecule encoding one or more cytokine molecules.

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(54) Title: COMBINATION THERAPY USING A CHIMERIC ANTIGEN RECEPTOR

(57) Abstract: The invention provides compositions and methods for treating diseases such as cancer. The invention also relates to a method of administering a chimeric antigen receptor (CAR) therapy and an additional therapeutic agent, e.g., one or more cytokine molecules, e.g., a virus comprising a nucleic acid molecule encoding one or more cytokine molecules.

COMBINATION THERAPY USING A CHIMERIC ANTIGEN RECEPTOR

RELATED APPLICATION

This application claims priority to U.S. Serial No. 62/624,707 filed January 31, 2018, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 31, 2019, is named N2067-7148WO_SL.txt and is 377,462 bytes in size.

FIELD OF THE INVENTION

The present invention relates generally to the use of cells, e.g., immune effector cells, engineered to express a Chimeric Antigen Receptor (CAR) in combination with one or more cytokine molecules, e.g., a virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding one or more cytokine molecules, to treat a disease, e.g., cancer.

BACKGROUND OF THE INVENTION

Recent developments using chimeric antigen receptor (CAR) modified T cell (CART) therapy, which relies on redirecting T cells to a suitable cell-surface molecule on cancer cells, show promising results in harnessing the power of the immune system to treat cancers (see, e.g., Sadelain et al., Cancer Discovery 3:388-398 (2013)).

Given the ongoing need for improved strategies for targeting diseases such as cancer, new compositions and methods for improving CART therapies are highly desirable.

SUMMARY OF THE INVENTION

This disclosure features, at least in part, compositions and methods of treating disorders such as cancer using immune effector cells (e.g., T cells or NK cells) that express a chimeric antigen receptor (CAR) molecule, e.g., a CAR molecule that binds to a tumor antigen, e.g., an antigen expressed on the surface of a solid tumor or a hematological tumor. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with one or more cytokine molecules (e.g., a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule). In one aspect, the invention features use of the CAR-

expressing cell therapy in combination with a TNFα molecule and an IL-2 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a TNFα molecule and an IL-7 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with an IL-7 molecule and an IL-2 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a TNFα molecule, an IL-2 molecule, and an IL-7 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding one or more cytokine molecules (e.g., a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule). In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding a TNFα molecule and an IL-2 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding a TNFα molecule and an IL-7 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding an IL-7 molecule and an IL-2 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding a TNFα molecule, an IL-2 molecule, and an IL-7 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a first virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding an IL-2 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a first virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding an IL-7 molecule. In one aspect, the invention features use of the CAR-expressing cell therapy in combination with a first virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding an IL-7 molecule, and a second virus, e.g., an oncolytic virus, e.g., an oncolytic adenovirus, comprising a nucleic acid molecule encoding an IL-2 molecule. In one embodiment, the virus, first virus, or second virus comprises an adenovirus vector, e.g., Ad5/3 vector.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to the antigen ("CAR-expressing cell"), wherein the subject

has received, is receiving, or is about to receive: (i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

In one embodiment, the antigen is chosen from CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC. In one embodiment, the antigen is mesothelin.

In one embodiment, the subject has received a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule. In one embodiment, the subject has received a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule. In one embodiment, the subject has received a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule. In one embodiment, the subject has received a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject: (i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising

a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule, wherein the subject has received, is receiving, or is about to receive a cell (e.g., a population of cells) that expresses a CAR molecule that binds to the antigen ("CAR-expressing cell").

In one embodiment, the antigen is chosen from CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC. In one embodiment, the antigen is mesothelin.

In one embodiment, the subject is about to receive the CAR-expressing cell.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject a first virus comprising a nucleic acid molecule encoding a TNFα molecule, wherein the subject has received, is receiving, or is about to receive: (i) a cell (e.g., a population of cells) that expresses a CAR molecule that binds to the antigen ("CAR-expressing cell"), and (ii) a second virus comprising a nucleic acid molecule encoding an IL-2 molecule or an IL-7 molecule. In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject a first virus comprising a nucleic acid molecule encoding an IL-2 molecule, wherein the subject has received, is receiving, or is about to receive: (i) a cell (e.g., a population of cells) that expresses a CAR molecule that binds to the antigen ("CAR-expressing cell"), and (ii) a second virus comprising a nucleic acid molecule encoding a TNFα molecule or an IL-7 molecule. In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject a first virus comprising a nucleic acid molecule encoding an IL-7 molecule, wherein the subject has received, is receiving, or is about to receive: (i) a cell (e.g., a population of cells) that expresses a CAR molecule that binds to the antigen ("CAR-expressing cell"), and (ii) a second virus comprising a nucleic acid molecule encoding a TNFα molecule or an IL-2 molecule.

In one embodiment, the antigen is chosen from CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC. In one embodiment, the antigen is mesothelin.

In one aspect, disclosed herein is a method of treating a subject having a cancer, wherein the cancer exhibits or is identified as exhibiting heterogeneous expression of an antigen, e.g., a tumor antigen, e.g., mesothelin, e.g., wherein less than 90%, 80%, 70%, 60%, or 50% of cells in the cancer express the antigen, comprising administering to the subject: a cell (e.g., a population of cells) that expresses a CAR molecule that binds to the antigen ("CAR-expressing cell"); and (i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

In one embodiment, the antigen is chosen from CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC. In one embodiment, the antigen is mesothelin.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject: a cell (e.g., a population of cells) that expresses a CAR molecule that binds to the antigen ("CAR-expressing cell"); and (i) a non-oncolytic virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first non-oncolytic virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second non-oncolytic virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a non-oncolytic virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the non-oncolytic virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a non-oncolytic first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second non-oncolytic virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

In one embodiment, the antigen is chosen from CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC. In one embodiment, the antigen is mesothelin.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with mesothelin expression, comprising administering to the subject: a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2; and (i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally

wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule .

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of an antigen, e.g., a tumor antigen, e.g., a method of treating a subject having a cancer, comprising administering to the subject: a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to the antigen ("CAR-expressing cell"); and (i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule .

In one aspect, disclosed herein is a method of providing an anti-cancer immune response in a subject having a cancer, comprising administering to the subject: a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2; and (i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; (ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule; (iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or (iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule .

In certain embodiments of the aforementioned aspects and embodiments, the virus (or the first virus and the second virus) are administered prior to the administration of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell), e.g., about 1, 2, 3, 4, or 5 days prior to the administration of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell).

In certain embodiments of the aforementioned aspects and embodiments, the virus (or the first virus and the second virus) are administered after the administration of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell), e.g., about 1, 2, 3, 4, or 5 days after the administration of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell).

In certain embodiments of the aforementioned aspects and embodiments, the first virus comprising the nucleic acid molecule encoding the TNFα molecule and the second virus comprising the nucleic acid molecule encoding the IL-2 molecule are administered simultaneously. In certain embodiments of the aforementioned aspects and embodiments, the first virus comprising the nucleic acid molecule encoding the TNFα molecule and the second virus comprising the nucleic acid molecule encoding the IL-7 molecule are administered simultaneously. In certain embodiments of the aforementioned aspects and embodiments, the first virus comprising the nucleic acid molecule encoding the IL-7 molecule and the second virus comprising the nucleic acid molecule encoding the IL-2 molecule are administered simultaneously.

In certain embodiments of the aforementioned aspects and embodiments, (i) the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell), and (ii) the virus (or the first virus and the second virus) are administered for a first treatment interval, wherein the first treatment interval comprises a single dose of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell), and a single dose of the virus (or the first virus and the second virus).

In one embodiment, the first treatment interval is initiated upon administration of the single dose of the virus (or the first virus and the second virus) and completed upon administration of the single dose of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell).

In one embodiment, the single dose of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) is administered, e.g., about 1, 2, 3, 4, or 5 days after the administration of the single dose of the virus (or the first virus and the second virus).

In one embodiment, the first treatment interval is repeated, e.g., one or more times, e.g., 1, 2, 3, 4, or 5 more times. In one embodiment, the first treatment interval is followed by one or more, e.g., 1, 2, 3, 4, or 5, subsequent treatment intervals. In one embodiment, the one or more subsequent treatment intervals are different from the first treatment interval.

In certain embodiments of the aforementioned aspects and embodiments, the virus (or the first virus and the second virus) is administered systemically or locally.

In one embodiment, the virus (or the first virus and the second virus) is administered locally. In one embodiment, the subject has a cancer and the virus (or the first virus and the second virus) is administered intratumorally.

In certain embodiments of the aforementioned aspects and embodiments, the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) is administered intravenously.

In certain embodiments of the aforementioned aspects and embodiments, the first virus and the second virus are the same virus. In certain embodiments of the aforementioned aspects and embodiments, the first virus and the second virus are different viruses. In one embodiment, the virus, the first virus, and/or the second virus are chosen from adenovirus, herpes simplex virus, retrovirus, parvovirus, vaccinia virus, sinbis virus, influenza virus, or RNA virus (e.g., reovirus, newcastle disease virus (NDV), measles virus, or vesicular stomatitis virus (VSV)). In one embodiment, the virus, the first virus, and/or the second virus are oncolytic virus, e.g., oncolytic adenovirus, oncolytic adeno-associated virus, oncolytic Herpes Simplex Virus (HSV), oncolytic parvovirus, oncolytic retrovirus, oncolytic lentivirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza virus, oncolytic reovirus, oncolytic Newcastle disease virus (NDV), oncolytic measles virus, oncolytic vesicular stomatitis virus (VSV), oncolytic poliovirus, oncolytic poxvirus, oncolytic Seneca Valley virus, oncolytic coxsackievirus, oncolytic enterovirus, oncolytic myxoma virus, or oncolytic maraba virus. In one embodiment, the virus, the first virus, and/or the second virus are oncolytic adenovirus. In one embodiment, the subject has cancer cells expressing desmoglein-2 (DSG-2). In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are disposed on an adenoviral vector. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-7 molecule are disposed on an adenoviral vector. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the IL-7 molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are disposed on an adenoviral vector. In one embodiment, the adenoviral vector is chosen from Ad5, Ad3 or Ad5/3. In one embodiment, the adenoviral vector is Ad5. In one embodiment, the adenoviral vector is Ad3. In one embodiment, the adenoviral vector is Ad5/3. In one embodiment, the adenoviral vector is Ad5/3 comprising an Ad5 nucleic acid backbone and Ad3 fiber knob or Ad5/3 chimeric fiber knob. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are disposed on an oncolytic adenoviral vector. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-7 molecule are disposed on an oncolytic adenoviral vector. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the IL-7 molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are disposed on an oncolytic adenoviral vector. In some embodiments, (i) the oncolytic adenoviral vector comprises an Ad5 nucleic acid backbone comprising Ad5/3 chimeric fiber knob, (ii) the oncolytic adenoviral vector comprises E2F promoter, e.g., E2F1

promoter, (iii) the oncolytic adenoviral vector comprises a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1, and/or (iv) the oncolytic adenoviral vector comprises a nucleic acid sequence deletion of viral gp19k and 6.7k reading frames. In some embodiments, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are in the place of the deleted gp19k/6.7k in the E3 region, e.g., resulting in replication-associated control of expression of the TNFα molecule and/or the IL-2 molecule under the viral E3 promoter. In some embodiments, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-7 molecule are in the place of the deleted gp19k/6.7k in the E3 region, e.g., resulting in replication-associated control of expression of the TNFα molecule and/or the IL-7 molecule under the viral E3 promoter. In some embodiments, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the IL-7 molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are in the place of the deleted gp19k/6.7k in the E3 region, e.g., resulting in replication-associated control of expression of the IL-7 molecule and/or the IL-2 molecule under the viral E3 promoter. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are disposed on an Ad3 oncolytic adenoviral vector. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule, the nucleic acid molecule encoding the TNFα molecule, and/or the nucleic acid molecule encoding the IL-7 molecule are disposed on an Ad3 oncolytic adenoviral vector. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule, the nucleic acid molecule encoding the IL-7 molecule, and/or the nucleic acid molecule encoding the IL-2 molecule are disposed on an Ad3 oncolytic adenoviral vector. In some embodiments, the Ad3 oncolytic adenoviral vector comprises: (i) a deletion in the E3 area, and (ii) a tumor specific promoter for expression of, e.g., the TNFα molecule and/or the IL-2 molecule, e.g., in the place of the deleted E3 area. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are encapsulated in a single viral particle. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are encapsulated in a single viral particle. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are encapsulated in a single viral particle.

In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA

molecule. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-2 molecule are disposed on separate nucleic acid molecules. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on separate nucleic acid molecules.

In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-7 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-7 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-7 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-7 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and/or the IL-7 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-7 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-7 molecule are disposed on separate nucleic acid molecules. In one embodiment, the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are disposed on separate nucleic acid molecules.

In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule comprises a sequence encoding the IL-7 molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the IL-7 molecule and the sequence encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule comprises a sequence encoding the IL-7 molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the IL-7 molecule and the sequence encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and/or the IL-2 molecule comprises a sequence encoding the IL-7 molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the IL-7 molecule and the sequence encoding the IL-2 molecule are disposed on separate nucleic acid molecules. In one embodiment, the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on separate nucleic acid molecules.

In certain embodiments of the aforementioned aspects and embodiments, the antigen is mesothelin, wherein the CAR-expressing cell expresses a CAR that binds to mesothein ("mesothelin CAR-expressing cell")

In certain embodiments of the aforementioned aspects and embodiments, the virus comprising a nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, the first virus comprising the nucleic acid molecule encoding the TNFα molecule, and/or the second virus comprising the nucleic acid molecule encoding the IL-2 molecule, have one, two, or all of the following properties:

(i) mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, activates the mesothelin CAR-expressing cell, e.g., at a level at least about 20, 50, 100, 150, or 200% higher than, e.g., mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, infected with an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of an activation marker (e.g., CD69), e.g., as assessed using methods described in Example 1 with respect to FIG. 1B or 1C,

(ii) mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, increases proliferation of the mesothelin

CAR-expressing cell, e.g., at a level at least about 20, 50, 100, 150, or 200% higher than, e.g., mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, infected with an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 1D or 1E, or

(iii) the lytic activity of the mesothelin CAR-expressing cell against mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, is increased by at least about 1, 2, 3, 4, or 5-fold, e.g., compared to the lytic activity of the mesothelin CAR-expressing cell against otherwise similar mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, that are not infected with the virus, or the first and/or second virus, e.g., as assessed using methods described in Example 1 with respect to FIG. 1A.

In certain embodiments of the aforementioned aspects and embodiments, the administration of the mesothelin CAR-expressing cell and (a) the virus comprising a nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, or (b) the first virus comprising the nucleic acid molecule encoding the TNFα molecule, and the second virus comprising the nucleic acid molecule encoding the IL-2 molecule, results in one or more (2, 3, 4, 5, 6, 7, 8, 9, or all) of the following properties:

(i) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, produces a reduction in tumor load, e.g., a reduction of at least about 0.5, 1, 2, 5, 10, or 200-fold, e.g., about 15, 20, 25, 30, 35, 40, or 45 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2B, 2C, 2D, or 6B.

(ii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases survival of the subject, e.g., by at least about 2, 5, 10, 20, 50, or 100-fold, e.g., about 20, 40, 60, 80, or 100 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2E,

(iii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, reduces tumor metastasis, e.g., tumor metastasis to the lung, by at least about 20, 40, 60, or 80%, e.g., about 20, 50, 100, or 150 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the

mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2F,

(iv) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases expression of a cytokine molecule, e.g., IFN-γ, by the mesothelin CAR-expressing cell, by at least about 1, 2, 3, or 4-fold, e.g., about 5, 10, 15, or 20 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 3E,

(v) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases infiltration of the mesothelin CAR-expressing cell into a tumor, e.g., by at least about 5, 10, 25, 50, 75, 100, 125, 150, 175, or 200-fold, e.g., about 2, 4, 6, 8, 10, 12, 13, 14, 16, 18, 20, 30, 40, or 50 days after the administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 4A, 4B, or 6C,

(vi) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases infiltration of endogenous T cells, e.g., CD4+ and/or CD8+ T cells, into a tumor, e.g., by at least about 1, 2, 5, 10, 20, 30, or 50-fold, e.g., about 15, 20, 25, 30, or 35 days after the administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 3A, 3B, 9B, or 6D,

(vii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, activates tumor infiltrating lymphocytes (TILs), e.g., by at least about 20, 30, 40, or 50%, e.g., about 10, 20, 30, or 40 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of

the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of an activation marker, e.g., as measured by expression of CD69 and/or CD25, e.g., as assessed using methods described in Example 1 with respect to FIG. 3D or 9C,

(viii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases M1 polarization of macrophages, e.g., by at least about 20, 30, 40, or 50%, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of CD80 and/or CD86 on macrophages, e.g., as assessed using methods described in Example 1 with respect to FIG. 6E,

(ix) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases maturation of dendritic cells, e.g., maturation of CD11c+ dendritic cells, e.g., by at least about 20, 30, 40, or 50%, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of CD80 and/or CD86 on dendritic cells, e.g., as assessed using methods described in Example 1 with respect to FIG. 10E, or

(x) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases the level of a chemokine in the subject, e.g., an immune-cell attractive chemokine, e.g., a TNF-α inducible chemokine, e.g., one, two, or all of: monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 10 (CXCL-10) and RANTES, by at least about 5, 10, 25, 50, 75, 100, 125, 150, 175, or 200-fold, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 6F or 10F.

In certain embodiments of the aforementioned aspects and embodiments,

(a) the virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(b) the first virus comprising a nucleic acid molecule encoding a TNFα molecule, and the second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(c) the virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) the first virus comprising a nucleic acid molecule encoding an IL-7 molecule and the second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule,

have one, two, or all of the following properties:

(i) mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, activates the mesothelin CAR-expressing cell, e.g., at a level at least about 20, 50, 100, 150, or 200% higher than, e.g., mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, infected with an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as measured by expression of an activation marker (e.g., CD69), e.g., as assessed using methods described in Example 1 with respect to FIG. 1B or 1C,

(ii) mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, increases proliferation of the mesothelin CAR-expressing cell, e.g., at a level at least about 20, 50, 100, 150, or 200% higher than, e.g., mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, infected with an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 1D or 1E, or

(iii) the lytic activity of the mesothelin CAR-expressing cell against mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, is increased by at least about 1, 2, 3, 4, or 5-fold, e.g., compared to the lytic activity of the mesothelin CAR-expressing cell against otherwise similar mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, that are not infected with the virus, or the first and/or second virus, e.g., as assessed using methods described in Example 1 with respect to FIG. 1A.

In certain embodiments of the aforementioned aspects and embodiments, the administration of the mesothelin CAR-expressing cell and

(a) the virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(b) the first virus comprising a nucleic acid molecule encoding a TNFα molecule, and the second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(c) the virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) the first virus comprising a nucleic acid molecule encoding an IL-7 molecule and the second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule,

results in one or more (2, 3, 4, 5, 6, 7, 8, 9, or all) of the following properties:

(i) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, produces a reduction in tumor load, e.g., a reduction of at least about 0.5, 1, 2, 5, 10, or 200-fold, e.g., about 15, 20, 25, 30, 35, 40, or 45 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2B, 2C, 2D, or 6B.

(ii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases survival of the subject, e.g., by at least about 2, 5, 10, 20, 50, or 100-fold, e.g., about 20, 40, 60, 80, or 100 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2E,

(iii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, reduces tumor metastasis, e.g., tumor metastasis to the lung, by at least about 20, 40, 60, or 80%, e.g., about 20, 50, 100, or 150 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2F,

(iv) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases expression of a cytokine molecule, e.g., IFN-γ, by the mesothelin CAR-expressing cell, by at least about 1, 2, 3, or 4-fold, e.g., about 5, 10, 15, or 20 days after administration of the virus (or the first and second viruses), e.g.,

compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 3E,

(v) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases infiltration of the mesothelin CAR-expressing cell into a tumor, e.g., by at least about 5, 10, 25, 50, 75, 100, 125, 150, 175, or 200-fold, e.g., about 2, 4, 6, 8, 10, 12, 13, 14, 16, 18, 20, 30, 40, or 50 days after the administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 4A, 4B, or 6C,

(vi) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases infiltration of endogenous T cells, e.g., CD4+ and/or CD8+ T cells, into a tumor, e.g., by at least about 1, 2, 5, 10, 20, 30, or 50-fold, e.g., about 15, 20, 25, 30, or 35 days after the administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 3A, 3B, 9B, or 6D,

(vii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, activates tumor infiltrating lymphocytes (TILs), e.g., by at least about 20, 30, 40, or 50%, e.g., about 10, 20, 30, or 40 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as measured by expression of an activation marker, e.g., as measured by expression of CD69 and/or CD25, e.g., as assessed using methods described in Example 1 with respect to FIG. 3D or 9C,

(viii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases M1 polarization of macrophages, e.g., by at least about 20, 30, 40, or 50%, e.g., about 1, 2, 3, 5, or 10 days after

administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as measured by expression of CD80 and/or CD86 on macrophages, e.g., as assessed using methods described in Example 1 with respect to FIG. 6E,

(ix) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases maturation of dendritic cells, e.g., maturation of CD11c+ dendritic cells, e.g., by at least about 20, 30, 40, or 50%, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as measured by expression of CD80 and/or CD86 on dendritic cells, e.g., as assessed using methods described in Example 1 with respect to FIG. 10E, or

(x) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases the level of a chemokine in the subject, e.g., an immune-cell attractive chemokine, e.g., a TNF-α inducible chemokine, e.g., one, two, or all of: monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 10 (CXCL-10) and RANTES, by at least about 5, 10, 25, 50, 75, 100, 125, 150, 175, or 200-fold, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule, the IL-2 molecule, and/or the IL-7 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 6F or 10F.

In one embodiment, the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2.

In one embodiment, the mesothelin binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 according to the HC CDR amino acid sequences in Table 4 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions,

insertions or deletions, *e.g.*, conserved substitutions), and a LC CDR1, a LC CDR2, and a LC CDR3 according to the LC CDR amino acid sequences in Table 5 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the mesothelin binding domain comprises:

(i) a heavy chain variable region (VH) of any mesothelin binding domain listed in Table 2 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

(ii) a light chain variable region (VL) of any mesothelin binding domain listed in Table 2 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the mesothelin binding domain comprises:

(i) an scFv of any mesothelin binding domain listed in Table 2 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), or

(ii) an amino acid sequence chosen from: SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 275, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, or SEQ ID NO: 62 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the CAR molecule comprises:

(i) a CAR sequence listed in Table 2 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1) (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), or

(ii) an amino acid sequence chosen from: SEQ ID NO: 67; SEQ ID NO: 73, SEQ ID NO: 278, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, or SEQ ID NO: 86 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1) (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the CAR molecule comprises:

(i) HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NOs: 138, 156, and 179, respectively; and LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NOs: 203, 227, and 251, respectively,

(ii) the amino acid sequence of SEQ ID NO: 43, or

(iii) the amino acid sequence of SEQ ID NO: 67 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1).

In one embodiment, the CAR molecule comprises:

(i) HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NOs: 144, 162, 185, respectively; and LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NOs: 209, 233, and 257, respectively,

(ii) the amino acid sequence of SEQ ID NO: 49, or

(iii) the amino acid sequence of SEQ ID NO: 73 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1).

In one embodiment, the CAR molecule comprises a transmembrane domain, optionally wherein the transmembrane domain comprises a transmembrane domain from a protein chosen from the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 or CD154, optionally wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the CAR molecule comprises an antigen binding domain that binds to the antigen, optionally wherein the antigen binding domain is connected to the transmembrane domain by a hinge region, optionally wherein the hinge region comprises an amino acid sequence chosen from SEQ ID NO: 2, 3, or 4 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the CAR molecule comprises a primary signaling domain, optionally wherein the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), FcεRI, DAP10, DAP12, or CD66d, optionally wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 9 or 10 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the CAR molecule comprises a costimulatory signaling domain, optionally wherein the costimulatory signaling domain comprises a functional signaling domain derived from a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signalling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor,

BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, or a ligand that specifically binds with CD83, optionally wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 7 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the CAR molecule comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta. In one embodiment, the CAR molecule comprises the amino acid sequence of SEQ ID NO: 7 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions) and the amino acid sequence of SEQ ID NO: 9 or 10 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), optionally wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10.

In one embodiment, the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) is a cell comprising a nucleic acid molecule encoding the CAR molecule, optionally wherein the nucleic acid molecule encoding the CAR molecule is an RNA molecule, e.g., an in vitro transcribed RNA molecule.

In one embodiment, the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) is a cell comprising a vector comprising a nucleic acid molecule encoding the CAR molecule, optionally wherein the vector is a lentiviral vector.

In one embodiment, the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) is a T cell (e.g., an autologous or allogeneic T cell) or an NK cell (e.g., an autologous or allogeneic NK cell).

In one embodiment, the subject has a cancer. In one embodiment, the subject has a solid tumor.

In one embodiment, the cancer is chosen from one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma , breast

cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof.

In one embodiment, the cancer is chosen from chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

In one embodiment, the cancer exhibits heterogeneous expression of the antigen, e.g., wherein less than 90%, 80%, 70%, 60%, or 50% of cells in the cancer express the antigen.

In one embodiment, the method further comprises administering an additional therapeutic agent, e.g., an anti-cancer agent.

In one embodiment, the subject is a mammal, e.g., a human. In one embodiment, the cell is a T cell or NK cell. In one embodiment, the cell is autologous to the subject. In one embodiment, the cell is allogeneic to the subject.

In one aspect, disclosed herein is a combination comprising:

(i) a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino

acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2; and

(ii) (a) a virus comprising a nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule;

(b) a first virus comprising a nucleic acid molecule encoding the TNFα molecule, and a second virus comprising a nucleic acid molecule encoding the IL-2 molecule;

(c) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule

for use in treating a disease associated with mesothelin expression, e.g., a cancer, in a subject.

In one aspect, disclosed herein is a composition (e.g., one or more compositions or dosage forms), comprising:

(i) a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2; and

(ii) (a) a virus comprising a nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule;

(b) a first virus comprising a nucleic acid molecule encoding the TNFα molecule, and a second virus comprising a nucleic acid molecule encoding the IL-2 molecule;

(c) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Headings, sub-headings or numbered or lettered elements, e.g., (a), (b), (i) etc, are presented merely for ease of reading. The use of headings or numbered or lettered elements in this document does not require the steps or elements be performed in alphabetical order or that the steps or elements are necessarily discrete from one another. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIGs. 1A-1E: Oncolytic adenovirus (OAd) expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2)) enhances activation, proliferation and lytic activity of mesothelin-redirected chimeric antigen receptor T cells (meso-CAR T cells). FIG. 1A: Kinetics of pancreatic ductal adenocarcinoma (PDA) tumor cell lysis incubated with the combination of OAd-TNFα-IL2 with meso-CAR T cells measured by the real-time xCELLigence cell analyzer. Means of cell index from triplicate wells are shown. Data are representative of three experiments from three different donors. FIG. 1B: Up-regulation of CD69 on T cells upon stimulation with PDA cell lines pre-infected with OAds. Histograms show CD69 expression of T cells at day 3 post co-culture with control media alone (Unstimulated) or co-culture with the indicated tumor cell lines pre-infected with either control media (media), parental OAd (OAd) or OAd-TNFa-IL2 (OAd-TNFα-IL2). Data are representative of three experiments from three different donors. FIG. 1C: Fold increase of %CD69 positive T cells from pooled data. Fold increase of percent CD69 positive T cells by co-culturing with tumor cell lines pre-treated either with OAd or OAd-TNFα-IL2 relative to those by co-culturing with cell lines pre-treated with control media (set to one) are shown. Means and SEM of pooled data from three experiments are shown. *, $p<0.05$; ****, $p<0.0001$ by one-way ANOVA with Turkey's post-hoc test. FIG. 1D: T cell proliferation upon the stimulation with tumor cell lines pre-infected with OAds. Using the same co-culture method as FIGs. 1B and 1C, T cell expansion was determined at day 5 by flow cytometry (FCM) and counting beads. Means and SD from triplicate wells are shown. Data are representative of four

experiments from three different donors. FIG. 1E: Relative fold expansion of T cells upon stimulation with tumor cell lines pre-infected with OAds. Fold expansion of T cells co-cultured with cell lines pre-treated with control media was set to one. Means and SEM of pooled data from four experiments are shown. *, $p<0.05$ by one-way ANOVA with Tukey's post-hoc test.

FIGs. 2A-2F: Oncolytic adenovirus (OAd) expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2)) enhances anti-tumor efficacy of mesothelin-redirected chimeric antigen receptor T cells (meso-CAR T cells) and improves survival in the pancreatic ductal adenocarcinoma (PDA) xenograft model. FIG. 2A: Experimental schematic. AsPC-1 tumor bearing mice were treated with either intratumoral injection of PBS, $0.95x10^9$ virus particle (vp) parental OAd (OAd) or OAd-TNFα-IL2 followed by intravenous injection of either PBS, $1x10^6$ meso-CAR T cells or human CD19-redirected CAR T cells (h19-CAR T cells) at day 3 after OAd injection. Tumor volumes were followed by caliper measurement. FIG. 2B: Tumor volumes by caliper measurements. Data are representative of two experiments from two different donors. Means and SEM are shown (n=7 or 8 each). *, $p<0.05$; ****, $p<0.0001$ by repeated measures two-ANOVA with Bonferroni correction. FIG. 2C: Water fall plots comparing baseline to the endpoint (day 41). Percent change from baseline to the endpoint is shown. Each bar represents an individual mouse. Data are from the experiment shown as FIG. 2B. FIG. 2D: Tumor volumes by caliper measurements. Data are representative of two experiments from two different donors. Means and SEM are shown (n=3 each for PBS group and n=5 each for the other groups). *, $p<0.05$ by two-way ANOVA with Bonferroni correction. FIG. 2E: Kaplan-Meier survival curve. Data are from the experiment shown as figure 2D. *, $p<0.05$; **, $p<0.01$ (vs. OAd-TNFa-IL2+meso-CAR T cell group) by Log-rank test. FIG. 2F: Combined OAd-TNFα-IL2 with meso-CAR T cells can prevent tumor metastasis. Representative lungs from OAd-mTNFα-IL2 + meso-CAR T cell group, OAd group and OAd + meso-CAR T cell group are shown. The two lungs with multiple metastasis shown here are from mice treated with OAd alone or combined OAd and meso-CAR T cells which were euthanized at day 102 due to weight loss (Center and right panel). The lung without metastasis is representative from mice treated with combined OAd-TNFα-IL2 and meso-CAR T cells (Left panel).

FIGs. 3A-3H: Oncolytic adenovirus (OAd) expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2)) induces robust T cell infiltration of tumors and enhances T cell functions. FIG. 3A: Analysis of CD8+ cell infiltration to the tumor at day 28 by immunohistochemistry (IHC). A group of mice was sacrificed and tumors were analyzed by IHC for CD8 staining. Representative tumors from the indicated treatment groups are shown. Original magnification, 20x; scale bar, 100μm. FIG. 3B: Quantification of TILs at day 28. The number of CD8+ TILs was quantified using Aperio ImageScope software. Number of CD8+ cells was normalized as percent CD8+ cells in total nucleated cells. Data are representative of two experiments from two

different donors. ***, p<0.001 by one-way ANOVA with Tukey's post-hoc test. FIG. 3C: Correlation between intensity of CD8+ TILs and tumor volumes. Number of CD8+ T cells (% of total cells) quantified from IHC against tumor sizes at day 28 are plotted. Each dot represents an individual mouse; a linear regression line and Pearson correlation coefficient (R) are shown. *, p<0.05 (D) Expression of activation markers on TILs at day 28. T cell activation markers, CD95 and CD25 on CD8+ TILs were analyzed by FCM. Data are representative of two experiments from two different donors. *, p<0.05. FIG. 3E: Cytokine profile of the bulk tumors at day 14. A group of mice was sacrificed at day 14. Pieces of tumors were homogenized and cytokines in the supernatant of the homogenate were analyzed by high-sensitivity LUMINEX assay. *, p<0.05; **, p<0.01; ***, p<0.001 by one-way ANOVA with Tukey's post-hoc test. FIG. 3F: Analysis of mesothelin expression by tumors by IHC at day 28. Mesothelin expression by tumor cells was analyzed by IHC (upper panels). Mesothelin positive area and staining intensity were analyzed with Aperio ImageScope software. Digital masks over the same fields as upper panels are shown in the lower panels. Original magnification, 20x; scale bar,100μm. FIG. 3G: Mesothelin expression by tumors is shown as percentage of mesothelin positive area. Three tumors (one from OAd-TNFα-IL2 group and two from OAd-TNFα-IL2 + meso-CAR T cell group) are not plotted as they achieved histological complete remission with no evaluable intact tumor areas. FIG. 3H: Correlation between mesothelin expression and tumor sizes. Area of mesothelin positive (%) are plotted against tumor size at day 28. Each dot represents an individual mouse and linear regression lines are shown. *, p<0.05; **, p<0.01. For vertical scatter grams, each dot represents an individual mouse and bars represents mean and SEM (FIGs. 3B, 3C, 3D, 3E and 3G).

FIGs. 4A-4C: Oncolytic adenovirus (OAd) expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2)) induces robust and persistent mesothelin-redirected chimeric antigen receptor T cell (meso-CAR T cell) accumulation in the tumor and improves T cell engraftment. FIGs. 4A and 4B: Trafficking of meso-CAR T cells by bioluminescence imaging (BLI). Using the same treatment schedule as FIG. 2A, luciferase labeled meso-CAR T cells (CBR-meso-CAR T cells) were tracked by BLI. Luminescence from tumor area was analyzed (FIG. 4B). Means and SEM are shown (n=5 each). ****, p<0.0001 (vs. OAd-TNFα-IL2 + meso-CAR T cell group at any time points between day 13 and day 28) by two-way repeated measures ANOVA with Bonferroni correction. FIG. 4C: CD3+ T cell counts in peripheral blood (PB). T cell number was determined by Trucount analysis. Means and SEM are shown (n=5 each). ***, p<0.001 (vs. OAd-TNFa-IL2 + meso-CAR T cell group) by repeated measures two-way ANOVA.

FIGs. 5A-5G: Intensity of functional T cell infiltration is associated with sustained tumor regression after mesothelin-redirected chimeric antigen receptor T cells (meso-CAR T cells) and oncolytic adenovirus expressing TNF-α and IL-2, Ad5/3-E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2) treatment in an AsPC-1 tumor xenograft immunodeficient mouse model. FIGs. 5A and 5B:

Tumor volumes by caliper measurements and water fall plots comparing baseline to day 57. Mice from the indicated two treatment groups were observed until day 57 and then sacrificed. Tumor volumes and water fall plots for the surviving mice (six of eight mice for meso-CAR T cell group and all of the seven mice for OAd-TNFα-IL2 + meso-CAR T cell group) are shown. Each dot and bar represent an individual mouse, and mean and SEM are shown for (FIG. 5A). FIG. 5C: Mesothelin expression on tumors at day 57. Mesothelin expression of the representative tumor from OAd-TNFα-IL2 + meso-CAR T cell group by immunohistochemistry (IHC) (upper panels) and digital masks (lower panels) on the same fields as upper panels are shown. The low power fields (LPF) show central necrosis and heterogeneity in mesothelin intensity (far left panels). Representative high-power fields (HPF) of mesothelin positive area (center panels) and mesothelin low-negative area (far right panels) from the tumor shown in the LPF. Original magnification, 20x; scale bar, 5 mm for LPF, 100 μm for HPF. FIG. 5D: Correlation between mesothelin expression and tumor size at day 57. Area of mesothelin positive area (%) against tumor volumes for individual mice are plotted. n.s., not significant. FIG. 5E: Correlation between density of CD3+ TILs and tumor volumes. Percent CD3+ cells to tumor cells (%) against tumor volumes are plotted. Each dot represents an individual mouse, and a linear regression line and Pearson correlation coefficient (R) are shown. *, $p<0.05$. FIG. 5F: Expression of Ki67 by tumor infiltrating lymphocytes (TILs). Ki67 expression by CD3+ TILs from surviving mice were analyzed by FCM. Columns are arranged in the order of tumor volumes at day 57 (left is the smallest and the right is the largest) and the tumor sizes are shown at the top of each column. FIG. 5G: Correlation between Ki67 expression by CD4+ and CD8+ TILs and tumor volumes. % Ki67 expression by CD4+ TILs or CD8+ TILs against tumor volumes are plotted. Each dot represents an individual mouse, and linear regression lines and Pearson correlation coefficient (R) are shown. *, $p<0.05$; **, $p<0.01$.

FIGs. 6A-6F: Combined mouse TNF-α and IL-2 delivered by adenoviruses with mouse mesothelin-redirected chimeric antigen receptor T cells (mmeso-CAR T cells) enables significant tumor suppression by enhancing both CAR-dependent and CAR-independent host immunity in a syngeneic pancreatic ductal adenocarcinoma (PDA)-engrafted immunocompetent mouse model. FIG. 6A: Experimental schematic. Established PDA7940b tumors were treated either with intratumoral injection of PBS, $1x10^9$ virus particle of control adenovirus (Ad-luc) or 1:1 mixture of Ad-mTNFα and Ad-mIL2 (total $1x10^9$vp) (Ad-mTNFα-mIL2) followed by intravenous injection of either PBS, $5x10^6$ mmeso-CAR T cells or human CD19 redirected chimeric antigen receptor T cells (h19-CAR T cells) at day 1 after Ad injection. Mice were preconditioned with Intraperitoneal injection of 120 mg/kg cyclophosphamide (Ctx) at 24 hours before the first T cell injection. Adenovirus and CAR T cell injections were repeated four times weekly. Tumor volumes were monitored by caliper measurement. FIG. 6B: Tumor volumes by caliper measurements. Means and SEM are shown (n=5 or 6 each). Data are representative of two experiments. *, $p<0.05$ by repeated measurements two-way ANOVA. FIG. 6C:

Trafficking of CAR T cells by bioluminescence (BLI). Luciferase labeled CAR T cells (CBR-CAR T cells) after the first single injection was tracked by BLI. Experiment was performed with the same schedule as FIG. 6A but T cell injection was performed just once. Luminescence from tumor area was analyzed. Means and SEM are shown (n=5 each). *, $p<0.05$; ***, $p<0.001$ (vs. Ad-mTNFα-mIL2 + mmeso-CAR T cell group) by repeated measurements two-way ANOVA with Bonferroni correction. FIG. 6D: Recruitment of donor derived tumor infiltrating lymphocytes (TILs) and host TILs at day 12. Number of CD4+ and CD8+ TILs were analyzed by FCM. Origin of T cells were determined by staining of CD45.1 (Donor) and CD45.2 (Host). Y-axis label: K, x1000. *, $p<0.05$; **, $p<0.01$ by one-way ANOVA with Tukey's post-hoc test. FIG. 6E: Phenotype of macrophages in tumors and spleens at day 1. CD80 and CD86 expression on tumor infiltrating macrophages and spleen macrophages was analyzed at 24 hours post intratumoral injection of either PBS, Ad-luc or Ad-mTNFα-mIL2 by FCM. *, $p<0.05$; **, $p<0.01$ by one-way ANOVA with Tukey's post-hoc test. FIG. 6F: Chemokine analysis of bulk tumors at day 1. A group of mice was sacrificed at day 1 post intratumoral injection of adenoviruses. Pieces of tumors were homogenized and chemokines in the supernatant of the homogenate were analyzed by LUMINEX assay. *, $p<0.05$; ***, $p<0.001$ by one-way ANOVA with Tukey's post-hoc test. For vertical scatter grams, each dot represents an individual mouse, and bars represent means and SEM. (FIGs. 6D, 6E and 6F).

FIGs. 7A-7D: Oncolytic adenovirus (OAd) delivers cytokine genes to pancreatic ductal adenocarcinoma (PDA) tumor cell lines and directly lyses target cells. FIG. 7A: Schematic representation of oncolytic adenovirus expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 or OAd-TNFα-IL2 for short) and its parental virus (Ad5/3-D24 or OAd for short). LITR, left inverted terminal repeats; RITR, right inverted terminal repeats; 100K, adenovirus 100K assembly protein; IRES, internal ribosome entry site; IL-2, IL-2 transgene; TNF-a, TNF-α transgene. FIG. 7B: Time course analysis of cytokine production by pancreatic tumor cell lines infected with OAd-TNFα-IL2. Twenty thousand tumor cells were infected with 30 virus particle (vp)/cell of OAd-TNFα-IL2 (total 250μl media) and culture supernatant was harvested at intervals from day 1 to day 7 after virus infection. Concentrations of TNF-α and IL-2 were analyzed by ELISA. Data are representative of two experiments. Means and SD from triplicate wells are shown. FIG. 7C: Kinetics of tumor cell lysis by oncolytic adenoviruses. Ten thousand PDA targets were infected either with OAd (upper panels) or OAd-TNFα-IL2 (lower panels) at the indicated titers. Cell index over six days was collected with xCELLigence real time cell analyzer. Means of values from triplicate wells are plotted. pfu, plaque forming unit. Data are representative from three experiments. FIG. 7D: Mesothelin expression by pancreatic cancer cell lines, BxPC-3, Capan-2 and AsPC-1 was analyzed by FCM.

FIG. 8: Adenovirus infects AsPC-1 tumors and induces necrosis. Adenovirus staining on tumors at day 14 after the injection of Oncolytic adenovirus (OAd) expressing TNF-α and IL-2 (Ad5/3-

E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2)) in an AsPC-1 xenograft NSG mouse model. A representative tumor treated with intratumoral injection of OAd-TNFα-IL2 using the same schedules and procedures as described in FIG. 2A is shown. Adenovirus positive cells are typically observed between intact tumor area and necrotic tumor area, which indicated that adenoviruses were gradually expanding while inducing tumor necrosis.

FIGs. 9A-9D: Oncolytic adenovirus (OAd) expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 (Ad5/3-OAd-TNFα-IL2)) induces robust T cell recruitment and infiltration to tumors and enhances T cell functions. Data are from the experiment shown in main FIGs. 3A-3H. FIG. 9A: Tumor volumes at day 14 and day 28. Tumor volumes by caliper measurements are shown. FIG. 9B: Number of CD4+ and CD8+ tumor infiltrating lymphocytes (TILs) at day 14 and day 28. TILs were analyzed by FCM at day 14 and day 28. Number of TILs was normalized to percent CD4+ or CD8+ cells in total nucleated cells. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by one-way ANOVA with Tukey's post-hoc test. FIG. 9C: Expression of activation markers on TILs at day 28. T cell activation markers, CD95 and CD25 on CD4+ TILs were analyzed by FCM. FIG. 9D: Cytokine levels in serum at day 14. Indicated human cytokines in mouse serum were analyzed by high-sensitivity LUMINEX assay. *, $p<0.05$; ***, $p<0.001$ by one-way ANOVA with Tukey's post-hoc test. For all scatter grams (FIGs. 9A, 9B, 9C and 9D), each dot represents an individual mouse, and bars represent means and SEM.

FIGs. 10A-10F: Development of new mouse mesothelin-redirected CAR T cells (mmeso-CAR T cells) and adenoviruses expressing mouse cytokines (Ad-mTNFα and Ad-mIL2) enabling assessment of the combination therapy of Ad-mTNFα-IL2 with CAR T cells in an immunocompetent setting. FIG. 10A: Schematic representation of mmeso-CAR expressed using standard gamma retrovirus technology. FIG. 10B: Surface expression of mmeso-CAR and control h19-CAR on mouse T cells. CAR expression by mouse splenic T cells was analyzed at day 5 after retroviral transduction to express CARs. Data are representative of at least four different T cell preparations. FIG. 10C: Kinetics of target cell killing by mmeso-CAR T cells and control human CD19-redirected CAR T cells (h19-CAR T cells) by xCELLigence real time cell analyzer. PDA7940b cells expressed high levels of mesothelin (FIG. 10C (part 1)). Five thousand PDA7940b cells were seeded in the e-plate. After 24 hours incubation, either control media, control h19-CAR T cells or mmeso-CAR T cells were added at the indicated E:T ratio. Cell index was recorded every 20 minutes (FIG. 10C (part 2)). Data are representative of at least four experiments from four different T cell preparations. Means of triplicate wells are shown. FIG. 10D: Cytokine production of PDA7940b cells infected with Ad-mTNFα-mIL2. Five thousand PDA7940b tumor cells were seeded to a 96 well plate and infected with Ad-mTNFa-mIL2 at the indicated concentrations (total 250µl media). Supernatant was harvested at 72 hours after the infection and cytokine levels were analyzed by ELISA. Data are representative of two experiments. Means and SD of triplicate wells are shown. FIG. 10E: CD80 and CD86 expression by DC at day 1 post intratumoral

adenovirus injection. CD80 and CD86 expression on DCs from tumors and spleen were analyzed by FCM. Data are from the experiment shown in FIG. 6E. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by one-way ANOVA with Tukey's post-hoc test. FIG. 10F: Chemokine (RANTES) analysis from tumors at day 1 port adenovirus injection. A group of mice was sacrificed at day 1 post intratumoral injection of adenoviruses. Pieces of tumors were homogenized and a chemokine, RANTES in the supernatant of the tumor homogenate were analyzed by LUMINEX assay. Data are from the experiment shown in FIG. 6F. For all scatter grams (FIGs. 10E and 10F), each dot represents an individual mouse, and mean and SEM are shown.

DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

The term "a" and "an" refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

Administered "in combination", as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two

treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

The term "TNFα" or "TNFα molecule" refers to Tumor necrosis factor ligand superfamily member 2 (TNFSF2, also known as DIF, TNFA, TNLG1F, or TNF-alpha), a multifunctional cytokine that belongs to the tumor necrosis factor (TNF) superfamily. GenBank No. NP_000585.2 and Swiss-Prot accession number P01375 provide exemplary human TNFα amino acid sequences. In some embodiments, TNFα or TNFα molecule is a naturally-existing TNFα or a functional variant or fragment thereof. In some embodiments, human TNFα has the following amino acid and nucleic acid sequences:

Tumor necrosis factor [Homo sapiens, NP_000585.2]

1 MSTESMIRDV ELAEEALPKK TGGPQGSRRC LFLSLFSFLI VAGATTLFCL LHFGVIGPQR
61 EEFPRDLSLI SPLAQAVRSS SRTPSDKPVA HVVANPQAEGQLQWLNRRAN ALLANGVELR
121 DNQLVVPSEG LYLIYSQVLF KGQGCPSTHV LLTHTISRIA VSYQTKVNLL SAIKSPCQRE
181 TPEGAEAKPW YEPIYLGGVF QLEKGDRLSA EINRPDYLDF AESGQVYFGI IAL (SEQ ID NO: 611)

Homo sapiens tumor necrosis factor (TNF), mRNA [NM_000594.3]

1 cagacgctcc ctcagcaagg acagcagagg accagctaag agggagagaa gcaactacag
61 acccccccctg aaaacaaccc tcagacgcca catcccctga caagctgcca ggcaggttct
121 cttcctctca catactgacc cacggctcca ccctctctcc cctggaaagg acaccatgag
181 cactgaaagc atgatccggg acgtggagct ggccgaggag gcgctcccca agaagacagg
241 ggggccccag ggctccaggc ggtgcttgtt cctcagcctc ttctccttcc tgatcgtggc
301 aggcgccacc acgctcttct gcctgctgca ctttggagtg atcggccccc agagggaaga
361 gttccccagg gacctctctc taatcagccc tctggcccag gcagtcagat catcttctcg
421 aaccccgagt gacaagcctg tagcccatgt tgtagcaaac cctcaagctg aggggcagct
481 ccagtggctg aaccgccggg ccaatgccct cctggccaat ggcgtggagc tgagagataa
541 ccagctggtg gtgccatcag agggcctgta cctcatctac tcccaggtcc tcttcaaggg
601 ccaaggctgc ccctccaccc atgtgctcct cacccacacc atcagccgca tcgccgtctc
661 ctaccagacc aaggtcaacc tcctctctgc catcaagagc ccctgccaga gggagacccc
721 agagggggct gaggccaagc cctggtatga gcccatctat ctgggagggg tcttccagct
781 ggagaagggt gaccgactca gcgctgagat caatcggccc gactatctcg actttgccga
841 gtctgggcag gtctactttg ggatcattgc cctgtgagga ggacgaacat ccaaccttcc
901 caaacgcctc ccctgcccca atccctttat taccccctcc ttcagacacc ctcaacctct
961 tctggctcaa aaagagaatt gggggcttag ggtcggaacc caagcttaga actttaagca
1021 acaagaccac cacttcgaaa cctgggattc aggaatgtgt ggcctgcaca gtgaagtgct
1081 ggcaaccact aagaattcaa actggggcct ccagaactca ctggggccta cagctttgat
1141 ccctgacatc tggaatctgg agaccaggga gcctttggtt ctggccagaa tgctgcagga

1201 cttgagaaga cctcacctag aaattgacac aagtggacct taggccttcc tctctccaga
1261 tgtttccaga cttccttgag acacggagcc cagccctccc catggagcca gctccctcta
1321 tttatgtttg cacttgtgat tatttattat ttatttatta tttatttatt tacagatgaa
1381 tgtatttatt tgggagaccg gggtatcctg ggggacccaa tgtaggagct gccttggctc
1441 agacatgttt tccgtgaaaa cggagctgaa caataggctg ttcccatgta gccccctggc
1501 ctctgtgcct tcttttgatt atgtttttta aaatatttat ctgattaagt tgtctaaaca
1561 atgctgattt ggtgaccaac tgtcactcat tgctgagcct ctgctcccca ggggagttgt
1621 gtctgtaatc gccctactat tcagtggcga gaaataaagt ttgcttagaa aagaaaaaaa
1681 aaaaaa (SEQ ID NO: 612)

The term "IL-2" or "IL-2 molecule" refers to interleukin-2 (also known as TCGF, or lymphokine), a secreted cytokine. GenBank No. NP_000577.2 and Swiss-Prot accession number P60568 provide exemplary human IL-2 amino acid sequences. In some embodiments, IL-2 or IL-2 molecule is a naturally-existing IL-2 or a functional variant or fragment thereof. In some embodiments, human IL-2 has the following amino acid and nucleic acid sequences:

Interleukin-2 precursor [Homo sapiens, NP_000577.2]
1 MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML
61 TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE
121 TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO: 613)

Homo sapiens interleukin 2 (IL2), mRNA [NM_000586.3]
1 agttccctat cactctcttt aatcactact cacagtaacc tcaactcctg ccacaatgta
61 caggatgcaa ctcctgtctt gcattgcact aagtcttgca cttgtcacaa acagtgcacc
121 tacttcaagt tctacaaaga aaacacagct acaactggag catttactgc tggatttaca
181 gatgattttg aatggaatta ataattacaa gaatcccaaa ctcaccagga tgctcacatt
241 taagttttac atgcccaaga aggccacaga actgaaacat cttcagtgtc tagaagaaga
301 actcaaacct ctggaggaag tgctaaattt agctcaaagc aaaaactttc acttaagacc
361 cagggactta atcagcaata tcaacgtaat agttctggaa ctaaagggat ctgaaacaac
421 attcatgtgt gaatatgctg atgagacagc aaccattgta gaatttctga acagatggat
481 taccttttgt caaagcatca tctcaacact gacttgataa ttaagtgctt cccacttaaa
541 acatatcagg ccttctattt atttaaatat ttaaatttta tatttattgt tgaatgtatg
601 gtttgctacc tattgtaact attattctta atcttaaaac tataaatatg gatcttttat
661 gattcttttt gtaagcccta ggggctctaa aatggtttca cttatttatc ccaaaatatt
721 tattattatg ttgaatgtta aatatagtat ctatgtagat tggttagtaa aactatttaa
781 taaatttgat aaatataaaa aaaaaaaaaa aaaaaaaaaa aa (SEQ ID NO: 614)

The term "IL-7" or "IL-7 molecule" refers to interleukin-7. GenBank No. NP_000871.1 and Swiss-Prot accession number P13232 provide exemplary human IL-7 amino acid sequences. In some embodiments, IL-7 or IL-7 molecule is a naturally-existing IL-7 or a functional variant or fragment thereof. In some embodiments, human IL-7 has the following amino acid and nucleic acid sequences:

Interleukin-7 isoform 1 precursor [Homo sapiens, NP_000871.1]

1 MFHVSFRYIF GLPPLILVLL PVASSDCDIE GKDGKQYESV LMVSIDQLLD SMKEIGSNCL
61 NNEFNFFKRH ICDANKEGMF LFRAARKLRQ FLKMNSTGDF DLHLLKVSEG TTILLNCTGQ
121 VKGRKPAALG EAQPTKSLEE NKSLKEQKKL NDLCFLKRLL QEIKTCWNKI LMGTKEH
(SEQ ID NO: 627)

Homo sapiens interleukin 7 (IL7), transcript variant 1, mRNA [NM_000880.4]

1 acacttgtgg cttccgtgca cacattaaca actcatggtt ctagctccca gtcgccaagc
61 gttgccaagg cgttgagaga tcatctggga agtcttttac ccagaattgc tttgattcag
121 gccagctggt ttttcctgcg gtgattcgga aattcgcgaa ttcctctggt cctcatccag
181 gtgcgcggga agcaggtgcc caggagagag gggataatga agattccatg ctgatgatcc
241 caaagattga acctgcagac caagcgcaaa gtagaaactg aaagtacact gctggcggat
301 cctacggaag ttatggaaaa ggcaaagcgc agagccacgc cgtagtgtgt gccgcccccc
361 ttgggatgga tgaaactgca gtcgcggcgt gggtaagagg aaccagctgc agagatcacc
421 ctgcccaaca cagactcggc aactccgcgg aagaccaggg tcctgggagt gactatgggc
481 ggtgagagct tgctcctgct ccagttgcgg tcatcatgac tacgcccgcc tcccgcagac
541 catgttccat gtttctttta ggtatatctt tggacttcct cccctgatcc ttgttctgtt
601 gccagtagca tcatctgatt gtgatattga aggtaaagat ggcaaacaat atgagagtgt
661 tctaatggtc agcatcgatc aattattgga cagcatgaaa gaaattggta gcaattgcct
721 gaataatgaa tttaactttt ttaaaagaca tatctgtgat gctaataagg aaggtatgtt
781 tttattccgt gctgctcgca agttgaggca atttcttaaa atgaatagca ctggtgattt
841 tgatctccac ttattaaaag tttcagaagg cacaacaata ctgttgaact gcactggcca
901 ggttaaagga agaaaaccag ctgccctggg tgaagcccaa ccaacaaaga gtttggaaga
961 aaataaatct ttaaaggaac agaaaaaact gaatgacttg tgtttcctaa agagactatt
1021 acaagagata aaaacttgtt ggaataaaat tttgatgggc actaaagaac actgaaaaat
1081 atggagtggc aatatagaaa cacgaacttt agctgcatcc tccaagaatc tatctgctta
1141 tgcagttttt cagagtggaa tgcttcctag aagttactga atgcaccatg gtcaaaacgg
1201 attagggcat ttgagaaatg catattgtat tactagaaga tgaatacaaa caatggaaac
1261 tgaatgctcc agtcaacaaa ctatttctta tatatgtgaa catttatcaa tcagtataat
1321 tctgtactga tttttgtaag acaatccatg taaggtatca gttgcaataa tacttctcaa
1381 acctgtttaa atatttcaag acattaaatc tatgaagtat ataatggttt caaagattca
1441 aaattgacat tgctttactg tcaaaataat tttatggctc actatgaatc tattatactg
1501 tattaagagt gaaaattgtc ttcttctgtg ctggagatgt tttagagtta acaatgatat
1561 atggataatg ccggtgagaa taagagagtc ataaacctta agtaagcaac agcataacaa
1621 ggtccaagat acctaaaaga gatttcaaga gatttaatta atcatgaatg tgtaacacag
1681 tgccttcaat aaatggtata gcaaatgttt tgacatgaaa aaaggacaat ttcaaaaaaa
1741 taaaataaaa taaaaataaa ttcacctagt ctaaggatgc taaaccttag tactgagtta

1801 cattgtcatt tatatagatt ataacttgtc taaataagtt tgcaatttgg gagatatatt
1861 tttaagataa taatatatgt ttacctttta attaatgaaa tatctgtatt taattttgac
1921 actatatctg tatataaaat attttcatac agcattacaa attgcttact ttggaataca
1981 tttctccttt gataaaataa atgagctatg tattaa (SEQ ID NO: 628)

The compositions and methods of the present invention encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, *e.g.*, sequences at least 85%, 90%, or 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid sequence that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity, for example, amino acid sequences that contain a common structural domain having at least about 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

In the context of a nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity, for example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

The term "variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence. In some embodiments, the variant is a functional variant.

The term "functional variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence, and is capable of having one or more activities of the reference amino acid sequence.

The term "Chimeric Antigen Receptor" or alternatively a "CAR" refers to a recombinant polypeptide construct comprising at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule as defined below. In some embodiments, the domains in the CAR polypeptide construct are in the same polypeptide chain, e.g., comprise a chimeric fusion protein. In some embodiments, the domains in the

CAR polypeptide construct are not contiguous with each other, e.g., are in different polypeptide chains, e.g., as provided in an RCAR as described herein.

In one aspect, the cytoplasmic signaling domain comprises a primary signaling domain (e.g., a primary signaling domain of CD3-zeta). In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule is chosen from 41BB (i.e., CD137), CD27, ICOS, and/or CD28. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen recognition domain, wherein the leader sequence is optionally cleaved from the antigen recognition domain (e.g., an scFv) during cellular processing and localization of the CAR to the cellular membrane.

A CAR that comprises an antigen binding domain (e.g., an scFv, a single domain antibody, or TCR (e.g., a TCR alpha binding domain or TCR beta binding domain)) that targets a specific tumor marker X, wherein X can be a tumor marker as described herein, is also referred to as XCAR. For example, a CAR that comprises an antigen binding domain that targets CD19 is referred to as CD19CAR. The CAR can be expressed in any cell, e.g., an immune effector cell as described herein (e.g., a T cell or an NK cell).

The term "signaling domain" refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule, which specifically binds with an antigen. Antibodies can be polyclonal or

monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules.

The term "antibody fragment" refers to at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, e.g., an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, scFv antibody fragments, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, and multi-specific molecules formed from antibody fragments such as a bivalent fragment comprising two or more, e.g., two, Fab fragments linked by a disulfide brudge at the hinge region, or two or more, e.g., two isolated CDR or other epitope binding fragments of an antibody linked. An antibody fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antibody fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3) (see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

The term "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 ("Chothia" numbering scheme), or a combination thereof. Under the Kabat numbering scheme, in some embodiments, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light

chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under the Chothia numbering scheme, in some embodiments, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3). In a combined Kabat and Chothia numbering scheme, in some embodiments, the CDRs correspond to the amino acid residues that are part of a Kabat CDR, a Chothia CDR, or both. For instance, in some embodiments, the CDRs correspond to amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in a VH, e.g., a mammalian VH, e.g., a human VH; and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in a VL, e.g., a mammalian VL, e.g., a human VL.

The portion of the CAR composition of the invention comprising an antibody or antibody fragment thereof may exist in a variety of forms, for example, where the antigen binding domain is expressed as part of a polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv), or e.g., a humanized antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one aspect, the antigen binding domain of a CAR composition of the invention comprises an antibody fragment. In a further aspect, the CAR comprises an antibody fragment that comprises an scFv.

As used herein, the term “binding domain” or "antibody molecule" (also referred to herein as “anti-target binding domain”) refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term “binding domain” or “antibody molecule” encompasses antibodies and antibody fragments. In an embodiment, an antibody molecule is a multispecific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. The term “antibody heavy chain,” refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

The term “antibody light chain,” refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

The term "recombinant antibody" refers to an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage or yeast expression system. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

The term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

The term "anti-tumor effect" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, decrease in tumor cell proliferation, decrease in tumor cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor in the first place.

The term "anti-cancer effect" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-cancer effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of cancer in the first

place. The term "anti-tumor effect" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, or a decrease in tumor cell survival. The term "autologous" refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

The term "allogeneic" refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically.

The term "xenogeneic" refers to a graft derived from an animal of a different species.

The term "cancer" refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. Preferred cancers treated by the methods described herein include multiple myeloma, Hodgkin's lymphoma or non-Hodgkin's lymphoma.

The terms "tumor" and "cancer" are used interchangeably herein, e.g., both terms encompass solid and liquid, e.g., diffuse or circulating, tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors.

"Derived from" as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connotate or include a process or source limitation on a first molecule that is derived from a second molecule. For example, in the case of an intracellular signaling domain that is derived from a CD3zeta molecule, the intracellular signaling domain retains sufficient CD3zeta structure such that is has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connotate or include a limitation to a particular process of producing the intracellular signaling domain, e.g., it does not mean that, to provide the intracellular signaling domain, one must start with a CD3zeta sequence and delete unwanted sequence, or impose mutations, to arrive at the intracellular signaling domain.

The phrase "disease associated with expression of an antigen, e.g., a tumor antigen" includes, but is not limited to, a disease associated with a cell which expresses the antigen (e.g., wild-type or mutant antigen) or condition associated with a cell which expresses the antigen (e.g., wild-type or mutant antigen) including, e.g., proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a noncancer

related indication associated with a cell which expresses the antigen (e.g., wild-type or mutant antigen). For the avoidance of doubt, a disease associated with expression of the antigen may include a condition associated with a cell which does not presently express the antigen, e.g., because expression of the antigen has been downregulated, e.g., due to treatment with a molecule targeting the antigen, but which at one time expressed the antigen. In some embodiments, the disease associated with expression of an antigen, e.g., a tumor antigen is a cancer (e.g., a solid cancer or a hematological cancer), a viral infection (e.g., HIV, a fungal infection, e.g., C. neoformans), an autoimmune disease (e.g. rheumatoid arthritis, system lupus erythematosus (SLE or lupus), pemphigus vulgaris, and Sjogren's syndrome; inflammatory bowel disease, ulcerative colitis; transplant-related allospecific immunity disorders related to mucosal immunity; and unwanted immune responses towards biologics (e.g., Factor VIII) where humoral immunity is important).

The phrase "disease associated with expression of mesothelin" includes, but is not limited to, a disease associated with expression of mesothelin or condition associated with cells which express mesothelin including, e.g., proliferative diseases such as a cancer or malignancy or a precancerous condition such as a mesothelial hyperplasia; or a noncancer related indication associated with cells which express mesothelin. Examples of various cancers that express mesothelin include but are not limited to, mesothelioma, lung cancer, ovarian cancer, pancreatic cancer, and the like.

The term "conservative sequence modifications" refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the functional assays described herein.

The term "stimulation," refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can

mediate altered expression of certain molecules, such as downregulation of TGF-β, and/or reorganization of cytoskeletal structures, and the like.

The term "stimulatory molecule," refers to a molecule expressed by a T cell that provides the primary cytoplasmic signaling sequence(s) that regulate primary activation of the TCR complex in a stimulatory way for at least some aspect of the T cell signaling pathway. In some embodiments, the ITAM-containing domain within the CAR recapitulates the signaling of the primary TCR independently of endogenous TCR complexes. In one aspect, the primary signal is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a "primary signaling domain") that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing primary cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS") , FcεRI and CD66d, DAP10 and DAP12. In a specific CAR of the invention, the intracellular signaling domain in any one or more CARS of the invention comprises an intracellular signaling sequence, e.g., a primary signaling sequence of CD3-zeta. The term "antigen presenting cell" or "APC" refers to an immune system cell such as an accessory cell (e.g., a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

An "intracellular signaling domain," as the term is used herein, refers to an intracellular portion of a molecule. In embodiments, the intracellular signal domain transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CART cell. Examples of immune effector function, e.g., in a CART cell, include cytolytic activity and helper activity, including the secretion of cytokines.

In an embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent simulation. In an embodiment, the

intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CART, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, CD66d, DAP10 and DAP12.

The term "zeta" or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" refers to CD247. Swiss-Prot accession number P20963 provides exemplary human CD3 zeta amino acid sequences. A "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" or a "TCR-zeta stimulatory domain" refers to a stimulatory domain of CD3-zeta or a variant thereof (e.g., a molecule having mutations, e.g., point mutations, fragments, insertions, or deletions). In one embodiment, the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or a variant thereof (e.g., a molecule having mutations, e.g., point mutations, fragments, insertions, or deletions). In one embodiment, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO: 9 or 10, or a variant thereof (e.g., a molecule having mutations, e.g., point mutations, fragments, insertions, or deletions).

The term "costimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Costimulatory molecules include, but are not limited to an MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100

(SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, and a ligand that specifically binds with CD83.

A costimulatory intracellular signaling domain refers to the intracellular portion of a costimulatory molecule.

The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

The term "4-1BB" refers to CD137 or Tumor necrosis factor receptor superfamily member 9. Swiss-Prot accession number P20963 provides exemplary human 4-1BB amino acid sequences. A "4-1BB costimulatory domain" refers to a costimulatory domain of 4-1BB, or a variant thereof (e.g., a molecule having mutations, e.g., point mutations, fragments, insertions, or deletions). In one embodiment, the "4-1BB costimulatory domain" is the sequence provided as SEQ ID NO: 7 or a variant thereof (e.g., a molecule having mutations, e.g., point mutations, fragments, insertions, or deletions).

"Immune effector cell," as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloic-derived phagocytes.

"Immune effector function or immune effector response," as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack of a target cell. E.g., an immune effector function or response refers a property of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

The term "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence. In some embodiments, expression comprises translation of an mRNA introduced into a cell.

The term "transfer vector" refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "transfer vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term "lentivirus" refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses.

The term "lentiviral vector" refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., Mol. Ther.

17(8): 1453–1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include but are not limited to, e.g., the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

The term "homologous" or "identity" refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof are human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, a humanized antibody/antibody fragment can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications can further refine and optimize antibody or antibody fragment performance. In general, the humanized antibody or antibody fragment thereof will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or a significant portion of the FR regions are those of a human immunoglobulin sequence. The humanized antibody or antibody fragment can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321: 522-525, 1986; Reichmann et al., Nature, 332: 323-329, 1988; Presta, Curr. Op. Struct. Biol., 2: 593-596, 1992.

"Fully human" refers to an immunoglobulin, such as an antibody or antibody fragment, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody or immunoglobulin.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

The term "operably linked" or "transcriptional control" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term "parenteral" administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

The term "nucleic acid," "nucleic acid molecule," "polynucleotide," or "polynucleotide molecule" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. In some embodiments, a "nucleic acid," "nucleic acid molecule," "polynucleotide," or "polynucleotide molecule" comprise a nucleotide/nucleoside derivative or analog. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions, e.g., conservative substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions, e.g., conservative substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino

acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term "promoter/regulatory sequence" refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "constitutive" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term "inducible" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term "tissue-specific" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The terms "cancer associated antigen" or "tumor antigen" interchangeably refers to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cancer cell, either entirely or as a fragment (e.g., MHC/peptide), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a tumor antigen is a marker expressed by both normal cells and cancer cells, e.g., a lineage marker, e.g., CD19 on B cells. In some embodiments, a tumor antigen is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some embodiments, a tumor antigen is a cell

surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a tumor antigen will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (e.g., MHC/peptide), and not synthesized or expressed on the surface of a normal cell. In some embodiments, the CARs of the present invention include CARs comprising an antigen binding domain (e.g., antibody or antibody fragment) that binds to a MHC presented peptide. Normally, peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules, and are recognized by T cell receptors (TCRs) on CD8 + T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, e.g., Sastry et al., J Virol. 2011 85(5):1935-1942; Sergeeva et al., Blood, 2011 117(16):4262-4272; Verma et al., J Immunol 2010 184(4):2156-2165; Willemsen et al., Gene Ther 2001 8(21) :1601-1608 ; Dao et al., Sci Transl Med 2013 5(176) :176ra33 ; Tassev et al., Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

The term "tumor-supporting antigen" or "cancer-supporting antigen" interchangeably refer to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cell that is, itself, not cancerous, but supports the cancer cells, e.g., by promoting their growth or survival e.g., resistance to immune cells. Exemplary cells of this type include stromal cells and myeloid-derived suppressor cells (MDSCs). The tumor-supporting antigen itself need not play a role in supporting the tumor cells so long as the antigen is present on a cell that supports cancer cells.

The term "flexible polypeptide linker" or "linker" as used in the context of an scFv refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-Ser)n, where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3. n=4, n=5 and n=6, n=7, n=8, n=9 and n=10 (SEQ ID NO: 606). In one embodiment, the flexible polypeptide linkers include, but are not limited to, (Gly4 Ser)4 (SEQ ID NO: 27) or (Gly4 Ser)3 (SEQ ID NO: 28). In another embodiment, the linkers include multiple repeats of (Gly2Ser), (GlySer) or (Gly3Ser) (SEQ ID NO: 29). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference.

As used herein, a 5' cap (also termed an RNA cap, an RNA 7-methylguanosine cap or an RNA m7G cap) is a modified guanine nucleotide that has been added to the "front" or 5' end of a eukaryotic

messenger RNA shortly after the start of transcription. The 5' cap consists of a terminal group which is linked to the first transcribed nucleotide. Its presence is critical for recognition by the ribosome and protection from RNases. Cap addition is coupled to transcription, and occurs co-transcriptionally, such that each influences the other. Shortly after the start of transcription, the 5' end of the mRNA being synthesized is bound by a cap-synthesizing complex associated with RNA polymerase. This enzymatic complex catalyzes the chemical reactions that are required for mRNA capping. Synthesis proceeds as a multi-step biochemical reaction. The capping moiety can be modified to modulate functionality of mRNA such as its stability or efficiency of translation.

As used herein, "in vitro transcribed RNA" refers to RNA, preferably mRNA, that has been synthesized in vitro. Generally, the in vitro transcribed RNA is generated from an in vitro transcription vector. The in vitro transcription vector comprises a template that is used to generate the in vitro transcribed RNA.

As used herein, a "poly(A)" is a series of adenosines attached by polyadenylation to the mRNA. In the preferred embodiment of a construct for transient expression, the polyA is between 50 and 5000 (SEQ ID NO: 30), preferably greater than 64, more preferably greater than 100, most preferably greater than 300 or 400. poly(A) sequences can be modified chemically or enzymatically to modulate mRNA functionality such as localization, stability or efficiency of translation.

As used herein, "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (often several hundred) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, "transient" refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (e.g., one or more therapeutic agents such as a CAR of the invention). In specific embodiments, the terms "treat", "treatment" and "treating" refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms "treat", "treatment" and "treating" -refer to the inhibition of the progression of a proliferative disorder, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms "treat", "treatment" and "treating" refer to the reduction or stabilization of tumor size or cancerous cell count.

The term "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase "cell surface receptor" includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the membrane of a cell.

The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).

The term, a "substantially purified" cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some aspects, the cells are cultured in vitro. In other aspects, the cells are not cultured in vitro.

The term "therapeutic" as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term "prophylaxis" as used herein means the prevention of or protective treatment for a disease or disease state.

In the context of the present invention, "tumor antigen" or "hyperproliferative disorder antigen" or "antigen associated with a hyperproliferative disorder" refers to antigens that are common to specific hyperproliferative disorders. In certain aspects, the hyperproliferative disorder antigens of the present invention are derived from, cancers including but not limited to primary or metastatic melanoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic

prostate cancer), ovarian cancer, pancreatic cancer, and the like, or a plasma cell proliferative disorder, e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome).

The term "transfected" or "transformed" or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The term "specifically binds," refers to an antibody, or a ligand, which recognizes and binds with a cognate binding partner (e.g., a stimulatory and/or costimulatory molecule present on a T cell) protein present in a sample, but which antibody or ligand does not substantially recognize or bind other molecules in the sample.

"Regulatable chimeric antigen receptor (RCAR)," as used herein, refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, an RCAR comprises at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule as defined herein in the context of a CAR molecule. In some embodiments, the set of polypeptides in the RCAR are not contiguous with each other, e.g., are in different polypeptide chains. In some embodiments, the RCAR includes a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain. In some embodiments, the RCAR is expressed in a cell (e.g., an immune effector cell) as described herein, e.g., an RCAR-expressing cell (also referred to herein as "RCARX cell"). In an embodiment the RCARX cell is a T cell, and is referred to as a RCART cell. In an embodiment the RCARX cell is an NK cell, and is referred to as a RCARN cell. The RCAR can provide the RCAR-expressing cell with specificity for a target cell, typically a cancer cell, and with regulatable intracellular signal generation or proliferation, which can optimize an immune effector property of the RCAR-expressing cell. In embodiments, an RCAR cell relies at least in part, on an antigen binding domain to provide specificity to a target cell that comprises the antigen bound by the antigen binding domain.

"Membrane anchor" or "membrane tethering domain", as that term is used herein, refers to a polypeptide or moiety, e.g., a myristoyl group, sufficient to anchor an extracellular or intracellular domain to the plasma membrane.

"Switch domain," as that term is used herein, e.g., when referring to an RCAR, refers to an entity, typically a polypeptide-based entity, that, in the presence of a dimerization molecule, associates with another switch domain. The association results in a functional coupling of a first entity linked to, e.g., fused to, a first switch domain, and a second entity linked to, e.g., fused to, a second switch domain. A first and second switch domain are collectively referred to as a dimerization switch. In embodiments, the first and second switch domains are the same as one another, e.g., they are polypeptides having the same primary amino acid sequence, and are referred to collectively as a homodimerization switch. In embodiments, the first and second switch domains are different from one another, e.g., they are polypeptides having different primary amino acid sequences, and are referred to collectively as a heterodimerization switch. In embodiments, the switch is intracellular. In embodiments, the switch is extracellular. In embodiments, the switch domain is a polypeptide-based entity, e.g., FKBP or FRB-based, and the dimerization molecule is small molecule, e.g., a rapalogue. In embodiments, the switch domain is a polypeptide-based entity, e.g., an scFv that binds a myc peptide, and the dimerization molecule is a polypeptide, a fragment thereof, or a multimer of a polypeptide, e.g., a myc ligand or multimers of a myc ligand that bind to one or more myc scFvs. In embodiments, the switch domain is a polypeptide-based entity, e.g., myc receptor, and the dimerization molecule is an antibody or fragments thereof, e.g., myc antibody.

"Dimerization molecule," as that term is used herein, e.g., when referring to an RCAR, refers to a molecule that promotes the association of a first switch domain with a second switch domain. In embodiments, the dimerization molecule does not naturally occur in the subject, or does not occur in concentrations that would result in significant dimerization. In embodiments, the dimerization molecule is a small molecule, e.g., rapamycin or a rapalogue, e.g, RAD001.

The term "bioequivalent" refers to an amount of an agent other than the reference compound (e.g., RAD001), required to produce an effect equivalent to the effect produced by the reference dose or reference amount of the reference compound (e.g., RAD001). In an embodiment the effect is the level of mTOR inhibition, e.g., as measured by P70 S6 kinase inhibition, e.g., as evaluated in an in vivo or in vitro assay, e.g., as measured by an assay described herein, e.g., the Boulay assay, or measurement of phosphorylated S6 levels by western blot. In an embodiment, the effect is alteration of the ratio of PD-1 positive/PD-1 negative T cells, as measured by cell sorting. In an embodiment a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of P70 S6 kinase inhibition as does the reference dose or reference amount of a reference compound. In an embodiment, a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level

of alteration in the ratio of PD-1 positive/PD-1 negative T cells as does the reference dose or reference amount of a reference compound.

The term "low, immune enhancing, dose" when used in conjuction with an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., RAD001 or rapamycin, or a catalytic mTOR inhibitor, refers to a dose of mTOR inhibitor that partially, but not fully, inhibits mTOR activity, e.g., as measured by the inhibition of P70 S6 kinase activity. Methods for evaluating mTOR activity, e.g., by inhibition of P70 S6 kinase, are discussed herein. The dose is insufficient to result in complete immune suppression but is sufficient to enhance the immune response. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in a decrease in the number of PD-1 positive T cells and/or an increase in the number of PD-1 negative T cells, or an increase in the ratio of PD-1 negative T cells/PD-1 positive T cells. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in an increase in the number of naive T cells. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in one or more of the following:

an increase in the expression of one or more of the following markers: $CD62L^{high}$, $CD127^{high}$, $CD27^{+}$, and BCL2, e.g., on memory T cells, e.g., memory T cell precursors;

a decrease in the expression of KLRG1, e.g., on memory T cells, e.g., memory T cell precursors; and

an increase in the number of memory T cell precursors, e.g., cells with any one or combination of the following characteristics: increased $CD62L^{high}$, increased $CD127^{high}$, increased $CD27^{+}$, decreased KLRG1, and increased BCL2;

wherein any of the changes described above occurs, e.g., at least transiently, e.g., as compared to a non-treated subject.

"Refractory" as used herein refers to a disease, e.g., cancer, that does not respond to a treatment. In embodiments, a refractory cancer can be resistant to a treatment before or at the beginning of the treatment. In other embodiments, the refractory cancer can become resistant during a treatment. A refractory cancer is also called a resistant cancer.

"Relapsed" or "relapse" as used herein refers to the return or reappearance of a disease (e.g., cancer) or the signs and symptoms of a disease such as cancer after a period of improvement or responsiveness, e.g., after prior treatment of a therapy, e.g., cancer therapy. The initial period of responsiveness may involve the level of cancer cells falling below a certain threshold, e.g., below 20%, 1%, 10%, 5%, 4%, 3%, 2%, or 1%. The reappearance may involve the level of cancer cells rising above a certain threshold, e.g., above 20%, 1%, 10%, 5%, 4%, 3%, 2%, or 1%. For example, e.g., in the context of B-ALL, the reappearance may involve, e.g., a reappearance of blasts in the blood, bone marrow (> 5%), or any extramedullary site, after a complete response. A complete response, in this context, may involve < 5% BM blast. More generally, in an embodiment, a response (e.g., complete

response or partial response) can involve the absence of detectable MRD (minimal residual disease). In an embodiment, the initial period of responsiveness lasts at least 1, 2, 3, 4, 5, or 6 days; at least 1, 2, 3, or 4 weeks; at least 1, 2, 3, 4, 6, 8, 10, or 12 months; or at least 1, 2, 3, 4, or 5 years.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98%, or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98%, and 98-99% identity. This applies regardless of the breadth of the range.

A "gene editing system" as the term is used herein, refers to a system, e.g., one or more molecules, that direct and effect an alteration, e.g., a deletion, of one or more nucleic acids at or near a site of genomic DNA targeted by said system. Gene editing systems are known in the art, and are described more fully below.

Various aspects of the compositions and methods herein are described in further detail below. Additional definitions are set out throughout the specification.

Detailed Description

Provided herein are compositions and methods for treating a disease such as cancer, by administering a cell comprising a chimeric antigen receptor (CAR) molecule, e.g., that targets a tumor antigen, in combination with a virus comprising a nucleic acid molecule encoding a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule. Exemplary components to generate a CAR and a CAR-expressing cell are disclosure herein. Exemplary viruses comprising a nucleic acid molecule encoding a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule are also described herein.

In embodiments, the combination therapy of a CAR-expressing cell (e.g., a mesothelin CAR-expressing cell) described herein and a virus comprising a nucleic acid molecule encoding a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule described herein results in one or more of the following: improved or increased anti-tumor activity of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell); increased proliferation or persistence of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell); improved or increased infiltration of the CAR-expressing cell (e.g.,

the mesothelin CAR-expressing cell); improved inhibition of tumor progression or metastasis; delay of tumor progression; inhibition or reduction in cancer cell proliferation; and/or reduction in tumor burden, e.g., tumor volume, or size.

As demonstrated in the examples provided herein, in some embodiments, administration of the virus comprising a nucleic acid molecule encoding a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule prior to administration of the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) results in increased therapeutic efficacy, e.g., increased inhibition of tumor progression and/or tumor growth, in some cancers, e.g., as compared to administration of the virus or the CAR-expressing cell (e.g., the mesothelin CAR-expressing cell) alone.

Virus comprising a nucleic acid molecule encoding one or more cytokine molecules

In one aspect, disclosed herein are methods using a virus comprising a nucleic acid molecule encoding one or more cytokine molecules.

In one embodiment, the one or more cytokine molecules are chosen from IFNα, IFNβ, IFNγ, complement C5a, IL-2, IL-7, TNFα, CD40L, IL12, IL-23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7, XCL2, or any combination thereof.

In one embodiment, the virus can be any virus suitable for treating a subject, e.g., a human.

In one embodiment, the virus is a virus, e.g., an oncolytic adenovirus, disclosed in US20150232880, herein incorporated by reference in its entirety. In one embodiment, the virus comprises a viral vector chosen from Ad5, Ad3 or Ad5/3 vector. In one embodiment, the vector is Ad5 vector. In one embodiment, the vector is Ad3 vector. In one embodiment, the vector is Ad5/3 vector. As used herein, the term "adenovirus serotype 5 (Ad5) nucleic acid backbone" refers to the genome of Ad5. "Ad3 nucleic acid backbone" refers to the genome of Ad3. "Ad5/3 vector" refers to a chimeric vector having parts of both Ad5 and Ad3 vectors. "Ad5/3 chimeric fiber knob" refers to a chimerism, wherein the knob part of the fiber is from Ad serotype 3, and the rest of the fiber is from Ad serotype 5. In one embodiment, the construct has the fiber knob from Ad3 while the remainder of the genome is from Ad5 (see FIGs. 17, 33 and 34 of US20150232880). The vectors may be modified in any way known in the art, e.g. by deleting, inserting, mutating or modifying any viral areas. The vectors are made tumor specific with regard to replication. In one embodiment, the adenoviral vector comprises modifications in E1, E3 and/or E4 such as insertion of tumor specific promoters (e.g., to drive E1),

deletions of areas (e.g., the constant region 2 of E1 as used in "D24", E3/gp19k, E3/6.7k) and insertion of transgenes. In one embodiment, a tumor specific oncolytic adenovirus is generated by engineering a 24 base pair deletion (D24) affecting the constant region 2 (CR2) of E1. In one embodiment, an oncolytic adenovirus is generated by engineering a gp19k/6.7K deletion in E3 (a deletion of 965 base pairs from the adenoviral E3A region). In a resulting adenoviral construct, both gp19k and 6.7K genes are deleted (Kanerva A et al. 2005, Gene Therapy 12, 87-94). Furthermore, fiber knob areas of the vector can be modified. In one embodiment of the invention, the adenoviral vector is Ad5/3 comprising an Ad5 nucleic acid backbone and Ad3 fiber knob or Ad5/3 chimeric fiber knob.

In one embodiment, the virus is an oncolytic virus. Suitable oncolytic viruses are known in the art, e.g., those described in Kaufman, Nat Rev Drug Discov. 2015; 14(9):642-662, which is incorporated by reference herein in its entirety. In some embodiments, the oncolytic virus specifically targets cancer cells, e.g., the oncolytic virus has no effect or a minimal effect on non-cancer cells. In some embodiments, the oncolytic virus selectively replicates in cancer cells. In embodiments, the oncolytic virus is capable of selectively replicating in and triggering the death of or slowing the growth of a cancer cell. An oncolytic virus includes but is not limited to an oncolytic adenovirus, oncolytic adeno-associated virus, oncolytic Herpes Simplex Virus (HSV), oncolytic parvovirus, oncolytic retrovirus, oncolytic lentivirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza virus, oncolytic reovirus, oncolytic Newcastle disease virus (NDV), oncolytic measles virus, oncolytic vesicular stomatitis virus (VSV), oncolytic poliovirus, oncolytic poxvirus, oncolytic Seneca Valley virus, oncolytic coxsackievirus, oncolytic enterovirus, oncolytic myxoma virus, or oncolytic maraba virus.

In some embodiments, the oncolytic virus is a recombinant oncolytic virus, such as those described in US2010/0178684, which is incorporated herein by reference in its entirety. In some embodiments, a recombinant oncolytic virus comprises a nucleic acid sequence (e.g., heterologous nucleic acid sequence) encoding an inhibitor of an immune or inflammatory response, e.g., as described in US2010/0178684, which is incorporated herein by reference in its entirety. In embodiments, the recombinant oncolytic virus, e.g., oncolytic NDV, comprises a pro-apoptotic protein (e.g., apoptin), a cytokine (e.g., GM-CSF, CSF, interferon-gamma, interleukin-2 (IL-2), tumor necrosis factor-alpha), an immunoglobulin (e.g., an antibody against ED-B firbonectin), tumor associated antigen, a bispecific adapter protein (e.g., bispecific antibody or antibody fragment directed against NDV HN protein and a T cell co-stimulatory receptor, such as CD3 or CD28; or fusion protein between human IL-2 and single chain antibody directed against NDV HN protein). See, e.g., Zamarin et al. Future Microbiol. 7.3(2012):347-67, incorporated herein by reference in its entirety. In some embodiments, the oncolytic virus is a chimeric oncolytic NDV described in US 8591881 B2, US 2012/0122185 A1, or US 2014/0271677 A1, each of which is incorporated herein by reference in their entireties.

In some embodiments, the oncolytic virus comprises a conditionally replicative adenovirus (CRAd), which is designed to replicate exclusively in cancer cells. See, e.g., Alemany et al. Nature Biotechnol. 18(2000):723-27. In some embodiments, an oncolytic adenovirus comprises one described in Table 1 on page 725 of Alemany et al., incorporated herein by reference in its entirety.

Exemplary oncolytic viruses include but are not limited to the following:

Group B Oncolytic Adenovirus (ColoAd1) (PsiOxus Therapeutics Ltd.) (see, e.g., Clinical Trial Identifier: NCT02053220);

ONCOS-102 (previously called CGTG-102), which is an adenovirus comprising granulocyte-macrophage colony stimulating factor (GM-CSF) (Oncos Therapeutics) (see, e.g., Clinical Trial Identifier: NCT01598129);

VCN-01, which is a genetically modified oncolytic human adenovirus encoding human PH20 hyaluronidase (VCN Biosciences, S.L.) (see, e.g., Clinical Trial Identifiers: NCT02045602 and NCT02045589);

Conditionally Replicative Adenovirus ICOVIR-5, which is a virus derived from wild-type human adenovirus serotype 5 (Had5) that has been modified to selectively replicate in cancer cells with a deregulated retinoblastoma/E2F pathway (Institut Català d'Oncologia) (see, e.g., Clinical Trial Identifier: NCT01864759);

Celyvir, which comprises bone marrow-derived autologous mesenchymal stem cells (MSCs) infected with ICOVIR5, an oncolytic adenovirus (Hospital Infantil Universitario Niño Jesús, Madrid, Spain/ Ramon Alemany) (see, e.g., Clinical Trial Identifier: NCT01844661);

CG0070, which is a conditionally replicating oncolytic serotype 5 adenovirus (Ad5) in which human E2F-1 promoter drives expression of the essential E1a viral genes, thereby restricting viral replication and cytotoxicity to Rb pathway-defective tumor cells (Cold Genesys, Inc.) (see, e.g., Clinical Trial Identifier: NCT02143804); or

DNX-2401 (formerly named Delta-24-RGD), which is an adenovirus that has been engineered to replicate selectively in retinoblastoma (Rb)-pathway deficient cells and to infect cells that express certain RGD-binding integrins more efficiently (Clinica Universidad de Navarra, Universidad de Navarra/ DNAtrix, Inc.) (see, e.g., Clinical Trial Identifier: NCT01956734).

In some embodiments, the oncolytic virus can express a detectable marker, e.g., a fluorescent molecule (e.g., fluorescein, Texas red, rhodamine, green fluorescent protein, and the like), an enzyme (e.g., horse radish peroxidase, alkaline phosphatase), a luminescent molecule (e.g., luciferase), a

radioactive molecule (e.g., 3H, 125I, 35S, 14C, or 32P), or calorimetric labels such as colloidal gold or colored beads.

In some embodiments, a virus, e.g., an oncolytic virus, described herein is administering by injection, e.g., subcutaneous, intra-arterial, intravenous, intramuscular, intrathecal, or intraperitoneal injection. In some embodiments, an oncolytic virus described herein is administered intratumorally, transdermally, transmucosally, orally, intranasally, subcutaneously, intra-arterially, intravenously, intramuscularly, intrathecally, or intraperitoneally, or via pulmonary administration.

Additional viruses that are useful in this invention include, but are not limited to retroviruses, adenoviruses, adeno- associated viruses, herpes viruses, or lentiviruses.

Chimeric antigen receptor (CAR)

In one aspect, disclosed herein are methods using a cell (e.g., a population of cells) that expresses a CAR molecule. In one aspect, an exemplary CAR construct comprises an optional leader sequence (e.g., a leader sequence described herein), an antigen binding domain (e.g., an antigen binding domain described herein), a hinge (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein), and an intracellular stimulatory domain (e.g., an intracellular stimulatory domain described herein). In one aspect, an exemplary CAR construct comprises an optional leader sequence (e.g., a leader sequence described herein), an extracellular antigen binding domain (e.g., an antigen binding domain described herein), a hinge (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein), an intracellular costimulatory signaling domain (e.g., a costimulatory signaling domain described herein) and/or an intracellular primary signaling domain (e.g., a primary signaling domain described herein).

Sequences of non-limiting examples of various components that can be part of a CAR molecule described herein, are listed in **Table 1**, where "aa" stands for amino acids, and "na" stands for nucleic acids that encode the corresponding peptide.

Table 1. Sequences of various components of CAR (aa – amino acid sequence, na – nucleic acid sequence).

SEQ ID NO	Description	Sequence
11	EF-1 promoter (na)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCC CCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGG TGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTC CCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTC TTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTC CCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACT TCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG

		GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTG AGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCA CCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTT GATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGG CCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGG GGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCG GCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGT GCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGC CCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGC TGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTG AGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATG TGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGC TTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGT TTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA
1	Leader (aa)	MALPVTALLLPLALLLHAARP
12	Leader (na)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTGCTGCTGCATG CCGCTAGACCC
2	CD 8 hinge (aa)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
13	CD8 hinge (na)	ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCG CAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCA GTGCACACGAGGGGGCTGGACTTCGCCTGTGAT
3	Ig4 hinge (aa)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGKM
14	Ig4 hinge (na)	GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGG GCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGAT CAGCCGGACCCCCGAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGA CCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCCGGGAGGAGCAGTTCAATAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAATACAAGTG TAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAA GGCCAAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCA AGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTT CTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA CAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTG TACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAGGGCAACGTCTTT AGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGC CTGAGCCTGTCCCTGGGCAAGATG
4	IgD hinge (aa)	RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQ EERETKTPECPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTW EVAGKVPTGGVEEGLLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPP QRLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQ REVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVSHEDSRTLL NASRSLEVSYVTDH
15	IgD hinge (na)	AGGTGGCCCGAAAGTCCCAAGGCCCAGGCATCTAGTGTTCCTACTGCACAG CCCCAGGCAGAAGGCAGCCTAGCCAAAGCTACTACTGCACCTGCCACTACG CGCAATACTGGCCGTGGCGGGGAGGAGAAGAAAAAGGAGAAAGAGAAAGA AGAACAGGAAGAGAGGGAGACCAAGACCCCTGAATGTCCATCCCATACCCA GCCGCTGGGCGTCTATCTCTTGACTCCCGCAGTACAGGACTTGTGGCTTAGA GATAAGGCCACCTTTACATGTTTCGTCGTGGGCTCTGACCTGAAGGATGCCC ATTTGACTTGGGAGGTTGCCGGAAAGGTACCCACAGGGGGGGTTGAGGAAG GGTTGCTGGAGCGCCATTCCAATGGCTCTCAGAGCCAGCACTCAAGACTCA

		CCCTTCCGAGATCCCTGTGGAACGCCGGGACCTCTGTCACATGTACTCTAAA TCATCCTAGCCTGCCCCCACAGCGTCTGATGGCCCTTAGAGAGCCAGCCGCC CAGGCACCAGTTAAGCTTAGCCTGAATCTGCTCGCCAGTAGTGATCCCCCAG AGGCCGCCAGCTGGCTCTTATGCGAAGTGTCCGGCTTTAGCCCGCCCAACAT CTTGCTCATGTGGCTGGAGGACCAGCGAGAAGTGAACACCAGCGGCTTCGC TCCAGCCCGGCCCCCACCCCAGCCGGGTTCTACCACATTCTGGGCCTGGAGT GTCTTAAGGGTCCCAGCACCACCTAGCCCCCAGCCAGCCACATACACCTGTG TTGTGTCCCATGAAGATAGCAGGACCCTGCTAAATGCTTCTAGGAGTCTGGA GGTTTCCTACGTGACTGACCATT
6	CD8 Transmembrane (aa)	IYIWAPLAGTCGVLLLSLVITLYC
17	CD8 Transmembrane (na)	ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCAC TGGTTATCACCCTTTACTGC
7	4-1BB intracellular domain (aa)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
18	4-1BB intracellular domain (na)	AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGA CCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAA GAAGAAGAAGGAGGATGTGAACTG
8	CD27 (aa)	QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP
19	CD27 (na)	AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCC CGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGC GACTTCGCAGCCTATCGCTCC
9	CD3-zeta (aa) (Q/K mutant)	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
20	CD3-zeta (na) (Q/K mutant)	AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCA GAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAA GGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATG GCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAA GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTA CGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC
10	CD3-zeta (aa) (NCBI Reference Sequence NM_000734.3)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
21	CD3-zeta (na) (NCBI Reference Sequence NM_000734.3)	AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCA GAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAA GGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATG GCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAA GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTA CGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC
36	CD28 Intracellular domain (amino acid sequence)	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
37	CD28 Intracellular domain (nucleotide sequence)	AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCC CGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGC GACTTCGCAGCCTATCGCTCC

38	ICOS Intracellular domain (amino acid sequence)	T K K K Y S S S V H D P N G E Y M F M R A V N T A K K S R L T D V T L
607	ICOS Intracellular domain (nucleotide sequence)	ACAAAAAAGAAGTATTCATCCAGTGTGCACGACCCTAACGGTGAATACATG TTCATGAGAGCAGTGAACACAGCCAAAAAATCCAGACTCACAGATGTGACC CTA
5	GS hinge/linker (aa)	GGGGSGGGGS
16	GS hinge/linker (na)	GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC
608	GS hinge/linker (na)	GGTGGCGGAGGTTCTGGAGGTGGGGGTTCC
25	linker	GGGGS
26	linker	(Gly-Gly-Gly-Gly-Ser)n, where n = 1-6, e.g., GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
27	linker	(Gly4 Ser)4
28	linker	(Gly4 Ser)3
29	linker	(Gly3Ser)
609	linker	(Gly-Gly-Gly-Ser)n where n is a positive integer equal to or greater than 1
606	linker	(Gly-Gly-Gly-Ser)n, where n = 1-10, e.g., GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS
610	linker	GSTSGSGKPGSGEGSTKG
30	polyA	$(A)_{5000}$ This sequence may encompass 50-5000 adenines.
31	polyT	$(T)_{100}$
32	polyT	$(T)_{5000}$ This sequence may encompass 50-5000 thymines.
33	polyA	$(A)_{5000}$ This sequence may encompass 100-5000 adenines.
34	polyA	$(A)_{400}$ This sequence may encompass 100-400 adenines.
35	polyA	$(A)_{2000}$ This sequence may encompass 50-2000 adenines.
22	PD1 CAR (aa)	pgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcrfrvtqlp ngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqtlvtttpaprpptpaptia sqplslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrf peeeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkma eayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr
23	PD-1 CAR (na) (PD1 ECD underlined)	atggccctccctgtcactgccctgcttctcccccctcgcactcctgctccacgccgctagaccacccggatggtttctggactctc cggatcgcccgtggaatcccccaaccttctcaccggcactcttggttgtgactgagggcgataatgcgaccttcacgtgctcgt tctccaacacctccgaatcattcgtgctgaactggtaccgcatgagcccgtcaaaccagaccgacaagctcgccgcgtttccg gaagatcggtcgcaaccgggacaggattgtcggttccgcgtgactcaactgccgaatggcagagacttccacatgagcgtgg tccgcgctaggcgaaacgactccgggacctacctgtgcggagccatctcgctggcgcctaaggcccaaatcaaagagagct tgagggccgaactgagagtgaccgagcgcagagctgaggtgccaactgcacatccatccccatcgcctcggcctgcgggg cagtttcagaccctggtcacgaccactccggcgccgcgcccaccgactccggccccaactatcgcgagccagccccgtcg ctgaggccggaagcatgccgccctgccgccggaggtgctgtgcatacccggggattggacttcgcatgcgacatctacattt gggctcctctcgccggaacttgtggcgtgctccttctgtccctggtcatcaccctgtactgcaagcggggtcggaaaaagcttct gtacattttcaagcagcccttcatgaggcccgtgcaaaccacccaggaggaggacggttgctcctgccggttccccgaagag gaagaaggaggttgcgagctgcgcgtgaagttctcccggagcgccgacgcccccgcctataagcagggccagaaccagct gtacaacgaactgaacctgggacggcgggaagagtacgatgtgctggacaagcggcgcggccgggaccccgaaatggg cgggaagcctagaagaaagaaccctcaggaaggcctgtataacgagctgcagaaggacaagatggccgaggcctactccg aaattgggatgaagggagagcggcggaggggaaaggggcacgacggcctgtaccaaggactgtccaccgccaccaagg acacatacgatgccctgcacatgcaggcccttcccctcgc

24	PD-1 CAR (aa) with signal (PD1 ECD underlined)	Malpvtalllplalllhaarppgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaaf pedrsqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagq fqtlvtttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkq pfmrpvqttqeedgcscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprr knpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr

CAR Antigen Binding Domain

In one aspect, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets a tumor antigen, e.g., a tumor antigen described herein. In some embodiments, the antigen binding domain binds to: CD19; CD123; CD22; CD30; CD171; CS-1; C-type lectin-like molecule-1, CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3; TNF receptor family member; B-cell maturation antigen (BCMA); Tn antigen ((Tn Ag) or (GalNAcα-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2; Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21; vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3; transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycoceramide (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6

complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1, melanoma antigen recognized by T cells 1; Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like, Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); or immunoglobulin lambda-like polypeptide 1 (IGLL1).

The antigen binding domain can be any domain that binds to an antigen, including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), or a fragment there of, e.g., single chain TCR, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For

example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

CAR Transmembrane domain

With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR is used, e.g., in one embodiment, the transmembrane domain may be from the same protein that the signaling domain, costimulatory domain or the hinge domain is derived from. In another aspect, the transmembrane domain is not derived from the same protein that any other domain of the CAR is derived from. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the cell surface of a CAR-expressing cell. In a different aspect, the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell.

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In one aspect, the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane domain(s) of, e.g., the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8 (e.g., CD8 alpha, CD8 beta), CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R α, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile),

CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, and NKG2C.

In some instances, the transmembrane domain can be attached to the extracellular region of the CAR, e.g., the antigen binding domain of the CAR, via a hinge, e.g., a hinge from a human protein. For example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge (e.g., an IgG4 hinge, an IgD hinge), a GS linker (e.g., a GS linker described herein), a KIR2DS2 hinge or a CD8a hinge. In one embodiment, the hinge or spacer comprises (e.g., consists of) the amino acid sequence of SEQ ID NO:2. In one aspect, the transmembrane domain comprises (e.g., consists of) a transmembrane domain of SEQ ID NO: 6.

In one aspect, the hinge or spacer comprises an IgG4 hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence SEQ ID NO:3.

In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence SEQ ID NO:14.

In one aspect, the hinge or spacer comprises an IgD hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence SEQ ID NO:4.

In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of SEQ ID NO:15.

In one aspect, the transmembrane domain may be recombinant, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In one aspect a triplet of phenylalanine, tryptophan and valine can be found at each end of a recombinant transmembrane domain.

Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling region of the CAR. A glycine-serine doublet provides a particularly suitable linker. For example, in one aspect, the linker comprises the amino acid sequence of GGGGSGGGGS (SEQ ID NO:5). In some embodiments, the linker is encoded by a nucleotide sequence of GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC (SEQ ID NO:16).

In one aspect, the hinge or spacer comprises a KIR2DS2 hinge and portions thereof.

Cytoplasmic domain

The cytoplasmic domain or region of the CAR includes an intracellular signaling domain. An intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been introduced.

Examples of intracellular signaling domains for use in a CAR described herein include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal

transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, e.g., a costimulatory domain).

A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary intracellular signaling domains that are of particular use in the invention include those of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d. In one embodiment, a CAR of the invention comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta, e.g., a CD3-zeta sequence described herein.

In one embodiment, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In an embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

Costimulatory Signaling Domain

The intracellular signalling domain of the CAR can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. In one embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of ICOS.

A costimulatory molecule can be a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such

molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp30, NKp44, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, NKG2D, NKG2C and PAG/Cbp.

The intracellular signaling sequences within the cytoplasmic portion of the CAR may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, for example, between 2 and 10 amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequence. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable linker.

In one aspect, the intracellular signaling domain is designed to comprise two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In one aspect, the signaling domain of 4-1BB is a signaling domain of SEQ ID NO: 7. In one aspect, the signaling domain of CD3-zeta is a signaling domain of SEQ ID NO: 9.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD27. In one aspect, the signaling domain of CD27 comprises an amino acid sequence of SEQ ID NO: 8. In one aspect, the signalling domain of CD27 is encoded by a nucleic acid sequence of SEQ ID NO: 19.

In one aspect, the CAR-expressing cell described herein can further comprise a second CAR, e.g., a second CAR that includes a different antigen binding domain, e.g., to the same target or a different target (e.g., a target other than a cancer associated antigen described herein or a different cancer associated antigen described herein, *e.g.*, CD19, CD33, CLL-1, CD34, FLT3, or folate receptor beta). In one embodiment, the second CAR includes an antigen binding domain to a target expressed the same cancer cell type as the cancer associated antigen. In one embodiment, the CAR-expressing cell comprises a first CAR that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a second CAR that targets a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. While not wishing to be bound by theory, placement of a costimulatory signaling domain, *e.g.*, 4-1BB, CD28, ICOS, CD27 or OX-40, onto the first CAR, and the primary signaling domain, *e.g.*, CD3 zeta, on the second CAR can limit the CAR activity to cells where both targets are expressed. In one embodiment, the CAR expressing cell comprises a first cancer associated antigen CAR that includes an antigen binding domain that binds a target antigen described herein, a transmembrane domain and a costimulatory domain and a second CAR that targets a different target antigen (e.g., an antigen expressed on that same cancer cell type as the first target antigen) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the CAR expressing cell comprises a first CAR that includes an antigen binding domain that binds a target antigen described herein, a transmembrane domain and a primary signaling domain and a second CAR that targets an antigen other than the first target antigen (e.g., an antigen expressed on the same cancer cell type as the first target antigen) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In another aspect, the disclosure features a population of CAR-expressing cells, e.g., CART cells. In some embodiments, the population of CAR-expressing cells comprises a mixture of cells expressing different CARs. For example, in one embodiment, the population of CART cells can include a first cell expressing a CAR having an antigen binding domain to a cancer associated antigen described herein, and a second cell expressing a CAR having a different antigen binding domain, e.g., an antigen binding domain to a different a cancer associated antigen described herein, e.g., an antigen binding domain to a cancer associated antigen described herein that differs from the cancer associate antigen bound by the antigen binding domain of the CAR expressed by the first cell. As another example, the population of CAR-expressing cells can include a first cell expressing a CAR that includes an antigen binding domain to a cancer associated antigen described herein, and a second cell expressing a CAR that includes an antigen binding domain to a target other than a cancer associate antigen as described herein. In one embodiment, the population of CAR-expressing cells includes, e.g., a first cell

expressing a CAR that includes a primary intracellular signaling domain, and a second cell expressing a CAR that includes a secondary signaling domain.

In another aspect, the disclosure features a population of cells wherein at least one cell in the population expresses a CAR having an antigen binding domain to a cancer associated antigen described herein, and a second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules, e.g., PD-1, can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD-1, PD-L1, CTLA4, TIM3, CEACAM (CEACAM-1, CEACAM-3, and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGF (e.g., TGFbeta). In one embodiment, the agent which inhibits an inhibitory molecule comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD-1, PD-L1, CTLA4, TIM3, CEACAM (CEACAM-1, CEACAM-3, and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGF beta, or a fragment of any of these, and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27, OX40 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD-1 or a fragment thereof, and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein).

CD19 CAR

In some embodiments, the CAR-expressing cell described herein is a CD19 CAR-expressing cell (e.g., a cell expressing a CAR that binds to human CD19).

In one embodiment, the antigen binding domain of the CD19 CAR has the same or a similar binding specificity as the FMC63 scFv fragment described in Nicholson et al. Mol. Immun. 34 (16-17): 1157-1165 (1997). In one embodiment, the antigen binding domain of the CD19 CAR includes the scFv fragment described in Nicholson et al. *Mol. Immun.* 34 (16-17): 1157-1165 (1997).

In some embodiments, the CD19 CAR includes an antigen binding domain (*e.g.*, a humanized antigen binding domain) according to Table 3 of WO2014/153270, incorporated herein by reference. WO2014/153270 also describes methods of assaying the binding and efficacy of various CAR constructs.

In one aspect, the parental murine scFv sequence is the CAR19 construct provided in PCT publication WO2012/079000 (incorporated herein by reference). In one embodiment, the anti-CD19 binding domain is a scFv described in WO2012/079000.

In one embodiment, the CAR molecule comprises the fusion polypeptide sequence provided as SEQ ID NO: 12 in PCT publication WO2012/079000, which provides an scFv fragment of murine origin that specifically binds to human CD19.

In one embodiment, the CD19 CAR comprises an amino acid sequence provided as SEQ ID NO: 12 in PCT publication WO2012/079000. In embodiment, the amino acid sequence is

(MALPVTALLLPLALLLHAARP)diqmtqttsslsaslgdrvtiscrasqdiskylnwyqqkpdgtvklliyhtsrlhsg vpsrfsgsgsgtdysltisnleqediatyfcqqgntlpytfgggtkleitggggsggggsggggsevklqesgpglvapsqslsvtctvsgvslpdyg vswirqpprkglewlgviwgsettyynsalksrltiikdnsksqvflkmnslqtddtaiyycakhyyyggsyamdywgqgtsvtvssttpaprp ptpaptiasqplslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeegg celrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdgl yqglstatkdtydalhmqalppr (SEQ ID NO: 624), or a sequence substantially homologous thereto. The optional sequence of the signal peptide is shown in capital letters and parenthesis.

In one embodiment, the amino acid sequence is:

Diqmtqttsslsaslgdrvtiscrasqdiskylnwyqqkpdgtvklliyhtsrlhsgvpsrfsgsgsgtdysltisnleqediatyfcqqgn tlpytfgggtkleitggggsggggsggggsevklqesgpglvapsqslsvtctvsgvslpdygvswirqpprkglewlgviwgsettyynsalksr ltiikdnsksqvflkmnslqtddtaiyycakhyyyggsyamdywgqgtsvtvssttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfa cdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgrre eydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr (SEQ ID NO: 625), or a sequence substantially homologous thereto.

In one embodiment, the CD19 CAR has the USAN designation TISAGENLECLEUCEL-T. In embodiments, CTL019 is made by a gene modification of T cells is mediated by stable insertion via transduction with a self-inactivating, replication deficient Lentiviral (LV) vector containing the CTL019 transgene under the control of the EF-1 alpha promoter. CTL019 can be a mixture of transgene positive and negative T cells that are delivered to the subject on the basis of percent transgene positive T cells.

In other embodiments, the CD19 CAR comprises an antigen binding domain (e.g., a humanized antigen binding domain) according to Table 3 of WO2014/153270, incorporated herein by reference.

Humanization of murine CD19 antibody is desired for the clinical setting, where the mouse-specific residues may induce a human-anti-mouse antigen (HAMA) response in patients who receive CART19 treatment, i.e., treatment with T cells transduced with the CAR19 construct. The production, characterization, and efficacy of humanized CD19 CAR sequences is described in International

Application WO2014/153270 which is herein incorporated by reference in its entirety, including Examples 1-5 (p. 115-159).

Any known CD19 CAR, e.g., the CD19 antigen binding domain of any known CD19 CAR, in the art can be used in accordance with the present disclosure. For example, LG-740; CD19 CAR described in the US Pat. No. 8,399,645; US Pat. No. 7,446,190; Xu et al., Leuk Lymphoma. 2013 54(2):255-260(2012); Cruz et al., Blood 122(17):2965-2973 (2013); Brentjens et al., Blood, 118(18):4817-4828 (2011); Kochenderfer et al., Blood 116(20):4099-102 (2010); Kochenderfer et al., Blood 122 (25):4129-39(2013); and 16th Annu Meet Am Soc Gen Cell Ther (ASGCT) (May 15-18, Salt Lake City) 2013, Abst 10.

Exemplary CD19 CARs include CD19 CARs described herein, e.g., in one or more tables described herein, or an anti-CD19 CAR described in Xu et al. Blood 123.24(2014):3750-9; Kochenderfer et al. Blood 122.25(2013):4129-39, Cruz et al. Blood 122.17(2013):2965-73, NCT00586391, NCT01087294, NCT02456350, NCT00840853, NCT02659943, NCT02650999, NCT02640209, NCT01747486, NCT02546739, NCT02656147, NCT02772198, NCT00709033, NCT02081937, NCT00924326, NCT02735083, NCT02794246, NCT02746952, NCT01593696, NCT02134262, NCT01853631, NCT02443831, NCT02277522, NCT02348216, NCT02614066, NCT02030834, NCT02624258, NCT02625480, NCT02030847, NCT02644655, NCT02349698, NCT02813837, NCT02050347, NCT01683279, NCT02529813, NCT02537977, NCT02799550, NCT02672501, NCT02819583, NCT02028455, NCT01840566, NCT01318317, NCT01864889, NCT02706405, NCT01475058, NCT01430390, NCT02146924, NCT02051257, NCT02431988, NCT01815749, NCT02153580, NCT01865617, NCT02208362, NCT02685670, NCT02535364, NCT02631044, NCT02728882, NCT02735291, NCT01860937, NCT02822326, NCT02737085, NCT02465983, NCT02132624, NCT02782351, NCT01493453, NCT02652910, NCT02247609, NCT01029366, NCT01626495, NCT02721407, NCT01044069, NCT00422383, NCT01680991, NCT02794961, or NCT02456207, each of which is incorporated herein by reference in its entirety.

BCMA CAR

In some embodiments, the CAR-expressing cell described herein is a BCMA CAR-expressing cell (e.g., a cell expressing a CAR that binds to human BCMA). Exemplary BCMA CARs can include sequences disclosed in Table 1 or 16 of WO2016/014565, incorporated herein by reference. The BCMA CAR construct can include an optional leader sequence; an optional hinge domain, e.g., a CD8 hinge domain; a transmembrane domain, e.g., a CD8 transmembrane domain; an intracellular domain, e.g., a 4-1BB intracellular domain; and a functional signaling domain, e.g., a CD3 zeta domain. In certain embodiments, the domains are contiguous and in the same reading frame to form a single fusion

protein. In other embodiments, the domain are in separate polypeptides, e.g., as in an RCAR molecule as described herein.

In some embodiments, the BCMA CAR molecule includes one or more CDRs, VH, VL, scFv, or full-length sequences of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1 disclosed in WO2016/014565, or a sequence substantially (e.g., 95-99%) identical thereto.

Additional exemplary BCMA-targeting sequences that can be used in the anti-BCMA CAR constructs are disclosed in WO 2017/021450, WO 2017/011804, WO 2017/025038, WO 2016/090327, WO 2016/130598, WO 2016/210293, WO 2016/090320, WO 2016/014789, WO 2016/094304, WO 2016/154055, WO 2015/166073, WO 2015/188119, WO 2015/158671, US 9,243,058, US 8,920,776, US 9,273,141, US 7,083,785, US 9,034,324, US 2007/0049735, US 2015/0284467, US 2015/0051266, US 2015/0344844, US 2016/0131655, US 2016/0297884, US 2016/0297885, US 2017/0051308, US 2017/0051252, US 2017/0051252, WO 2016/020332, WO 2016/087531, WO 2016/079177, WO 2015/172800, WO 2017/008169, US 9,340,621, US 2013/0273055, US 2016/0176973, US 2015/0368351, US 2017/0051068, US 2016/0368988, and US 2015/0232557, herein incorporated by reference in their entirety. In some embodiments, additional exemplary BCMA CAR constructs are generated using the VH and VL sequences from PCT Publication WO2012/0163805 (the contents of which are hereby incorporated by reference in its entirety).

CD20 CAR

In some embodiments, the CAR-expressing cell described herein is a CD20 CAR-expressing cell (e.g., a cell expressing a CAR that binds to human CD20). In some embodiments, the CD20 CAR-expressing cell includes an antigen binding domain according to WO2016/164731 and PCT/US2017/055627, incorporated herein by reference. Exemplary CD20-binding sequences or CD20 CAR sequences are disclosed in, e.g., Tables 1-5 of PCT/US2017/055627. In some embodiments, the CD20 CAR comprises a CDR, variable region, scFv, or full-length sequence of a CD20 CAR disclosed in PCT/US2017/055627 or WO2016/164731.

CD22 CAR

In some embodiments, the CAR-expressing cell described herein is a CD22 CAR-expressing cell (e.g., a cell expressing a CAR that binds to human CD22). In some embodiments, the CD22 CAR-expressing cell includes an antigen binding domain according to WO2016/164731 and PCT/US2017/055627, incorporated herein by reference. Exemplary CD22-binding sequences or CD22 CAR sequences are disclosed in, e.g., Tables 6A, 6B, 7A, 7B, 7C, 8A, 8B, 9A, 9B, 10A, and 10B of WO2016/164731 and Tables 6-10 of PCT/US2017/055627. In some embodiments, the CD22 CAR sequences comprise a CDR, variable region, scFv or full-length sequence of a CD22 CAR disclosed in PCT/US2017/055627 or WO2016/164731.

EGFR CAR

In some embodiments, the CAR-expressing cell described herein is an EGFR CAR-expressing cell (e.g., a cell expressing a CAR that binds to human EGFR). In some embodiments, the CAR-expressing cell described herein is an EGFRvIII CAR-expressing cell (e.g., a cell expressing a CAR that binds to human EGFRvIII). Exemplary EGFRvIII CARs can include sequences disclosed in WO2014/130657, e.g., Table 2 of WO2014/130657, incorporated herein by reference.

Exemplary EGFRvIII-binding sequences or EGFR CAR sequences may comprise a CDR, a variable region, an scFv, or a full-length CAR sequence of a EGFR CAR disclosed in WO2014/130657.

Mesothelin CAR

In some embodiments, the CAR-expressing cell described herein is a mesothelin CAR-expressing cell (e.g., a cell expressing a CAR that binds to human mesothelin). Exemplary mesothelin CARs can include sequences disclosed in WO2015090230 and WO2017112741, e.g., Tables 2, 3, 4, and 5 of WO2017112741, incorporated herein by reference.

Exemplary mesothelin CAR constructs disclosed herein comprise a scFv (e.g., a human scFv) as disclosed in Table 2 or 3 herein, optionally preceded with an optional leader sequence (e.g., SEQ ID NO:1 and SEQ ID NO:12 for exemplary leader amino acid and nucleotide sequences, respectively). The sequences of the scFv fragments (amino acid sequences of SEQ ID NOs: 39-62) are provided herein in Table 2. The mesothelin CAR construct can further include an optional hinge domain, e.g., a CD8 hinge domain (e.g., including the amino acid sequence of SEQ ID NO: 2 or encoded by a nucleic acid sequence of SEQ ID NO:13); a transmembrane domain, e.g., a CD8 transmembrane domain (e.g., including the amino acid sequence of SEQ ID NO: 6 or encoded by the nucleotide sequence of SEQ ID NO: 17); an intracellular domain, e.g., a 4-1BB intracellular domain (e.g., including the amino acid sequence of SEQ ID NO: 7 or encoded by the nucleotide sequence of SEQ ID NO: 18; and a functional

signaling domain, e.g., a CD3 zeta domain (e.g., including amino acid sequence of SEQ ID NO: 9 or 10, or encoded by the nucleotide sequence of SEQ ID NO: 20 or 21). In certain embodiments, the domains are contiguous with and in the same reading frame to form a single fusion protein. In other embodiments, the domain are in separate polypeptides, e.g., as in an RCAR molecule as described herein.

In certain embodiments, the full length mesothelin CAR molecule includes the amino acid sequence of, or is encoded by the nucleotide sequence of, M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, or ss1, provided in Table 2 or 3, or a sequence substantially identical (e.g., 95-99% identical thereto, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

In certain embodiments, the mesothelin CAR molecule, or the mesothelin antigen binding domain, includes the scFv amino acid sequence of, or is encoded by the nucleotide sequence of, M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, or ss1, provided in Table 2 or 3, or a sequence substantially identical (e.g., 95-99% identical thereto, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

In certain embodiments, the mesothelin CAR molecule, or the mesothelin antigen binding domain, includes the heavy chain variable region and/or the light chain variable region of M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, or ss1, provided in Table 2, or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

In certain embodiments, the mesothelin CAR molecule, or the mesothelin antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3) of M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, or ss1, provided in Table 4; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, or ss1, provided in Table 5; or a sequence substantially identical (e.g., 95-99% identical, or up to 5, 4, 3, 2, or 1 amino acid changes) to any of the aforesaid sequences.

The sequences of CDR sequences of the scFv domains are shown in Table 4 for the heavy chain variable domains and in Table 5 for the light chain variable domains.

The amino acid and nucleic acid sequences of the mesothelin scFv domains and mesothelin CAR molecules are provided in Table 2 (amino acid sequences) and Table 3 (nucleic acid sequences). In one embodiment, the mesothelin CAR molecule includes a leader sequence described herein, e.g., as

underlined in the sequences provided in Table 2. In one embodiment, the mesothelin CAR molecule does not include a leader sequence.

Table 2. Amino Acid Sequences of Human scFvs and CARs (bold underline is the leader sequence and grey box is a linker sequence). In the case of the scFvs, the remaining amino acids are the heavy chain variable region and light chain variable regions, with each of the HC CDRs (HC CDR1, HC CDR2, HC CDR3) and LC CDRs (LC CDR1, LC CDR2, LCCDR3) underlined). In the case of the CARs, the further remaining amino acids are the remaining amino acids of the CARs.)

SEQ ID NO:	Description	Amino Acid Sequence
39	M1 (ScFv domain)	QVQLQQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGRINPNSGGTNY AQKFQGRVTMTRDTSISTAYMELSRLRSEDTAVYYCARGRYYGMDVWGQGTMVTVSSGGG GSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATISCRASQSVSSNFAWYQQRPGQA PRLLIYDASNRATGIPPRFSGSGSGTDFTLTISSLEPEDFAAYYCHQRSNWLYTFGQGTK VDIK
63	M1 (full) >ZA53-27BC (M1 ZA53-27BC R001-A11 126161)	**MALPVTALLLPLALLLHAARP**QVQLQQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQ APGQGLEWMGRINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSEDTAVYYCARG RYYGMDVWGQGTMVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATIS CRASQSVSSNFAWYQQRPGQAPRLLIYDASNRATGIPPRFSGSGSGTDFTLTISSLEPED FAAYYCHQRSNWLYTFGQGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
40	M2 (ScFv domain)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNY AQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDLRRTVVTPRAYYGMDVWGQGT TVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRVTITCQASQDISNSLN WYQQKAGKAPKLLIYDASTLETGVPSRFSGSGSGTDFSFTISSLQPEDIATYYCQQHDNL PLTFGQGTKVEIK
64	M2 (full) >FA56-26RC (M2 FA56-26RC R001-A10 126162)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQ APGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARD LRRTVVTPRAYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSA SVGDRVTITCQASQDISNSLNWYQQKAGKAPKLLIYDASTLETGVPSRFSGSGSGTDFSF TISSLQPEDIATYYCQQHDNLPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR
41	M3 (ScFv domain)	QVQLVQSGAEVKKPGAPVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNY AQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGEWDGSYYYDYWGQGTLVTVSS GGGGSGGGGSGGGGSGGGGSDIVLTQTPSSLSASVGDRVTITCRASQSINTYLNWYQHKP GKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSFSPLTFGGG TKLEIK
65	M3 >VA58-21LC (M3 VA58-21LC R001-A1 126163)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVKKPGAPVKVSCKASGYTFTGYYMHWVRQ APGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARG EWDGSYYYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVLTQTPSSLSASVGDRV TITCRASQSINTYLNWYQHKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQSFSPLTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD

		ALHMQALPPR
42	M4 (ScFv domain)	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQVPGKGLVWVSRINTDGSTTTY ADSVEGRFTISRDNAKNTLYLQMNSLRDDDTAVYYCVGGHWAVWGQGTTVTVSSGGGGSG GGGSGGGGSGGGGSDIQMTQSPSTLSASVGDRVTITCRASQSISDRLAWYQQKPGKAPKL LIYKASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFAVYYCQQYGHLPMYTFGQGTKVE IK
66	M4 >DP37-07IC (M4 DP37-07IC R001-C6 126164)	**MALPVTALLLPLALLLHAARP**QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQ VPGKGLVWVSRINTDGSTTTYADSVEGRFTISRDNAKNTLYLQMNSLRDDDTAVYYCVGG HWAVWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSTLSASVGDRVTITCRA SQSISDRLAWYQQKPGKAPKLLIYKASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFAV YYCQQYGHLPMYTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
43	M5 (ScFv domain)	QVQLVQSGAEVEKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGWINPNSGGTNY AQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCASGWDFDYWGQGTLVTVSSGGGGS GGGGSGGGGSGGGGSDIVMTQSPSSLSASVGDRVTITCRASQSIRYYLSWYQQKPGKAPK LLIYTASILQNGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQTYTTPDFGPGTKVEI K
67	M5 >XP31-20LC (M5 XP31-20LC R001-B4 126165)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVEKPGASVKVSCKASGYTFTDYYMHWVRQ APGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCASG WDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQSPSSLSASVGDRVTITCR ASQSIRYYLSWYQQKPGKAPKLLIYTASILQNGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCLQTYTTPDFGPGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR
44	M6 (ScFv domain)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSY AQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYRLIAVAGDYYYYGMDVWGQGT MVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQGVGRWLA WYQQKPGTAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTINNLQPEDFATYYCQQANSF PLTFGGGTRLEIK
68	M6 >FE10-06ID (M6 46FE10-06ID R001-A4 126166)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQ APGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARY RLIAVAGDYYYYGMDVWGQGTMVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSA SVGDRVTITCRASQGVGRWLAWYQQKPGTAPKLLIYAASTLQSGVPSRFSGSGSGTDFTL TINNLQPEDFATYYCQQANSFPLTFGGGTRLEIKTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR
45	M7 (ScFv domain)	QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARWKVSSSSPAFDYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERAILSCRASQSVYTKYLGWYQQ KPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQHYGGSPLIT FGQGTRLEIK
69	M7 >VE12-01CD (M7 VE12-01CD R001-A5 126167)	**MALPVTALLLPLALLLHAARP**QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQ APGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARW KVSSSSPAFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGER AILSCRASQSVYTKYLGWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINR LEPEDFAVYYCQHYGGSPLITFGQGTRLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRP AAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR

46	M8 (ScFv domain)	QVQLQQSGAEVKKPGASVKVSCKTSGYPFTGYSLHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDHYGGNSLFYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSSISASVGDTVSITCRASQDSGTWLAWYQQKPGKAPNLLMYDASTLEDGVPSRFSGSASGTEFTLTVNRLQPEDSATYYCQQYNSYPLTFGGGTKVDIK
70	M8 >LE13-05XD (M8 LE13-05XD R001-E5 126168)	**MALPVTALLLPLALLLHAARP**QVQLQQSGAEVKKPGASVKVSCKTSGYPFTGYSLHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDHYGGNSLFYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSSISASVGDTVSITCRASQDSGTWLAWYQQKPGKAPNLLMYDASTLEDGVPSRFSGSASGTEFTLTVNRLQPEDSATYYCQQYNSYPLTFGGGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
47	M9 (ScFv domain)	QVQLVQSGAEVKKPGASVEVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTGYAQKFQGRVTMTRDTSTSTVHMELSSLRSEDTAVYYCARGGYSSSSDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPPSLSASVGDRVTITCRASQDISSALAWYQQKPGTPPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFSSYPLTFGGGTRLEIK
71	M9 >BE15-00SD (M9 BE15-00SD R001-A3 126169)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVKKPGASVEVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTGYAQKFQGRVTMTRDTSTSTVHMELSSLRSEDTAVYYCARGGYSSSSDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPPSLSASVGDRVTITCRASQDISSALAWYQQKPGTPPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFSSYPLTFGGGTRLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
48	M10 (ScFv domain)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARVAGGIYYYYGMDVWGQGTTITVSSGGGGSGGGGSGGGGSGGGGSDIVMTQTPDSLAVSLGERATISCKSSHSVLYNRNNKNYLAWYQQKPGQPPKLLFYWASTRKSGVPDRFSGSGSGTDFTLTISSLQPEDFATYFCQQTQTFPLTFGQGTRLEIN
72	M10 >RE16-05MD (M10 RE16-05MD R001-D10 126170)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARVAGGIYYYYGMDVWGQGTTITVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIVMTQTPDSLAVSLGERATISCKSSHSVLYNRNNKNYLAWYQQKPGQPPKLLFYWASTRKSGVPDRFSGSGSGTDFTLTISSLQPEDFATYFCQQTQTFPLTFGQGTRLEINTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
49	M11 (ScFv domain)	QVQLQQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQNFQGRVTMTRDTSISTAYMELRRLRSDDTAVYYCASGWDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIRMTQSPSSLSASVGDRVTITCRASQSIRYYLSWYQQKPGKAPKLLIYTASILQNGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQTYTTPDFGPGTKVEIK
73	M11 >NE10-19WD (M11 NE10-19WD R001-G2 126171)	**MALPVTALLLPLALLLHAARP**QVQLQQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQNFQGRVTMTRDTSISTAYMELRRLRSDDTAVYYCASGWDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIRMTQSPSSLSASVGDRVTITCRASQSIRYYLSWYQQKPGKAPKLLIYTASILQNGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQTYTTPDFGPGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

50	M12 (ScFv domain)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGRINPNSGGTNY AQKFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARTTTSYAFDIWGQGTMVTVSSGG GGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRVTITCRASQSISTWLAWYQQKPGK APNLLIYKASTLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNTYSPYTFGQG TKLEIK
74	M12 >DE12-14RD (M12 DE12-14RD R001-G9 126172)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQ APGQGLEWMGRINPNSGGTNYAQKFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCART TTSYAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRVTI TCRASQSISTWLAWYQQKPGKAPNLLIYKASTLESGVPSRFSGSGSGTEFTLTISSLQPD DFATYYCQQYNTYSPYTFGQGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
51	M13 (ScFv domain)	QVQLVQSGGGLVKPGGSLRLSCEASGFIFSDYYMGWIRQAPGKGLEWVSYIGRSGSSMYY ADSVKGRFTFSRDNAKNSLYLQMNSLRAEDTAVYYCAASPVVAATEDFQHWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSDIVMTQTPATLSLSPGERATLSCRASQSVTSNYLAWYQQ KPGQAPRLLLFGASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAMYYCQQYGSAPVTF GQGTKLEIK
75	M13 >TE13-19LD (M13 TE13-19LD R002-C3 126173)	**MALPVTALLLPLALLLHAARP**QVQLVQSGGGLVKPGGSLRLSCEASGFIFSDYYMGWIRQ APGKGLEWVSYIGRSGSSMYYADSVKGRFTFSRDNAKNSLYLQMNSLRAEDTAVYYCAAS PVVAATEDFQHWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIVMTQTPATLSLSPGER ATLSCRASQSVTSNYLAWYQQKPGQAPRLLLFGASTRATGIPDRFSGSGSGTDFTLTINR LEPEDFAMYYCQQYGSAPVTFGQGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
52	M14 (ScFv domain)	QVQLVQSGAEVRAPGASVKISCKASGFTFRGYYIHWVRQAPGQGLEWMGIINPSGGSRAY AQKFQGRVTMTRDTSTSTVYMELSSLRSDDTAMYYCARTASCGGDCYYLDYWGQGTLVTV SSGGGGSGGGGSGGGGSGGGGSDIQMTQSPPTLSASVGDRVTITCRASENVNIWLAWYQQ KPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISSLQPDDFATYYCQQYQSYPLTF GGGTKVDIK
76	M14 >BS83-95ID (M14 BS83-95ID R001-E8 126174)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVRAPGASVKISCKASGFTFRGYYIHWVRQ APGQGLEWMGIINPSGGSRAYAQKFQGRVTMTRDTSTSTVYMELSSLRSDDTAMYYCART ASCGGDCYYLDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPPTLSASVGD RVTITCRASENVNIWLAWYQQKPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISS LQPDDFATYYCQQYQSYPLTFGGGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
53	M15 (ScFv domain)	QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIGY ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKDGSSSWSWGYFDYWGQGTLVTV SSGGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRTTCQGDALRSYYASWYQQKPGQAP MLVIYGKNNRPSGIPDRFSGSDSGDTASLTITGAQAEDEADYYCNSRDSSGYPVFGTGTK VTVL
77	M15 >HS86-94XD (M15 HS86-94XD NT 127553)	**MALPVTALLLPLALLLHAARP**QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQ APGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKD GSSSWSWGYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRTTC QGDALRSYYASWYQQKPGQAPMLVIYGKNNRPSGIPDRFSGSDSGDTASLTITGAQAEDE ADYYCNSRDSSGYPVFGTGTKVTVLTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
54	M16 (ScFv	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSTGY

	domain)	ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDSSSWYGGGSAFDIWGQGTMVT VSSGGGGSGGGGSGGGGSSSELTQEPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQA PVLVIFGRSRRPSGIPDRFSGSSSGNTASLIITGAQAEDEADYYCNSRDNTANHYVFGTG TKLTVL
78	M16 >XS87-99RD (M16 XS87-99RD NT 127554)	**MALPVTALLLPLALLLHAARP**EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQ APGKGLEWVSGISWNSGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKD SSSWYGGGSAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSSSELTQEPAVSVALGQTVRIT CQGDSLRSYYASWYQQKPGQAPVLVIFGRSRRPSGIPDRFSGSSSGNTASLIITGAQAED EADYYCNSRDNTANHYVFGTGTKLTVLTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
55	M17 (ScFv domain)	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSTGY ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDSSSWYGGGSAFDIWGQGTMVT VSSGGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQA PVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRGSSGNHYVFGTG TKVTVL
79	M17 >NS89-94MD (M17 NS89-94MD NT 127555)	**MALPVTALLLPLALLLHAARP**EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQ APGKGLEWVSGISWNSGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKD SSSWYGGGSAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSSSELTQDPAVSVALGQTVRIT CQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAED EADYYCNSRGSSGNHYVFGTGTKVTVLTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
56	M18 (ScFv domain)	QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQAPGKGLVWVSRINSDGSSTSY ADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRTGWVGSYYYYMDVWGKGTTVTV SSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYQ QKPGQPPRLLIYDVSTRATGIPARFSGGGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPW TFGQGTKVEIK
80	M18 >DS90-09HD (M18 DS90-09HD R003-A05 127556)	**MALPVTALLLPLALLLHAARP**QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMHWVRQ APGKGLVWVSRINSDGSSTSYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRT GWVGSYYYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGE RATLSCRASQSVSSNYLAWYQQKPGQPPRLLIYDVSTRATGIPARFSGGGSGTDFTLTIS SLEPEDFAVYYCQQRSNWPPWTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACR PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
57	M19 (ScFv domain)	QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGYSRYYYYGMDVWGQGTTVTVS SGGGGSGGGGSGGGGSGGGGSEIVMTQSPATLSLSPGERAILSCRASQSVYTKYLGWYQQ KPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQHYGGSPLIT FGQGTKVDIK
81	M19 >TS92-04BD (M19 TS92-04BD R003-C06 127557)	**MALPVTALLLPLALLLHAARP**QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQ APGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKG YSRYYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSEIVMTQSPATLSLSPGER AILSCRASQSVYTKYLGWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTINR LEPEDFAVYYCQHYGGSPLITFGQGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRP AAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
58	M20 (ScFv domain)	QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKREAAAGHDWYFDLWGRGTLVTV

		SSGGGGSGGGGSGGGGSGGGGSDIRVTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSIPLTF GQGTKVEIK
82	M20 (full) >JS93-08WD (M20 JS93-08WD R003-E07 127558)	**MALPVTALLLPLALLLHAARP**QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQ APGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKR EAAAGHDWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIRVTQSPSSLSASVGD RVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQSYSIPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
59	M21 (ScFv domain)	QVQLVQSWAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSY AQKFQGRVTMTRDTSTSTVYMELSNLRSEDTAVYYCARSPRVTTGYFDYWGQGTLVTVSS GGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKP GKAPKLLIYKASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYSSYPLTFGG GTRLEIK
83	M21 (full CAR)	**MALPVTALLLPLALLLHAARP**QVQLVQSWAEVKKPGASVKVSCKASGYTFTSYYMHWVRQ APGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSNLRSEDTAVYYCARS PRVTTGYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRV TITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESGVPSRFSGSGSGTEFTLTISSLQ PDDFATYYCQQYSSYPLTFGGGTRLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
60	M22 (ScFv domain)	QVQLVQSGAEVRRPGASVKISCRASGDTSTRHYIHWLRQAPGQGPEWMGVINPTTGPATG SPAYAQMLQGRVTMTRDTSTRTVYMELRSLRFEDTAVYYCARSVVGRSAPYYFDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQGISDYSA WYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISYLQSEDFATYYCQQYYSY PLTFGGGTKVDIK
84	M22 (full CAR)	**MALPVTALLLPLALLLHAARP**QVQLVQSGAEVRRPGASVKISCRASGDTSTRHYIHWLRQ APGQGPEWMGVINPTTGPATGSPAYAQMLQGRVTMTRDTSTRTVYMELRSLRFEDTAVYY CARSVVGRSAPYYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSA SVGDRVTITCRASQGISDYSAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTL TISYLQSEDFATYYCQQYYSYPLTFGGGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR
61	M23 (ScFv domain)	QVQLQQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQGLEWMGIINPSGGYTTY AQKFQGRLTMTRDTSTSTVYMELSSLRSEDTAVYYCARIRSCGGDCYYFDNWGQGTLVTV SSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRVTITCRASENVNIWLAWYQQ KPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISSLQPDDFATYYCQQYQSYPLTF GGGTKVDIK
85	M23 (full CAR)	**MALPVTALLLPLALLLHAARP**QVQLQQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQ APGQGLEWMGIINPSGGYTTYAQKFQGRLTMTRDTSTSTVYMELSSLRSEDTAVYYCARI RSCGGDCYYFDNWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGD RVTITCRASENVNIWLAWYQQKPGKAPKLLIYKSSSLASGVPSRFSGSGSGAEFTLTISS LQPDDFATYYCQQYQSYPLTFGGGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
62	M24 (ScFv domain)	QITLKESGPALVKPTQTLTLTCTFSGFSLSTAGVHVGWIRQPPGKALEWLALISWADDKR YRPSLRSRLDITRVTSKDQVVLSMTNMQPEDTATYYCALQGFDGYEANWGPGTLVTVSSG GGGSGGGGSGGGGSGGGGSDIVMTQSPSSLSASAGDRVTITCRASRGISSALAWYQQKPG

		KPPKLLIYDASSLESGVPSRFSGSGSGTDFTLTIDSLEPEDFATYYCQQSYSTPWTFGQG TKVDIK
86	M24 (full CAR)	**MALPVTALLLPLALLLHAARP**QITLKESGPALVKPTQTLTLTCTFSGFSLSTAGVHVGWI RQPPGKALEWLALISWADDKRYRPSLRSRLDITRVTSKDQVVLSMTNMQPEDTATYYCAL QGFDGYEANWGPGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQSPSSLSASAGDRVT ITCRASRGISSALAWYQQKPGKPPKLLIYDASSLESGVPSRFSGSGSGTDFTLTIDSLEP EDFATYYCQQSYSTPWTFGQGTKVDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
275	Ss1 (scFv domain)	QVQLQQSGPELEKPGASVKISCKASGYSFTGYTMNWVKQSHGKSLEWIGLITPYNGASS YNQKFRGKATLTVDKSSSTAYMDLLSLTSEDSAVYFCARGGYDGRGFDYWGQGTTVTVS SGGGGSGGGGSGGGGSDIELTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSP KRWIYDTSKLASGVPGRFSGSGSGNSYSLTISSVEAEDDATYYCQQWSGYPLTFGAGTK LEI
278	Ss1 (full CAR)	**MALPVTALLLPLALLLHAARP**QVQLQQSGPELEKPGASVKISCKASGYSFTGYTMNWVK QSHGKSLEWIGLITPYNGASSYNQKFRGKATLTVDKSSSTAYMDLLSLTSEDSAVYFCA RGGYDGRGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPAIMSASPGEKVTMT CSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPGRFSGSGSGNSYSLTISSVEAED DATYYCQQWSGYPLTFGAGTKLEITTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCELRVKFSRSADAPA

Table 3: Nucleic Acid Sequences encoding CAR molecules (the leader sequence is underlined)

SEQ ID NO:	Desc.	Nucleic Acid Sequence
87	M1 (ScFv domain) >ZA53-27BC (M1)	CAAGTCCAACTGCAGCAGTCAGGAGCGGAAGTGAAGAAACCAGGAGCGTCAGTCAAAGTGTCGTGCAAGGCTAGCGGCTAC ACCTTCACCGGCTACTACATGCACTGGGTTCGACAGGCTCCAGGGCAGGGTCTGGAGTGGATGGGCCGCATCAACCCGAAT TCCGGTGGGACTAACTACGCCCAGAAGTTCCAGGGAAGAGTGACCATGACTAGGGACACGTCGATCAGCACTGCGTACATG GAACTGAGCCGCCTGCGGTCCGAGGATACTGCCGTCTACTACTGCGCACGCGGAAGGTACTATGGAATGGACGTGTGGGGC CAAGGGACTATGGTGACTGTGAGCTCGGGAGGGGGAGGCTCCGGTGGCGGGGGATCAGGAGGAGGAGGATCAGGGGGAGGA GGTTCCGAAATTGTCCTCACCCAGAGCCCGGCAACCCTCTCACTTTCCCCGGGAGAGCGCGCAACCATCTCTTGCCGGGCT AGCCAATCCGTGTCGTCCAATTTCGCCTGGTACCAGCAACGGCCGGGACAAGCCCCTAGACTCCTGATCTACGACGCCAGC AACAGAGCGACTGGAATTCCTCCACGCTTTTCGGGATCAGGCTCCGGTACCGACTTCACCCTGACTATCTCGTCGCTCGAA CCCGAGGATTTCGCCGCCTACTACTGTCATCAGCGGTCGAACTGGTTGTATACGTTTGGCCAGGGCACCAAGGTGGATATC AAG
111	M1 (Full) >ZA53-27BC (M1)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTGCAGCAG TCAGGAGCGGAAGTGAAGAAACCAGGAGCGTCAGTCAAAGTGTCGTGCAAGGCTAGCGGCTACACCTTCACCGGCTACTA CATGCACTGGGTTCGACAGGCTCCAGGGCAGGGTCTGGAGTGGATGGGCCGCATCAACCCGAATTCCGGTGGGACTAACT ACGCCCAGAAGTTCCAGGGAAGAGTGACCATGACTAGGGACACGTCGATCAGCACTGCGTACATGGAACTGAGCCGCCTG CGGTCCGAGGATACTGCCGTCTACTACTGCGCACGCGGAAGGTACTATGGAATGGACGTGTGGGGCCAAGGGACTATGGT GACTGTGAGCTCGGGAGGGGGAGGCTCCGGTGGCGGGGGATCAGGAGGAGGAGGATCAGGGGGAGGAGGTTCCGAAATTG TCCTCACCCAGAGCCCGGCAACCCTCTCACTTTCCCCGGGAGAGCGCGCAACCATCTCTTGCCGGGCTAGCCAATCCGTG TCGTCCAATTTCGCCTGGTACCAGCAACGGCCGGGACAAGCCCCTAGACTCCTGATCTACGACGCCAGCAACAGAGCGAC TGGAATTCCTCCACGCTTTTCGGGATCAGGCTCCGGTACCGACTTCACCCTGACTATCTCGTCGCTCGAACCCGAGGATT TCGCCGCCTACTACTGTCATCAGCGGTCGAACTGGTTGTATACGTTTGGCCAGGGCACCAAGGTGGATATCAAGACCACT ACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACC CGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTT GCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAA CCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTG CGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCA ATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGG GGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTC ACATGCAGGCCCTGCCGCCTCGG
88	M2 (ScFv domain)	CAAGTCCAACTCGTCCAGTCAGGAGCAGAAGTCAAGAAACCAGGTGCTAGCGTGAAAGTGTCGTGCAAGGCGTCGGGATAC ACTTTCACCGGATACTAC ATGCACTGGGTCCGCCAGGCCCCCGGACAAGGACTGGAATGGATGGGCTGGATCAACCCGAATAGCGGGGGAACTAATTA CGCCCAGAAGTTTCAGGGACGAGTGACCATGACCCGCGATACCTCTATCTCGACCGCCTACATGGAGCTCTCCAGACTGC GCTCCGACGATACTGCAGTGTACTACTGCGCCCGGGACCTGAGGCGGACTGTGGTTACTCCTCGCGCCTATTATGGCATG

	>FA56-26RC (M2)	GACGTGTGGGGCCAAGGAACTACTGTGACTGTGAGCTCGGGAGGCGGTGGGTCAGGCGGAGGAGGGTCGGGCGGTGGTGG CTCGGGAGGGGGAGGAAGCGACATTCAACTTACGCAGAGCCCGTCAACCCTGTCAGCGTCAGTGGGAGATCGGGTGACCA TCACGTGTCAGGCCAGCCAGGATATCTCCAACTCGCTCAACTGGTACCAGCAAAAGGCGGGTAAAGCTCCGAAGCTGCTG ATCTACGACGCTTCCACCCTCGAGACTGGAGTCCCATCCAGATTTTCCGGGTCAGGAAGCGGCACCGATTTCTCCTTCAC CATTTCGTCCTTGCAACCGGAGGACATCGCAACCTACTACTGCCAGCAGCATGACAACTTGCCTCTGACGTTCGGGCAGG GCACCAAGGTGGAAATCAAG
112	M2 (Full) >FA56-26RC (M2)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTCCAG TCAGGAGCAGAAGTCAAGAAACCAGGTGCTAGCGTGAAAGTGTCGTGCAAGGCGTCGGGATACACTTTCACCGGATACTAC ATGCACTGGGTCCGCCAGGCCCCCGGACAAGGACTGGAATGGATGGGCTGGATCAACCCGAATAGCGGGGGAACTAATTA CGCCCAGAAGTTTCAGGGACGAGTGACCATGACCCGCGATACCTCTATCTCGACCGCCTACATGGAGCTCTCCAGACTGC GCTCCGACGATACTGCAGTGTACTACTGCGCCCGGGACCTGAGGCGGACTGTGGTTACTCCTCGCGCCTATTATGGCATG GACGTGTGGGGCCAAGGAACTACTGTGACTGTGAGCTCGGGAGGCGGTGGGTCAGGCGGAGGAGGGTCGGGCGGTGGTGG CTCGGGAGGGGGAGGAAGCGACATTCAACTTACGCAGAGCCCGTCAACCCTGTCAGCGTCAGTGGGAGATCGGGTGACCA TCACGTGTCAGGCCAGCCAGGATATCTCCAACTCGCTCAACTGGTACCAGCAAAAGGCGGGTAAAGCTCCGAAGCTGCTG ATCTACGACGCTTCCACCCTCGAGACTGGAGTCCCATCCAGATTTTCCGGGTCAGGAAGCGGCACCGATTTCTCCTTCAC CATTTCGTCCTTGCAACCGGAGGACATCGCAACCTACTACTGCCAGCAGCATGACAACTTGCCTCTGACGTTCGGGCAGG GCACCAAGGTGGAAATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTG TCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTA CATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGA AGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGG TTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACC CAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAA GCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGC CACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
89	M3 (ScFv domain) >VA58-21LC (M3)	CAAGTCCAACTCGTCCAA TCAGGAGCGGAAGTCAAAAAGCCCGGAGCTCCAGTGAAAGTGTCATGCAAGGCCTCCGGCTACACCTTCACCGGTTACTA TATGCACTGGGTGCGGCAGGCCCCGGGCCAGGGGTTGGAATGGATGGGATGGATCAATCCAAACTCGGGTGGGACTAACT ACGCCCAGAAGTTCCAAGGACGGGTGACCATGACTAGGGACACCTCGATCTCCACCGCATACATGGAGCTTAGCAGACTC CGCTCCGACGATACCGCAGTCTACTATTGCGCGCGGGGAGAGTGGGACGGATCGTACTACTACGATTACTGGGGCCAGGG AACTCTGGTGACTGTTTCCTCGGGTGGAGGAGGTTCAGGCGGAGGCGGCTCGGGCGGGGGAGGATCTGGAGGAGGAGGGT CCGACATTGTGCTGACCCAAACTCCTTCGTCCCTGTCGGCCAGCGTGGGCGACCGCGTGACGATTACGTGCAGAGCTAGC CAATCCATCAATACTTACCTCAACTGGTACCAGCATAAGCCGGGGAAAGCACCAAAGCTGCTGATCTACGCCGCCTCATC CTTGCAGAGCGGTGTGCCTTCACGCTTTAGCGGATCGGGATCGGGAACGGATTTCACCCTGACTATCAGCTCCCTCCAGC CGGAGGATTTTGCGACCTACTACTGTCAGCAGAGCTTCTCACCGCTGACTTTCGGCGGCGGGACCAAGCTGGAAATCAAG
113	M3 (Full) >VA58-21LC (M3)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTCCAA TCAGGAGCGGAAGTCAAAAAGCCCGGAGCTCCAGTGAAAGTGTCATGCAAGGCCTCCGGCTACACCTTCACCGGTTACTA TATGCACTGGGTGCGGCAGGCCCCGGGCCAGGGGTTGGAATGGATGGGATGGATCAATCCAAACTCGGGTGGGACTAACT ACGCCCAGAAGTTCCAAGGACGGGTGACCATGACTAGGGACACCTCGATCTCCACCGCATACATGGAGCTTAGCAGACTC CGCTCCGACGATACCGCAGTCTACTATTGCGCGCGGGGAGAGTGGGACGGATCGTACTACTACGATTACTGGGGCCAGGG AACTCTGGTGACTGTTTCCTCGGGTGGAGGAGGTTCAGGCGGAGGCGGCTCGGGCGGGGGAGGATCTGGAGGAGGAGGGT CCGACATTGTGCTGACCCAAACTCCTTCGTCCCTGTCGGCCAGCGTGGGCGACCGCGTGACGATTACGTGCAGAGCTAGC CAATCCATCAATACTTACCTCAACTGGTACCAGCATAAGCCGGGGAAAGCACCAAAGCTGCTGATCTACGCCGCCTCATC CTTGCAGAGCGGTGTGCCTTCACGCTTTAGCGGATCGGGATCGGGAACGGATTTCACCCTGACTATCAGCTCCCTCCAGC CGGAGGATTTTGCGACCTACTACTGTCAGCAGAGCTTCTCACCGCTGACTTTCGGCGGCGGGACCAAGCTGGAAATCAAG **ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTT AAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGG CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACG AACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTAT GAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACG CTCTTCACATGCAGGCCCTGCCGCCTCGG
90	M4 (ScFv domain) >DP37-07IC (M4)	CAAGTGCAACTCGTTGAA TCAGGTGGAGGTTTGGTGCAACCCGGAGGATCTCTCAGACTGTCGTGTGCGGCGTCCGGGTTCACCTTTTCGTCCTACTG GATGCACTGGGTGCGCCAGGTGCCGGGAAAAGGACTGGTGTGGGTGTCCAGAATCAACACCGACGGGTCAACGACTACCT ACGCAGATAGCGTGGAAGGTCGGTTCACCATTTCGCGGGACAACGCTAAAAACACTCTGTACCTTCAGATGAATTCACTG CGCGATGACGACACCGCAGTCTACTACTGCGTCGGTGGACACTGGGCGGTCTGGGGACAGGGAACTACGGTGACTGTGTC CAGCGGCGGGGGAGGAAGCGGCGGAGGGGGGAGCGGAGGCGGAGGATCAGGAGGAGGCGGCTCCGATATCCAGATGACCC AGTCGCCATCGACCCTCTCCGCTAGCGTGGGGGATAGGGTCACTATCACTTGCCGAGCCAGCCAATCCATTAGCGACCGG CTTGCCTGGTACCAACAGAAACCTGGAAAGGCCCCGAAGCTGCTCATCTACAAGGCCTCGTCACTGGAGTCGGGAGTCCC GTCCCGCTTTTCCGGCTCGGCTCAGGCACCGAGTTCACTCTGACCATCTCGAGCCTGCAGCCGGACGATTTCGCCGTGT ATTACTGCCAGCAATACGGACATCTCCCAATGTACACGTTCGGTCAGGGCACCAAGGTCGAAATCAAG
114	M4 >DP37-07IC	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAACTCGTTGAA TCAGGTGGAGGTTTGGTGCAACCCGGAGGATCTCTCAGACTGTCGTGTGCGGCGTCCGGGTTCACCTTTTCGTCCTACTG GATGCACTGGGTGCGCCAGGTGCCGGGAAAAGGACTGGTGTGGGTGTCCAGAATCAACACCGACGGGTCAACGACTACCT ACGCAGATAGCGTGGAAGGTCGGTTCACCATTTCGCGGGACAACGCTAAAAACACTCTGTACCTTCAGATGAATTCACTG CGCGATGACGACACCGCAGTCTACTACTGCGTCGGTGGACACTGGGCGGTCTGGGGACAGGGAACTACGGTGACTGTGTC

	(M4)	CAGCGGCGGGGGAGGAAGCGGCGGAGGGGGGAGCGGAGGCGGAGGATCAGGAGGAGGCGGCTCCGATATCCAGATGACCC AGTCGCCATCGACCCTCTCCGCTAGCGTGGGGGATAGGGTCACTATCACTTGCCGAGCCAGCCAATCCATTAGCGACCGG CTTGCCTGGTACCAACAGAAACCTGGAAAGGCCCCGAAGCTGCTCATCTACAAGGCCTCGTCACTGGAGTCGGGAGTCCC GTCCCGCTTTTCCGGCTCGGGCTCAGGCACCGAGTTCACTCTGACCATCTCGAGCCTGCAGCCGGACGATTTCGCCGTGT ATTACTGCCAGCAATACGGACATCTCCCAATGTACACGTTCGGTCAGGGCACCAAGGTCGAAATCAAG**ACCACTACC**CCA GCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGC TGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGG TCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTC ATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACT GCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTG GTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACG CAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATGC AGGCCCTGCCGCCTCGG
91	M5 (ScFv domain) >XP31-20LC (M5)	CAAGTCCAACTCGTTCAATCAGGCGCAGAAGTCGAAAAGCCCGGAGCATCAGTCAAAGTCTCTTGCAAGGCTTCCGGCTAC ACCTTCACGGACTACTAC ATGCACTGGGTGCGCCAGGCTCCAGGCCAGGGACTGGAGTGGATGGGATGGATCAACCCGAATTCCGGGGGAACTAACTA CGCCCAGAAGTTTCAGGGCCGGGTGACTATGACTCGCGATACCTCGATCTCGACTGCGTACATGGAGCTCAGCCGCCTCC GGTCGGACGATACCGCCGTGTACTATTGTGCGTCGGGATGGGACTTCGACTACTGGGGGCAGGGCACTCTGGTCACTGTG TCAAGCGGAGGAGGTGGATCAGGTGGAGGTGGAAGCGGGGGAGGAGGTTCCGGCGGCGGAGGATCAGATATCGTGATGAC GCAATCGCCTTCCTCGTTGTCCGCATCCGTGGGAGACAGGGTGACCATTACTTGCAGAGCGTCCCAGTCCATTCGGTACT ACCTGTCGTGGTACCAGCAGAAGCCGGGGAAAGCCCCAAAACTGCTTATCTATACTGCCTCGATCCTCCAAAACGGCGTG CCATCAAGATTCAGCGGTTCGGGCAGCGGGACCGACTTTACCCTGACTATCAGCAGCCTGCAGCCGGAAGATTTCGCCAC GTACTACTGCCTGCAAACCTACACCACCCCGGACTTCGGACCTGGAACCAAGGTGGAGATCAAG
115	M5 (Full) >XP31-20LC (M5)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTTCAA TCAGGCGCAGAAGTCGAAAAGCCCGGAGCATCAGTCAAAGTCTCTTGCAAGGCTTCCGGCTACACCTTCACGGACTACTAC ATGCACTGGGTGCGCCAGGCTCCAGGCCAGGGACTGGAGTGGATGGGATGGATCAACCCGAATTCCGGGGGAACTAACTA CGCCCAGAAGTTTCAGGGCCGGGTGACTATGACTCGCGATACCTCGATCTCGACTGCGTACATGGAGCTCAGCCGCCTCC GGTCGGACGATACCGCCGTGTACTATTGTGCGTCGGGATGGGACTTCGACTACTGGGGGCAGGGCACTCTGGTCACTGTG TCAAGCGGAGGAGGTGGATCAGGTGGAGGTGGAAGCGGGGGAGGAGGTTCCGGCGGCGGAGGATCAGATATCGTGATGAC GCAATCGCCTTCCTCGTTGTCCGCATCCGTGGGAGACAGGGTGACCATTACTTGCAGAGCGTCCCAGTCCATTCGGTACT ACCTGTCGTGGTACCAGCAGAAGCCGGGGAAAGCCCCAAAACTGCTTATCTATACTGCCTCGATCCTCCAAAACGGCGTG CCATCAAGATTCAGCGGTTCGGGCAGCGGGACCGACTTTACCCTGACTATCAGCAGCCTGCAGCCGGAAGATTTCGCCAC GTACTACTGCCTGCAAACCTACACCACCCCGGACTTCGGACCTGGAACCAAGGTGGAGATCAAG**ACCACTACC**CCAGCAC CGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGT GGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCT GCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGA GGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGC GTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCG GAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCC AAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGA AGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGC CCTGCCGCCTCGG
92	M6 (ScFv domain) >FE10-06ID (M6)	CAAGTGCAACTCGTCCAGTCAGGTGCAGAAGTGAAGAAACCCGGAGCGTCAGTCAAAGTGTCATGCAAGGCGTCAGGCTAC ACCTTCACCAGCTACTAC ATGCACTGGGTGCGGCAGGCCCCAGGCCAAGGCTTGGAGTGGATGGGAATCATTAACCCGTCAGGAGGCTCCACCTCCTA CGCCCAGAAGTTTCAGGGAAGAGTGACGATGACTCGGGATACGTCGACCTCGACCGTGTACATGGAACTGAGCTCGCTGC GCTCCGAGGACACTGCTGTGTACTACTGCGCACGGTACAGACTCATTGCCGTGGCAGGAGACTACTACTACTATGGCATG GACGTCTGGGGGCAGGGCACTATGGTCACTGTGTCGTCCGGCGGAGGAGGCTCGGGTGGAGGAGGTAGCGGAGGAGGGGG AAGCGGAGGGGGGGGCTCCGATATCCAGATGACTCAGTCGCCTTCCTCCGTGTCGGCCTCGGTTGGAGATCGCGTCACCA TCACTTGTCGAGCTTCCCAAGGAGTCGGTAGGTGGCTGGCGTGGTACCAGCAAAAGCCGGGAACTGCCCCGAAGCTCCTG ATCTACGCGGCTAGCACCCTGCAGTCGGGAGTGCCATCCCGCTTCAGCGGATCTGGGTCAGGTACCGACTTCACCCTTAC GATCAACAATCTCCAGCCGGAGGACTTTGCCACCTATTACTGCCAACAGGCCAACAGCTTCCCTCTGACTTTCGGAGGGG GCACTCGCCTGGAAATCAAG
116	M6 (Full) >FE10-06ID (M6)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAACTCGTCCAG TCAGGTGCAGAAGTGAAGAAACCCGGAGCGTCAGTCAAAGTGTCATGCAAGGCGTCAGGCTACACCTTCACCAGCTACTAC ATGCACTGGGTGCGGCAGGCCCCAGGCCAAGGCTTGGAGTGGATGGGAATCATTAACCCGTCAGGAGGCTCCACCTCCTA CGCCCAGAAGTTTCAGGGAAGAGTGACGATGACTCGGGATACGTCGACCTCGACCGTGTACATGGAACTGAGCTCGCTGC GCTCCGAGGACACTGCTGTGTACTACTGCGCACGGTACAGACTCATTGCCGTGGCAGGAGACTACTACTACTATGGCATG GACGTCTGGGGGCAGGGCACTATGGTCACTGTGTCGTCCGGCGGAGGAGGCTCGGGTGGAGGAGGTAGCGGAGGAGGGGG AAGCGGAGGGGGGGGCTCCGATATCCAGATGACTCAGTCGCCTTCCTCCGTGTCGGCCTCGGTTGGAGATCGCGTCACCA TCACTTGTCGAGCTTCCCAAGGAGTCGGTAGGTGGCTGGCGTGGTACCAGCAAAAGCCGGGAACTGCCCCGAAGCTCCTG ATCTACGCGGCTAGCACCCTGCAGTCGGGAGTGCCATCCCGCTTCAGCGGATCTGGGTCAGGTACCGACTTCACCCTTAC GATCAACAATCTCCAGCCGGAGGACTTTGCCACCTATTACTGCCAACAGGCCAACAGCTTCCCTCTGACTTTCGGAGGGG GCACTCGCCTGGAAATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTG TCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTA CATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGA AGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGG TTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACC CAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAA

		GCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGC CACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
93	M7 (ScFv domain) >VE12-01CD (M7)	CAAGTGCAATTGGTTCAA TCAGGAGGAGGAGTGGTGCAACCTGGAAGATCTCTCAGACTGTCGTGTGCGGCATCGGGATTCACTTTCTCATCATACGC AATGCACTGGGTCCGCCAGGCCCCGGGCAAAGGCTTGGAATGGGTGGCGGTCATTTCATACGACGGCTCGAACAAGTACT ACGCTGACAGCGTGAAGGGACGCTTTACTATTTCCCGGGACAATTCGAAGAACACTCTGTACCTCCAGATGAACTCCCTT AGGGCTGAGGACACCGCCGTCTACTACTGCGCACGCTGGAAAGTGTCGTCCAGCTCCCCAGCTTTTGACTACTGGGGACA GGGAACCCTTGTGACCGTGTCGTCCGGTGGAGGGGGAAGCGGCGGAGGGGGATCAGGTGGCGGCGGATCGGGAGGCGGGG GATCAGAAATCGTGCTGACTCAGTCCCCGGCCACGCTGTCTCTCAGCCCGGGAGAGAGAGCGATCCTGTCCTGCCGCGCC TCGCAGAGCGTGTACACTAAGTACCTGGGGTGGTACCAGCAGAAACCGGGTCAAGCGCCTCGGCTGCTGATCTACGATGC CTCCACCCGGGCCACCGGAATCCCCGATCGGTTCTCCGGCAGCGGCTCGGGAACTGATTTCACGCTGACCATCAATCGCC TGGAGCCGGAAGATTTCGCCGTCTATTACTGCCAGCATTACGGCGGGAGCCCACTCATCACCTTCGGTCAAGGAACCCGA CTCGAAATCAAG
117	M7 (Full) >VE12-01CD (M7)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAATTGGTTCAA TCAGGAGGAGGAGTGGTGCAACCTGGAAGATCTCTCAGACTGTCGTGTGCGGCATCGGGATTCACTTTCTCATCATACGC AATGCACTGGGTCCGCCAGGCCCCGGGCAAAGGCTTGGAATGGGTGGCGGTCATTTCATACGACGGCTCGAACAAGTACT ACGCTGACAGCGTGAAGGGACGCTTTACTATTTCCCGGGACAATTCGAAGAACACTCTGTACCTCCAGATGAACTCCCTT AGGGCTGAGGACACCGCCGTCTACTACTGCGCACGCTGGAAAGTGTCGTCCAGCTCCCCAGCTTTTGACTACTGGGGACA GGGAACCCTTGTGACCGTGTCGTCCGGTGGAGGGGGAAGCGGCGGAGGGGGATCAGGTGGCGGCGGATCGGGAGGCGGGG GATCAGAAATCGTGCTGACTCAGTCCCCGGCCACGCTGTCTCTCAGCCCGGGAGAGAGAGCGATCCTGTCCTGCCGCGCC TCGCAGAGCGTGTACACTAAGTACCTGGGGTGGTACCAGCAGAAACCGGGTCAAGCGCCTCGGCTGCTGATCTACGATGC CTCCACCCGGGCCACCGGAATCCCCGATCGGTTCTCCGGCAGCGGCTCGGGAACTGATTTCACGCTGACCATCAATCGCC TGGAGCCGGAAGATTTCGCCGTCTATTACTGCCAGCATTACGGCGGGAGCCCACTCATCACCTTCGGTCAAGGAACCCGA CTCGAAATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGG CCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTG CTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGA GGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACC AGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATG GGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAG CGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGG ACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
94	M8 (ScFv domain) >LE13-05XD (M8)	CAAGTCCAACTCCAGCAG TCAGGTGCAGAAGTCAAAAAGCCAGGAGCATCCGTGAAGGTTTCGTGCAAGACTTCCGGCTACCCTTTTACCGGGTACTC CCTCCATTGGGTGAGACAAGCACCGGGCCAGGGACTGGAGTGGATGGGATGGATCAACCCAAATTCGGGCGGCACCAACT ATGCGCAGAAGTTCCAGGGACGGGTGACCATGACTCGCGACACTTCGATCTCCACTGCCTACATGGAGCTGTCCCGCTTG AGATCTGACGACACGGCCGTCTACTACTGCGCCCGGGATCACTACGGAGGTAATTCGCTGTTCTACTGGGGGCAGGGAAC CCTTGTGACTGTGTCCTCGGGTGGTGGAGGGTCAGGAGGCGGAGGCTCAGGGGGAGGAGGTAGCGGAGGAGGCGGATCAG ACATCCAACTGACCCAGTCACCATCCTCCATCTCGGCTAGCGTCGGAGACACCGTGTCGATTACTTGTAGGGCCTCCCAA GACTCAGGGACGTGGCTGGCGTGGTATCAGCAAAAACCGGGCAAAGCTCCGAACCTGTTGATGTACGACGCCAGCACCCT CGAAGATGGAGTGCCTAGCCGCTTCAGCGGAAGCGCCTCGGGCACTGAATTCACGCTGACTGTGAATCGGCTCCAGCCGG AGGATTCGGCGACCTACTACTGCCAGCAGTACAACAGCTACCCCCTGACCTTTGGAGGCGGGACCAAGGTGGATATCAAG
118	M8 (Full) >LE13-05XD (M8)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCCAGCAG TCAGGTGCAGAAGTCAAAAAGCCAGGAGCATCCGTGAAGGTTTCGTGCAAGACTTCCGGCTACCCTTTTACCGGGTACTC CCTCCATTGGGTGAGACAAGCACCGGGCCAGGGACTGGAGTGGATGGGATGGATCAACCCAAATTCGGGCGGCACCAACT ATGCGCAGAAGTTCCAGGGACGGGTGACCATGACTCGCGACACTTCGATCTCCACTGCCTACATGGAGCTGTCCCGCTTG AGATCTGACGACACGGCCGTCTACTACTGCGCCCGGGATCACTACGGAGGTAATTCGCTGTTCTACTGGGGGCAGGGAAC CCTTGTGACTGTGTCCTCGGGTGGTGGAGGGTCAGGAGGCGGAGGCTCAGGGGGAGGAGGTAGCGGAGGAGGCGGATCAG ACATCCAACTGACCCAGTCACCATCCTCCATCTCGGCTAGCGTCGGAGACACCGTGTCGATTACTTGTAGGGCCTCCCAA GACTCAGGGACGTGGCTGGCGTGGTATCAGCAAAAACCGGGCAAAGCTCCGAACCTGTTGATGTACGACGCCAGCACCCT CGAAGATGGAGTGCCTAGCCGCTTCAGCGGAAGCGCCTCGGGCACTGAATTCACGCTGACTGTGAATCGGCTCCAGCCGG AGGATTCGGCGACCTACTACTGCCAGCAGTACAACAGCTACCCCCTGACCTTTGGAGGCGGGACCAAGGTGGATATCAAG **ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTT AAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGG CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACG AACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTAT GAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACG CTCTTCACATGCAGGCCCTGCCGCCTCGG
95	M9 (ScFv domain) >BE15-00SD	CAAGTGCAACTCGTCCAG TCAGGTGCAGAAGTGAAGAAACCAGGAGCGTCCGTCGAAGTGTCGTGTAAGGCGTCCGGCTACACTTTCACCTCGTACTA CATGCACTGGGTGCGGCAGGCCCCGGGACAAGGCCTCGAATGGATGGGAATCATCAACCCGAGCGGAGGCTCGACTGGTT ACGCCCAGAAGTTCCAGGGAAGGGTGACGATGACCCGCGATACCTCGACTTCGACCGTTCATATGGAGCTCTCGTCCCTG CGGAGCGAGGACACTGCTGTCTACTATTGCGCGCGGGGAGGATACTCTAGCTCCTCCGATGCATTTGACATTTGGGGCCA GGGAACTATGGTGACCGTGTCATCAGGCGGAGGTGGATCAGGAGGAGGAGGGTCGGGAGGGGGAGGCAGCGGCGGGGGTG GGTCGGACATTCAGATGACGCAGTCCCCTCCTAGCCTGAGCGCCTCGGTGGGTGACAGAGTGACCATCACTTGCAGAGCC TCGCAAGACATCTCCTCCGCATTGGCTTGGTACCAGCAAAAGCCGGGCACTCCGCCGAAACTGCTCATCTACGATGCCTC CTCACTGGAGTCAGGAGTCCCATCTCGCTTCTCGGGGTCAGGAAGCGGCACCGATTTTACCCTTACCATCTCCAGCCTGC AGCCCGAGGACTTCGCCACGTACTACTGCCAACAGTTCAGCTCCTACCCACTGACCTTCGGGGGCGGAACTCGCCTGGAA

	(M9)	ATCAAG
119	M9 (Full) >BE15-00SD (M9)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAACTCGTCCAG TCAGGTGCAGAAGTGAAGAAACCAGGAGCGTCCGTCGAAGTGTCGTGTAAGGCGTCCGGCTACACTTTCACCTCGTACTA CATGCACTGGGTGCGGCAGGCCCCGGGACAAGGCCTCGAATGGATGGGAATCATCAACCCGAGCGGAGGCTCGACTGGTT ACGCCCAGAAGTTCCAGGGAAGGGTGACGATGACCCGCGATACCTCGACTTCGACCGTTCATATGGAGCTCTCGTCCCTG CGGAGCGAGGACACTGCTGTCTACTATTGCGCGCGGGGAGGATACTCTAGCTCCTCCGATGCATTTGACATTTGGGGCCA GGGAACTATGGTGACCGTGTCATCAGGCGGAGGTGGATCAGGAGGAGGAGGGTCGGGAGGGGGAGGCAGCGGCGGGGGTG GGTCGGACATTCAGATGACGCAGTCCCCTCCTAGCCTGAGCGCCTCGGTGGGTGACAGAGTGACCATCACTTGCAGAGCC TCGCAAGACATCTCCTCCGCATTGGCTTGGTACCAGCAAAAGCCGGGCACTCCGCCGAAACTGCTCATCTACGATGCCTC CTCACTGGAGTCAGGAGTCCCATCTCGCTTCTCGGGGTCAGGAAGCGGCACCGATTTTACCCTTACCATCTCCAGCCTGC AGCCCGAGGACTTCGCCACGTACTACTGCCAACAGTTCAGCTCCTACCCACTGACCTTCGGGGGCGGAACTCGCCTGGAA ATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGA GGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCT GGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTAC ATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGA GGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCT ACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGG AAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGAT TGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCT ATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
96	M10 (ScFv domain) >RE16-05MD (M10)	CAAGTGCAACTCGTCCAGAGCGGAGCAGAAGTCAAGAAGCCAGGAGCGTCAGTGAAAGTGTCATGCAAGGCCAGCGGCTAT ACCTTTACTTCGTATGGG ATCTCCTGGGTGCGGCAGGCACCGGGCCAAGGACTGGAGTGGATGGGATGGATCTCAGCCTACAACGGTAACACCAACTA CGCCCAGAAGCTGCAAGGACGCGTGACCATGACTACTGATACGAGCACCTCCACTGCCTACATGGAATTGCGGTCCCTTC GGTCGGACGATACTGCTGTGTACTACTGCGCAAGAGTCGCCGGAGGGATCTACTACTACTACGGCATGGACGTCTGGGGA CAGGGAACCACCATTACGGTGTCGAGCGGAGGGGGAGGCTCGGGGGGAGGAGGAAGCGGAGGTGGCGGCTCCGGGGGCGG CGGATCGGACATTGTGATGACCCAGACTCCTGACTCCCTGGCTGTTTCGTTGGGAGAGCGCGCGACTATCTCGTGTAAGT CCAGCCACTCAGTCCTGTACAATCGCAATAACAAGAACTACCTCGCGTGGTACCAGCAAAAACCGGGTCAGCCGCCTAAA CTCCTGTTCTACTGGGCCTCCACCAGAAAGAGCGGGGTGCCAGATCGATTCTCTGGATCAGGATCAGGTACCGACTTTAC GCTGACCATCTCGTCCCTGCAGCCGGAGGATTTCGCGACTTACTTCTGCCAGCAGACTCAGACTTTCCCCCTCACCTTCG GTCAAGGCACCAGGCTGGAAATCAAT
120	M10 (Full) >RE16-05MD (M10)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAACTCGTCCAG AGCGGAGCAGAAGTCAAGAAGCCAGGAGCGTCAGTGAAAGTGTCATGCAAGGCCAGCGGCTATACCTTTACTTCGTATGGG ATCTCCTGGGTGCGGCAGGCACCGGGCCAAGGACTGGAGTGGATGGGATGGATCTCAGCCTACAACGGTAACACCAACTA CGCCCAGAAGCTGCAAGGACGCGTGACCATGACTACTGATACGAGCACCTCCACTGCCTACATGGAATTGCGGTCCCTTC GGTCGGACGATACTGCTGTGTACTACTGCGCAAGAGTCGCCGGAGGGATCTACTACTACTACGGCATGGACGTCTGGGGA CAGGGAACCACCATTACGGTGTCGAGCGGAGGGGGAGGCTCGGGGGGAGGAGGAAGCGGAGGTGGCGGCTCCGGGGGCGG CGGATCGGACATTGTGATGACCCAGACTCCTGACTCCCTGGCTGTTTCGTTGGGAGAGCGCGCGACTATCTCGTGTAAGT CCAGCCACTCAGTCCTGTACAATCGCAATAACAAGAACTACCTCGCGTGGTACCAGCAAAAACCGGGTCAGCCGCCTAAA CTCCTGTTCTACTGGGCCTCCACCAGAAAGAGCGGGGTGCCAGATCGATTCTCTGGATCAGGATCAGGTACCGACTTTAC GCTGACCATCTCGTCCCTGCAGCCGGAGGATTTCGCGACTTACTTCTGCCAGCAGACTCAGACTTTCCCCCTCACCTTCG GTCAAGGCACCAGGCTGGAAATCAAT**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAG CCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGA TATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCG GTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCA TGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAA GCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGAC GGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATG GCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAG CACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
97	M11 (ScFv domain) >NE10-19WD (M11)	CAAGTCCAATTGCAGCAGAGCGGAGCAGAAGTGAAGAAGCCAGGAGCGTCAGTCAAAGTGTCGTGTAAGGCGTCAGGATAC ACCTTCACGGGATACTAC ATGCACTGGGTGCGCCAGGCCCCGGGCCAAGGACTCGAGTGGATGGGCTGGATCAACCCTAACTCTGGAGGCACCAACTA CGCCCAGAATTTCCAAGGCAGAGTGACCATGACCCGGGACACCTCCATCTCGACTGCCTATATGGAACTGCGGCGGCTGC GCTCGGACGATACTGCTGTGTATTACTGCGCCAGCGGCTGGGACTTTGACTACTGGGGACAGGGTACTCTGGTGACTGTT TCCTCGGGAGGAGGCGGATCGGGTGGAGGAGGTAGCGGGGGAGGGGGGTCGGGAGGCGGAGGCAGCGATATTCGCATGAC TCAATCGCCGTCCTCCCTGAGCGCTAGCGTGGGAGATCGAGTCACCATCACTTGCAGAGCGTCACAGTCGATTCGCTACT ACCTGTCCTGGTACCAGCAGAAACCGGGAAAGGCACCAAAGCTTCTGATCTACACGGCCTCCATCCTGCAAAATGGTGTC CCATCAAGGTTCTCCGGGTCAGGGAGCGGCACTGACTTCACTCTCACCATCTCCTCACTCCAGCCCGAGGACTTTGCAAC CTACTACTGCCTCCAGACGTACACCACCCCGGATTTCGGTCCTGGAACCAAGGTGGAAATCAAA
121	M11 (Full) >NE10-19WD	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAATTGCAGCAG AGCGGAGCAGAAGTGAAGAAGCCAGGAGCGTCAGTCAAAGTGTCGTGTAAGGCGTCAGGATACACCTTCACGGGATACTAC ATGCACTGGGTGCGCCAGGCCCCGGGCCAAGGACTCGAGTGGATGGGCTGGATCAACCCTAACTCTGGAGGCACCAACTA CGCCCAGAATTTCCAAGGCAGAGTGACCATGACCCGGGACACCTCCATCTCGACTGCCTATATGGAACTGCGGCGGCTGC GCTCGGACGATACTGCTGTGTATTACTGCGCCAGCGGCTGGGACTTTGACTACTGGGGACAGGGTACTCTGGTGACTGTT TCCTCGGGAGGAGGCGGATCGGGTGGAGGAGGTAGCGGGGGAGGGGGGTCGGGAGGCGGAGGCAGCGATATTCGCATGAC TCAATCGCCGTCCTCCCTGAGCGCTAGCGTGGGAGATCGAGTCACCATCACTTGCAGAGCGTCACAGTCGATTCGCTACT

	(M11)	ACCTGTCCTGGTACCAGCAGAAACCGGGAAAGGCACCAAAGCTTCTGATCTACACGGCCTCCATCCTGCAAAATGGTGTC CCATCAAGGTTCTCCGGGTCAGGGAGCGGCACTGACTTCACTCTCACCATCTCCTCACTCCAGCCCGAGGACTTTGCAAC CTACTACTGCCTCCAGACGTACACCACCCCGGATTTCGGTCCTGGAACCAAGGTGGAAATCAAA**ACCACTACC**CCAGCAC CGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGT GGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCT GCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGA GGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGC GTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCG GAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCC AAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGA AGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGC CCTGCCGCCTCGG
98	M12 (ScFv domain) >DE12-14RD (M12)	CAAGTCCAACTCGTCCAA AGCGGAGCAGAAGTCAAAAAGCCAGGAGCGTCGGTGAAAGTGTCTTGCAAAGCCAGCGGCTACACCTTCACGGGTTACTA CATGCACTGGGTGCGCCAGGCGCCGGGCCAGGGGCTGGAGTGGATGGGCCGGATTAACCCTAACAGCGGGGGAACTAATT ACGCTCAGAAGTTCCAGGGTAGAGTCACCATGACTACGGACACTTCCACTTCCACCGCCTATATGGAACTGCGCTCCCTC CGCTCAGATGATACTGCCGTGTATTACTGCGCGCGGACTACCACGTCATACGCATTTGACATCTGGGGCCAGGGAACTAT GGTGACCGTGAGCTCGGGCGGAGGCGGTTCAGGGGGAGGAGGAAGCGGAGGAGGAGGATCGGGAGGAGGTGGCTCCGATA TCCAGCTGACTCAGTCCCCGAGCACCCTGTCGGCGTCGGTGGGGGACAGGGTTACCATCACCTGTAGAGCTTCCCAATCC ATTTCGACTTGGCTGGCCTGGTACCAGCAAAAGCCGGGAAAGGCCCCTAATTTGCTTATCTACAAGGCATCGACCCTCGA AAGCGGTGTGCCCTCCCGGTTTTCGGGATCAGGATCAGGGACCGAGTTCACCCTGACCATCTCATCCCTCCAGCCGGACG ACTTCGCCACTTACTACTGCCAGCAGTACAACACCTACTCGCCATACACTTTCGGCCAAGGCACCAAGCTGGAGATCAAG
122	M12 (Full) >DE12-14RD (M12)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTCCAA AGCGGAGCAGAAGTCAAAAAGCCAGGAGCGTCGGTGAAAGTGTCTTGCAAAGCCAGCGGCTACACCTTCACGGGTTACTA CATGCACTGGGTGCGCCAGGCGCCGGGCCAGGGGCTGGAGTGGATGGGCCGGATTAACCCTAACAGCGGGGGAACTAATT ACGCTCAGAAGTTCCAGGGTAGAGTCACCATGACTACGGACACTTCCACTTCCACCGCCTATATGGAACTGCGCTCCCTC CGCTCAGATGATACTGCCGTGTATTACTGCGCGCGGACTACCACGTCATACGCATTTGACATCTGGGGCCAGGGAACTAT GGTGACCGTGAGCTCGGGCGGAGGCGGTTCAGGGGGAGGAGGAAGCGGAGGAGGAGGATCGGGAGGAGGTGGCTCCGATA TCCAGCTGACTCAGTCCCCGAGCACCCTGTCGGCGTCGGTGGGGGACAGGGTTACCATCACCTGTAGAGCTTCCCAATCC ATTTCGACTTGGCTGGCCTGGTACCAGCAAAAGCCGGGAAAGGCCCCTAATTTGCTTATCTACAAGGCATCGACCCTCGA AAGCGGTGTGCCCTCCCGGTTTTCGGGATCAGGATCAGGGACCGAGTTCACCCTGACCATCTCATCCCTCCAGCCGGACG ACTTCGCCACTTACTACTGCCAGCAGTACAACACCTACTCGCCATACACTTTCGGCCAAGGCACCAAGCTGGAGATCAAG **ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTT AAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGG CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACG AACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTAT GAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACG CTCTTCACATGCAGGCCCTGCCGCCTCGG
99	M13 (ScFv domain) >TE13-19LD (M13)	CAAGTTCAACTCGTGCAATCAGGTGGAGGACTCGTCAAACCCGGAGGATCATTGAGACTGTCATGCGAAGCGAGCGGTTTT ATCTTCTCCGATTACTAT ATGGGATGGATTCGGCAGGCCCCGGGAAAGGGACTCGAATGGGTGTCATACATCGGAAGGTCAGGCTCGTCCATGTACTA CGCAGACTCGGTGAAAGGCAGATTCACCTTTAGCCGGGACAACGCCAAGAATTCCCTCTACTTGCAGATGAACAGCCTGC GAGCCGAGGATACTGCTGTCTACTACTGTGCCGCGTCGCCGGTGGTGGCAGCTACTGAAGATTTCCAGCACTGGGGACAG GGAACTCTGGTCACGGTGTCGAGCGGTGGGGGCGGAAGCGGAGGCGGAGGATCGGGCGGCGGAGGTTCGGGGGGGGGAGG GTCTGACATCGTGATGACCCAAACCCCAGCCACCCTGAGCCTCTCCCCTGGAGAGCGCGCGACTCTTTCGTGCCGCGCTT CCCAGTCAGTGACCAGCAATTACTTGGCTTGGTACCAACAGAAGCCGGGACAGGCGCCACGGCTGCTGCTTTTTGGTGCC AGCACTCGCGCCACCGGAATCCCGGATCGCTTCTCGGGCTCAGGGTCCGGGACGGACTTCACCCTGACTATCAACCGGCT GGAACCTGAGGACTTCGCGATGTACTACTGCCAGCAGTACGGCTCCGCACCAGTCACTTTCGGACAAGGCACCAAGCTGG AGATCAAG
123	M13 (Full) >TE13-19LD (M13)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTTCAACTCGTGCAA TCAGGTGGAGGACTCGTCAAACCCGGAGGATCATTGAGACTGTCATGCGAAGCGAGCGGTTTTATCTTCTCCGATTACTAT ATGGGATGGATTCGGCAGGCCCCGGGAAAGGGACTCGAATGGGTGTCATACATCGGAAGGTCAGGCTCGTCCATGTACTA CGCAGACTCGGTGAAAGGCAGATTCACCTTTAGCCGGGACAACGCCAAGAATTCCCTCTACTTGCAGATGAACAGCCTGC GAGCCGAGGATACTGCTGTCTACTACTGTGCCGCGTCGCCGGTGGTGGCAGCTACTGAAGATTTCCAGCACTGGGGACAG GGAACTCTGGTCACGGTGTCGAGCGGTGGGGGCGGAAGCGGAGGCGGAGGATCGGGCGGCGGAGGTTCGGGGGGGGGAGG GTCTGACATCGTGATGACCCAAACCCCAGCCACCCTGAGCCTCTCCCCTGGAGAGCGCGCGACTCTTTCGTGCCGCGCTT CCCAGTCAGTGACCAGCAATTACTTGGCTTGGTACCAACAGAAGCCGGGACAGGCGCCACGGCTGCTGCTTTTTGGTGCC AGCACTCGCGCCACCGGAATCCCGGATCGCTTCTCGGGCTCAGGGTCCGGGACGGACTTCACCCTGACTATCAACCGGCT GGAACCTGAGGACTTCGCGATGTACTACTGCCAGCAGTACGGCTCCGCACCAGTCACTTTCGGACAAGGCACCAAGCTGG AGATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCG GAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCC TCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGT ACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCT CTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCG

		GGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACAC CTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
100	M14 (ScFv domain) >BS83-95ID (M14)	CAAGTCCAACTCGTCCAGTCGGGAGCAGAAGTTAGAGCACCAGGAGCGTCAGTGAAAATCTCATGCAAGGCCTCGGGCTTC ACGTTCCGCGGATACTAC ATCCACTGGGTGCGCCAAGCCCCGGGTCAGGGATTGGAGTGGATGGGAATCATTAACCCATCAGGAGGGAGCCGGGCTTA CGCGCAGAAGTTCCAGGGACGCGTCACTATGACCCGAGATACTTCCACCTCGACTGTGTACATGGAACTCTCGTCCCTGA GGTCCGACGACACTGCGATGTATTACTGTGCTCGGACTGCCAGCTGCGGTGGGGACTGTTACTACCTCGATTACTGGGGC CAGGGAACTCTGGTGACCGTGTCCAGCGGAGGTGGCGGGTCAGGGGGTGGCGGAAGCGGAGGCGGCGGTTCAGGCGGAGG AGGCTCGGACATCCAAATGACGCAATCGCCGCCTACCCTGAGCGCTTCCGTGGGAGATCGGGTGACCATTACTTGCAGAG CATCCGAGAACGTCAATATCTGGCTGGCCTGGTACCAACAGAAGCCGGGGAAGGCCCCTAAACTGCTGATCTACAAGTCG AGCAGCCTTGCCTCTGGAGTGCCCTCCCGCTTCTCGGGCTCGGGATCAGGAGCGGAATTCACCCTCACCATCTCCTCCCT GCAGCCAGATGACTTTGCCACCTACTACTGCCAGCAGTACCAGAGCTATCCGTTGACCTTTGGGGGAGGCACTAAAGTGG ACATCAAG
124	M14 (Full) >BS83-95ID (M14)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTCCAG TCGGGAGCAGAAGTTAGAGCACCAGGAGCGTCAGTGAAAATCTCATGCAAGGCCTCGGGCTTCACGTTCCGCGGATACTAC ATCCACTGGGTGCGCCAAGCCCCGGGTCAGGGATTGGAGTGGATGGGAATCATTAACCCATCAGGAGGGAGCCGGGCTTA CGCGCAGAAGTTCCAGGGACGCGTCACTATGACCCGAGATACTTCCACCTCGACTGTGTACATGGAACTCTCGTCCCTGA GGTCCGACGACACTGCGATGTATTACTGTGCTCGGACTGCCAGCTGCGGTGGGGACTGTTACTACCTCGATTACTGGGGC CAGGGAACTCTGGTGACCGTGTCCAGCGGAGGTGGCGGGTCAGGGGGTGGCGGAAGCGGAGGCGGCGGTTCAGGCGGAGG AGGCTCGGACATCCAAATGACGCAATCGCCGCCTACCCTGAGCGCTTCCGTGGGAGATCGGGTGACCATTACTTGCAGAG CATCCGAGAACGTCAATATCTGGCTGGCCTGGTACCAACAGAAGCCGGGGAAGGCCCCTAAACTGCTGATCTACAAGTCG AGCAGCCTTGCCTCTGGAGTGCCCTCCCGCTTCTCGGGCTCGGGATCAGGAGCGGAATTCACCCTCACCATCTCCTCCCT GCAGCCAGATGACTTTGCCACCTACTACTGCCAGCAGTACCAGAGCTATCCGTTGACCTTTGGGGGAGGCACTAAAGTGG ACATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCG GAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCC TCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGT ACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCT CTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCG GGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACAC CTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
101	M15 (ScFv domain) >HS86-94XD (M15)	CAAGTTCAACTCGTTCAA TCAGGTGGAGGACTCGTGCAACCAGGAAGATCACTCAGACTCAGCTGCGCCGCGTCGGGATTCACTTTCGATGACTACGC AATGCACTGGGTGCGGCAGGCCCCGGGCAAAGGACTGGAATGGGTGAGCGGAATTAGCTGGAACTCGGGGTCCATCGGGT ACGCCGACTCGGTGAAGGGACGCTTTACGATCTCCCGGGACAATGCCAAGAACTCCCTGTATTTGCAGATGAACTCCTTG AGGGCTGAGGACACCGCCGTGTACTACTGCGCTAAAGATGGATCATCGTCCTGGTCCTGGGGATACTTCGATTACTGGGG CCAGGGCACTCTGGTGACCGTGTCGTCAGGCGGTGGAGGGTCGGGCGGAGGAGGTAGCGGAGGCGGAGGGAGCAGCTCTG AACTGACCCAAGACCCGGCGGTGTCGGTCGCCCTTGGTCAGACTGTGCGGACTACCTGTCAGGGGGACGCGCTGCGCTCG TACTACGCTTCATGGTACCAGCAGAAGCCCGGACAGGCACCTATGCTGGTCATCTACGGAAAGAATAACCGCCCATCCGG CATCCCGGATCGCTTCTCGGGTTCGGACAGCGGCGACACCGCATCCCTGACGATCACTGGAGCGCAGGCCGAGGATGAAG CCGACTACTACTGCAATTCCCGAGATTCAAGCGGCTACCCTGTGTTTGGGACCGGAACTAAGGTCACCGTCCTG
125	M15 (Full) >HS86-94XD (M15)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTTCAACTCGTTCAA TCAGGTGGAGGACTCGTGCAACCAGGAAGATCACTCAGACTCAGCTGCGCCGCGTCGGGATTCACTTTCGATGACTACGC AATGCACTGGGTGCGGCAGGCCCCGGGCAAAGGACTGGAATGGGTGAGCGGAATTAGCTGGAACTCGGGGTCCATCGGGT ACGCCGACTCGGTGAAGGGACGCTTTACGATCTCCCGGGACAATGCCAAGAACTCCCTGTATTTGCAGATGAACTCCTTG AGGGCTGAGGACACCGCCGTGTACTACTGCGCTAAAGATGGATCATCGTCCTGGTCCTGGGGATACTTCGATTACTGGGG CCAGGGCACTCTGGTGACCGTGTCGTCAGGCGGTGGAGGGTCGGGCGGAGGAGGTAGCGGAGGCGGAGGGAGCAGCTCTG AACTGACCCAAGACCCGGCGGTGTCGGTCGCCCTTGGTCAGACTGTGCGGACTACCTGTCAGGGGGACGCGCTGCGCTCG TACTACGCTTCATGGTACCAGCAGAAGCCCGGACAGGCACCTATGCTGGTCATCTACGGAAAGAATAACCGCCCATCCGG CATCCCGGATCGCTTCTCGGGTTCGGACAGCGGCGACACCGCATCCCTGACGATCACTGGAGCGCAGGCCGAGGATGAAG CCGACTACTACTGCAATTCCCGAGATTCAAGCGGCTACCCTGTGTTTGGGACCGGAACTAAGGTCACCGTCCTGACCACT ACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACC CGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTT GCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAA CCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTG CGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCA ATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGG GGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTC ACATGCAGGCCCTGCCGCCTCGG
102	M16 (ScFv domain)	GAAGTGCAACTCGTGGAA TCTGGTGGAGGACTTGTGCAACCTGGAAGATCGTTGAGACTCTCATGTGCTGCCTCCGGGTTCACCTTTGACGACTACGC CATGCACTGGGTGCGCCAGGCACCAGGAAAGGGTCTGGAGTGGGTTTCGGGTATCTCGTGGAACTCCGGGAGCACTGGCT ACGCTGATTCGGTGAAAGGCCGGTTTACCATCTCCCGAGACAATGCGAAGAATTCCCTCTATCTGCAGATGAACAGCCTC CGGGCCGAGGATACTGCCCTGTACTACTGCGCCAAGGATAGCTCATCATGGTACGGAGGTGGATCGGCTTTCGATATCTG GGGCCAGGGCACGATGGTCACCGTGTCCTCGGGGGGCGGAGGCTCCGGGGGAGGAGGTAGCGGAGGAGGAGGATCGAGCT

	>XS87-99RD (M16)	CAGAGTTGACTCAAGAACCCGCAGTGTCCGTGGCACTGGGCCAAACCGTCAGGATCACTTGCCAGGGAGACAGCCTGAGG TCGTACTACGCGTCCTGGTACCAGCAGAAGCCGGGACAGGCCCCGGTCCTGGTCATTTTCGGACGCTCAAGACGCCCATC GGGCATCCCGGACCGGTTCAGCGGAAGCTCCTCGGGAAACACCGCGTCACTTATCATTACCGGCGCACAGGCTGAGGACG AAGCGGATTACTACTGCAACTCCCGCGACAATACTGCCAACCATTACGTGTTCGGGACCGGAACGAAACTGACTGTCCTG
126	M16 (Full) >XS87-99RD (M16)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAAGTGCAACTCGTGGAA TCTGGTGGAGGACTTGTGCAACCTGGAAGATCGTTGAGACTCTCATGTGCTGCCTCCGGGTTCACCTTTGACGACTACGC CATGCACTGGGTGCGCCAGGCACCAGGAAAGGGTCTGGAGTGGGTTTCGGGTATCTCGTGGAACTCCGGGAGCACTGGCT ACGCTGATTCGGTGAAAGGCCGGTTTACCATCTCCCGAGACAATGCGAAGAATTCCCTCTATCTGCAGATGAACAGCCTC CGGGCCGAGGATACTGCCCTGTACTACTGCGCCAAGGATAGCTCATCATGGTACGGAGGTGGATCGGCTTTCGATATCTG GGGCCAGGGCACGATGGTCACCGTGTCCTCGGGGGGCGGAGGCTCCGGGGGAGGAGGTAGCGGAGGAGGAGGATCGAGCT CAGAGTTGACTCAAGAACCCGCAGTGTCCGTGGCACTGGGCCAAACCGTCAGGATCACTTGCCAGGGAGACAGCCTGAGG TCGTACTACGCGTCCTGGTACCAGCAGAAGCCGGGACAGGCCCCGGTCCTGGTCATTTTCGGACGCTCAAGACGCCCATC GGGCATCCCGGACCGGTTCAGCGGAAGCTCCTCGGGAAACACCGCGTCACTTATCATTACCGGCGCACAGGCTGAGGACG AAGCGGATTACTACTGCAACTCCCGCGACAATACTGCCAACCATTACGTGTTCGGGACCGGAACGAAACTGACTGTCCTG **ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTT AAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGG CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACG AACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTAT GAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACG CTCTTCACATGCAGGCCCTGCCGCCTCGG
103	M17 (ScFv domain) >NS89-94MD (M17)	GAAGTTCAATTGGTGGAA TCTGGAGGAGGACTTGTGCAACCCGGTAGATCTCTGAGACTGTCCTGTGCGGCATCGGGATTCACCTTCGACGACTACGC TATGCACTGGGTGAGACAAGCCCCTGGAAAAGGACTGGAGTGGGTGTCAGGCATCTCCTGGAATAGCGGGTCCACTGGAT ACGCCGATTCGGTCAAGGGTCGCTTCACCATTTCCCGGGACAATGCCAAGAACTCCCTGTACCTTCAAATGAACTCCCTC CGGGCCGAGGATACCGCCCTCTACTACTGCGCCAAAGACAGCTCGTCATGGTATGGCGGAGGGTCGGCATTTGACATCTG GGGACAGGGAACTATGGTGACTGTGTCATCAGGAGGCGGCGGAAGCGGCGGCGGCGGGTCCGGCGGAGGAGGGTCGTCCA GCGAACTCACCCAAGATCCAGCAGTGAGCGTCGCGCTGGGCCAGACCGTCAGGATCACGTGCCAGGGAGATTCACTGCGC TCATACTACGCGTCCTGGTACCAGCAGAAGCCGGGGCAGGCCCCGGTCCTCGTGATCTACGGAAAGAACAACCGCCCGTC GGGTATCCCAGACCGCTTTTCGGGTAGCTCCAGCGGAAATACGGCTAGCCTGACCATCACTGGAGCACAGGCTGAGGATG AAGCGGACTACTACTGCAATTCGCGGGGCTCATCGGGGAACCATTACGTGTTCGGAACTGGTACCAAGGTGACTGTCCTG
127	M17 (Full) >NS89-94MD (M17)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCGAAGTTCAATTGGTGGAA TCTGGAGGAGGACTTGTGCAACCCGGTAGATCTCTGAGACTGTCCTGTGCGGCATCGGGATTCACCTTCGACGACTACGC TATGCACTGGGTGAGACAAGCCCCTGGAAAAGGACTGGAGTGGGTGTCAGGCATCTCCTGGAATAGCGGGTCCACTGGAT ACGCCGATTCGGTCAAGGGTCGCTTCACCATTTCCCGGGACAATGCCAAGAACTCCCTGTACCTTCAAATGAACTCCCTC CGGGCCGAGGATACCGCCCTCTACTACTGCGCCAAAGACAGCTCGTCATGGTATGGCGGAGGGTCGGCATTTGACATCTG GGGACAGGGAACTATGGTGACTGTGTCATCAGGAGGCGGCGGAAGCGGCGGCGGCGGGTCCGGCGGAGGAGGGTCGTCCA GCGAACTCACCCAAGATCCAGCAGTGAGCGTCGCGCTGGGCCAGACCGTCAGGATCACGTGCCAGGGAGATTCACTGCGC TCATACTACGCGTCCTGGTACCAGCAGAAGCCGGGGCAGGCCCCGGTCCTCGTGATCTACGGAAAGAACAACCGCCCGTC GGGTATCCCAGACCGCTTTTCGGGTAGCTCCAGCGGAAATACGGCTAGCCTGACCATCACTGGAGCACAGGCTGAGGATG AAGCGGACTACTACTGCAATTCGCGGGGCTCATCGGGGAACCATTACGTGTTCGGAACTGGTACCAAGGTGACTGTCCTG **ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTT AAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGG CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACG AACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTAT GAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACG CTCTTCACATGCAGGCCCTGCCGCCTCGG
104	M18 (ScFv domain) >DS90-09HD (M18)	CAAGTGCAGCTCGTTCAATCAGGCGGAGGACTCGTTCAACCAGGAGGATCATTGCGACTCTCATGTGCGGCCTCTGGATTC ACGTTTAGCTCATATTGG ATGCACTGGGTGCGGCAGGCGCCGGGGAAAGGTCTGGTGTGGGTCAGCCGCATCAACTCAGACGGCTCCTCGACTTCGTA CGCCGACTCCGTGAAGGGACGCTTTACCATTTCCCGCGACAACGCCAAGAATACCCTTTACCTTCAGATGAACTCCCTCC GCGCTGAGGATACCGCCGTGTACTACTGCGTGAGGACTGGCTGGGTCGGCAGCTACTACTACTACATGGACGTGTGGGGC AAAGGAACTACTGTCACCGTGTCAAGCGGCGGTGGAGGTTCCGGCGGGGGAGGATCGGGGGGGGGCGGATCGGGTGGCGG AGGATCGGAGATCGTGTTGACCCAGTCGCCGGGAACCCTGTCGCTGTCGCCTGGGGAGAGAGCAACTCTGTCCTGCCGGG CTTCCCAGTCGGTGTCGAGCAATTACCTGGCATGGTACCAACAGAAGCCGGGACAGCCGCCACGCCTGCTGATCTATGAC GTGTCAACTCGGGCAACTGGAATCCCTGCGCGGTTCAGCGGCGGAGGGAGCGGTACCGATTTCACCCTGACTATTTCCTC CCTCGAACCAGAAGATTTCGCCGTCTACTACTGCCAGCAGAGAAGCAACTGGCCGCCCTGGACGTTCGGACAAGGAACCA AGGTCGAAATCAAG

128	M18 (Full) >DS90-09HD (M18)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAGCTCGTTCAA TCAGGCGGAGGACTCGTTCAACCAGGAGGATCATTGCGACTCTCATGTGCGGCCTCTGGATTCACGTTTAGCTCATATTGG ATGCACTGGGTGCGGCAGGCGCCGGGGAAAGGTCTGGTGTGGGTCAGCCGCATCAACTCAGACGGCTCCTCGACTTCGTA CGCCGACTCCGTGAAGGGACGCTTTACCATTTCCCGCGACAACGCCAAGAATACCCTTTACCTTCAGATGAACTCCCTCC GCGCTGAGGATACCGCCGTGTACTACTGCGTGAGGACTGGCTGGGTCGGCAGCTACTACTACTACATGGACGTGTGGGGC AAAGGAACTACTGTCACCGTGTCAAGCGGCGGTGGAGGTTCCGGCGGGGGAGGATCGGGGGGGGGCGGATCGGGTGGCGG AGGATCGGAGATCGTGTTGACCCAGTCGCCGGGAACCCTGTCGCTGTCGCCTGGGGAGAGAGCAACTCTGTCCTGCCGGG CTTCCCAGTCGGTGTCGAGCAATTACCTGGCATGGTACCAACAGAAGCCGGGACAGCCGCCACGCCTGCTGATCTATGAC GTGTCAACTCGGGCAACTGGAATCCCTGCGCGGTTCAGCGGCGGAGGGAGCGGTACCGATTTCACCCTGACTATTTCCTC CCTCGAACCAGAAGATTTCGCCGTCTACTACTGCCAGCAGAGAAGCAACTGGCCGCCCTGGACGTTCGGACAAGGAACCA AGGTCGAAATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTG CGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTG GGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGC TGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCA GAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAA CCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAA TGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTAT AGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAA GGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
105	M19 (ScFv domain) >TS92-04BD (M19)	CAAGTGCAATTGGTTCAA TCAGGAGGAGGAGTCGTGCAGCCCGGAAGATCGTTGAGACTGTCATGTGCCGCGAGCGGCTTTACTTTCTCAAGCTACGG AATGCATTGGGTGCGACAGGCTCCGGGAAAAGGACTGGAATGGGTCGCAGTGATCTCATACGACGGCTCGAACAAGTACT ACGCCGACTCCGTCAAGGGTCGGTTCACGATTTCGCGCGATAATTCCAAGAACACTCTGTACCTCCAAATGAACAGCCTC CGGGCAGAGGACACCGCCGTCTACTACTGCGCTAAGGGATACTCGCGCTACTACTACTATGGAATGGATGTGTGGGGCCA GGGAACTACCGTGACGGTGTCGTCCGGCGGCGGTGGGTCGGGCGGAGGCGGATCAGGTGGAGGTGGAAGCGGAGGAGGAG GGAGCGAAATCGTCATGACTCAGTCCCCTGCTACCCTTTCTCTGTCGCCGGGAGAAAGAGCCATCCTGAGCTGCCGGGCC TCCCAGAGCGTGTACACCAAATACCTGGGATGGTACCAGCAGAAGCCGGGGCAGGCACCAAGGCTCCTGATCTACGATGC GTCCACCCGCGCGACTGGTATCCCAGACCGCTTTTCCGGCTCGGGGTCAGGGACTGACTTCACCCTTACTATCAATCGGC TCGAGCCTGAGGATTTCGCCGTGTATTACTGCCAGCACTACGGAGGGTCCCCGCTGATTACCTTCGGCCAAGGCACCAAA GTGGACATCAAG
129	M19 (Full) >TS92-04BD (M19)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAATTGGTTCAA TCAGGAGGAGGAGTCGTGCAGCCCGGAAGATCGTTGAGACTGTCATGTGCCGCGAGCGGCTTTACTTTCTCAAGCTACGG AATGCATTGGGTGCGACAGGCTCCGGGAAAAGGACTGGAATGGGTCGCAGTGATCTCATACGACGGCTCGAACAAGTACT ACGCCGACTCCGTCAAGGGTCGGTTCACGATTTCGCGCGATAATTCCAAGAACACTCTGTACCTCCAAATGAACAGCCTC CGGGCAGAGGACACCGCCGTCTACTACTGCGCTAAGGGATACTCGCGCTACTACTACTATGGAATGGATGTGTGGGGCCA GGGAACTACCGTGACGGTGTCGTCCGGCGGCGGTGGGTCGGGCGGAGGCGGATCAGGTGGAGGTGGAAGCGGAGGAGGAG GGAGCGAAATCGTCATGACTCAGTCCCCTGCTACCCTTTCTCTGTCGCCGGGAGAAAGAGCCATCCTGAGCTGCCGGGCC TCCCAGAGCGTGTACACCAAATACCTGGGATGGTACCAGCAGAAGCCGGGGCAGGCACCAAGGCTCCTGATCTACGATGC GTCCACCCGCGCGACTGGTATCCCAGACCGCTTTTCCGGCTCGGGGTCAGGGACTGACTTCACCCTTACTATCAATCGGC TCGAGCCTGAGGATTTCGCCGTGTATTACTGCCAGCACTACGGAGGGTCCCCGCTGATTACCTTCGGCCAAGGCACCAAA GTGGACATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGG CCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTG CTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGA GGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACC AGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATG GGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAG CGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGG ACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
106	M20 (ScFv domain) >JS93-08WD (M20)	CAAGTGCAACTTGTTCAATCAGGAGGAGGACTCGTTCAACCCGGAGGATCACTGCGACTCTCATGTGCAGCGTCGGGGTTC ACCTTCTCCAGCTACGCA ATGTCCTGGGTGCGCCAAGCCCCTGGAAAAGGCCTGGAGTGGGTGTCGGCCATCTCTGGGAGCGGGGGATCAACTTACTA CGCTGACTCCGTCAAGGGCCGCTTTACCATCTCCCGGGACAACAGCAAGAACACTCTCTATCTCCAGATGAACTCGCTGA GAGCCGAAGATACCGCTGTCTACTACTGCGCGAAGAGAGAAGCTGCCGCAGGGCACGATTGGTACTTCGACTTGTGGGGC AGGGGCACCCTTGTGACCGTGTCCTCCGGTGGAGGCGGATCAGGAGGTGGGGGATCGGGTGGAGGAGGAAGCGGAGGCGG CGGTTCGGACATTCGCGTCACCCAGTCACCGAGCTCCCTCAGCGCATCGGTGGGCGACCGGGTCACTATCACTTGCCGGG CGTCCCAGTCGATCTCATCGTATCTGAATTGGTACCAGCAGAAACCGGGAAAGGCGCCGAAGCTGTTGATCTACGCTGCC AGCTCCCTGCAGTCGGGTGTGCCATCACGCTTTTCCGGCTCGGGATCGGGAACCGATTTCACTCTGACGATCTCTAGCCT GCAGCCAGAAGATTTCGCCACTTACTACTGCCAGCAGTCCTACAGCATCCCTCTGACTTTCGGACAAGGGACGAAAGTGG AGATTAAG
130	M20 (Full) >JS93-08WD (M20	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTGCAACTTGTTCAA TCAGGAGGAGGACTCGTTCAACCCGGAGGATCACTGCGACTCTCATGTGCAGCGTCGGGGTTCACCTTCTCCAGCTACGCA ATGTCCTGGGTGCGCCAAGCCCCTGGAAAAGGCCTGGAGTGGGTGTCGGCCATCTCTGGGAGCGGGGGATCAACTTACTA CGCTGACTCCGTCAAGGGCCGCTTTACCATCTCCCGGGACAACAGCAAGAACACTCTCTATCTCCAGATGAACTCGCTGA GAGCCGAAGATACCGCTGTCTACTACTGCGCGAAGAGAGAAGCTGCCGCAGGGCACGATTGGTACTTCGACTTGTGGGGC AGGGGCACCCTTGTGACCGTGTCCTCCGGTGGAGGCGGATCAGGAGGTGGGGGATCGGGTGGAGGAGGAAGCGGAGGCGG CGGTTCGGACATTCGCGTCACCCAGTCACCGAGCTCCCTCAGCGCATCGGTGGGCGACCGGGTCACTATCACTTGCCGGG CGTCCCAGTCGATCTCATCGTATCTGAATTGGTACCAGCAGAAACCGGGAAAGGCGCCGAAGCTGTTGATCTACGCTGCC AGCTCCCTGCAGTCGGGTGTGCCATCACGCTTTTCCGGCTCGGGATCGGGAACCGATTTCACTCTGACGATCTCTAGCCT

	)	GCAGCCAGAAGATTTCGCCACTTACTACTGCCAGCAGTCCTACAGCATCCCTCTGACTTTCGGACAAGGGACGAAAGTGG AGATTAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCG GAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCC TCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGT ACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCT CTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCG GGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACAC CTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
107	M21 (ScFv domain) >ZS95-03QD (M21)	CAAGTCCAACTCGTTCAGTCATGGGCAGAAGTCAAGAAACCCGGTGCAAGCGTCAAAGTGTCGTGTAAGGCCTCCGGCTAC ACTTTCACTTCCTACTAC ATGCACTGGGTGCGCCAAGCCCCGGGACAGGGCCTTGAATGGATGGGCATCATCAACCCATCAGGAGGTTCCACGAGCTA CGCGCAGAAGTTCCAGGGGAGAGTGACGATGACTAGAGATACCTCCACGAGCACCGTCTACATGGAGCTGTCGAATCTGC GGTCAGAGGACACTGCTGTGTATTACTGCGCGCGCTCCCCGCGGGTGACCACTGGCTACTTTGACTACTGGGGACAAGGG ACCCTGGTGACCGTCAGCTCGGGAGGCGGAGGATCGGGAGGTGGAGGGTCCGGTGGAGGCGGCTCTGGAGGAGGCGGGTC GGACATTCAATTGACCCAGAGCCCATCCACCCTCTCAGCCTCGGTGGGGGATAGGGTGACTATCACTTGCCGGGCCTCCC AGTCAATTTCCAGCTGGCTGGCTTGGTACCAGCAAAAGCCTGGAAAGGCACCGAAGCTCCTGATCTACAAGGCCTCATCT CTGGAATCAGGAGTGCCTTCGCGCTTCAGCGGAAGCGGCTCGGGAACTGAGTTTACCCTGACCATCTCGAGCCTGCAGCC AGATGACTTCGCGACCTATTACTGCCAGCAGTACTCGTCCTACCCGTTGACTTTCGGAGGAGGTACCCGCCTCGAAATCA AA
131	M21 (Full) >ZS95-03QD (M21)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTTCAG TCATGGGCAGAAGTCAAGAAACCCGGTGCAAGCGTCAAAGTGTCGTGTAAGGCCTCCGGCTACACTTTCACTTCCTACTAC ATGCACTGGGTGCGCCAAGCCCCGGGACAGGGCCTTGAATGGATGGGCATCATCAACCCATCAGGAGGTTCCACGAGCTA CGCGCAGAAGTTCCAGGGGAGAGTGACGATGACTAGAGATACCTCCACGAGCACCGTCTACATGGAGCTGTCGAATCTGC GGTCAGAGGACACTGCTGTGTATTACTGCGCGCGCTCCCCGCGGGTGACCACTGGCTACTTTGACTACTGGGGACAAGGG ACCCTGGTGACCGTCAGCTCGGGAGGCGGAGGATCGGGAGGTGGAGGGTCCGGTGGAGGCGGCTCTGGAGGAGGCGGGTC GGACATTCAATTGACCCAGAGCCCATCCACCCTCTCAGCCTCGGTGGGGGATAGGGTGACTATCACTTGCCGGGCCTCCC AGTCAATTTCCAGCTGGCTGGCTTGGTACCAGCAAAAGCCTGGAAAGGCACCGAAGCTCCTGATCTACAAGGCCTCATCT CTGGAATCAGGAGTGCCTTCGCGCTTCAGCGGAAGCGGCTCGGGAACTGAGTTTACCCTGACCATCTCGAGCCTGCAGCC AGATGACTTCGCGACCTATTACTGCCAGCAGTACTCGTCCTACCCGTTGACTTTCGGAGGAGGTACCCGCCTCGAAATCA AA**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCA TGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGC TGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCT TTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAA GGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAA CGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGC CGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGT ATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGA CGCTCTTCACATGCAGGCCCTGCCGCCTCGG
108	M22 (ScFv domain) >PS96-08LD (M22)	CAAGTCCAACTCGTCCAGTCCGGTGCAGAAGTCAGAAGGCCAGGAGCAAGCGTGAAGATCTCGTGTAGAGCGTCAGGAGAC ACCAGCACTCGCCATTAC ATCCACTGGCTGCGCCAGGCTCCGGGCCAAGGGCCGGAGTGGATGGGTGTGATCAACCCGACTACGGGACCGGCTACCGG AAGCCCTGCGTACGCACAGATGCTGCAGGGACGGGTGACTATGACCCGCGATACTAGCACTAGGACCGTGTACATGGAAC TCCGCTCGTTGCGGTTCGAAGATACCGCCGTCTACTACTGCGCCCGGTCCGTGGTGGGCCGAAGCGCCCCTTACTACTTC GATTACTGGGGACAGGGCACTCTGGTGACCGTTAGCTCCGGTGGGGGAGGCTCGGGTGGAGGCGGATCGGGAGGAGGAG CAGCGGTGGAGGGGGATCGGACATTCAGATGACCCAGTCACCCTCCTCCCTCTCAGCCTCGGTCGGGGACCGGGTGACCA TTACGTGCAGAGCCTCACAAGGGATCTCGGACTACTCCGCCTGGTACCAGCAGAAACCGGGAAAAGCGCCAAAGCTCCTG ATCTACGCCGCGAGCACCCTGCAATCAGGAGTGCCATCGCGCTTTTCTGGATCGGGCTCAGGGACTGACTTCACGCTGAC TATCTCCTACCTTCAGTCCGAGGATTTCGCTACCTACTACTGCCAACAGTATTACTCCTATCCCCTGACCTTTGGCGGAG GCACTAAGGTGGACATCAAG
132	M22 (Full) >PS96-08LD (M22)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCGTCCAG TCCGGTGCAGAAGTCAGAAGGCCAGGAGCAAGCGTGAAGATCTCGTGTAGAGCGTCAGGAGACACCAGCACTCGCCATTAC ATCCACTGGCTGCGCCAGGCTCCGGGCCAAGGGCCGGAGTGGATGGGTGTGATCAACCCGACTACGGGACCGGCTACCGG AAGCCCTGCGTACGCACAGATGCTGCAGGGACGGGTGACTATGACCCGCGATACTAGCACTAGGACCGTGTACATGGAAC TCCGCTCGTTGCGGTTCGAAGATACCGCCGTCTACTACTGCGCCCGGTCCGTGGTGGGCCGAAGCGCCCCTTACTACTTC GATTACTGGGGACAGGGCACTCTGGTGACCGTTAGCTCCGGTGGGGGAGGCTCGGGTGGAGGCGGATCGGGAGGAGGAG CAGCGGTGGAGGGGGATCGGACATTCAGATGACCCAGTCACCCTCCTCCCTCTCAGCCTCGGTCGGGGACCGGGTGACCA TTACGTGCAGAGCCTCACAAGGGATCTCGGACTACTCCGCCTGGTACCAGCAGAAACCGGGAAAAGCGCCAAAGCTCCTG ATCTACGCCGCGAGCACCCTGCAATCAGGAGTGCCATCGCGCTTTTCTGGATCGGGCTCAGGGACTGACTTCACGCTGAC TATCTCCTACCTTCAGTCCGAGGATTTCGCTACCTACTACTGCCAACAGTATTACTCCTATCCCCTGACCTTTGGCGGAG GCACTAAGGTGGACATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTG TCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTA CATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGA AGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGG TTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACC CAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAA GCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGC

		CACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
109	M23 (ScFv domain) >XH66-84HE (M23)	CAAGTCCAACTCCAGCAATCGGGAGCAGAAGTCAAGAAACCAGGCGCATCGGTGAAAGTGTCGTGTAAGGCGTCAGGGTAC ACCTTCACCAACTACTAT ATGCACTGGGTGCGCCAGGCTCCAGGCCAGGGGTTGGAGTGGATGGGGATCATCAATCCGTCAGGTGGCTACACCACTTA CGCTCAGAAGTTCCAGGGACGCCTCACTATGACTCGCGATACTAGCACCTCCACGGTGTACATGGAACTGTCATCGCTGA GGTCCGAAGATACCGCCGTCTACTACTGCGCACGGATCAGATCCTGCGGAGGAGATTGTTACTACTTTGACAACTGGGGA CAGGGCACCCTTGTTACTGTGTCATCGGGAGGAGGGGGAAGCGGAGGAGGTGGATCAGGCGGCGGTGGCAGCGGGGGCGG AGGATCGGACATTCAGCTGACTCAGTCCCCCTCCACTTTGTCGGCCAGCGTGGGAGACAGAGTGACCATCACTTGCCGGG CGTCCGAGAACGTCAATATCTGGCTGGCCTGGTACCAGCAAAAGCCTGGAAAAGCCCCGAAGCTGCTCATCTATAAGTCA TCCAGCCTGGCGTCTGGTGTGCCGTCGCGGTTCTCCGGCAGCGGGAGCGGAGCCGAGTTCACTCTCACCATTTCGAGCCT TCAACCGGACGATTTCGCCACCTACTACTGCCAGCAGTACCAATCCTACCCTCTGACGTTTGGAGGTGGAACCAAGGTGG ACATCAAG
133	M23 (Full) >XH66-84HE (M23)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAGTCCAACTCCAGCAA TCGGGAGCAGAAGTCAAGAAACCAGGCGCATCGGTGAAAGTGTCGTGTAAGGCGTCAGGGTACACCTTCACCAACTACTAT ATGCACTGGGTGCGCCAGGCTCCAGGCCAGGGGTTGGAGTGGATGGGGATCATCAATCCGTCAGGTGGCTACACCACTTA CGCTCAGAAGTTCCAGGGACGCCTCACTATGACTCGCGATACTAGCACCTCCACGGTGTACATGGAACTGTCATCGCTGA GGTCCGAAGATACCGCCGTCTACTACTGCGCACGGATCAGATCCTGCGGAGGAGATTGTTACTACTTTGACAACTGGGGA CAGGGCACCCTTGTTACTGTGTCATCGGGAGGAGGGGGAAGCGGAGGAGGTGGATCAGGCGGCGGTGGCAGCGGGGGCGG AGGATCGGACATTCAGCTGACTCAGTCCCCCTCCACTTTGTCGGCCAGCGTGGGAGACAGAGTGACCATCACTTGCCGGG CGTCCGAGAACGTCAATATCTGGCTGGCCTGGTACCAGCAAAAGCCTGGAAAAGCCCCGAAGCTGCTCATCTATAAGTCA TCCAGCCTGGCGTCTGGTGTGCCGTCGCGGTTCTCCGGCAGCGGGAGCGGAGCCGAGTTCACTCTCACCATTTCGAGCCT TCAACCGGACGATTTCGCCACCTACTACTGCCAGCAGTACCAATCCTACCCTCTGACGTTTGGAGGTGGAACCAAGGTGG ACATCAAG**ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCG GAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCC TCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGT ACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCT CTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCG GGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACAC CTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
110	M24 (ScFv domain) >NH67-89CE (M24)	CAAATCACTCTGAAAGAA TCTGGACCGGCCCTGGTTAAGCCGACTCAAACGCTCACCCTTACTTGCACCTTCAGCGGATTCTCACTCAGCACTGCTGG TGTGCACGTCGGATGGATTAGACAGCCGCCTGGAAAGGCCCTGGAATGGCTCGCCCTCATCTCCTGGGCCGATGACAAGA GATACAGGCCCTCGCTTCGATCCCGGTTGGACATTACCCGGGTGACCTCGAAAGATCAGGTGGTGCTCTCAATGACCAAT ATGCAGCCGGAGGACACCGCTACGTACTACTGCGCACTGCAAGGATTTGACGGCTACGAGGCTAACTGGGGACCAGGTAC TCTGGTCACCGTGAGCTCCGGCGGGGGAGGATCAGGCGGGGGGGGGTCAGGAGGCGGAGGCTCCGGTGGAGGAGGATCGG ATATCGTCATGACCCAGTCCCCAAGCTCGCTGAGCGCGTCAGCGGGCGACCGCGTGACTATCACTTGCCGGGCCAGCCGC GGCATCTCCTCCGCACTGGCGTGGTACCAGCAGAAGCCTGGAAAACCGCCAAAGCTCCTGATCTATGCCTCCAGCCT GGAGTCAGGTGTCCCCAGCCGCTTCTCGGGTTCGGGCTCGGGAACCGACTTCACTTTGACCATCGACTCGCTGGAACCGG AAGATTTCGCAACCTACTACTGTCAGCAGTCCTACTCGACCCCTTGGACTTTTGGACAAGGGACGAAGGTGGACATCAAG
134	M24 (Full) >NH67-89CE (M24)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCGGCCCCAAATCACTCTGAAAGAA TCTGGACCGGCCCTGGTTAAGCCGACTCAAACGCTCACCCTTACTTGCACCTTCAGCGGATTCTCACTCAGCACTGCTGG TGTGCACGTCGGATGGATTAGACAGCCGCCTGGAAAGGCCCTGGAATGGCTCGCCCTCATCTCCTGGGCCGATGACAAGA GATACAGGCCCTCGCTTCGATCCCGGTTGGACATTACCCGGGTGACCTCGAAAGATCAGGTGGTGCTCTCAATGACCAAT ATGCAGCCGGAGGACACCGCTACGTACTACTGCGCACTGCAAGGATTTGACGGCTACGAGGCTAACTGGGGACCAGGTAC TCTGGTCACCGTGAGCTCCGGCGGGGGAGGATCAGGCGGGGGGGGGTCAGGAGGCGGAGGCTCCGGTGGAGGAGGATCGG ATATCGTCATGACCCAGTCCCCAAGCTCGCTGAGCGCGTCAGCGGGCGACCGCGTGACTATCACTTGCCGGGCCAGCCGC GGCATCTCCTCCGCACTGGCGTGGTACCAGCAGAAGCCTGGAAAACCGCCAAAGCTCCTGATCTATGATGCCTCCAGCCT GGAGTCAGGTGTCCCCAGCCGCTTCTCGGGTTCGGGCTCGGGAACCGACTTCACTTTGACCATCGACTCGCTGGAACCGG AAGATTTCGCAACCTACTACTGTCAGCAGTCCTACTCGACCCCTTGGACTTTTGGACAAGGGACGAAGGTGGACATCAAG **ACCACTACC**CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTT AAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGG CGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACG AACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTAT GAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACG CTCTTCACATGCAGGCCCTGCCGCCTCGG
279	Ss1 (scFv domain)	CAAGTCCAGCTCCAGCAGTCGGGCCCAGAGTTGGAGAAGCCTGGGGCGAGCGTGAAGAT CTCATGCAAAGCCTCAGGCTACTCCTTTACTGGATACACGATGAATTGGGTGAAACAGT CGCATGGAAAGTCACTGGAATGGATCGGTCTGATTACGCCCTACAACGGCGCCTCCAGC TACAACCAGAAGTTCAGGGGAAAGGCGACCCTTACTGTCGACAAGTCGTCAAGCACCGC CTACATGGACCTCCTGTCCCTGACCTCCGAAGATAGCGCGGTCTACTTTTGTGCACGCG GAGGTTACGATGGACGGGGATTCGACTACTGGGGCCAGGGAACCACTGTCACCGTGTCG AGCGGAGGCGGAGGGAGCGGAGGAGGAGGCAGCGGAGGTGGAGGGTCGGATATCGAACT

		CACTCAGTCCCCAGCAATCATGTCCGCTTCACCGGGAGAAAAGGTGACCATGACTTGCT CGGCCTCCTCGTCCGTGTCATACATGCACTGGTACCAACAAAAATCGGGGACCTCCCCT AAGAGATGGATCTACGATACCAGCAAACTGGCTTCAGGCGTGCCGGGACGCTTCTCGGG TTCGGGGAGCGGAAATTCGTATTCGTTGACCATTTCGTCCGTGGAAGCCGAGGACGACG CAACTTATTACTGCCAACAGTGGTCAGGCTACCCGCTCACTTTCGGAGCCGGCACTAAG CTGGAGATC
280	Ss1 (full)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGCCGCTCG GCCCCAAGTCCAGCTCCAGCAGTCGGGCCCAGAGTTGGAGAAGCCTGGGGCGAGCGTGA AGATCTCATGCAAAGCCTCAGGCTACTCCTTTACTGGATACACGATGAATTGGGTGAAA CAGTCGCATGGAAAGTCACTGGAATGGATCGGTCTGATTACGCCCTACAACGGCGCCTC CAGCTACAACCAGAAGTTCAGGGGAAAGGCGACCCTTACTGTCGACAAGTCGTCAAGCA CCGCCTACATGGACCTCCTGTCCCTGACCTCCGAAGATAGCGCGGTCTACTTTTGTGCA CGCGGAGGTTACGATGGACGGGGATTCGACTACTGGGGCCAGGGAACCACTGTCACCGT GTCGAGCGGAGGCGGAGGGAGCGGAGGAGGAGGCAGCGGAGGTGGAGGGTCGGATATCG AACTCACTCAGTCCCCAGCAATCATGTCCGCTTCACCGGGAGAAAAGGTGACCATGACT TGCTCGGCCTCCTCGTCCGTGTCATACATGCACTGGTACCAACAAAAATCGGGGACCTC CCCTAAGAGATGGATCTACGATACCAGCAAACTGGCTTCAGGCGTGCCGGGACGCTTCT CGGGTTCGGGGAGCGGAAATTCGTATTCGTTGACCATTTCGTCCGTGGAAGCCGAGGAC GACGCAACTTATTACTGCCAACAGTGGTCAGGCTACCCGCTCACTTTCGGAGCCGGCAC TAAGCTGGAGATCACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCG CCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTG CATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTAC TTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGA AGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAG GACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAA ATTCAGCCGCAGCGCAGATGCTCCAGCC

Table 4. Amino acid sequences for the heavy chain (HC) CDR1, CDR2, and CDR3 regions of human anti-mesothelin scFvs

Descrip.	HC-CDR1	SEQ ID NO:	HC-CDR2	SEQ ID NO:	HC-CDR3	SEQ ID NO:
M1	GYTFTGYYMH	136	RINPNSGGTNYAQKFQG	155	GRYYGMDV	175
M2	GYTFTGYYMH	136	WINPNSGGTNYAQKFQG	156	DLRRTVVTPRAYYGMDV	176
M3	GYTFTGYYMH	136	WINPNSGGTNYAQKFQG	156	GEWDGSYYYDY	177
M4	GFTFSSYWMH	137	RINTDGSTTTYADSVEG	157	GHWAV	178
M5	GYTFTDYYMH	138	WINPNSGGTNYAQKFQG	156	GWDFDY	179
M6	GYTFTSYYMH	139	IINPSGGSTSYAQKFQ	158	YRLIAVAGDYYYYGMDV	180
M7	GFTFSSYAMH	140	VISYDGSNKYYADSVKG	274	WKVSSSSPAFDY	181
M8	GYPFTGYSLH	141	WINPNSGGTNYAQKFQG	159	DHYGGNSLFY	182
M9	GYTFTSYYMH	142	IINPSGGSTGYAQKFQG	160	GGYSSSSDAFDI	183
M10	GYTFTSYGIS	143	WISAYNGNTNYAQKLQ	161	VAGGIYYYYGMDV	184
M11	GYTFTGYYMH	144	WINPNSGGTNYAQNFQG	162	GWDFDY	185
M12	GYTFTGYYMH	144	RINPNSGGTNYAQKFQG	163	TTTSYAFDI	186
M13	GFIFSDYYMG	145	YIGRSGSSMYYADSVKG	164	SPVVAATEDFQH	187
M14	GFTFRGYYIH	146	IINPSGGSRAYAQKFQG	165	TASCGGDCYYLDY	188
M15	GFTFDDYAMH	147	GISWNSGSIGYADSVK	166	DGSSSWSWGYFDY	189
M16	GFTFDDYAMH	147	GISWNSGSTGYADSVKG	167	DSSSWYGGGSAFDI	190
M17	GFTFDDYAMH	147	GISWNSGSTGYADSVKG	167	DSSSWYGGGSAFDI	191
M18	GFTFSSYWMH	148	RINSDGSSTSYADSVKG	168	TGWVGSYYYYMDV	192
M19	GFTFSSYGMH	149	VISYDGSNKYYADSVKG	169	GYSRYYYYGMDV	193
M20	GFTFSSYAMS	150	AISGSGGSTYYADSVKG	170	REAAAGHDWYFDL	194
M21	GYTFTSYYMH	151	IINPSGGSTSYAQKFQG	171	SPRVTTGYFDY	195
M22	GDTSTRHYIH	152	VINPTTGPATGSPAYAQMLQG	172	SVVGRSAPYYFDY	196

M23	GYTFTNYYMH	153	IINPSGGYTTYAQKFQG	173	IRSCGGDCYYFDN	197
M24	GFSLSTAGVHVG	154	LISWADDKRYRPSLRS	174	QGFDGYEAN	198
Ss1	GYSFTGYTMN	281	LITPYNGASSYNQKFRG	282	GGYDGRGFDY	283

Table 5. Amino acid sequences for the light chain (LC) CDR1, CDR2, and CDR3 regions of human anti-mesothelin scFvs

Description	LC-CDR1	SEQ ID NO:	LC-CDR2	SEQ ID NO:	LC-CDR3	SEQ ID NO:
M1	RASQSVSSNFA	199	DASNRAT	223	HQRSNWLYT	247
M2	QASQDISNSLN	200	DASTLET	224	QQHDNLPLT	248
M3	RASQSINTYLN	201	AASSLQS	225	QQSFSPLT	249
M4	RASQSISDRLA	202	KASSLES	226	QQYGHLPMYT	250
M5	RASQSIRYYLS	203	TASILQN	227	LQTYTTPD	251
M6	RASQGVGRWLA	204	AASTLQS	228	QQANSFPLT	252
M7	RASQSVYTKYLG	205	DASTRAT	229	QHYGGSPLIT	253
M8	RASQDSGTWLA	206	DASTLED	230	QQYNSYPLT	254
M9	RASQDISSALA	207	DASSLES	231	QQFSSYPLT	255
M10	KSSHSVLYNRNNKNYLA	208	WASTRKS	232	QQTQTFPLT	256
M11	RASQSIRYYLS	209	TASILQN	233	LQTYTTPD	257
M12	RASQSISTWLA	210	KASTLES	234	QQYNTYSPYT	258
M13	RASQSVTSNYLA	211	GASTRAT	235	QQYGSAPVT	259
M14	RASENVNIWLA	212	KSSSLAS	236	QQYQSYPLT	260
M15	QGDALRSYYAS	213	GKNNRPS	237	NSRDSSGYPV	261
M16	QGDSLRSYYAS	214	GRSRRPS	238	NSRDNTANHYV	262
M17	QGDSLRSYYAS	215	GKNNRPS	239	NSRGSSGNHYV	263
M18	RASQSVSSNYLA	216	DVSTRAT	240	QQRSNWPPWT	264
M19	RASQSVYTKYLG	217	DASTRAT	241	QHYGGSPLIT	265
M20	RASQSISSYLN	218	AASSLQS	242	QQSYSIPLT	266
M21	RASQSISSWLA	219	KASSLES	243	QQYSSYPLT	267
M22	RASQGISDYS	220	AASTLQS	244	QQYYSYPLT	268
M23	RASENVNIWLA	221	KSSSLAS	245	QQYQSYPLT	269
M24	RASRGISSALA	222	DASSLES	246	QQSYSTPWT	270
Ss1	SASSSVSYMH	284	DTSKLAS	285	QQWSGYPLT	286

Bispecific CARs

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, e.g., different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable domain sequence

and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv, or fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for a second epitope.

In certain embodiments, the antibody molecule is a multi-specific (*e.g.*, a bispecific or a trispecific) antibody molecule. Protocols for generating bispecific or heterodimeric antibody molecules are known in the art; including but not limited to, for example, the "knob in a hole" approach described in, *e.g.*, US 5731168; the electrostatic steering Fc pairing as described in, *e.g.*, WO 09/089004, WO 06/106905 and WO 2010/129304; Strand Exchange Engineered Domains (SEED) heterodimer formation as described in, *e.g.*, WO 07/110205; Fab arm exchange as described in, *e.g.*, WO 08/119353, WO 2011/131746, and WO 2013/060867; double antibody conjugate, *e.g.*, by antibody cross-linking to generate a bi-specific structure using a heterobifunctional reagent having an amine-reactive group and a sulfhydryl reactive group as described in, *e.g.*, US 4433059; bispecific antibody determinants generated by recombining half antibodies (heavy-light chain pairs or Fabs) from different antibodies through cycle of reduction and oxidation of disulfide bonds between the two heavy chains, as described in, *e.g.*, US 4444878; trifunctional antibodies, *e.g.*, three Fab' fragments cross-linked through sulfhdryl reactive groups, as described in, *e.g.*, US5273743; biosynthetic binding proteins, *e.g.*, pair of scFvs cross-linked through C-terminal tails preferably through disulfide or amine-reactive chemical cross-linking, as described in, *e.g.*, US5534254; bifunctional antibodies, *e.g.*, Fab fragments with different binding specificities dimerized through leucine zippers (*e.g.*, c-fos and c-jun) that have replaced the constant domain, as described in, *e.g.*, US5582996; bispecific and oligospecific mono-and oligovalent receptors, *e.g.*, VH-CH1 regions of two antibodies (two Fab fragments) linked through a polypeptide spacer between the CH1 region of one antibody and the VH region of the other antibody typically with associated light chains, as described in, *e.g.*, US5591828; bispecific DNA-antibody conjugates, *e.g.*, crosslinking of antibodies or Fab fragments through a double stranded piece of DNA, as described in, *e.g.*, US5635602; bispecific fusion proteins, *e.g.*, an expression construct containing two scFvs with a hydrophilic helical peptide linker between them and a full constant region, as described in, *e.g.*, US5637481; multivalent and multispecific binding proteins, *e.g.*, dimer of polypeptides having first domain with binding region of Ig heavy chain variable region, and second domain with binding region

of Ig light chain variable region, generally termed diabodies (higher order structures are also encompassed creating for bispecifc, trispecific, or tetraspecific molecules, as described in, *e.g.*, US5837242; minibody constructs with linked VL and VH chains further connected with peptide spacers to an antibody hinge region and CH3 region, which can be dimerized to form bispecific/multivalent molecules, as described in, *e.g.*, US5837821; VH and VL domains linked with a short peptide linker (*e.g.*, 5 or 10 amino acids) or no linker at all in either orientation, which can form dimers to form bispecific diabodies; trimers and tetramers, as described in, *e.g.*, US5844094; String of VH domains (or VL domains in family members) connected by peptide linkages with crosslinkable groups at the C-terminus futher associated with VL domains to form a series of FVs (or scFvs), as described in, *e.g.*, US5864019; and single chain binding polypeptides with both a VH and a VL domain linked through a peptide linker are combined into multivalent structures through non-covalent or chemical crosslinking to form, *e.g.*, homobivalent, heterobivalent, trivalent, and tetravalent structures using both scFV or diabody type format, as described in, *e.g.*, US5869620. Additional exemplary multispecific and bispecific molecules and methods of making the same are found, for example, in US5910573, US5932448, US5959083, US5989830, US6005079, US6239259, US6294353, US6333396, US6476198, US6511663, US6670453, US6743896, US6809185, US6833441, US7129330, US7183076, US7521056, US7527787, US7534866, US7612181, US2002004587A1, US2002076406A1, US2002103345A1, US2003207346A1, US2003211078A1, US2004219643A1, US2004220388A1, US2004242847A1, US2005003403A1, US2005004352A1, US2005069552A1, US2005079170A1, US2005100543A1, US2005136049A1, US2005136051A1, US2005163782A1, US2005266425A1, US2006083747A1, US2006120960A1, US2006204493A1, US2006263367A1, US2007004909A1, US2007087381A1, US2007128150A1, US2007141049A1, US2007154901A1, US2007274985A1, US2008050370A1, US2008069820A1, US2008152645A1, US2008171855A1, US2008241884A1, US2008254512A1, US2008260738A1, US2009130106A1, US2009148905A1, US2009155275A1, US2009162359A1, US2009162360A1, US2009175851A1, US2009175867A1, US2009232811A1, US2009234105A1, US2009263392A1, US2009274649A1, EP346087A2, WO0006605A2, WO02072635A2, WO04081051A1, WO06020258A2, WO2007044887A2, WO2007095338A2, WO2007137760A2, WO2008119353A1, WO2009021754A2, WO2009068630A1, WO9103493A1, WO9323537A1, WO9409131A1, WO9412625A2, WO9509917A1, WO9637621A2, WO9964460A1. The contents of the above-referenced applications are incorporated herein by reference in their entireties.

Within each antibody or antibody fragment (e.g., scFv) of a bispecific antibody molecule, the VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_1) upstream of its VL (VL_1) and the

downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_2) upstream of its VH (VH_2), such that the overall bispecific antibody molecule has the arrangement VH_1-VL_1-VL_2-VH_2. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_1) upstream of its VH (VH_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_2) upstream of its VL (VL_2), such that the overall bispecific antibody molecule has the arrangement VL_1-VH_1-VH_2-VL_2. Optionally, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), e.g., between VL_1 and VL_2 if the construct is arranged as VH_1-VL_1-VL_2-VH_2, or between VH_1 and VH_2 if the construct is arranged as VL_1-VH_1-VH_2-VL_2. The linker may be a linker as described herein, e.g., a (Gly_4-Ser)n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 26). In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. Optionally, a linker is disposed between the VL and VH of the first scFv. Optionally, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers can be the same or different. Accordingly, in some embodiments, a bispecific CAR comprises VLs, VHs, and optionally one or more linkers in an arrangement as described herein.

In one aspect, the bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence, e.g., a scFv, which has binding specificity for mesothelin, e.g., comprises a scFv as described herein, e.g., as described in Table 2 or 3, or comprises the light chain CDRs and/or heavy chain CDRs from a mesothelin scFv described herein, and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope on a different antigen. In some aspects the second immunoglobulin variable domain sequence has binding specificity for an antigen other than mesothelin, e.g., an antigen expressed by a cancer or tumor cell. In some aspects, the second immunoglobulin variable domain sequence has binding specificity for an antigen selected from a target other than mesothelin on stroma cells, e.g., FAP; a target other than mesothelin on prostate cancer cells, e.g., androgen receptor, OR51E2, PSMA, PSCA, PDGRF-β, TARP, GloboH, MAD-CT-1, or MAD-CT-2; a target other than mesothelin on ovararian cancer cells, e.g., Tn, PRSS21, CD171, Lewis Y, folate receptor α, claudin6, GloboH, or sperm protein 17, e.g., a target other than mesothelin on lung cancer cells, e.g.,VEGF, HER3, IGF-1R, EGFR, DLL4, or Trop-2.

Chimeric TCR

In one aspect, the mesothelin antibodies and antibody fragments of the present invention (for example, those disclosed in Tables 2 or 3) can be grafted to one or more constant domain of a T cell receptor ("TCR") chain, for example, a TCR alpha or TCR beta chain, to create an chimeric TCR that binds specificity to mesothelin. Without being bound by theory, it is believed that chimeric TCRs will

signal through the TCR complex upon antigen binding. For example, a mesothelin scFv as disclosed herein, can be grafted to the constant domain, e.g., at least a portion of the extracellular constant domain, the transmembrane domain and the cytoplasmic domain, of a TCR chain, for example, the TCR alpha chain and/or the TCR beta chain. As another example, a mesothelin antibody fragment, for example a VL domain as described herein, can be grafted to the constant domain of a TCR alpha chain, and a mesothelin antibody fragment, for example a VH domain as described herein, can be grafted to the constant domain of a TCR beta chain (or alternatively, a VL domain may be grafted to the constant domain of the TCR beta chain and a VH domain may be grafted to a TCR alpha chain). As another example, the CDRs of a mesothelin antibody or antibody fragment, e.g., the CDRs of a mesothelin antibody or antibody fragment as described in Tables 4 or 5 may be grafted into a TCR alpha and/or beta chain to create a chimeric TCR that binds specifically to mesothelin. For example, the LCDRs disclosed herein may be grafted into the variable domain of a TCR alpha chain and the HCDRs disclosed herein may be grafted to the variable domain of a TCR beta chain, or vice versa. Such chimeric TCRs may be produced by methods known in the art (For example, Willemsen RA et al, Gene Therapy 2000; 7: 1369–1377; Zhang T et al, Cancer Gene Ther 2004; 11: 487–496; Aggen et al, Gene Ther. 2012 Apr;19(4):365-74).

Natural Killer Cell Receptor (NKR) CARs

In an embodiment, the CAR molecule described herein comprises one or more components of a natural killer cell receptor (NKR), thereby forming an NKR-CAR. The NKR component can be a transmembrane domain, a hinge domain, or a cytoplasmic domain from any of the following natural killer cell receptors: killer cell immunoglobulin-like receptor (KIR), e.g., KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, DIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DP1, and KIR3DP1; natural cyotoxicity receptor (NCR), e.g., NKp30, NKp44, NKp46; signaling lymphocyte activation molecule (SLAM) family of immune cell receptors, e.g., CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10; Fc receptor (FcR), e.g., CD16, and CD64; and Ly49 receptors, e.g., LY49A, LY49C. The NKR-CAR molecules described herein may interact with an adaptor molecule or intracellular signaling domain, e.g., DAP12. Exemplary configurations and sequences of CAR molecules comprising NKR components are described in International Publication No. WO2014/145252, the contents of which are hereby incorporated by reference.

Split CAR

In some embodiments, the CAR-expressing cell described herein, uses a split CAR. The split CAR approach is described in more detail in publications WO2014/055442 and WO2014/055657, incorporated herein by reference. Briefly, a split CAR system comprises a cell expressing a first CAR

having a first antigen binding domain and a costimulatory domain (e.g., 41BB), and the cell also expresses a second CAR having a second antigen binding domain and an intracellular signaling domain (e.g., CD3 zeta). When the cell encounters the first antigen, the costimulatory domain is activated, and the cell proliferates. When the cell encounters the second antigen, the intracellular signaling domain is activated and cell-killing activity begins. Thus, the CAR-expressing cell is only fully activated in the presence of both antigens. In embodiments the first antigen binding domain recognizes the tumor antigen or B cell antigen described herein, e.g., comprises an antigen binding domain described herein, and the second antigen binding domain recognizes a second antigen, e.g., a second tumor antigen or a second B cell antigen described herein.

Co-expression of CAR with Other Molecules or Agents

Co-expression of a Second CAR

In one aspect, the CAR-expressing cell described herein can further comprise a second CAR, e.g., a second CAR that includes a different antigen binding domain, e.g., to the same target (mesothelin) or a different target (e.g., a target other than mesothelin on stroma cells, e.g., FAP; a target other than mesothelin on prostate cancer cells, e.g., androgen receptor, OR51E2, PSMA, PSCA, PDGRF-β, TARP, GloboH, MAD-CT-1, or MAD-CT-2; a target other than mesothelin on ovararian cancer cells, e.g., Tn, PRSS21, CD171, Lewis Y, folate receptor α, claudin6, GloboH, or sperm protein 17, e.g., a target other than mesothelin on lung cancer cells, e.g.,VEGF, HER3, IGF-1R, EGFR, DLL4, or Trop-2). In one embodiment, the CAR-expressing cell comprises a first CAR that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a second CAR that targets a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. Placement of a costimulatory signaling domain, e.g., 4-1BB, CD28, CD27, OX-40 or ICOS, onto the first CAR, and the primary signaling domain, e.g., CD3 zeta, on the second CAR can limit the CAR activity to cells where both targets are expressed. In one embodiment, the CAR expressing cell comprises a first mesothelin CAR that includes a mesothelin binding domain, a transmembrane domain and a costimulatory domain and a second CAR that targets an antigen other than mesothelin (e.g., a target other than mesothelin on stroma cells, e.g., FAP; a target other than mesothelin on prostate cancer cells, e.g., androgen receptor, OR51E2, PSMA, PSCA, PDGRF-β, TARP, GloboH, MAD-CT-1, or MAD-CT-2; a target other than mesothelin on ovararian cancer cells, e.g., Tn, PRSS21, CD171, Lewis Y, folate receptor α, claudin6, GloboH, or sperm protein 17, e.g., a target other than mesothelin on lung cancer cells, e.g.,VEGF, HER3, IGF-1R, EGFR, DLL4, or Trop-2) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the CAR expressing cell comprises a first mesothelin CAR that includes a mesothelin binding domain, a transmembrane domain and a primary signaling domain and a second CAR that targets an antigen other

than mesothelin (e.g., a target other than mesothelin on stroma cells, e.g., FAP; a target other than mesothelin on prostate cancer cells, e.g., androgen receptor, OR51E2, PSMA, PSCA, PDGRF-β, TARP, GloboH, MAD-CT-1, or MAD-CT-2; a target other than mesothelin on ovararian cancer cells, e.g., Tn, PRSS21, CD171, Lewis Y, folate receptor α, claudin6, GloboH, or sperm protein 17, e.g., a target other than mesothelin on lung cancer cells, e.g.,VEGF, HER3, IGF-1R, EGFR, DLL4, or Trop-2) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In one embodiment, the CAR-expressing cell comprises a mesothelin CAR described herein and an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that binds an antigen found on normal cells but not cancer cells, e.g., normal cells that also express mesothelin. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1, PD-L1, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, or TGFR beta.

In one embodiment, when the CAR-expressing cell comprises two or more different CARs, the antigen binding domains of the different CARs can be such that the antigen binding domains do not interact with one another. For example, a cell expressing a first and second CAR can have an antigen binding domain of the first CAR, e.g., as a fragment, e.g., an scFv, that does not form an association with the antigen binding domain of the second CAR, e.g., the antigen binding domain of the second CAR is a VHH.

In some embodiments, the antigen binding domain comprises a single domain antigen binding (SDAB) molecules include molecules whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain variable domains, binding molecules naturally devoid of light chains, single domains derived from conventional 4-chain antibodies, engineered domains and single domain scaffolds other than those derived from antibodies. SDAB molecules may be any of the art, or any future single domain molecules. SDAB molecules may be derived from any species including, but not limited to mouse, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. This term also includes naturally occurring single domain antibody molecules from species other than Camelidae and sharks.

In one aspect, an SDAB molecule can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain

molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) Protein Sci. 14:2901-2909.

According to another aspect, an SDAB molecule is a naturally occurring single domain antigen binding molecule known as heavy chain devoid of light chains. Such single domain molecules are disclosed in WO 9404678 and Hamers-Casterman, C. et al. (1993) Nature 363:446-448, for example. For clarity reasons, this variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from Camelidae species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain; such VHHs are within the scope of the invention.

The SDAB molecules can be recombinant, CDR-grafted, humanized, camelized, de-immunized and/or in vitro generated (e.g., selected by phage display).

It has also been discovered, that cells having a plurality of chimeric membrane embedded receptors comprising an antigen binding domain that interactions between the antigen binding domain of the receptors can be undesirable, e.g., because it inhibits the ability of one or more of the antigen binding domains to bind its cognate antigen. Accordingly, disclosed herein are cells having a first and a second non-naturally occurring chimeric membrane embedded receptor comprising antigen binding domains that minimize such interactions. Also disclosed herein are nucleic acids encoding a first and a second non-naturally occurring chimeric membrane embedded receptor comprising an antigen binding domains that minimize such interactions, as well as methods of making and using such cells and nucleic acids. In an embodiment the antigen binding domain of one of the first and the second non-naturally occurring chimeric membrane embedded receptor, comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

In some embodiments, the claimed invention comprises a first and second CAR, wherein the antigen binding domain of one of the first and the second CAR does not comprise a variable light domain and a variable heavy domain. In some embodiments, the antigen binding domain of one of the first and the second CAR is an scFv, and the other is not an scFv. In some embodiments, the antigen binding domain of one of the first and the second CAR comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of the first and the second CAR comprises a nanobody. In some embodiments, the antigen binding domain of one of the first and the second CAR comprises a camelid VHH domain.

In some embodiments, the antigen binding domain of one of the first and thesecond CAR comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey

single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of the first and the second CAR comprises an scFv, and the other comprises a nanobody. In some embodiments, the antigen binding domain of one of the first and the second CAR comprises comprises an scFv, and the other comprises a camelid VHH domain.

In some embodiments, when present on the surface of a cell, binding of the antigen binding domain of the first CAR to its cognate antigen is not substantially reduced by the presence of the second CAR. In some embodiments, binding of the antigen binding domain of the first CAR to its cognate antigen in the presence of the second CAR is 85%, 90%, 95%, 96%, 97%, 98% or 99% of binding of the antigen binding domain of the first CAR to its cognate antigen in the absence of the second CAR.

In some embodiments, when present on the surface of a cell, the antigen binding domains of the first and the second CAR, associate with one another less than if both were scFv antigen binding domains. In some embodiments, the antigen binding domains of the first and the second CAR, associate with one another 85%, 90%, 95%, 96%, 97%, 98% or 99% less than if both were scFv antigen binding domains.

Co-expression of an Agent that Enhances CAR Activity

In another aspect, the CAR-expressing cell described herein can further express another agent, e.g., an agent that enhances the activity or fitness of a CAR-expressing cell.

For example, in one embodiment, the agent can be an agent which inhibits a molecule that modulates or regulates, e.g., inhibits, T cell function. In some embodiments, the molecule that modulates or regulates T cell function is an inhibitory molecule. Inhibitory molecules, e.g., PD1, can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, or TGFR beta.

In embodiments, an agent, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA; or e.g., an inhibitory protein or system, e.g., a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), e.g., as described herein, can be used to inhibit expression of a molecule that modulates or regulates, e.g., inhibits, T-cell function in the CAR-expressing cell. In an embodiment the agent is an shRNA, e.g., an shRNA described herein. In an embodiment, the agent that modulates or regulates, e.g., inhibits, T-cell function is inhibited within a CAR-expressing cell. For example, a dsRNA molecule that inhibits expression of a molecule that modulates or regulates, e.g., inhibits, T-cell function is linked to the nucleic acid that encodes a component, e.g., all of the components, of the CAR.

In one embodiment, the agent which inhibits an inhibitory molecule comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, or TGFR beta, or a fragment of any of these (e.g., at least a portion of an extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of an extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein). PD1 is an inhibitory member of the CD28 family of receptors that also includes CD28, CTLA-4, ICOS, and BTLA. PD-1 is expressed on activated B cells, T cells and myeloid cells (Agata et al. 1996 Int. Immunol 8:765-75). Two ligands for PD1, PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD1 (Freeman et a. 2000 J Exp Med 192:1027-34; Latchman et al. 2001 Nat Immunol 2:261-8; Carter et al. 2002 Eur J Immunol 32:634-43). PD-L1 is abundant in human cancers (Dong et al. 2003 J Mol Med 81:281-7; Blank et al. 2005 Cancer Immunol. Immunother 54:307-314; Konishi et al. 2004 Clin Cancer Res 10:5094). Immune suppression can be reversed by inhibiting the local interaction of PD1 with PD-L1.

In one embodiment, the agent comprises the extracellular domain (ECD) of an inhibitory molecule, e.g., Programmed Death 1 (PD1), can be fused to a transmembrane domain and intracellular signaling domains such as 41BB and CD3 zeta (also referred to herein as a PD1 CAR). In one embodiment, the PD1 CAR, when used in combinations with a mesothelin CAR described herein, improves the persistence of the T cell. In one embodiment, the CAR is a PD1 CAR comprising the extracellular domain of PD1 indicated as underlined in SEQ ID NO: 24 and a signal sequence at amino acids 1-21 of SEQ ID NO:24. In one embodiment, the PD1 CAR comprises the amino acid sequence of SEQ ID NO:24.

In one embodiment, the PD1 CAR without the N-terminal signal sequence comprises the amino acid sequence provided of SEQ ID NO:22.

In one embodiment, the agent comprises a nucleic acid sequence encoding the PD1 CAR with the N-terminal signal sequence, e.g., the PD1 CAR described herein. In one embodiment, the nucleic acid sequence for the PD1 CAR is shown in Table 1, with the PD1 ECD underlined in SEQ ID NO: 23.

In another example, in one embodiment, the agent which enhances the activity of a CAR-expressing cell can be a costimulatory molecule or costimulatory molecule ligand. Examples of costimulatory molecules include MHC class I molecule, BTLA and a Toll ligand receptor, as well as OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, and a ligand that specifically binds with CD83., e.g., as described herein. Examples of costimulatory molecule ligands include CD80, CD86, CD40L, ICOSL, CD70, OX40L, 4-1BBL, GITRL, and LIGHT. In embodiments, the costimulatory molecule ligand is a ligand for a costimulatory molecule different from the costimulatory molecule domain of the CAR. In embodiments, the costimulatory molecule ligand is a ligand for a costimulatory molecule that is the same as the costimulatory molecule domain of the CAR. In an embodiment, the costimulatory molecule ligand is 4-1BBL. In an embodiment, the costimulatory ligand is CD80 or CD86. In an embodiment, the costimulatory molecule ligand is CD70. In embodiments, a CAR-expressing immune effector cell described herein can be further engineered to express one or more additional costimulatory molecules or costimulatory molecule ligands.

Co-expression of CAR with a Chemokine Receptor

In embodiments, the CAR-expressing cell described herein, e.g., mesothelin CAR-expressing cell, further comprises a chemokine receptor molecule. Transgenic expression of chemokine receptors CCR2b or CXCR2 in T cells enhances trafficking to CCL2- or CXCL1-secreting solid tumors including melanoma and neuroblastoma (Craddock et al., *J Immunother*. 2010 Oct; 33(8):780-8 and Kershaw et al., *Hum Gene Ther*. 2002 Nov 1; 13(16):1971-80). Thus, without wishing to be bound by theory, it is believed that chemokine receptors expressed in CAR-expressing cells that recognize chemokines secreted by tumors, e.g., solid tumors, can improve homing of the CAR-expressing cell to the tumor, facilitate the infiltration of the CAR-expressing cell to the tumor, and enhances antitumor efficacy of the CAR-expressing cell. The chemokine receptor molecule can comprise a naturally occurring or recombinant chemokine receptor or a chemokine-binding fragment thereof. A chemokine receptor molecule suitable for expression in a CAR-expressing cell (e.g., CAR-Tx) described herein include a CXC chemokine receptor (e.g., CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, or CXCR7), a

CC chemokine receptor (e.g., CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, or CCR11), a CX3C chemokine receptor (e.g., CX3CR1), a XC chemokine receptor (e.g., XCR1), or a chemokine-binding fragment thereof. In one embodiment, the chemokine receptor molecule to be expressed with a CAR described herein is selected based on the chemokine(s) secreted by the tumor. In one embodiment, the CAR-expressing cell described herein further comprises, e.g., expresses, a CCR2b receptor or a CXCR2 receptor. In an embodiment, the CAR described herein and the chemokine receptor molecule are on the same vector or are on two different vectors. In embodiments where the CAR described herein and the chemokine receptor molecule are on the same vector, the CAR and the chemokine receptor molecule are each under control of two different promoters or are under the control of the same promoter.

RNA Transfection

Disclosed herein are methods for producing an in vitro transcribed RNA CAR. The present invention also includes a CAR encoding RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection can involve in vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO: 35). RNA so produced can efficiently transfect different kinds of cells. In one aspect, the template includes sequences for the CAR.

In one aspect the CAR is encoded by a messenger RNA (mRNA). In one aspect the mRNA encoding the CAR is introduced into an immune effector cell, e.g., a T cell or a NK cell, for production of a CAR-expressing cell (e.g., CART cell or CAR-expressing NK cell).

In one embodiment, the in vitro transcribed RNA CAR can be introduced to a cell as a form of transient transfection. The RNA is produced by in vitro transcription using a polymerase chain reaction (PCR)-generated template. DNA of interest from any source can be directly converted by PCR into a template for in vitro mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any other appropriate source of DNA. The desired temple for in vitro transcription is a CAR of the present invention. For example, the template for the RNA CAR comprises an extracellular region comprising a single chain variable domain of an anti-tumor antibody; a hinge region, a transmembrane domain (e.g., a transmembrane domain of CD8a); and a cytoplasmic region that includes an intracellular signaling domain, e.g., comprising the signaling domain of CD3-zeta and the signaling domain of 4-1BB.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one embodiment, the nucleic acid can include some or all of the 5' and/or 3' untranslated regions (UTRs). The nucleic acid can include exons and introns. In one embodiment, the DNA to be used for PCR is a human nucleic acid sequence. In another embodiment, the DNA to be used for PCR is a human nucleic acid sequence including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

PCR is used to generate a template for in vitro transcription of mRNA which is used for transfection. Methods for performing PCR are well known in the art. Primers for use in PCR are designed to have regions that are substantially complementary to regions of the DNA to be used as a template for the PCR. "Substantially complementary," as used herein, refers to sequences of nucleotides where a majority or all of the bases in the primer sequence are complementary, or one or more bases are non-complementary, or mismatched. Substantially complementary sequences are able to anneal or hybridize with the intended DNA target under annealing conditions used for PCR. The primers can be designed to be substantially complementary to any portion of the DNA template. For example, the primers can be designed to amplify the portion of a nucleic acid that is normally transcribed in cells (the open reading frame), including 5' and 3' UTRs. The primers can also be designed to amplify a portion of a nucleic acid that encodes a particular domain of interest. In one embodiment, the primers are designed to amplify the coding region of a human cDNA, including all or portions of the 5' and 3' UTRs. Primers useful for PCR can be generated by synthetic methods that are well known in the art. "Forward primers" are primers that contain a region of nucleotides that are substantially complementary to nucleotides on the DNA template that are upstream of the DNA sequence that is to be amplified. "Upstream" is used herein to refer to a location 5, to the DNA sequence to be amplified relative to the coding strand. "Reverse primers" are primers that contain a region of nucleotides that are substantially complementary to a double-stranded DNA template that are downstream of the DNA sequence that is to be amplified. "Downstream" is used herein to refer to a location 3' to the DNA sequence to be amplified relative to the coding strand.

Any DNA polymerase useful for PCR can be used in the methods disclosed herein. The reagents and polymerase are commercially available from a number of sources.

Chemical structures with the ability to promote stability and/or translation efficiency may also be used. The RNA preferably has 5' and 3' UTRs. In one embodiment, the 5' UTR is between one and

3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the nucleic acid of interest. Alternatively, UTR sequences that are not endogenous to the nucleic acid of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the nucleic acid of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous nucleic acid. Alternatively, when a 5' UTR that is not endogenous to the nucleic acid of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments the 5' UTR can be 5'UTR of an RNA virus whose RNA genome is stable in cells. In other embodiments various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

To enable synthesis of RNA from a DNA template without the need for gene cloning, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added to the 5' end of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product upstream of the open reading frame that is to be transcribed. In one preferred embodiment, the promoter is a T7 polymerase promoter, as described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

In a preferred embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatameric product which is not suitable for expression in eukaryotic cells. The transcription of plasmid DNA linearized at the end of

the 3' UTR results in normal sized mRNA which is not effective in eukaryotic transfection even if it is polyadenylated after transcription.

On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270:1485-65 (2003).

The conventional method of integration of polyA/T stretches into a DNA template is molecular cloning. However polyA/T sequence integrated into plasmid DNA can cause plasmid instability, which is why plasmid DNA templates obtained from bacterial cells are often highly contaminated with deletions and other aberrations. This makes cloning procedures not only laborious and time consuming but often not reliable. That is why a method which allows construction of DNA templates with polyA/T 3' stretch without cloning highly desirable.

The polyA/T segment of the transcriptional DNA template can be produced during PCR by using a reverse primer containing a polyT tail, such as 100T tail (SEQ ID NO: 31) (size can be 50-5000 T (SEQ ID NO: 32)), or after PCR by any other method, including, but not limited to, DNA ligation or in vitro recombination. Poly(A) tails also provide stability to RNAs and reduce their degradation. Generally, the length of a poly(A) tail positively correlates with the stability of the transcribed RNA. In one embodiment, the poly(A) tail is between 100 and 5000 adenosines (SEQ ID NO: 33).

Poly(A) tails of RNAs can be further extended following in vitro transcription with the use of a poly(A) polymerase, such as E. coli polyA polymerase (E-PAP). In one embodiment, increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides (SEQ ID NO: 34) results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the attachment of different chemical groups to the 3' end can increase mRNA stability. Such attachment can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP analogs can be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogs can further increase the stability of the RNA.

5' caps on also provide stability to RNA molecules. In a preferred embodiment, RNAs produced by the methods disclosed herein include a 5' cap. The 5' cap is provided using techniques known in the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7:1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

The RNAs produced by the methods disclosed herein can also contain an internal ribosome entry site (IRES) sequence. The IRES sequence may be any viral, chromosomal or artificially designed sequence which initiates cap-independent ribosome binding to mRNA and facilitates the initiation of translation. Any solutes suitable for cell electroporation, which can contain factors facilitating cellular

permeability and viability such as sugars, peptides, lipids, proteins, antioxidants, and surfactants can be included.

RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendort, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as "gene guns" (see, for example, Nishikawa, et al. Hum Gene Ther., 12(8):861-70 (2001).

Non-viral delivery methods

In some aspects, non-viral methods can be used to deliver a nucleic acid encoding a CAR described herein into a cell or tissue or a subject.

In some embodiments, the non-viral method includes the use of a transposon (also called a transposable element). In some embodiments, a transposon is a piece of DNA that can insert itself at a location in a genome, for example, a piece of DNA that is capable of self-replicating and inserting its copy into a genome, or a piece of DNA that can be spliced out of a longer nucleic acid and inserted into another place in a genome. For example, a transposon comprises a DNA sequence made up of inverted repeats flanking genes for transposition.

Exemplary methods of nucleic acid delivery using a transposon include a Sleeping Beauty transposon system (SBTS) and a piggyBac (PB) transposon system. See, e.g., Aronovich et al. Hum. Mol. Genet. 20.R1(2011):R14-20; Singh et al. Cancer Res. 15(2008):2961–2971; Huang et al. Mol. Ther. 16(2008):580–589; Grabundzija et al. Mol. Ther. 18(2010):1200–1209; Kebriaei et al. Blood. 122.21(2013):166; Williams. Molecular Therapy 16.9(2008):1515–16; Bell et al. Nat. Protoc. 2.12(2007):3153-65; and Ding et al. Cell. 122.3(2005):473-83, all of which are incorporated herein by reference.

The SBTS includes two components: 1) a transposon containing a transgene and 2) a source of transposase enzyme. The transposase can transpose the transposon from a carrier plasmid (or other donor DNA) to a target DNA, such as a host cell chromosome/genome. For example, the transposase binds to the carrier plasmid/donor DNA, cuts the transposon (including transgene(s)) out of the plasmid, and inserts it into the genome of the host cell. See, e.g., Aronovich et al. *supra.*

Exemplary transposons include a pT2-based transposon. See, e.g., Grabundzija et al. Nucleic Acids Res. 41.3(2013):1829-47; and Singh et al. Cancer Res. 68.8(2008): 2961–2971, all of which are

incorporated herein by reference. Exemplary transposases include a Tc1/mariner-type transposase, e.g., the SB10 transposase or the SB11 transposase (a hyperactive transposase which can be expressed, e.g., from a cytomegalovirus promoter). See, e.g., Aronovich et al.; Kebriaei et al.; and Grabundzija et al., all of which are incorporated herein by reference.

Use of the SBTS permits efficient integration and expression of a transgene, e.g., a nucleic acid encoding a CAR described herein. Provided herein are methods of generating a cell, e.g., T cell or NK cell, that stably expresses a CAR described herein, e.g., using a transposon system such as SBTS.

In accordance with methods described herein, in some embodiments, one or more nucleic acids, e.g., plasmids, containing the SBTS components are delivered to a cell (e.g., T or NK cell). For example, the nucleic acid(s) are delivered by standard methods of nucleic acid (e.g., plasmid DNA) delivery, e.g., methods described herein, e.g., electroporation, transfection, or lipofection. In some embodiments, the nucleic acid contains a transposon comprising a transgene, e.g., a nucleic acid encoding a CAR described herein. In some embodiments, the nucleic acid contains a transposon comprising a transgene (e.g., a nucleic acid encoding a CAR described herein) as well as a nucleic acid sequence encoding a transposase enzyme. In other embodiments, a system with two nucleic acids is provided, e.g., a dual-plasmid system, e.g., where a first plasmid contains a transposon comprising a transgene, and a second plasmid contains a nucleic acid sequence encoding a transposase enzyme. For example, the first and the second nucleic acids are co-delivered into a host cell.

In some embodiments, cells, e.g., T or NK cells, are generated that express a CAR described herein by using a combination of gene insertion using the SBTS and genetic editing using a nuclease (e.g., Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, or engineered meganuclease re-engineered homing endonucleases).

In some embodiments, use of a non-viral method of delivery permits reprogramming of cells, e.g., T or NK cells, and direct infusion of the cells into a subject. Advantages of non-viral vectors include but are not limited to the ease and relatively low cost of producing sufficient amounts required to meet a patient population, stability during storage, and lack of immunogenicity.

Nucleic Acid Constructs Encoding a CAR

The present invention also provides nucleic acid molecules encoding one or more CAR constructs described herein. In one aspect, the nucleic acid molecule is provided as a messenger RNA transcript. In one aspect, the nucleic acid molecule is provided as a DNA construct.

Accordingly, in one aspect, the invention pertains to an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain, a

transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, e.g., a costimulatory signaling domain and/or a primary signaling domain, e.g., zeta chain.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be produced synthetically, rather than cloned.

Vectors

The present invention also provides vectors in which a DNA of the present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. A retroviral vector may also be, e.g., a gammaretroviral vector. A gammaretroviral vector may include, e.g., a promoter, a packaging signal (ψ), a primer binding site (PBS), one or more (e.g., two) long terminal repeats (LTR), and a transgene of interest, e.g., a gene encoding a CAR. A gammaretroviral vector may lack viral structural gens such as gag, pol, and env. Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other gammaretroviral vectors are described, e.g., in Tobias Maetzig et al., "Gammaretroviral Vectors: Biology, Technology and Application" Viruses. 2011 Jun; 3(6): 677–713.

In another embodiment, the vector comprising the nucleic acid encoding the desired CAR of the invention is an adenoviral vector (A5/35). In another embodiment, the expression of nucleic acids encoding CARs can be accomplished using of transposons such as sleeping beauty, CRISPR, CAS9, and zinc finger nucleases. See below June et al. 2009*Nature Reviews Immunology* 9.10: 704-716, is incorporated herein by reference.

In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno- associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

An example of a promoter that is capable of expressing a CAR transgene in a mammalian T cell is the EF1a promoter. The native EF1a promoter drives expression of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the

ribosome. The EF1a promoter has been extensively used in mammalian expression plasmids and has been shown to be effective in driving CAR expression from transgenes cloned into a lentiviral vector. See, e.g., Milone et al., Mol. Ther. 17(8): 1453–1464 (2009).

Another example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1α promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

Another example of a promoter is the phosphoglycerate kinase (PGK) promoter. In embodiments, a truncated PGK promoter (e.g., a PGK promoter with one or more, e.g., 1, 2, 5, 10, 100, 200, 300, or 400, nucleotide deletions when compared to the wild-type PGK promoter sequence) may be desired. The nucleotide sequences of exemplary PGK promoters are provided below.

WT PGK Promoter

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAA
GGCAAATAGTGCAGGCCGTGCGGCGCTTGGCGTTCCTTGGAAGGGCTGAATCCCCGCCTCGTCCTTC
GCAGCGGCCCCCCGGGTGTTCCCATCGCCGCTTCTAGGCCCACTGCGACGCTTGCCTGCACTTCTTA
CACGCTCTGGGTCCCAGCCGCGGCGACGCAAAGGGCCTTGGTGCGGGTCTCGTCGGCGCAGGGACGC
GTTTGGGTCCCGACGGAACCTTTTCCGCGTTGGGGTTGGGGCACCATAAGCT
(SEQ ID NO: 615)

Exemplary truncated PGK Promoters:

PGK100:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTG

(SEQ ID NO: 616)

PGK200:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACG
(SEQ ID NO: 617)

PGK300:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAA
GGCAAATAGTGCAGGCCGTGCGGCGCTTGGCGTTCCTTGGAAGGGCTGAATCCCCG
(SEQ ID NO: 618)

PGK400:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAA
GGCAAATAGTGCAGGCCGTGCGGCGCTTGGCGTTCCTTGGAAGGGCTGAATCCCCGCCTCGTCCTTC
GCAGCGGCCCCCCGGGTGTTCCCATCGCCGCTTCTAGGCCCACTGCGACGCTTGCCTGCACTTCTTA
CACGCTCTGGGTCCCAGCCG
(SEQ ID NO: 619)

A vector may also include, e.g., a signal sequence to facilitate secretion, a polyadenylation signal and transcription terminator (e.g., from Bovine Growth Hormone (BGH) gene), an element allowing episomal replication and replication in prokaryotes (e.g. SV40 origin and ColE1 or others known in the art) and/or elements to allow selection (e.g., ampicillin resistance gene and/or zeocin marker).

In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is

manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter- driven transcription.

In one embodiment, the vector can further comprise a nucleic acid encoding a second CAR. In one embodiment, the second CAR includes an antigen binding domain to a target expressed on acute myeloid leukemia cells, such as, e.g., CD123, CD34, CLL-1, folate receptor beta, or FLT3; or a target expressed on a B cell, e.g., CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a. In one embodiment, the vector comprises a nucleic acid sequence encoding a first CAR that specifically binds a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a nucleic acid encoding a second CAR that specifically binds a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain.

In one embodiment, the vector comprises a nucleic acid encoding a CAR described herein and a nucleic acid encoding an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that binds an antigen found on normal cells but not cancer cells. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta.

In embodiments, the vector may comprise two or more nucleic acid sequences encoding a CAR, e.g., a CAR described herein and a second CAR, e.g., an inhibitory CAR or a CAR that specifically binds to a different antigen. In such embodiments, the two or more nucleic acid sequences encoding the CAR are encoded by a single nucleic molecule in the same frame and as a single polypeptide chain. In this aspect, the two or more CARs, can, e.g., be separated by one or more peptide cleavage sites. (e.g., an auto-cleavage site or a substrate for an intracellular protease). Examples of peptide cleavage sites include the following, wherein the GSG residues are optional:

T2A: (GSG) E G R G S L L T C G D V E E N P G P (SEQ ID NO: 620)

P2A: (GSG) A T N F S L L K Q A G D V E E N P G P (SEQ ID NO: 621)

E2A: (GSG) Q C T N Y A L L K L A G D V E S N P G P (SEQ ID NO: 622)

F2A: (GSG) V K Q T L N F D L L K L A G D V E S N P G P (SEQ ID NO: 623)

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g. , an artificial membrane vesicle). Other methods of state-of-the-art targeted delivery of nucleic acids are available, such as delivery of polynucleotides with targeted nanoparticles or other suitable sub-micron sized delivery system.

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a

liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, MO; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, NY); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

The present invention further provides a vector comprising a CAR encoding nucleic acid molecule. In one aspect, a CAR vector can be directly transduced into a cell, *e.g.,* a T cell or NK cell.

In one aspect, the vector is a cloning or expression vector, *e.g.,* a vector including, but not limited to, one or more plasmids (*e.g.*, expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes), retroviral and lentiviral vector constructs. In one aspect, the vector is capable of expressing the CAR construct in mammalian T cells or NK cells. In one aspect, the mammalian T cell is a human T cell. In one aspect, the mammalian NK cell is a human NK cell.

Sources of cells

Prior to expansion and genetic modification, a source of cells, e.g., immune effector cells (e.g., T cells or NK cells), is obtained from a subject. The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors.

In certain aspects of the present invention, any number of immune effector cell (e.g., T cell or NK cell) lines available in the art, may be used. In certain aspects of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one aspect of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative aspect, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations.

Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

It is recognized that the methods of the application can utilize culture media conditions comprising 5% or less, for example 2%, human AB serum, and employ known culture media conditions and compositions, for example those described in Smith *et al.*, "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement" *Clinical & Translational Immunology* (2015) 4, e31; doi:10.1038/cti.2014.31.

In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLLTM gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3+, CD4+, CD8+, CD45RA+, and/or CD45RO+T cells, can be further isolated by positive or negative selection techniques. For example, in one aspect, T cells are isolated by incubation with anti-CD3/anti-CD28 (e.g., 3x28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one aspect, the time period is about 30 minutes. In a further aspect, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further aspect, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred aspect, the time period is 10 to 24 hours. In one aspect, the incubation time period is 24 hours. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain aspects, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain aspects, it

may be desirable to enrich for or positively select for regulatory T cells which typically express CD4+, CD25+, CD62Lhi, GITR+, and FoxP3+. In certain aspects, it may be desirable to enrich for cells that are CD127low. Alternatively, in certain aspects, T regulatory cells are depleted by anti-C25 conjugated beads or other similar method of selection.

The methods described herein can include, e.g., selection of a specific subpopulation of immune effector cells, e.g., T cells, that are a T regulatory cell-depleted population, CD25+ depleted cells, using, e.g., a negative selection technique, e.g., described herein. Preferably, the population of T regulatory depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

In one embodiment, T regulatory cells, e.g., CD25+ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. In one embodiment, the anti-CD25 antibody, or fragment thereof, or CD25-binding ligand is conjugated to a substrate, e.g., a bead, or is otherwise coated on a substrate, e.g., a bead. In one embodiment, the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate as described herein.

In one embodiment, the T regulatory cells, e.g., CD25+ T cells, are removed from the population using CD25 depletion reagent from Miltenyi™. In one embodiment, the ratio of cells to CD25 depletion reagent is 1e7 cells to 20 uL, or 1e7 cells to15 uL, or 1e7 cells to 10 uL, or 1e7 cells to 5 uL, or 1e7 cells to 2.5 uL, or 1e7 cells to 1.25 uL. In one embodiment, e.g., for T regulatory cells, e.g., CD25+ depletion, greater than 500 million cells/ml is used. In a further aspect, a concentration of cells of 600, 700, 800, or 900 million cells/ml is used.

In one embodiment, the population of immune effector cells to be depleted includes about 6 x 10^9 CD25+ T cells. In other aspects, the population of immune effector cells to be depleted include about 1 x 10^9 to 1x 10^{10} CD25+ T cell, and any integer value in between. In one embodiment, the resulting population T regulatory depleted cells has 2 x 10^9 T regulatory cells, e.g., CD25+ cells, or less (e.g., 1 x 10^9, 5 x 10^8, 1 x 10^8, 5 x 10^7, 1 x 10^7, or less CD25+ cells).

In one embodiment, the T regulatory cells, e.g., CD25+ cells, are removed from the population using the CliniMAC system with a depletion tubing set, such as, e.g., tubing 162-01. In one embodiment, the CliniMAC system is run on a depletion setting such as, e.g., DEPLETION2.1.

Without wishing to be bound by a particular theory, decreasing the level of negative regulators of immune cells (e.g., decreasing the number of unwanted immune cells, e.g., T_{REG} cells), in a subject prior to apheresis or during manufacturing of a CAR-expressing cell product can reduce the risk of subject relapse. For example, methods of depleting T_{REG} cells are known in the art. Methods of decreasing T_{REG} cells include, but are not limited to, cyclophosphamide, anti-GITR antibody (an anti-GITR antibody described herein), CD25-depletion, and combinations thereof.

In some embodiments, the manufacturing methods comprise reducing the number of (e.g., depleting) T_{REG} cells prior to manufacturing of the CAR-expressing cell. For example, manufacturing methods comprise contacting the sample, e.g., the apheresis sample, with an anti-GITR antibody and/or an anti-CD25 antibody (or fragment thereof, or a CD25-binding ligand), e.g., to deplete T_{REG} cells prior to manufacturing of the CAR-expressing cell (e.g., T cell, NK cell) product.

In an embodiment, a subject is pre-treated with one or more therapies that reduce T_{REG} cells prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, methods of decreasing T_{REG} cells include, but are not limited to, administration to the subject of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof. Administration of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof, can occur before, during or after an infusion of the CAR-expressing cell product.

In an embodiment, a subject is pre-treated with cyclophosphamide prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, a subject is pre-treated with an anti-GITR antibody prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment.

In one embodiment, the population of cells to be removed are neither the regulatory T cells or tumor cells, but cells that otherwise negatively affect the expansion and/or function of CART cells, e.g. cells expressing CD14, CD11b, CD33, CD15, or other markers expressed by potentially immune suppressive cells. In one embodiment, such cells are envisioned to be removed concurrently with regulatory T cells and/or tumor cells, or following said depletion, or in another order.

The methods described herein can include more than one selection step, e.g., more than one depletion step. Enrichment of a T cell population by negative selection can be accomplished, e.g., with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail can include antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8.

The methods described herein can further include removing cells from the population which express a tumor antigen, e.g., a tumor antigen that does not comprise CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CD11b, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted, and tumor antigen depleted cells that are suitable for expression of a CAR, e.g., a CAR

described herein. In one embodiment, tumor antigen expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-tumor antigen antibody, or fragment thereof, can be attached to the same substrate, e.g., bead, which can be used to remove the cells or an anti-CD25 antibody, or fragment thereof, or the anti-tumor antigen antibody, or fragment thereof, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the tumor antigen expressing cells is sequential, and can occur, e.g., in either order.

Also provided are methods that include removing cells from the population which express a check point inhibitor, e.g., a check point inhibitor described herein, e.g., one or more of PD1+ cells, LAG3+ cells, and TIM3+ cells, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted cells, and check point inhibitor depleted cells, e.g., PD1+, LAG3+ and/or TIM3+ depleted cells. Exemplary check point inhibitors include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta. In embodiments, the checkpoint inhibitor is PD1 or PD-L1. In one embodiment, check point inhibitor expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-check point inhibitor antibody, or fragment thereof, can be attached to the same bead which can be used to remove the cells, or an anti-CD25 antibody, or fragment thereof, and the anti-check point inhibitor antibody, or fragment there, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the check point inhibitor expressing cells is sequential, and can occur, e.g., in either order.

In one embodiment, a T cell population can be selected that expresses one or more of IFN-γ, TNFα, IL-17A, IL-7, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other appropriate molecules, e.g., other cytokines. Methods for screening for cell expression can be determined, e.g., by the methods described in PCT Publication No.: WO 2013/126712.

For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain aspects, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (e.g., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one aspect, a concentration of 2 billion cells/ml is used. In one aspect, a concentration of 1 billion cells/ml is used. In a further aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150

million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (e.g., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In a related aspect, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute concentrations. In one aspect, the concentration of cells used is 5 X 10e6/ml. In other aspects, the concentration used can be from about 1 X 10^5/ml to 1 X 10^6/ml, and any integer value in between.

In other aspects, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature.

T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C or in liquid nitrogen.

In certain aspects, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as immune effector cells, e.g., T cells or NK cells, isolated and frozen for later use in

cell therapy, e.g., T cell therapy, for any number of diseases or conditions that would benefit from cell therapy, e.g., T cell therapy, such as those described herein. In one aspect a blood sample or an apheresis is taken from a generally healthy subject. In certain aspects, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain aspects, the immune effector cells (e.g., T cells or NK cells) may be expanded, frozen, and used at a later time. In certain aspects, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further aspect, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

In a further aspect of the present invention, T cells are obtained from a patient directly following treatment that leaves the subject with functional T cells. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain aspects, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

In one embodiment, the immune effector cells expressing a CAR molecule, e.g., a CAR molecule described herein, are obtained from a subject that has received a low, immune enhancing dose of an mTOR inhibitor. In an embodiment, the population of immune effector cells, e.g., T cells, to be engineered to express a CAR, are harvested after a sufficient time, or after sufficient dosing of the low, immune enhancing, dose of an mTOR inhibitor, such that the level of PD1 negative immune effector cells, e.g., T cells, or the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive

immune effector cells, e.g., T cells, in the subject or harvested from the subject has been, at least transiently, increased.

In other embodiments, population of immune effector cells, e.g., T cells, which have, or will be engineered to express a CAR, can be treated ex vivo by contact with an amount of an mTOR inhibitor that increases the number of PD1 negative immune effector cells, e.g., T cells or increases the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells.

In one embodiment, a T cell population is diaglycerol kinase (DGK)-deficient. DGK-deficient cells include cells that do not express DGK RNA or protein, or have reduced or inhibited DGK activity. DGK-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent DGK expression. Alternatively, DGK-deficient cells can be generated by treatment with DGK inhibitors described herein.

In one embodiment, a T cell population is Ikaros-deficient. Ikaros-deficient cells include cells that do not express Ikaros RNA or protein, or have reduced or inhibited Ikaros activity, Ikaros-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent Ikaros expression. Alternatively, Ikaros-deficient cells can be generated by treatment with Ikaros inhibitors, e.g., lenalidomide.

In embodiments, a T cell population is DGK-deficient and Ikaros-deficient, e.g., does not express DGK and Ikaros, or has reduced or inhibited DGK and Ikaros activity. Such DGK and Ikaros-deficient cells can be generated by any of the methods described herein.

In an embodiment, the NK cells are obtained from the subject. In another embodiment, the NK cells are an NK cell line, e.g., NK-92 cell line (Conkwest).

Modifications of CAR cells, including allogeneic CAR cells

In embodiments described herein, the immune effector cell can be an allogeneic immune effector cell, e.g., T cell or NK cell. For example, the cell can be an allogeneic T cell, e.g., an allogeneic T cell lacking expression of a functional T cell receptor (TCR) and/or human leukocyte antigen (HLA), e.g., HLA class I and/or HLA class II, and/or beta-2 microglobulin (β_2m). Compositions of allogeneic CAR and methods thereof have been described in, e.g., pages 227-237 of WO 2016/014565, incorporated herein by reference in its entirety.

In some embodiments, a cell, e.g., a T cell or a NK cell, is modified to reduce the expression of a TCR, and/or HLA, and/or β_2m, and/or an inhibitory molecule described herein (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA,

BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta), using, e.g., a method described herein, e.g., siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR) transcription-activator like effector nuclease (TALEN), or zinc finger endonuclease (ZFN).

In some embodiments, a cell, e.g., a T cell or a NK cell is engineered to express a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. In one embodiment, such modification improves persistence of the cell in a patient.

Activation and Expansion of T Cells

T cells may be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

Generally, the T cells of the invention may be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either CD4+ T cells or CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody can be used. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

In certain aspects, the primary stimulatory signal and the costimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in "cis" formation) or to separate surfaces (i.e., in "trans" formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one aspect, the agent providing the costimulatory

signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain aspects, both agents can be in solution. In one aspect, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

In one aspect, the two agents are immobilized on beads, either on the same bead, i.e., "cis," or to separate beads, i.e., "trans." By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the costimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one aspect, a 1:1 ratio of each antibody bound to the beads for CD4+ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular aspect an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one aspect, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, i.e., the ratio of CD3:CD28 is less than one. In certain aspects of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular aspect, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further aspect, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred aspect, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet one aspect, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain aspects the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further aspects the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio

being at least 1:1 particles per T cell. In one aspect, a ratio of particles to cells of 1:1 or less is used. In one particular aspect, a preferred particle: cell ratio is 1:5. In further aspects, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one aspect, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular aspect, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In one aspect, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type. In one aspect, the most typical ratios for use are in the neighborhood of 1:1, 2:1 and 3:1 on the first day.

In further aspects of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative aspect, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further aspect, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one aspect the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, for example PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain aspects, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one aspect, a concentration of about 10 billion cells/ml, 9 billion/ml, 8 billion/ml, 7 billion/ml, 6 billion/ml, 5 billion/ml, or 2 billion cells/ml is used. In one aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is

used. In further aspects, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have therapeutic value and would be desirable to obtain in certain aspects. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In one embodiment, cells transduced with a nucleic acid encoding a CAR, e.g., a CAR described herein, are expanded, e.g., by a method described herein. In one embodiment, the cells are expanded in culture for a period of several hours (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18, 21 hours) to about 14 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days). In one embodiment, the cells are expanded for a period of 4 to 9 days. In one embodiment, the cells are expanded for a period of 8 days or less, e.g., 7, 6 or 5 days. In one embodiment, the cells, e.g., a CAR cell described herein, are expanded in culture for 5 days, and the resulting cells are more potent than the same cells expanded in culture for 9 days under the same culture conditions. Potency can be defined, e.g., by various T cell functions, e.g. proliferation, target cell killing, cytokine production, activation, migration, or combinations thereof. In one embodiment, the cells, e.g., a CAR cell described herein, expanded for 5 days show at least a one, two, three or four fold increase in cells doublings upon antigen stimulation as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., the cells expressing a CAR described herein, are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN-γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., a CAR cell described herein, expanded for 5 days show at least a one, two, three, four, five, ten fold or more increase in pg/ml of proinflammatory cytokine production, e.g., IFN-γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions.

In one aspect of the present invention, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In one aspect, the mixture may be cultured for 21 days. In one aspect of the invention the beads and the T cells are cultured together for about eight days. In one aspect, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives for the growth of cells known

to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO_2).

In one embodiment, the cells are expanded in an appropriate media (e.g., media described herein) that includes one or more interleukin that result in at least a 200-fold (e.g., 200-fold, 250-fold, 300-fold, 350-fold) increase in cells over a 14 day expansion period, e.g., as measured by a method described herein such as flow cytometry. In one embodiment, the cells are expanded in the presence of IL-15 and/or IL-7 (e.g., IL-15 and IL-7).

In embodiments, methods described herein, e.g., CAR-expressing cell manufacturing methods, comprise removing T regulatory cells, e.g., CD25+ T cells, from a cell population, e.g., using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. Methods of removing T regulatory cells, e.g., CD25+ T cells, from a cell population are described herein. In embodiments, the methods, e.g., manufacturing methods, further comprise contacting a cell population (e.g., a cell population in which T regulatory cells, such as CD25+ T cells, have been depleted; or a cell population that has previously contacted an anti-CD25 antibody, fragment thereof, or CD25-binding ligand) with IL-15 and/or IL-7. For example, the cell population (e.g., that has previously contacted an anti-CD25 antibody, fragment thereof, or CD25-binding ligand) is expanded in the presence of IL-15 and/or IL-7.

In some embodiments a CAR-expressing cell described herein is contacted with a composition comprising a interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide e.g., hetIL-15, during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a IL-15 polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a combination of both a IL-15 polypeptide and a IL-15 Ra polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising hetIL-15 during the manufacturing of the CAR-expressing cell, e.g., ex vivo.

In one embodiment the CAR-expressing cell described herein is contacted with a composition comprising hetIL-15 during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising an IL-15 polypeptide during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising both an IL-15 polypeptide and an IL-15Ra polypeptide during ex vivo expansion. In one embodiment the contacting results in the survival and proliferation of a lymphocyte subpopulation, e.g., CD8+ T cells.

T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (TH, CD4+) that is greater than the cytotoxic or suppressor T cell population. Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of TC cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell population comprising predominately of TH cells may be advantageous. Similarly, if an antigen-specific subset of TC cells has been isolated it may be beneficial to expand this subset to a greater degree.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

Once a CAR is constructed, various assays can be used to evaluate the activity of the molecule, such as but not limited to, the ability to expand T cells following antigen stimulation, sustain T cell expansion in the absence of re-stimulation, and anti-cancer activities in appropriate in vitro and animal models. Assays to evaluate the effects of a CAR are described in further detail below

Western blot analysis of CAR expression in primary T cells can be used to detect the presence of monomers and dimers. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, T cells (1:1 mixture of $CD4^+$ and $CD8^+$ T cells) expressing the CARs are expanded *in vitro* for more than 10 days followed by lysis and SDS-PAGE under reducing conditions. CARs containing the full length TCR-ζ cytoplasmic domain and the endogenous TCR-ζ chain are detected by western blotting using an antibody to the TCR-ζ chain. The same T cell subsets are used for SDS-PAGE analysis under non-reducing conditions to permit evaluation of covalent dimer formation.

In vitro expansion of CAR^+ T cells following antigen stimulation can be measured by flow cytometry. For example, a mixture of $CD4^+$ and $CD8^+$ T cells are stimulated with αCD3/αCD28 aAPCs followed by transduction with lentiviral vectors expressing GFP under the control of the promoters to be

analyzed. Exemplary promoters include the CMV IE gene, EF-1α, ubiquitin C, or phosphoglycerokinase (PGK) promoters. GFP fluorescence is evaluated on day 6 of culture in the $CD4^+$ and/or $CD8^+$ T cell subsets by flow cytometry. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Alternatively, a mixture of $CD4^+$ and $CD8^+$ T cells are stimulated with αCD3/αCD28 coated magnetic beads on day 0, and transduced with CAR on day 1 using a bicistronic lentiviral vector expressing CAR along with eGFP using a 2A ribosomal skipping sequence. Cultures are re-stimulated with antigen-expressing cells, such as multiple myeloma cell lines or K562 expressing the antigen, following washing. Exogenous IL-2 is added to the cultures every other day at 100 IU/ml. GFP^+ T cells are enumerated by flow cytometry using bead-based counting. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009).

Sustained CAR^+ T cell expansion in the absence of re-stimulation can also be measured. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, mean T cell volume (fl) is measured on day 8 of culture using a Coulter Multisizer III particle counter, a Nexcelom Cellometer Vision or Millipore Scepter, following stimulation with αCD3/αCD28 coated magnetic beads on day 0, and transduction with the indicated CAR on day 1.

Animal models can also be used to measure a CART activity. For example, xenograft model using human antigen-specific CAR^+ T cells to treat a primary human multiple myeloma in immunodeficient mice can be used. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, after establishment of MM, mice are randomized as to treatment groups. Different numbers of CART cells can be injected into immunodeficient mice bearing MM. Animals are assessed for disease progression and tumor burden at weekly intervals. Survival curves for the groups are compared using the log-rank test. In addition, absolute peripheral blood $CD4^+$ and $CD8^+$ T cell counts 4 weeks following T cell injection in the immunodeficient mice can also be analyzed. Mice are injected with multiple myeloma cells and 3 weeks later are injected with T cells engineered to express CAR, e.g., by a bicistronic lentiviral vector that encodes the CAR linked to eGFP. T cells are normalized to 45–50% input GFP^+ T cells by mixing with mock-transduced cells prior to injection, and confirmed by flow cytometry. Animals are assessed for leukemia at 1-week intervals. Survival curves for the CAR^+ T cell groups are compared using the log-rank test.

Assessment of cell proliferation and cytokine production has been previously described, *e.g.*, at Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, assessment of CAR-mediated proliferation is performed in microtiter plates by mixing washed T cells with K562 cells expressing the antigen or other antigen-expressing myeloma cells are irradiated with gamma-radiation prior to use. Anti-CD3 (clone OKT3) and anti- CD28 (clone 9.3) monoclonal antibodies are added to cultures with KT32-BBL cells to serve as a positive control for stimulating T-cell proliferation since these signals

support long-term CD8+ T cell expansion *ex vivo*. T cells are enumerated in cultures using CountBright™ fluorescent beads (Invitrogen, Carlsbad, CA) and flow cytometry as described by the manufacturer. CAR+ T cells are identified by GFP expression using T cells that are engineered with eGFP-2A linked CAR-expressing lentiviral vectors. For CAR+ T cells not expressing GFP, the CAR+ T cells are detected with biotinylated recombinant antigen protein and a secondary avidin-PE conjugate. CD4+ and CD8+ expression on T cells are also simultaneously detected with specific monoclonal antibodies (BD Biosciences). Cytokine measurements are performed on supernatants collected 24 hours following re-stimulation using the human TH1/TH2 cytokine cytometric bead array kit (BD Biosciences, San Diego, CA) according the manufacturer's instructions. Fluorescence is assessed using a FACScalibur flow cytometer, and data is analyzed according to the manufacturer's instructions.

Cytotoxicity can be assessed by a standard 51Cr-release assay. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, target cells (e.g., K562 lines expressing the antigen and primary multiple myeloma cells) are loaded with 51Cr (as NaCrO4, New England Nuclear, Boston, MA) at 37°C for 2 hours with frequent agitation, washed twice in complete RPMI and plated into microtiter plates. Effector T cells are mixed with target cells in the wells in complete RPMI at varying ratios of effector cell:target cell (E:T). Additional wells containing media only (spontaneous release, SR) or a 1% solution of triton-X 100 detergent (total release, TR) are also prepared. After 4 hours of incubation at 37°C, supernatant from each well is harvested. Released 51Cr is then measured using a gamma particle counter (Packard Instrument Co., Waltham, MA). Each condition is performed in at least triplicate, and the percentage of lysis is calculated using the formula: % Lysis = (ER– SR) / (TR – SR), where ER represents the average 51Cr released for each experimental condition. Alternatively, cytotoxicity can also be assessed using a Bright-Glo™ Luciferase Assay.

Imaging technologies can be used to evaluate specific trafficking and proliferation of CARs in tumor-bearing animal models. Such assays have been described, for example, in Barrett *et al.*, Human Gene Therapy 22:1575-1586 (2011). Briefly, NOD/SCID/$\gamma c^{-/-}$ (NSG) mice or other immunodeficient are injected IV with multiple myeloma cells followed 7 days later with CART cells 4 hour after electroporation with the CAR constructs. The T cells are stably transfected with a lentiviral construct to express firefly luciferase, and mice are imaged for bioluminescence. Alternatively, therapeutic efficacy and specificity of a single injection of CAR+ T cells in a multiple myeloma xenograft model can be measured as the following: NSG mice are injected with multiple myeloma cells transduced to stably express firefly luciferase, followed by a single tail-vein injection of T cells electroporated with CAR construct days later. Animals are imaged at various time points post injection. For example, photon-density heat maps of firefly luciferasepositive tumors in representative mice at day 5 (2 days before treatment) and day 8 (24 hr post CAR+ PBLs) can be generated.

Alternatively, or in combination to the methods disclosed herein, methods and compositions for one or more of: detection and/or quantification of CAR-expressing cells (*e.g.*, *in vitro* or *in vivo* (e.g., clinical monitoring)); immune cell expansion and/or activation; and/or CAR-specific selection, that involve the use of a CAR ligand, are disclosed. In one exemplary embodiment, the CAR ligand is an antibody that binds to the CAR molecule, e.g., binds to the extracellular antigen binding domain of CAR (e.g., an antibody that binds to the antigen binding domain, e.g., an anti-idiotypic antibody; or an antibody that binds to a constant region of the extracellular binding domain). In other embodiments, the CAR ligand is a CAR antigen molecule (e.g., a CAR antigen molecule as described herein).

In one aspect, a method for detecting and/or quantifying CAR-expressing cells is disclosed. For example, the CAR ligand can be used to detect and/or quantify CAR-expressing cells *in vitro* or *in vivo* (e.g., clinical monitoring of CAR-expressing cells in a patient, or dosing a patient). The method includes:

providing the CAR ligand (optionally, a labelled CAR ligand, e.g., a CAR ligand that includes a tag, a bead, a radioactive or fluorescent label);

acquiring the CAR-expressing cell (e.g., acquiring a sample containing CAR-expressing cells, such as a manufacturing sample or a clinical sample);

contacting the CAR-expressing cell with the CAR ligand under conditions where binding occurs, thereby detecting the level (e.g., amount) of the CAR-expressing cells present. Binding of the CAR-expressing cell with the CAR ligand can be detected using standard techniques such as FACS, ELISA and the like.

In another aspect, a method of expanding and/or activating cells (e.g., immune effector cells) is disclosed. The method includes:

providing a CAR-expressing cell (e.g., a first CAR-expressing cell or a transiently expressing CAR cell);

contacting said CAR-expressing cell with a CAR ligand, e.g., a CAR ligand as described herein), under conditions where immune cell expansion and/or proliferation occurs, thereby producing the activated and/or expanded cell population.

In certain embodiments, the CAR ligand is present on (e.g., is immobilized or attached to a substrate, e.g., a non-naturally occurring substrate). In some embodiments, the substrate is a non-cellular substrate. The non-cellular substrate can be a solid support chosen from, e.g., a plate (e.g., a microtiter plate), a membrane (e.g., a nitrocellulose membrane), a matrix, a chip or a bead. In

embodiments, the CAR ligand is present in the substrate (e.g., on the substrate surface). The CAR ligand can be immobilized, attached, or associated covalently or non-covalently (e.g., cross-linked) to the substrate. In one embodiment, the CAR ligand is attached (e.g., covalently attached) to a bead. In the aforesaid embodiments, the immune cell population can be expanded *in vitro* or *ex vivo*. The method can further include culturing the population of immune cells in the presence of the ligand of the CAR molecule, e.g., using any of the methods described herein.

In other embodiments, the method of expanding and/or activating the cells further comprises addition of a second stimulatory molecule, e.g., CD28. For example, the CAR ligand and the second stimulatory molecule can be immobilized to a substrate, e.g., one or more beads, thereby providing increased cell expansion and/or activation.

In yet another aspect, a method for selecting or enriching for a CAR expressing cell is provided. The method includes contacting the CAR expressing cell with a CAR ligand as described herein; and selecting the cell on the basis of binding of the CAR ligand.

In yet other embodiments, a method for depleting, reducing and/or killing a CAR expressing cell is provided. The method includes contacting the CAR expressing cell with a CAR ligand as described herein; and targeting the cell on the basis of binding of the CAR ligand, thereby reducing the number, and/or killing, the CAR-expressing cell. In one embodiment, the CAR ligand is coupled to a toxic agent (e.g., a toxin or a cell ablative drug). In another embodiment, the anti-idiotypic antibody can cause effector cell activity, e.g., ADCC or ADC activities.

Exemplary anti-CAR antibodies that can be used in the methods disclosed herein are described, e.g., in WO 2014/190273 and by Jena et al., "Chimeric Antigen Receptor (CAR)-Specific Monoclonal Antibody to Detect CD19-Specific T cells in Clinical Trials", PLOS March 2013 8:3 e57838, the contents of which are incorporated by reference. In one embodiment, the anti-idiotypic antibody molecule recognizes an anti-CD19 antibody molecule, e.g., an anti-CD19 scFv. For instance, the anti-idiotypic antibody molecule can compete for binding with the CD19-specific CAR mAb clone no. 136.20.1 described in Jena et al., PLOS March 2013 8:3 e57838; may have the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3, using the Kabat definition, the Chothia definition, or a combination of tthe Kabat and Chothia definitions) as the CD19-specific CAR mAb clone no. 136.20.1; may have one or more (e.g., 2) variable regions as the CD19-specific CAR mAb clone no. 136.20.1, or may comprise the CD19-specific CAR mAb clone no. 136.20.1. In some embodiments, the anti-idiotypic antibody was made according to a method described in Jena et al. In another embodiment, the anti-idiotypic antibody molecule is an anti-idiotypic antibody molecule described in WO 2014/190273. In some

embodiments, the anti-idiotypic antibody molecule has the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3) as an antibody molecule of WO 2014/190273 such as 136.20.1; may have one or more (e.g., 2) variable regions of an antibody molecule of WO 2014/190273, or may comprise an antibody molecule of WO 2014/190273 such as 136.20.1. In other embodiments, the anti-CAR antibody binds to a constant region of the extracellular binding domain of the CAR molecule, e.g., as described in WO 2014/190273. In some embodiments, the anti-CAR antibody binds to a constant region of the extracellular binding domain of the CAR molecule, e.g., a heavy chain constant region (e.g., a CH2-CH3 hinge region) or light chain constant region. For instance, in some embodiments the anti-CAR antibody competes for binding with the 2D3 monoclonal antibody described in WO 2014/190273, has the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3) as 2D3, or has one or more (e.g., 2) variable regions of 2D3, or comprises 2D3 as described in WO 2014/190273.

In some aspects and embodiments, the compositions and methods herein are optimized for a specific subset of T cells, e.g., as described in US Serial No. 62/031,699 filed July 31, 2014, the contents of which are incorporated herein by reference in their entirety. In some embodiments, the optimized subsets of T cells display an enhanced persistence compared to a control T cell, e.g., a T cell of a different type (e.g., $CD8^+$ or $CD4^+$) expressing the same construct.

In some embodiments, a $CD4^+$ T cell comprises a CAR described herein, which CAR comprises an intracellular signaling domain suitable for (e.g., optimized for, e.g., leading to enhanced persistence in) a $CD4^+$ T cell, e.g., an ICOS domain. In some embodiments, a $CD8^+$ T cell comprises a CAR described herein, which CAR comprises an intracellular signaling domain suitable for (e.g., optimized for, e.g., leading to enhanced persistence of) a $CD8^+$ T cell, e.g., a 4-1BB domain, a CD28 domain, or another costimulatory domain other than an ICOS domain.

In an aspect, described herein is a method of treating a subject, e.g., a subject having cancer. The method includes administering to said subject, an effective amount of:

1) a $CD4^+$ T cell comprising a CAR (the CAR^{CD4+})
comprising:
an antigen binding domain, e.g., an antigen binding domain described herein;
a transmembrane domain; and
an intracellular signaling domain, e.g., a first costimulatory domain, e.g., an ICOS domain; and

2) a $CD8^+$ T cell comprising a CAR (the CAR^{CD8+}) comprising:
an antigen binding domain, e.g., an antigen binding domain described herein;

a transmembrane domain; and

an intracellular signaling domain, e.g., a second costimulatory domain, e.g., a 4-1BB domain, a CD28 domain, or another costimulatory domain other than an ICOS domain;

wherein the CAR^{CD4+} and the CAR^{CD8+} differ from one another.

Optionally, the method further includes administering:

3) a second CD8+ T cell comprising a CAR (the second CAR^{CD8+}) comprising:

an antigen binding domain, e.g., an antigen binding domain described herein;

a transmembrane domain; and

an intracellular signaling domain, wherein the second CAR^{CD8+} comprises an intracellular signaling domain, e.g., a costimulatory signaling domain, not present on the CAR^{CD8+}, and, optionally, does not comprise an ICOS signaling domain.

Other assays, including those that are known in the art can also be used to evaluate the CAR constructs of the invention.

Therapeutic Application

Methods using Biomarkers for Evaluating CAR-Effectiveness, Subject Suitability, or Sample Suitability

In another aspect, the invention features a method of evaluating or monitoring the effectiveness of a CAR-expressing cell therapy in a subject (e.g., a subject having a cancer). The method includes acquiring a value of effectiveness to the CAR therapy, subject suitability, or sample suitability, wherein said value is indicative of the effectiveness or suitability of the CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, the subject is evaluated prior to receiving, during, or after receiving, the CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, a responder (e.g., a complete responder) has, or is identified as having, a greater level or activity of one, two, or more (all) of GZMK, PPF1BP2, or naïve T cells as compared to a non-responder.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater level or activity of one, two, three, four, five, six, seven, or more (e.g., all) of IL22, IL-2RA, IL-21, IRF8, IL8, CCL17, CCL22, effector T cells, or regulatory T cells, as compared to a responder.

In an embodiment, a relapser is a patient having, or who is identified as having, an increased level of expression of one or more of (e.g., 2, 3, 4, or all of) the following genes, compared to non relapsers: MIR199A1, MIR1203, uc021ovp, ITM2C, and HLA-DQB1 and/or a decreased levels of expression of one or more of (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all of) the following genes, compared

to non relapsers: PPIAL4D, TTTY10, TXLNG2P, MIR4650-1, KDM5D, USP9Y, PRKY, RPS4Y2, RPS4Y1, NCRNA00185, SULT1E1, and EIF1AY.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of an immune cell exhaustion marker, *e.g.*, one, two or more immune checkpoint inhibitors (e.g., PD-1, PD-L1, TIM-3 and/or LAG-3). In one embodiment, a non-responder has, or is identified as having, a greater percentage of PD-1, PD-L1, or LAG-3 expressing immune effector cells (e.g., CD4+ T cells and/or CD8+ T cells) (e.g., CAR-expressing CD4+ cells and/or CD8+ T cells) compared to the percentage of PD-1 or LAG-3 expressing immune effector cells from a responder.

In one embodiment, a non-responder has, or is identified as having, a greater percentage of immune cells having an exhausted phenotype, *e.g.*, immune cells that co-express at least two exhaustion markers, *e.g.*, co-expresses PD-1, PD-L1 and/or TIM-3. In other embodiments, a non-responder has, or is identified as having, a greater percentage of immune cells having an exhausted phenotype, *e.g.*, immune cells that co-express at least two exhaustion markers, *e.g.*, co-expresses PD-1 and LAG-3.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of PD-1/ PD-L1+/LAG-3+ cells in the CAR-expressing cell population compared to a responder (e.g., a complete responder) to the CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, a partial responder has, or is identified as having, a higher percentages of PD-1/ PD-L1+/LAG-3+ cells, than a responder, in the CAR-expressing cell population.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, an exhausted phenotype of PD1/ PD-L1+ CAR+ and co-expression of LAG3 in the CAR-expressing cell population.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of PD-1/ PD-L1+/TIM-3+ cells in the CAR-expressing cell population compared to the responder (e.g., a complete responder).

In some embodiments of any of the methods disclosed herein, a partial responders has, or is identified as having, a higher percentage of PD-1/ PD-L1+/TIM-3+ cells, than responders, in the CAR-expressing cell population.

In some embodiments of any of the methods disclosed herein, the presence of CD8+ CD27+ CD45RO- T cells in an apheresis sample is a positive predictor of the subject response to a CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, a high percentage of PD1+ CAR+ and LAG3+ or TIM3+ T cells in an apheresis sample is a poor prognostic predictor of the subject response to a CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, the responder (e.g., the complete or partial responder) has one, two, three or more (or all) of the following profile:

(i) has a greater number of CD27+ immune effector cells compared to a reference value, e.g., a non-responder number of CD27+ immune effector cells;

(ii) (i) has a greater number of CD8+ T cells compared to a reference value, e.g., a non-responder number of CD8+ T cells;

(iii) has a lower number of immune cells expressing one or more checkpoint inhibitors, e.g., a checkpoint inhibitor chosen from PD-1, PD-L1, LAG-3, TIM-3, or KLRG-1, or a combination, compared to a reference value, e.g., a non-responder number of cells expressing one or more checkpoint inhibitors; or

(iv) has a greater number of one, two, three, four or more (all) of resting T_{EFF} cells, resting T_{REG} cells, naïve CD4 cells, unstimulated memory cells or early memory T cells, or a combination thereof, compared to a reference value, e.g., a non-responder number of resting T_{EFF} cells, resting T_{REG} cells, naïve CD4 cells, unstimulated memory cells or early memory T cells.

In some embodiments of any of the methods disclosed herein, the cytokine level or activity of (vi) is chosen from one, two, three, four, five, six, seven, eight, or more (or all) of cytokine CCL20/MIP3a, IL17A, IL6, GM-CSF, IFN-γ, IL10, IL13, IL2, IL21, IL4, IL5, IL9 or TNFα, or a combination thereof. The cytokine can be chosen from one, two, three, four or more (all) of IL-17a, CCL20, IL2, IL6, or TNFa. In one embodiment, an increased level or activity of a cytokine is chosen from one or both of IL-17a and CCL20, is indicative of increased responsiveness or decreased relapse.

In embodiments, the responder, a non-responder, a relapser or a non-relapser identified by the methods herein can be further evaluated according to clinical criteria. For example, a complete responder has, or is identified as, a subject having a disease, e.g., a cancer, who exhibits a complete response, e.g., a complete remission, to a treatment. A complete response may be identified, e.g., using the NCCN Guidelines®, or Cheson et al, J Clin Oncol 17:1244 (1999) and Cheson et al., "Revised Response Criteria for Malignant Lymphoma", J Clin Oncol 25:579-586 (2007) (both of which are incorporated by reference herein in their entireties), as described herein. A partial responder has, or is identified as, a subject having a disease, e.g., a cancer, who exhibits a partial response, e.g., a partial remission, to a treatment. A partial response may be identified, e.g., using the NCCN Guidelines®, or Cheson criteria as described herein. A non-responder has, or is identified as, a subject having a disease, e.g., a cancer, who does not exhibit a response to a treatment, e.g., the patient has stable disease or progressive disease. A non-responder may be identified, e.g., using the NCCN Guidelines®, or Cheson criteria as described herein.

Alternatively, or in combination with the methods disclosed herein, responsive to said value, performing one, two, three four or more of:

administering e.g., to a responder or a non-relapser, a CAR-expressing cell therapy;

administered an altered dosing of a CAR-expressing cell therapy;

altering the schedule or time course of a CAR-expressing cell therapy;

administering, e.g., to a non-responder or a partial responder, an additional agent in combination with a CAR-expressing cell therapy, e.g., a checkpoint inhibitor, e.g., a checkpoint inhibitor described herein;

administering to a non-responder or partial responder a therapy that increases the number of younger T cells in the subject prior to treatment with a CAR-expressing cell therapy;

modifying a manufacturing process of a CAR-expressing cell therapy, e.g., enriching for younger T cells prior to introducing a nucleic acid encoding a CAR, or increasing the transduction efficiency, e.g., for a subject identified as a non-responder or a partial responder;

administering an alternative therapy, e.g., for a non-responder or partial responder or relapser; or

if the subject is, or is identified as, a non-responder or a relapser, decreasing the T_{REG} cell population and/or T_{REG} gene signature, *e.g.*, by one or more of CD25 depletion, administration of cyclophosphamide, anti-GITR antibody, or a combination thereof.

In certain embodiments, the subject is pre-treated with an anti-GITR antibody. In certain embodiment, the subject is treated with an anti-GITR antibody prior to infusion or re-infusion.

Combination Therapies

A CAR-expressing cell described herein may be used in combination with other known agents and therapies. Administered "in combination", as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to

a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

A CAR-expressing cell described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the CAR-expressing cell described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

The CAR therapy and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The CAR therapy can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

When administered in combination, the CAR therapy and the additional agent (e.g., second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, e.g., as a monotherapy. In certain embodiments, the administered amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, e.g., as a monotherapy. In other embodiments, the amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, that results in a desired effect (e.g., treatment of cancer) is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent used individually, e.g., as a monotherapy, required to achieve the same therapeutic effect.

In some embodiments, the invention discloses a combination therapy including a CAR-expressing cell therapy described herein, a virus comprising a nucleic acid molecule encoding a TNFα molecule, an IL-2 molecule, and/or an IL-7 molecule, and an additional therapeutic agent.

PD-1 inhibitor

In some embodiments, the additional therapeutic agent is a PD-1 inhibitor. In some embodiments, the PD-1 inhibitor is chosen from PDR001 (Novartis), Nivolumab (Bristol-Myers Squibb), Pembrolizumab (Merck & Co), Pidilizumab (CureTech), MEDI0680 (Medimmune),

REGN2810 (Regeneron), TSR-042 (Tesaro), PF-06801591 (Pfizer), BGB-A317 (Beigene), BGB-108 (Beigene), INCSHR1210 (Incyte), or AMP-224 (Amplimmune).

In one embodiment, the PD-1 inhibitor is an anti-PD-1 antibody molecule. In one embodiment, the PD-1 inhibitor is an anti-PD-1 antibody molecule as described in US 2015/0210769, published on July 30, 2015, entitled "Antibody Molecules to PD-1 and Uses Thereof," incorporated by reference in its entirety. In one embodiment, the anti-PD-1 antibody molecule comprises the CDRs, variable regions, heavy chains and/or light chains of BAP049-Clone-E or BAP049-Clone-B disclosed in US 2015/0210769. The antibody molecules described herein can be made by vectors, host cells, and methods described in US 2015/0210769, incorporated by reference in its entirety.

In one embodiment, the anti-PD-1 antibody molecule is Nivolumab (Bristol-Myers Squibb), also known as MDX-1106, MDX-1106-04, ONO-4538, BMS-936558, or OPDIVO®. Nivolumab (clone 5C4) and other anti-PD-1 antibodies are disclosed in US 8,008,449 and WO 2006/121168, incorporated by reference in their entirety. In one embodiment, the anti-PD-1 antibody molecule is Pembrolizumab (Merck & Co), also known as Lambrolizumab, MK-3475, MK03475, SCH-900475, or KEYTRUDA®. Pembrolizumab and other anti-PD-1 antibodies are disclosed in Hamid, O. *et al.* (2013) *New England Journal of Medicine* 369 (2): 134–44, US 8,354,509, and WO 2009/114335, incorporated by reference in their entirety. In one embodiment, the anti-PD-1 antibody molecule is Pidilizumab (CureTech), also known as CT-011. Pidilizumab and other anti-PD-1 antibodies are disclosed in Rosenblatt, J. *et al.* (2011) *J Immunotherapy* 34(5): 409-18, US 7,695,715, US 7,332,582, and US 8,686,119, incorporated by reference in their entirety. In one embodiment, the anti-PD-1 antibody molecule is MEDI0680 (Medimmune), also known as AMP-514. MEDI0680 and other anti-PD-1 antibodies are disclosed in US 9,205,148 and WO 2012/145493, incorporated by reference in their entirety. In one embodiment, the anti-PD-1 antibody molecule is REGN2810 (Regeneron). In one embodiment, the anti-PD-1 antibody molecule is PF-06801591 (Pfizer). In one embodiment, the anti-PD-1 antibody molecule is BGB-A317 or BGB-108 (Beigene). In one embodiment, the anti-PD-1 antibody molecule is INCSHR1210 (Incyte), also known as INCSHR01210 or SHR-1210. In one embodiment, the anti-PD-1 antibody molecule is TSR-042 (Tesaro), also known as ANB011.

Further known anti-PD-1 antibody molecules include those described, *e.g.*, in WO 2015/112800, WO 2016/092419, WO 2015/085847, WO 2014/179664, WO 2014/194302, WO 2014/209804, WO 2015/200119, US 8,735,553, US 7,488,802, US 8,927,697, US 8,993,731, and US 9,102,727, incorporated by reference in their entirety.

In one embodiment, the PD-1 inhibitor is a peptide that inhibits the PD-1 signaling pathway, *e.g.*, as described in US 8,907,053, incorporated by reference in its entirety. In one embodiment, the PD-1 inhibitor is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1

binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In one embodiment, the PD-1 inhibitor is AMP-224 (B7-DCIg (Amplimmune), *e.g.*, disclosed in WO 2010/027827 and WO 2011/066342, incorporated by reference in their entirety).

PD-L1 Inhibitors

In some embodiments, the additional therapeutic agent is a PD-L1 inhibitor. In some embodiments, the PD-L1 inhibitor is chosen from FAZ053 (Novartis), Atezolizumab (Genentech/Roche), Avelumab (Merck Serono and Pfizer), Durvalumab (MedImmune/AstraZeneca), or BMS-936559 (Bristol-Myers Squibb).

In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody molecule. In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody molecule as disclosed in US 2016/0108123, published on April 21, 2016, entitled "Antibody Molecules to PD-L1 and Uses Thereof," incorporated by reference in its entirety. In one embodiment, the anti-PD-L1 antibody molecule comprises the CDRs, variable regions, heavy chains and/or light chains of BAP058-Clone O or BAP058-Clone N disclosed in US 2016/0108123.

In one embodiment, the anti-PD-L1 antibody molecule is Atezolizumab (Genentech/Roche), also known as MPDL3280A, RG7446, RO5541267, YW243.55.S70, or TECENTRIQ™. Atezolizumab and other anti-PD-L1 antibodies are disclosed in US 8,217,149, incorporated by reference in its entirety. In one embodiment, the anti-PD-L1 antibody molecule is Avelumab (Merck Serono and Pfizer), also known as MSB0010718C. Avelumab and other anti-PD-L1 antibodies are disclosed in WO 2013/079174, incorporated by reference in its entirety. In one embodiment, the anti-PD-L1 antibody molecule is Durvalumab (MedImmune/AstraZeneca), also known as MEDI4736. Durvalumab and other anti-PD-L1 antibodies are disclosed in US 8,779,108, incorporated by reference in its entirety. In one embodiment, the anti-PD-L1 antibody molecule is BMS-936559 (Bristol-Myers Squibb), also known as MDX-1105 or 12A4. BMS-936559 and other anti-PD-L1 antibodies are disclosed in US 7,943,743 and WO 2015/081158, incorporated by reference in their entirety.

Further known anti-PD-L1 antibodies include those described, *e.g.*, in WO 2015/181342, WO 2014/100079, WO 2016/000619, WO 2014/022758, WO 2014/055897, WO 2015/061668, WO 2013/079174, WO 2012/145493, WO 2015/112805, WO 2015/109124, WO 2015/195163, US 8,168,179, US 8,552,154, US 8,460,927, and US 9,175,082, incorporated by reference in their entirety.

LAG-3 Inhibitors

In some embodiments, the additional therapeutic agent is a LAG-3 inhibitor. In some embodiments, the LAG-3 inhibitor is chosen from LAG525 (Novartis), BMS-986016 (Bristol-Myers Squibb), or TSR-033 (Tesaro).

In one embodiment, the LAG-3 inhibitor is an anti-LAG-3 antibody molecule. In one embodiment, the LAG-3 inhibitor is an anti-LAG-3 antibody molecule as disclosed in US 2015/0259420, published on September 17, 2015, entitled "Antibody Molecules to LAG-3 and Uses Thereof," incorporated by reference in its entirety. In one embodiment, the anti-LAG-3 antibody molecule comprises the CDRs, variable regions, heavy chains and/or light chains of BAP050-Clone I or BAP050-Clone J disclosed in US 2015/0259420.

In one embodiment, the anti-LAG-3 antibody molecule is BMS-986016 (Bristol-Myers Squibb), also known as BMS986016. BMS-986016 and other anti-LAG-3 antibodies are disclosed in WO 2015/116539 and US 9,505,839, incorporated by reference in their entirety. In one embodiment, the anti-LAG-3 antibody molecule is TSR-033 (Tesaro). In one embodiment, the anti-LAG-3 antibody molecule is IMP731 or GSK2831781 (GSK and Prima BioMed). IMP731 and other anti-LAG-3 antibodies are disclosed in WO 2008/132601 and US 9,244,059, incorporated by reference in their entirety. In one embodiment, the anti-LAG-3 antibody molecule is IMP761 (Prima BioMed).

Further known anti-LAG-3 antibodies include those described, *e.g.*, in WO 2008/132601, WO 2010/019570, WO 2014/140180, WO 2015/116539, WO 2015/200119, WO 2016/028672, US 9,244,059, US 9,505,839, incorporated by reference in their entirety.

In one embodiment, the anti-LAG-3 inhibitor is a soluble LAG-3 protein, *e.g.*, IMP321 (Prima BioMed), *e.g.*, as disclosed in WO 2009/044273, incorporated by reference in its entirety.

TIM-3 Inhibitors

In some embodiments, the additional therapeutic agent is a TIM-3 inhibitor. In some embodiments, the TIM-3 inhibitor is MGB453 (Novartis) or TSR-022 (Tesaro).

In one embodiment, the TIM-3 inhibitor is an anti-TIM-3 antibody molecule. In one embodiment, the TIM-3 inhibitor is an anti-TIM-3 antibody molecule as disclosed in US 2015/0218274, published on August 6, 2015, entitled "Antibody Molecules to TIM-3 and Uses Thereof," incorporated by reference in its entirety. In one embodiment, the anti-TIM-3 antibody molecule comprises the CDRs, variable regions, heavy chains and/or light chains of ABTIM3-hum11 or ABTIM3-hum03 disclosed in US 2015/0218274.

In one embodiment, the anti-TIM-3 antibody molecule is TSR-022 (AnaptysBio/Tesaro). In one embodiment, the anti-TIM-3 antibody molecule comprises one or more of the CDR sequences (or

collectively all of the CDR sequences), the heavy chain or light chain variable region sequence, or the heavy chain or light chain sequence of APE5137 or APE5121. APE5137, APE5121, and other anti-TIM-3 antibodies are disclosed in WO 2016/161270, incorporated by reference in its entirety. In one embodiment, the anti-TIM-3 antibody molecule is the antibody clone F38-2E2.

Further known anti-TIM-3 antibodies include those described, *e.g.*, in WO 2016/111947, WO 2016/071448, WO 2016/144803, US 8,552,156, US 8,841,418, and US 9,163,087, incorporated by reference in their entirety.

Chemotherapeutic agents

In some embodiments, the additional therapeutic agent is a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzamab, gemtuzumab, rituximab, tositumomab), an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (e.g., fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

General Chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa,

tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

Exemplary alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

Exemplary mTOR inhibitors include, e.g., temsirolimus; ridaforolimus (formally known as deferolimus, (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*, 23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and N^2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-

arginylglycyl-L-α-aspartylL-serine- (SEQ ID NO: 626), inner salt (SF1126, CAS 936487-67-1), and XL765.

Exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ, CAS 951209-71-5, available from IRX Therapeutics).

Exemplary anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

Exemplary vinca alkaloids include, e.g., vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

Exemplary proteosome inhibitors include bortezomib (Velcade®); carfilzomib (PX-171-007, (*S*)-4-Methyl-*N*-((*S*)-1-(((*S*)-4-methyl-1-((*R*)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((*S*)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and *O*-Methyl-*N*-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-*O*-methyl-*N*-[(1*S*)-2-[(2*R*)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]- L-serinamide (ONX-0912).

Biopolymer delivery methods

In some embodiments, one or more CAR-expressing cells as disclosed herein can be administered or delivered to the subject via a biopolymer scaffold, e.g., a biopolymer implant. Biopolymer scaffolds can support or enhance the delivery, expansion, and/or dispersion of the CAR-expressing cells described herein. A biopolymer scaffold comprises a biocompatible (e.g., does not substantially induce an inflammatory or immune response) and/or a biodegradable polymer that can be naturally occurring or synthetic.

Examples of suitable biopolymers include, but are not limited to, agar, agarose, alginate, alginate/calcium phosphate cement (CPC), beta-galactosidase (β-GAL), (1 ,2,3,4,6-pentaacetyl a-D-galactose), cellulose, chitin, chitosan, collagen, elastin, gelatin, hyaluronic acid collagen, hydroxyapatite, poly(3-hydroxybutyrate-co-3-hydroxy-hexanoate) (PHBHHx), poly(lactide),

poly(caprolactone) (PCL), poly(lactide-co-glycolide) (PLG), polyethylene oxide (PEO), poly(lactic-co-glycolic acid) (PLGA), polypropylene oxide (PPO), polyvinyl alcohol) (PVA), silk, soy protein, and soy protein isolate, alone or in combination with any other polymer composition, in any concentration and in any ratio. The biopolymer can be augmented or modified with adhesion- or migration-promoting molecules, e.g., collagen-mimetic peptides that bind to the collagen receptor of lymphocytes, and/or stimulatory molecules to enhance the delivery, expansion, or function, e.g., anti-cancer activity, of the cells to be delivered. The biopolymer scaffold can be an injectable, e.g., a gel or a semi-solid, or a solid composition.

In some embodiments, CAR-expressing cells described herein are seeded onto the biopolymer scaffold prior to delivery to the subject. In embodiments, the biopolymer scaffold further comprises one or more additional therapeutic agents described herein (e.g., another CAR-expressing cell, an antibody, or a small molecule) or agents that enhance the activity of a CAR-expressing cell, e.g., incorporated or conjugated to the biopolymers of the scaffold. In embodiments, the biopolymer scaffold is injected, e.g., intratumorally, or surgically implanted at the tumor or within a proximity of the tumor sufficient to mediate an anti-tumor effect. Additional examples of biopolymer compositions and methods for their delivery are described in Stephan et al., *Nature Biotechnology*, 2015, 33:97-101; and WO2014/110591.

Pharmaceutical compositions and treatments

Pharmaceutical compositions of the present invention may comprise a CAR-expressing cell, e.g., a plurality of CAR-expressing cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are in one aspect formulated for intravenous administration.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

In one embodiment, the pharmaceutical composition is substantially free of, e.g., there are no detectable levels of a contaminant, e.g., selected from the group consisting of endotoxin, mycoplasma, replication competent lentivirus (RCL), p24, VSV-G nucleic acid, HIV gag, residual anti-CD3/anti-

CD28 coated beads, mouse antibodies, pooled human serum, bovine serum albumin, bovine serum, culture media components, vector packaging cell or plasmid components, a bacterium and a fungus. In one embodiment, the bacterium is at least one selected from the group consisting of Alcaligenes faecalis, Candida albicans, Escherichia coli, Haemophilus influenza, Neisseria meningitides, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, and Streptococcus pyogenes group A.

When "an immunologically effective amount," "an anti-tumor effective amount," "a tumor-inhibiting effective amount," or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, in some instances 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. of Med. 319:1676, 1988).

In certain aspects, it may be desired to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present invention, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain aspects, T cells can be activated from blood draws of from 10cc to 400cc. In certain aspects, T cells are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, or 100cc.

The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient trans arterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one aspect, the T cell compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In one aspect, the CAR-expressing cell (e.g., T cell or NK cell) compositions of the present invention are administered by i.v. injection. The compositions of CAR-expressing cells (e.g., T cells or NK cells) may be injected directly into a tumor, lymph node, or site of infection.

In a particular exemplary aspect, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted ex vivo to select and/or isolate the cells of interest, e.g., immune effector

cells (e.g., T cells or NK cells). These immune effector cell (e.g., T cell or NK cell) isolates may be expanded by methods known in the art and treated such that one or more CAR constructs of the invention may be introduced, thereby creating a CAR-expressing cell (e.g., CAR T cell or CAR-expressing NK cell)of the invention. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR-expressing cells (e.g., CAR T cells or NK cells) of the present invention. In an additional aspect, expanded cells are administered before or following surgery.

In embodiments, lymphodepletion is performed on a subject, e.g., prior to administering one or more cells that express a CAR described herein. In embodiments, the lymphodepletion comprises administering one or more of melphalan, cytoxan, cyclophosphamide, and fludarabine.

The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. The dose for CAMPATH, for example, will generally be in the range 1 to about 100 mg for an adult patient, usually administered daily for a period between 1 and 30 days. The preferred daily dose is 1 to 10 mg per day although in some instances larger doses of up to 40 mg per day may be used (described in U.S. Patent No. 6,120,766).

In one embodiment, the CAR is introduced into immune effector cells (e.g., T cells or NK cells), e.g., using in vitro transcription, and the subject (e.g., human) receives an initial administration of CAR immune effector cells (e.g., T cells or NK cells)of the invention, and one or more subsequent administrations of the CAR immune effector cells (e.g., T cells or NK cells) of the invention, wherein the one or more subsequent administrations are administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR immune effector cells (e.g., T cells or NK cells) of the invention are administered to the subject (e.g., human) per week, e.g., 2, 3, or 4 administrations of the CAR immune effector cells (e.g., T cells or NK cells) of the invention are administered per week. In one embodiment, the subject (e.g., human subject) receives more than one administration of the CAR immune effector cells (e.g., T cells or NK cells) per week (e.g., 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR immune effector cells (e.g., T cells or NK cells) administrations, and then one or more additional administration of the CAR immune effector cells (e.g., T cells or NK cells) (e.g., more than one administration of the CAR immune effector cells (e.g., T cells or NK cells) per week) is administered to the subject. In another embodiment, the subject (e.g., human subject) receives more than one cycle of CAR immune effector cells (e.g., T cells or NK cells), and the

time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR immune effector cells (e.g., T cells or NK cells) are administered every other day for 3 administrations per week. In one embodiment, the CAR immune effector cells (e.g., T cells or NK cells) of the invention are administered for at least two, three, four, five, six, seven, eight or more weeks.

In one aspect, CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) are generated using lentiviral viral vectors, such as lentivirus. CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) generated that way will have stable CAR expression.

In one aspect, CAR-expressing cells, e.g., CARTs, are generated using a viral vector such as a gammaretroviral vector, e.g., a gammaretroviral vector described herein. CARTs generated using these vectors can have stable CAR expression.

In one aspect, CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) transiently express CAR vectors for 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days after transduction. Transient expression of CARs can be effected by RNA CAR vector delivery. In one aspect, the CAR RNA is transduced into the cell, e.g., T cell or NK cell, by electroporation.

A potential issue that can arise in patients being treated using transiently expressing CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) (particularly with murine scFv bearing CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells)) is anaphylaxis after multiple treatments.

Without being bound by this theory, it is believed that such an anaphylactic response might be caused by a patient developing humoral anti-CAR response, i.e., anti-CAR antibodies having an anti-IgE isotype. It is thought that a patient's antibody producing cells undergo a class switch from IgG isotype (that does not cause anaphylaxis) to IgE isotype when there is a ten to fourteen day break in exposure to antigen.

If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR therapy (such as those generated by RNA transductions), CAR-expressing cell (e.g., CART or CAR-expressing NK cell) infusion breaks should not last more than ten to fourteen days.

EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited

to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compositions of the present invention and practice the claimed methods. The following working examples specifically point out various aspects of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: Treatment of pancreatic cancer with combined mesothelin-redirected chimeric antigen receptor T cells and cytokine-armed oncolytic adenoviruses

Abstract

Pancreatic ductal adenocarcinoma (PDA) is characterized by its highly immunosuppressive tumor microenvironment (TME) that limits T cell infiltration and induces T cell hypofunction. Mesothelin-redirected chimeric antigen receptor T cell (meso-CAR T cell) therapy has shown feasibility and some efficacy in clinical trials but antitumor efficacy remains modest. This study tested the hypothesis that combined meso-CAR T cells with an oncolytic adenovirus expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 or OAd-TNFα-IL2 for short) would improve efficacy. OAd-TNFα-IL2 enhanced the anti-tumor efficacy of meso-CAR T cells in immunodeficient mice engrafted with human PDA and efficacy was associated with robustly increased tumor infiltrating lymphocytes (TILs), enhancing and prolonging CAR T cell function. Importantly, the combined therapy prevented metastasis, indicating a systemic effect of the intratumorally injected OAd-TNFα-IL2 and meso-CAR T cells. This study also evaluated this approach in a syngeneic mouse tumor model by combining adenovirus expressing murine TNF-α and IL-2 (Ad-mTNFα-mIL2) and mouse CAR T cells. This approach induced significant tumor regression in mice engrafted with highly aggressive and immunosuppressive PDA tumors. In contrast, multiple dosing of CAR T cells failed to suppress tumor growth. Ad-mTNFα-mIL2 increased both CAR T cell and host T cell infiltration to the tumor and altered host tumor immune status with M1 polarization of macrophages and increased dendritic cell maturation. These findings indicate that combining cytokine-armed oncolytic adenovirus to enhance the efficacy of CAR T cell therapy is a promising approach to overcome the immunosuppressive TME by inducing both CAR-dependent and CAR-independent host immunity for the treatment of PDA.

Introduction

CAR T cell therapy has shown significant efficacy in patients with CD19-positive acute lymphoblastic leukemia (Grupp SA, et al. The New England journal of medicine. 2013;368(16):1509-18) and lymphoma (Kochenderfer JN, et al. J Clin Oncol. 2015;33(6):540-9; Locke FL, et al. 2017;25(1):285-95). However, CAR T cell efficacy remains disappointing in the setting of solid tumors

(Beatty GL, and O'Hara M. Chimeric antigen receptor-modified T cells for the treatment of solid tumors: Defining the challenges and next steps. Pharmacol Ther. 2016; Newick K, Moon E, and Albelda SM. Mol Ther Oncolytics. 2016;3:16006). There are several factors that can potentially limit the efficacy of CAR T cell therapy in solid tumors and particularly, in pancreatic cancer. First, in solid tumors there are no ideal CAR targets like CD19: most tumor associated antigens are not uniformly expressed in all tumor cells, which can likely lead to tumor escape (O'Rourke DM, et al. Science translational medicine. 2017;9(399)). Moreover, in pancreatic cancer the TME is particularly immunosuppressive, inhibiting T cell infiltration and functions (Liyanage UK, et al. J Immunol. 2002;169(5):2756-61; Mukherjee P, et al. Glycoconj J. 2001;18(11-12):931-42; Moon EK, et al. Clin Cancer Res. 2014;20(16):4262-73). Therefore, developing strategies to address tumor immunosuppression and heterogeneity would represent a vertical advance in the field.

Mesothelin is a promising target for CAR T cell therapy as it is overexpressed in the majority of pancreatic cancers, mesotheliomas, ovarian cancers, and some lung cancers and it is not expressed on T cells (Hassan R, and Ho M. Eur J Cancer. 2008;44(1):46-53; Morello A, Sadelain M, and Adusumilli PS. Cancer discovery. 2016;6(2):133-46). In previous work, meso-CAR T cells were shown to be effective in mesothelioma xenograft models (Carpenito C, et al. Proc Natl Acad Sci U S A. 2009;106(9):3360-5). A phase I clinical trial using T cells engineered to express an anti-mesothelin CAR showed stable disease in two out of six patients (NCT01897415) (Beatty GL, et al. Journal of Clinical Oncology. 2015;33(15_suppl):3007-), however there is a clear unmet medical need to improve responses in PDA and in patients with other solid tumors.

Oncolytic viruses represent highly promising agents for the treatment of solid tumors, and an oncolytic herpes virus expressing GM-CSF was approved by the US FDA for the therapy of advanced melanoma based on therapeutic benefit demonstrated in a clinical study (Andtbacka RH, et al. J Clin Oncol. 2015;33(25):2780-8). Oncolytic adenoviruses (OAds) can be programmed to specifically target, replicate in and kill cancer cells while sparing normal cells. The release of virus progeny results in an exponential increase of the virus inoculum, which can cause direct tumor debulking while providing danger signals necessary to awaken the immune system (Lichty BD, et al. Nature reviews Cancer. 2014;14(8):559-67). Importantly, OAds can be genetically modified to express therapeutic transgenes selectively in the TME (Siurala M, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2016;24(8):1435-43; Nishio N, et al. Cancer Res. 2014;74(18):5195-205; Tanoue K, et al. Cancer Res. 2017;77(8):2040-51; Rosewell Shaw A, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2017). The feasibility and safety of OAds in human patients have been demonstrated in clinical trials (Kim KH, et al. Gynecol Oncol. 2013;130(3):518-24; Ranki T, et al. Journal for immunotherapy of cancer. 2016;4:17).

Another possible strategy to modulate the TME in favor of adoptive T cell therapy is the local administration of recombinant cytokines. In this regard, it was previously demonstrated that local tumor delivery of tumor necrosis factor-alpha (TNF-α) and Interleukin-2 (IL-2) can enhance the anti-tumor efficacy of adoptively transferred OT-I cells (Tähtinen S, et al. PLoS One. 2015;10(6):e0131242). These two cytokines were selected from a panel of cytokines that are used in clinical oncology in the US and Europe. When combined, TNF-α and IL-2 provide non-overlapping synergistic effects. TNF-α appears to be responsible for immunological danger signaling and T-cell trafficking, while IL-2 activates and propagates T-cells locally (Siurala M, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2016;24(8):1435-43).

Here OAd was engineered to express TNF-α and IL-2 within the TME and tested in combination with CAR T cells targeting mesothelin. It was tested if OAds expressing cytokines could improve the efficacy of CAR T cell therapy by: (i) enhancing and sustaining T cell function and trafficking to the tumor microenvironment, (ii) overcoming tumor heterogeneity in antigen expression, and (iii) reducing tumor immunosuppression.

Results

Combined OAd-TNFα-IL2 and meso-CAR T cells efficiently lyse target tumor cells

OAds were modified to express TNF-α and IL-2 (OAd-TNFα-IL2) (Havunen R, et al. Mol Ther Oncolytics. 2017;4:77-86) (FIG. 7A). Cytokine production and cell lysis induced by infection of PDA tumor lines were tested first. Pancreatic tumor cell lines infected with OAd-TNFα-IL2 secreted large amounts of cytokines and tumor cell lysis was induced in dose-dependent manner (FIGs. 7B and 7C). Incorporation of cytokine transgenes did not enhance the lytic activity of OAds but rather modestly decreased the lytic activity in vitro (FIG. 7C). This was not surprising as the additional payload possibly decreases the efficiency of virus replication.

Subsequently, it was tested whether OAd-TNFα-IL2 enhances the lytic activity of meso-CAR T cells using real time cell analysis. Target cell lines BxPC-3, Capan-2, AsPC-1 expressing various levels of mesothelin were tested; mesothelin was highly positive in Capan-2, medium positive in AsPC-1 but dim in BxPC-3 (FIG. 7D). BxPC-3 cells expressed very low levels of mesothelin and were resistant to meso-CAR T cells; meso-CAR T cell alone suppressed BxPC-3 cell growth transiently but the cells eventually started growing again. However, when OAd-TNFα-IL2 was combined, meso-CAR T cells efficiently lysed all three target tumor cells (FIG. 1A). Meso-CAR T cells suppressed Capan-2 tumor cells slowly. The combined OAd-TNFα-IL2 with meso-CAR T cells induced substantially more rapid lysis of Capan-2 cells (FIG. 1A). Meso-CAR T cells lysed AsPC-1 cells rapidly and there was no additional benefit of OAd-TNFα-IL2 combination therapy in this in vitro assay (FIG. 1A).

OAd-TNFα-IL2 activates T cells and induces T cell proliferation

To test how OAd-TNFα-IL2 enhances the killing activity of meso-CAR T cells, T cell proliferation and upregulation of early T cell activation marker CD69 upon co-incubation with OAd pre-infected tumor cell lines were analyzed. Consistent with the enhanced killing activity (FIG. 1A), CD69 upregulation was poorest when stimulated by BxPC-3 cells, while moderate with Capan-2 cells and the highest with AsPC-1 cells in the absence of OAd-TNFα-IL2 (FIGs. 1B and 1C). However, OAd-TNFα-IL2 induced enhanced CAR T cell responses, especially when the CAR T cells were stimulated with BxPC-3 cells. Similar to CD69 up-regulation, OAd-TNFα-IL2 pre-infection significantly improved CAR T cell proliferation when cultured with the PDA tumor cells (FIGs. 1D and 1E). Thus, OAd-TNFα-IL2 increased target cell killing by meso-CAR T cells presumably by enhancing the function of meso-CAR T cells. Importantly, the most significant enhancement of T cell responses was observed when mesothelin low expressing and meso-CAR T resistant BxPC-3 cells were targeted, suggesting that OAd-TNFα-IL2 can be used to augment CAR T-mediated killing, particularly when target antigen expression is limiting.

Combination of OAd-TNFα-IL2 with meso-CAR T cells causes tumor regression in an AsPC-1 tumor xenograft NSG mouse model.

To evaluate whether OAd-TNFα-IL2 improves the antitumor efficacy of meso-CAR T cells, combined OAd with CAR T cell therapy was tested in an AsPC-1 xenograft NSG mouse model (FIG. 2A). Meso-CAR T monotherapy suppressed tumor growth moderately and OAd-TNFα-IL2 monotherapy failed to suppress tumor growth although infection was confirmed in tumor IHC (FIG. 7D). On the other hand, combined OAd-TNFα-IL2 with meso-CAR T cells efficiently suppressed tumor growth and achieved higher rate of tumor regressions at the endpoint (FIGs. 2B and 2C). To determine the benefit of cytokine transgenes, the parental OAd and OAd-TNFα-IL2 in combination with meso-CAR T cells were compared in the same mouse model as FIG. 2B. OAd and OAd-TNFα-IL2 monotherapy similarly reduced tumor growth and mice injected with OAd had modestly improved survival compared to OAd-TNFα-IL2 monotherapy (FIGs. 2D and 2E), which may be because baseline killing activity of parental OAd is higher than that of OAd-TNFα-IL2 (FIG. 7C). However, importantly, only OAd-TNFα-IL2 enhanced the tumor regression by meso-CAR T cells and improved survival while parental OAd failed to induce additional efficacy of meso-CAR T cells. These results suggested that the encoded cytokines have clear benefit to enhance the in vivo antitumor efficacy of CAR T cells, enabling the regression of established PDA tumors that fail to respond to CAR T cell monotherapy.

Furthermore, mice treated with OAd, OAd-TNFα-IL2 or even the combination of parental OAd and meso-CAR T developed tumor metastasis to the lungs even if primary tumors were controlled (FIG. 2F). However, no mice treated with combined OAd-TNFα-IL2 and meso-CAR T died of tumor metastasis. These results suggest that locally activated meso-CAR T cells in tumor site by OAd-TNFα-

IL2 have the potential to target tumors systemically or to prevent PDA cells from egressing from tumors.

OAd-TNFα-IL2 increases tumor infiltrating T cells

The magnitude of T cell infiltration has a strong impact on the natural history of many types of cancer (Talmadge JE. Semin Cancer Biol. 2011;21(2):131-8). To determine how parental OAd and OAd-TNFα-IL2 affect tumor infiltrating T cells (TILs), NSG animals were treated as in FIG. 2A, and groups of mice were sacrificed at days 14 and 28 for analysis of TILs and tumors. Consistent with the experiment in FIG. 2A, tumors treated with the combination of OAd-TNFα-IL2 and meso-CAR T tend to be smaller in volume on day 28 (FIG. 9A). In histopathological and FCM analysis, tumors treated with the combination of OAd-TNFα-IL2 with meso-CAR T were infiltrated with significantly more CD4 and CD8 positive T cells compared to those treated with meso-CAR T monotherapy or in combination with the parental OAd (FIGs. 3A and 3B and FIG. 9B). The number of CD8+ TILs in IHC was inversely correlated with tumor volume in mice treated with OAd-TNFα-IL2 and meso-CAR T but did not correlate in any other treatment group (FIG. 3C).

OAd-TNFα-IL2 activates TILs and induces responses of T cells to the tumor

The function of TILs also has a strong impact on the outcome of cancer treatments (Talmadge JE. Semin Cancer Biol. 2011;21(2):131-8). Expression of activation markers by TILs was analyzed at day 28. CD8+ TILs in tumors treated with meso-CAR T in combination with OAd-TNFα-IL2 as well as parental OAd expressed higher activation markers CD95 and CD25 compared to meso-CAR T monotherapy (FIG. 3D) with the same trend in CD4+ TILs (FIG. 9C), which indicates that OAd and OAd-TNFα-IL2 activated TILs.

Subsequently, the cytokine profile of bulk tumors was analyzed for two purposes. The first was to assess whether OAd-TNFα-IL2 successfully delivered cytokine genes to the tumor and made tumor cells produce the corresponding cytokines, and the second was to assess whether T cells in the tumors are functional and responding to the tumors. As a note, TNF-α and IL-2 are potentially derived either from tumors infected with OAd-TNFα-IL-2, meso-CAR T cells or both, while human IFN-γ is expected to be produced only by meso-CAR T cells in this mouse model. As expected, TNF-α and IL-2 were detectable from tumors treated with OAd-TNFα-IL2 monotherapy, which indicates vector-mediated secretion of cytokine genes expressed in the PDA tumor cells (FIG. 3E). The levels of all cytokines (TNF-α, IL-2 and IFN-γ) were very low or undetectable in tumors treated with meso-CAR T monotherapy (FIG. 3E), which indicated that T cells in the tumors were hypofunctional and/or that the absolute number of CAR T cells responding to the tumor cells was low. On the other hand, higher levels of all three cytokines were detected from tumors treated with the combination of OAd-TNFα-IL2 with meso-CAR T cells than meso-CAR T cell monotherapy (FIG. 3E). The same trend was confirmed in serum, indicating that systemic levels of cytokines were produced by this procedure (FIG. 9D). While it

was impossible to separate the relative contribution of CAR T cell derived IL-2 and TNF-α from OAd delivered IL-2 and TNF-α, the systemic levels of IFN-γ which should be derived only from T cells, indicated that the CAR T cell function was enhanced.

Combined OAd-TNFα-IL2 with meso-CAR T cells induces decreased mesothelin intensity, which is associated with anti-tumor efficacy.

Target antigen decrease by adoptive cell therapies is an indicator of enhanced on-target effect after adoptive transfer with TCR modified T cells (Stromnes IM, et al. Cancer cell. 2015;28(5):638-52). To address how combining OAd-TNFα-IL2 with meso-CAR T cells affects target antigen expression, mesothelin levels on tumors were quantified. Meso-CAR T cells alone or in combination therapy induced decreases in mesothelin intensity within tumors at day 28, which is consistent with selection for tumor cell variants expressing lower levels of mesothelin (FIGs. 3F and 3G). Meso-CAR T cells induced the most significant decrease in mesothelin expression when combined with OAd-TNFα-IL2, and the mesothelin decrease correlated with anti-tumor efficacy (FIG. 3H). These results suggested that OAd-TNFα-IL2 enhanced on-target tumor lytic activity of meso-CAR T cells, which is associated with improved tumor regression.

OAd-TNFα-IL2 induces robust and sustained meso-CAR T cell accumulation to tumors

As flow cytometry and histological analysis at days 14 and 28 indicated that Ad-TNFα-IL2 increases CAR T cell recruitment (FIGs. 3A and 3B and FIG. 9B), T cell trafficking assays were performed to determine the precise kinetics of meso-CAR T cell distribution. As early as day 2 after the injection, meso-CAR T cells in combination with OAd-TNFα-IL2 started to show higher accumulation to the tumor site and reached a two-log higher accumulation compared with parental OAd at day 13 (FIGs. 4A and B). OAd-TNFα-IL2 also enhanced T cell engraftment in peripheral blood with the peak at day 21 (FIG. 4C). Interestingly, meso-CAR T cell expansion was transient in peripheral blood (PB) (FIG. 4C), while meso-CAR T cells persisted at the tumor site with sustained high-level accumulation for at least 50 days (FIGs. 4A and 4B). These results indicate that the enhanced proliferation of CAR T cells by OAd-TNFα-IL-2 is due to recognition of tumor associated mesothelin rather than xenogeneic antigens and GVHD.

T cell factors dominate the causes of tumor resistance rather than target antigen loss in meso-CAR T therapies

Target antigen loss and T cell hypofunction or insufficient tumor infiltration are major causes of tumor relapse for adoptive cell therapies (O'Rourke DM, et al. Science translational medicine. 2017;9(399)). To explore the causes of tumor resistance, tumors and TILs were analyzed late after treatment. The mice treated with meso-CAR T monotherapy and mice treated with combined OAd-TNFα-IL2 with meso-CAR T cells were sacrificed on day 57 in the experiment shown in FIGs. 2B and 2C. Five out of seven mice from meso-CAR T group and all mice out of seven mice from OAd-TNFα-

IL2+ meso-CAR T cell group were surviving at day 57. Four tumors from the OAd-TNFα-IL2+ meso-CAR T cell group had sustained regression, while three other tumors showed regrowth (FIGs. 5A and 5B). The tumors retained mesothelin expression, but the distribution was heterogenous with areas of negative or low expression (FIG. 5C). However, the residual mesothelin intensity did not correlate with tumor regression on day 57 (FIG. 5D), unlike on day 28 (FIG. 3H). On the other hand, the density of CD3+ TILs still clearly correlated with anti-tumor efficacy at this later time point (FIG. 5E). By flow cytometry, both CD4+ and CD8+ CAR T cells were recovered from the tumors (FIG. 5F). The fraction of CD4+ TILs expressing Ki67+ was inversely correlated with tumor volume (FIG. 5G). These results indicate that loss of mesothelin expression and CAR T cell hypofunction may both contribute to tumor recurrence and it is likely that loss of function or induction of exhaustion may be a major factor explaining delayed tumor progression in this model.

Syngeneic immunocompetent mouse PDA model to test combination therapy with CAR T cells and adenovirus expressing cytokines

Human xenograft NSG mouse models are useful tools to define the anti-tumor efficacy of new treatments. However, they lack a functional immune system and do not faithfully reproduce the human TME (Shultz LD, Ishikawa F, and Greiner DL. Nature reviews Immunology. 2007;7(2):118-30), which prevents evaluation of mechanisms of OAd therapy other than direct enhancement of CAR T cells. Therefore, engineered mouse T cells were established expressing an anti-mouse mesothelin CAR with mouse 4-1BB and murine CD3-ζ signaling domains (mmeso-CAR T) (FIGs. 10A and 10B). In vitro the mmeso-CAR T cells effectively lysed PDA7940b cells derived from the genetically engineered KrasLSL.G12D/+p53R172H/+ mouse model, while control h19-CAR T cells did not (FIG. 10C).

Established mouse pancreatic tumors are resistant to mouse-meso-CAR T cells but combining Ad-mTNFα-mIL2 enables tumor regression.

The anti-tumor efficacy of mmeso-CAR T cells was tested in combination with an adenovirus expressing murine TNF-α and murine IL-2 in immunocompetent mice engrafted with syngeneic PDA7940b tumor (FIG. 6A). Non-replicative serotype 5 adenovirus coding for murine TNF-α (Ad-mTNFα) and murine IL-2 (Ad-mIL2) with CMV promoters was used to deliver cytokine genes to mouse tumors, recognizing that murine cells are non-permissive for human adenoviral replication (Siurala M, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2016;24(8):1435-43). These viruses could infect PDA7940 cells and induce cytokine production in a dose-dependent manner in vitro (FIG. 10D). Established PDA7940b tumors were highly aggressive and even multiple weekly dosing of mmeso-CAR T cell infusions failed to suppress tumor growth. In contrast, combined Ad-mTNFα-mIL2 (one to one ratio mixture of Ad-mTNFα and Ad-mIL2) with mmeso-CAR T cells had robust antitumor efficacy even though control Ad-luc did not significantly

enhance the anti-tumor efficacy of mmeso-CAR T cells (FIG. 6B). Interestingly, Ad-mTNFα-mIL2 monotherapy or in combination with control h19-CAR T also showed partial antitumor efficacy, highlighting the importance of therapeutic transgenes in an immunocompetent setting which likely activate endogenous adaptive and innate antitumor activity.

Ad-mTNFα-mIL2 recruits both adoptively transferred meso-CAR T cells and host T cells to PDA tumors

It has been reported that mouse PDA tumors are "cold tumors" with low level T cell infiltration which is associated with poor responses to immunotherapies (Hingorani SR, et al. Cancer cell. 2005;7(5):469-83). To determine whether Ad-mTNFα-mIL2 could improve T cell infiltration into the tumor bed, CAR T cells were tracked after the first injection by BLI using CBR labeled CAR T cells. Meso-CAR T cells alone showed transient low-level engraftment. In contrast, Ad-mTNFα-IL2 induced robustly higher meso-CAR T accumulation that peaked on day 6 after injection (FIG. 6C). Ad-mTNFα-mIL2 also induced low level h19-CAR T cell accumulation although h19-CAR T cell alone did not accumulate in the tumor (FIG. 6C). TILs were also analyzed at day 12 by FCM using the same experimental schedule (FIG. 6A). Tumors were poorly infiltrated with adoptively transferred T cells and host T cells after mmeso-CAR T cell monotherapy. In contrast, Ad-mTNFα-mIL2 induced significantly higher donor and host CD4+ and CD8+ T cell infiltration in the tumor (FIG. 6D).

Ad-mTNFα-mIL2 alters host immune status and induces M1 polarization of macrophages and DC maturation

It has been reported that KPC tumors faithfully reproduce the highly immunosuppressive phenotype of human PDA (Hingorani SR, et al. Cancer cell. 2005;7(5):469-83). The above results suggested that mIL-2 and mTNF-α delivered by adenoviruses enhanced the antitumor effect of adoptively transferred mmeso-CAR T cells that may be additionally augmented by CAR-independent host immunity. M1 macrophages are critical components involved in innate antitumor immunity (Mantovani A, et al. Nature reviews Clinical oncology. 2017;14(7):399-416). To assess how Ad-mTNFα-mIL2 alters host immune suppression, the phenotypes of macrophages and dendritic cells (DCs) were analyzed. Ad-mTNFα-mIL2 clearly induced upregulation of CD80 and CD86 expression from F4/80+ macrophages both in tumors and spleens on day 1 after intratumoral injection (FIG. 6E), which is consistent with M1 polarization. In contrast, injection of control Ad-luc did not induce upregulation of CD80 and CD86. Moreover Ad-TNFα-mIL2 also induced CD11c+ DC maturation assessed by CD80 and CD86 upregulation both in tumors and spleen (FIG. 10E).

Ad-mTNFa-mIL2 creates TME with high immune cell attractive chemokines.

Chemokines are secondary pro-inflammatory mediators that are induced by primary pro-inflammatory mediators such as interleukins or tumor necrosis factors and have critical roles for recruitment of immune cells (Nagarsheth N, Wicha MS, and Zou W. Nature reviews Immunology. 2017;17(9):559-72). The alteration of chemokine expression in tumors was investigated at day 1 after

adenovirus injection. Ad-mTNFα-mIL2 but not Ad-luc clearly increased immune-cell attractive chemokines; monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 10 (CXCL-10) and RANTES (FIG. 6F and FIG. 10F), which are reported as TNF-α inducible chemokines and function to attract immune cells including T cells, NK cells, macrophages and DCs (Nakasone Y, et al. The American journal of pathology. 2012;180(1):365-74; Narumi S, et al. Cytokine. 2000;12(7):1007-16; Wolf G, et al. Kidney international. 1993;44(4):795-804). These results suggested that in addition to direct efficacy of mTNF-α and mIL-2 delivered by adenoviruses, secondarily-induced chemokines also contribute to recruit adoptively transferred CAR T cells and host immune cells to the tumors.

Taken together, these results suggest that Ad-mTNFα-mIL2 has the potential to enhance the efficacy of mmeso-CAR T therapy by altering the host immune status to a more proinflammatory anti-tumor state and by inducing both CAR-dependent and CAR-independent immune reactions against pancreatic cancer.

Discussion

The central issues for adoptive cell therapies against solid tumors are poor T cell infiltration, hypofunction of T cells in the tumors and tumor heterogeneity (Beatty GL, and O'Hara M. Chimeric antigen receptor-modified T cells for the treatment of solid tumors: Defining the challenges and next steps. Pharmacol Ther. 2016; Newick K, Moon E, and Albelda SM. Mol Ther Oncolytics. 2016;3:16006; O'Rourke DM, et al. Science translational medicine. 2017;9(399)). PDA is characterized by a strongly immunosuppressive TME, which can limit the efficacy of adoptively transferred T cells (Liyanage UK, et al. J Immunol. 2002;169(5):2756-61; Mukherjee P, et al. Glycoconj J. 2001;18(11-12):931-42; Moon EK, et al. Clin Cancer Res. 2014;20(16):4262-73). The goals of the present study were to establish effective therapy against PDA by combining two promising immunotherapeutic approaches, OAd expressing cytokines and CAR T cells and to reveal the mechanisms of synergy and resistance to this combination therapy in PDA in syngeneic and xenogeneic experiments. This work confirms and extends work by Nishio and colleagues, who found that OAd armed with RANTES and IL-15 augmented apoptosis in tumor cells exposed to CAR-T cells, while the intratumoral release of both RANTES and IL-15 attracted CAR-T cells and promoted their local survival, respectively, and increasing the overall survival of neuroblastoma bearing mice (Tanoue K, et al. Cancer Res. 2017;77(8):2040-51).

It has been reported that PDA typically has few TILs whereas the lymphocytic populations are predominantly found in the stroma surrounding the tumor mass (Wachsmann MB, Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2012;60(4):643-63), and recent studies suggest that the T cells in long term survivors with pancreatic cancer target neoantigens (Balachandran VP, et al. Nature. 2017;551(7681):512-6). The presence of

high number of TILs and extensive infiltration are major indicators of favorable patient prognosis and positive therapeutic responses in treating several solid tumors, including colorectal cancer (Huh JW, Lee JH, and Kim HR. Archives of surgery (Chicago, Ill : 1960). 2012;147(4):366-72), lung cancer (Zeng DQ, et al. Oncotarget. 2016;7(12):13765-81), and ovarian carcinomas (James FR, et al. BMC cancer. 2017;17(1):657; Li J, et al. Oncotarget. 2017;8(9):15621-31). Although it is not clear yet whether the intensity of CAR TILs directly correlates with its efficacy or patient outcome in solid tumors, it is reasonable to assume that augmentation of CAR-TILs will enhance antitumor efficacy. It was demonstrated here that OAd-TNFα-IL2 induced robust CAR T cell infiltration, which was clearly associated with enhanced antitumor efficacy. TNF-α is reported to induce T cell attractive chemokines (Son DS, et al. Journal of inflammation (London, England). 2013;10(1):25) and IL-2 itself has ability to induce proliferation and chemotaxis of T cells (Robbins RA, et al. J Lab Clin Med. 1986;108(4):340-5). Moreover, in the context of local delivery with adenovirus, TNF-α appears to mediate potent immunological danger signaling and trafficking of a T-cells, while IL-2 can sustain activity of T cells and NK cells (Siurala M, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2016;24(8):1435-43). Taken together the main mechanism of the combination cytokine-armed OAd plus CAR T cell therapy was associated with robustly enhanced and sustained T cell function in the tumor.

The mechanisms of the combination therapy were further investigated by focusing on target antigen expression. O'Rourke et al. reported from the first in human study of epidermal growth factor receptor variant III (EGFRvIII) redirected CAR T cell therapy against glioblastoma (GBM) that most of patients had specific loss or decrease of EGFRvIII expression in tumors resected after CAR T cell infusion (O'Rourke DM, et al. Science translational medicine. 2017;9(399)). This finding supports the idea that additional therapies to enhance epitope spreading such as this approach to combine OAds with CAR T may be needed to prevent antigen escape. It was found here that mesothelin intensity in the TME was a predictor of response early after treatment but not late at the time of tumor recurrence, where T cell factors such as the intensity of T cell infiltration and enhanced T cell function was an important factor. A unique advantage of the combined OAd and CAR T cell therapy is that OAds recognize and infect tumor cells using different antigens from the ones that CAR T cells typically recognize. The 5/3 chimeric OAds that were used herein recognize desmoglein-2 (DSG-2) (Wang H, et al. Nat Med. 2011;17(1):96-104), whereas CAR T cells recognize mesothelin. It is likely that OAds can target and suppress mesothelin low or negative tumor cells. Indeed, it was observed that OAd-TNFα-IL2 not only enhanced function of meso-CAR T cells but also directly suppressed tumor growth independently of meso-CAR T cells. From these observations, meso-CAR T cells and OAd-TNFα-IL2 are an attractive combination to improve the treatment of PDA by overcoming T cell hypofunction and tumor heterogeneity in target antigen expression.

Whether OAd-TNFα-IL2 could enhance the efficacy of meso-CAR T cells was investigated in an AsPC-1 tumor xenograft NSG mouse model. Even though parental OAd combined with meso-CAR T or parental OAd monotherapy could suppress primary tumors to some extent, these mice typically died of tumor metastasis even in cases where primary tumors were being controlled. On the other hand, mice treated with combined OAd-TNFα-IL2 and meso-CAR T cells did not develop tumor metastasis. These results suggested that locally activated meso-CAR T cells at the primary tumor site exerted systemic immunosurveillance to prevent tumor progression. This is an important aspect of this combination therapy, as patients frequently die from tumor metastasis in PDA (Ryan DP, Hong TS, and Bardeesy N. The New England journal of medicine. 2014;371(11):1039-49).

Regarding the immunocompetent model, although replication deficient adenoviruses do not directly lyse infected tumor cells, Ad-mTNFα-mIL2 rapidly and aggressively reduced tumor growth after virus injection. These results indicate that host innate immunity such as macrophages, DCs and NK cells contributed to tumor control. It was observed that Ad-mTNFα-mIL2 induced infiltration of non-CAR host T cells in addition to adoptively transferred donor CAR T cells. Ad-mTNFα-mIL2 modulated the tumor immunosuppression by promoting M1 polarization of macrophages and maturation of DCs. As it was previously found that OAd therapy can prime T cells that recognize additional tumor antigens via T cell receptors (TCR-T cells) in the ovalbumin expressing B16 melanoma mouse model (Tähtinen S, et al. Cancer Immunol Res. 2015;3(8):915-25), this reprogramming of the TME is expected to prime TCR-T cells that recognize tumor neoantigens by epitope spreading. Thus, the immunocompetent mouse model revealed that adenovirus could enhance the efficacy of CAR T cell therapy not only by directly enhancing CAR T cell functions but also by inducing CAR-independent immunity of host cells perhaps by eliciting neoantigen responses to overcome tumor heterogeneity and tumor escape caused by target antigen loss.

One limitation of this study is that it used established PDA tumor lines and primary PDA tumor xenografts have not been tested. In addition, NSG mice do not have a complete immune system and human xenograft models do not reproduce the human TME (Shultz LD, et al. Nature reviews Immunology. 2007;7(2):118-30). Recent studies indicated that the main impact of oncolytic polio and herpes virus therapy is its immune modulating effects (Brown MC, et al. Science translational medicine. 2017;9(408); Yin J, et al. Frontiers in oncology. 2017;7:136). The lack of an intact immune system in NSG mice may overlook these important immunological aspects of OAd therapy. Therefore, this combination therapy was tested in a fully immunocompetent setting. Our newly established mouse mesothelin redirected CAR T cells enabled testing the mmeso-CAR T cell therapies in a fully immunocompetent setting. This is the first report demonstrating that mouse CAR T cells targeting native syngeneic mouse tumor antigens in solid tumors augment the antitumor efficacy of adenoviral

delivery of cytokine transgenes. Even in the highly immunosuppressive PDA TME, Ad-mTNFα-mIL2 successfully enhanced the antitumor efficacy with mmeso-CAR T cells.

In summary, this study describes a novel combination therapy of oncolytic adenovirus expressing TNF-α and IL-2 with meso-CAR T cells in the treatment of PDA. Meso-CAR T cells failed to work effectively in PDA tumors as monotherapy, but combining of OAds expressing TNF-α and IL-2 enabled effective meso-CAR T cell therapy by modulating the immunosuppressive TME and inducing CAR-dependent and CAR-independent host immunities. In addition to the preclinical data reported here, the safety profiles of the same platform of OAds used in these experiments have already been evaluated as monotherapies in several clinical trials (Kim KH, et al. Gynecol Oncol. 2013;130(3):518-24; Ranki T, et al. Journal for immunotherapy of cancer. 2016;4:17), and provide a compelling rationale for CAR T cell combination therapy targeting PDA.

Methods

Generation of mesothelin-redirected human CAR T cells

Anti-mesothelin CAR containing the CD3-ζ signaling domain and the 4-1BB co-stimulatory domain were generated as previously described (Carpenito C, et al. Proc Natl Acad Sci U S A. 2009;106(9):3360-5). T cells from normal donors were transduced with lentivirus to express anti-mesothelin CAR.

Generation of mouse mesothelin-redirected mouse CAR T cells

Mmeso-CAR was constructed by fusing anti-mesothelin scFv to a mouse CD3-ζ signaling domain and a mouse 4-1BB co-stimulatory domain. The CAR was cloned into MSGV vector and packaged in the Plat E cell line to obtain the retrovirus. For the T cell transduction, spleens were harvested from CD45.1 donor mice and T cells were purified with mouse T cell selection beads (Stemcell Technologies Vancouver, Canada). Purified mouse T cells were activated with anti-mouse CD3 and CD28 antibody coated beads (Dynabeads, ThermoFisher, Waltham, MA) at a 2:1 ratio of bead:cell and then transduced with retroviral vector MSGV for CAR expression on the recombinant human fibronectin (Retronectin, Takara Bio USA, Mountain View, CA) coated plates at day 3 post beads stimulation. Recombinant mouse IL-2 (50 U/ml) was supplemented at day 1 and then complemented as fresh media containing 50 U/ml IL-2 every day. Mouse T cells were harvested and subjected to the in vivo experiments at day 5.

Cell lines

BxPC-3, Capan-2 and AsPC-1 cell lines were obtained from the American Type Culture Collection (ATCC) and authenticated by the University of Arizona Genetics Core. PDA7940b cell line which was established from KrasLSL.G12D/+p53R172H/+ (KPC) mouse pancreatic tumor model (Hingorani SR, et al. Cancer cell. 2005;7(5):469-83) was kindly provided by Dr. Gregory Beatty, the

University of Pennsylvania. All cell lines were tested for the presence of mycoplasma contamination (MycoAlert Mycoplasma Detection Kit, Lonza). BxPC-3 and AsPC-1 were maintained in culture with DMEM/F12 (1:1) (Gibco, LifeTechnologies, Grand Island, NY) supplemented with 20% FBS (Seradigm, Providence, UT) and 50IU/ml penicillin/streptomycin (Gibco, LifeTechnologies). Capan-2 and PDA7940b were maintained in culture with DMEM (Gibco, LifeTechnologies) supplemented with 10% FBS and 50IU/ml penicillin/streptomycin.

Adenovirus construction

The oncolytic adenovirus that has a 24-base pair deletion in constant region 2 of the E1A gene and chimeric serotype 5 shaft and serotype 3 knob (Ad5/3-D24 (OAd) was constructed and produced as has been described previously (Kanerva A, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2003;8(3):449-58). Parental OAd was modified by adding a tumor specific E2F1 promoter driving an E1 gene deleted at the retinoblastoma protein binding site (Δ24) and further modified by encoding TNF-α and IL-2 genes to deliver cytokine genes to target tumor cells (Ad5/3-E2F-D24-TNFa-IRES-IL2 or OAd-TNFa-IL2 for short) (Havunen R, et al. Mol Ther Oncolytics. 2017;4:77-86) (FIG. 7A). Replication-incompetent adenovirus serotype 5 expressing luciferase (Ad-luc) and adenovirus serotype 5 expressing murine TNF-α and murine IL-2 with the cytomegalovirus (CMV) promoter (Ad-mTNFa and Ad-mIL2, respectively) were constructed as described previously (Siurala M, et al. Molecular therapy: the journal of the American Society of Gene Therapy. 2016;24(8):1435-43; Tähtinen S, et al. Cancer Immunol Res. 2015;3(8):915-25).

Killing assay using xCELLigence real time cell analyzer (RTCA) and In vitro co-culture assay

Kinetic analysis of tumor cell lysis was performed using xCELLigence real time cell analyzer (ACEA Biosciences, San Diego, CA) as previously described (Kho D, et al. Biosensors (Basel). 2015;5(2):199-222). Ten thousand tumor cells were seeded to the e-plate. After 24-hour culture, tumor cells were infected with 30 virus particle (vp) /cell OAd-TNFα-IL2 or control media. After another 24-hour culture, T cells or control media were added. Cell index was recorded every 20 minutes. For coculture assay, tumor cells were seeded in 48-well plates and infected either with control media or 30 virus particle vp /cell of OAd or OAd-TNFα-IL2. After 24 hours, either meso-CAR T cells or control media were added at the 1:1 of effector:target ratio. The expression of an activation marker CD69 on T cells was analyzed at 72 hours. Total T cell number was determined at day 5 by flow cytometry (FCM) using CountBright fluorescent beads (Invitrogen).

Study approval

The University of Pennsylvania Institutional Animal Care and Use Committee approved all animal experiments and all animal procedures were performed in animal facility at the University of Pennsylvania in accordance with Federal and Institutional Animal Care and Use Committee requirements.

Mouse experiments

NOD-SCID-γ-chain-/- (NSG) and C57BL/6 (B6) (CD45.1 donor and CD45.2 recipient) mice were purchased from Jackson Laboratories. For the human pancreatic tumor xenograft model, NSG mice were subcutaneously injected with $2x10^6$ AsPC-1 cells in total 100µl PBS with 50% Matrigel (Corning, Corning, NY) into the right flanks. When the mean of tumor volumes reached $200mm^3$, mice were treated with either intratumoral injection of PBS, $0.95x10^9$ vp OAd or $3x10^9$ vp OAd-TNFα-IL2 in 50µl PBS followed by intravenous injection of either PBS, $1x10^6$ meso-CAR T cells or control h19-CAR T cells at day 3 after OAd injection ($0.95x10^9$ vp of OAd is equivalent to $3x10^9$ vp of OAd-TNFα-IL2 in the plaque formation unit). For the syngeneic mouse PDA tumor engrafted model, B6 mice were subcutaneously injected with $5x10^5$ PDA7940b cells in 100µl PBS in the right flanks. Established PDA7940b tumors were treated either with intratumoral injection of PBS, $1x10^9$ virus particle of control adenovirus (Ad-luc) or 1:1 mixture of Ad-mTNFa and Ad-mIL2 (total $1x10^9$vp) followed by intravenous injection of either PBS, $5x10^6$ mmeso-CAR T cells or human CD19 redirected chimeric antigen receptor mouse T cells (h19-CAR T cells) at day 1 after Ad injection. Mice were preconditioned with intraperitoneal injection of 120 mg/kg cyclophosphamide (Ctx) at 24 hours before the first T cell injection. Adenovirus and CAR T cell injections were repeated four times weekly. Tumor volumes were monitored by caliper measurement.

Tumor and peripheral blood analysis

Tumor dimensions were measured with calipers and the volumes were calculated; volume = length x $width^2/2$. Peripheral blood (PB) was obtained by retro-orbital bleeding or cardiac puncture and cell numbers of each subsets (CD3, CD4, CD8) were quantified using TruCount tubes (BD Biosciences, San Jose, CA). All experiments were performed in a blind, randomized fashion.

Tumor processing for FCM

AsPC-1 tumors were mechanically diced and then pushed through a 70 µm strainer twice using a syringe plunger and washed with RPMI. PDA7940b tumors were mechanically diced and dissociated by incubating in RPMI media with 100U/ml Collagenase I (Gibco, Life technologies) and 100 U/ml Collagenase IV (Gibco, Life technologies) at 37°C for 30 minutes. Dissociated cells were passed through a 70µm cell strainer twice and washed with RPMI media. Cells were then used for FCM analysis.

Tumor homogenate preparation for cytokine assay

Tumor pieces were homogenized with 300 µl ice-cold PBS supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) in Lysing Matrix D 2ml tubes (MP Biomedicals, Solon, OH) using FastPrepTM FP120 (Thermo Savant, Woburn, MA). Tumor homogenate was centrifuged and supernatant was analyzed by high sensitivity LUMINEX assay per manufacturer's instructions (Merck Millipore, Burlington, MA).

Immunohistochemistry and quantification of the staining

Immunohistochemistry (IHC) was performed on paraformaldehyde fixed and paraffin embedded samples. Tumors were cut on a microtome and stained according to the standard protocols. For adenovirus detection, sections were incubated overnight at 4°C with rabbit anti-adenovirus type 2/5 E1A antibody (polyclonal, sc-430, Santa Cruz Biotechnology, Dallas, Tx) at 1:200 dilution, and then incubated with polymer-HRP conjugated anti-rabbit antibody (DAKO, Hamburg, Germany) followed by diaminobenzidine substrate to develop the colorimetric reaction. CD8 was stained with Rabbit anti-CD8 Ab (polyclonal, RB-9009-P0, Thermo Fisher Scientific), mesothelin was stained with mouse mesothelin Ab (Ab-1, MS-1320-S0, Thermo Fisher Scientific) Stained slides were scanned by 20x magnification. The number of CD8 positive cells and mesothelin intensity were quantified with Aperio ImageScope software (Leica biosystems, Wetzlar Germany).

T cell trafficking assay

CAR T cell trafficking assays were performed as previously described using click beetle luciferase green (CBG) labeled CAR T cells (Barrett DM, Human gene therapy. 2013;24(8):717-27). Bioluminescent imaging was performed using a Xenogen IVIS-200 Spectrum camera and analyzed with LivingImage software (Caliper LifeSciences, Hopkinton, MA).

Flow cytometry and antibodies

For FCM analysis, antibodies specific for human CD45 (2D1) and mouse CD3 (17A2) were purchased from Affymetrix. Antibodies specific for human CD3 (OKT3), human CD4 (OKT4) human CD69 (FN50), human CD95 (DX2), mouse CD45.1 (A20), mouse CD45.2 (104), mouse CD3 (17A2), mouse CD11c (N418), mouse F4/80 (BM8) and CD80 (16-10A1) were purchased from BioLegend. Antibodies specific for human CD45 (HI30), human CD8 (SK1), human CD25 (2A3), human Ki67 (B56), mouse CD45 (30-F11), mouse CD4 (RM4-5), mouse CD8a (53-6.7), mouse NK-1.1(PK136), mouse CD11b (M1/70), mouse Ly6C (AL-21), mouse Ly6G (1A8) and mouse CD86 (GL1) were purchased from BD Bioscience. An antibody specific for human-mesothelin (K1) was purchased from Covance. Expression of meso-CAR on human T cells was detected with biotinylated goat anti-mouse IgG (specific for scFv of murine origin) (Jackson ImmunoResearch, West Grove, PA). Expression of mmeso-CAR on mouse T cells was detected with biotinylated goat anti-human IgG specific for scFv of human origin) (Jackson ImmunoResearch). Cells were stained for viability with violet amine-reactive viability dye (Invitrogen, Frederick, MD). Surface markers were stained in PBS containing 2% FBS. Intracellular (nuclear) staining was performed using the Foxp3/Transcription Factor Staining Kit (Affymetrix, Santa Clara, CA) per manufacturer's instructions. Mouse tumor and spleen samples were stained after Fc blocking using purified rat anti-mouse CD16/32 antibody (BD Bioscience). All data were collected by the Fortessa LSRII cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo ver.10 software (TreeStar, Eugene, OR).

Statistics

Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software, San Diego, CA). Two-tailed Student t test was used to compare the two groups and one-way ANOVA with Tukey's post-hoc test was used to compare three or more groups. Repeated measures two-way ANOVA with Bonferroni correction was used to compare the effect of multiple levels of two factors with multiple observations at each level (for tumor volumes, luminescence and T cell engraftment data). Strength of relationship between two factors was presented as Pearson's correlation coefficient. Pearson's R values are shown. Survival curves were drawn using the Kaplan–Meier method and the difference of two curves were compared with Log-rank test. P values of <0.05 were considered significant.

Example 2: Combined mesothelin-redirected chimeric antigen receptor T cells with cytokine-armed oncolytic adenoviruses for the treatment of pancreatic cancer

Pancreatic ductal adenocarcinoma (PDA) is characterized by its highly immunosuppressive tumor microenvironment (TME) that can limit T cell infiltration and induce T cell hypofunction. Mesothelin-redirected CAR T cell (meso-CAR T cell) therapy has shown feasibility and some efficacy in clinical trials but antitumor efficacy remains modest. This study tested the hypothesis that combined meso-CAR T cell therapy with an oncolytic adenovirus expressing TNF-α and IL-2 (Ad5/3-E2F-D24-TNFα-IRES-IL2 or TILT-123) would improve efficacy in syngeneic and xenogeneic mouse pancreatic tumor models. Ad5/3-E2F-D24-TNFα-IRES-IL2 enhanced the anti-tumor efficacy of human meso-CAR T cells in immune-deficient mice engrafted with human PDA and efficacy was associated with robustly increased tumor infiltrating lymphocytes (TILs) and enhanced CAR T cell function. Importantly, the combined therapy prevented metastasis while neither therapy alone prevented metastasis, indicating a systemic effect of the intratumorally injected Ad5/3-E2F-D24-TNFα-IRES-IL2 in combination with intravenously injected meso-CAR T cells. Combining Ad5/3-E2F-D24-TNFα-IRES-IL2 with meso-CAR T cells also induced mesothelin downregulation in tumors, indicating enhanced on-target CAR T cell activity. Further analyses of TILs at later phases of treatment revealed that Ki67 positive CD3+ TILs are associated with sustained tumor regression. We also evaluated this approach in a syngeneic mouse tumor model by combining adenovirus expressing murine TNF-α and murine IL-2 (Ad-mTNFα-mIL2) and newly established mouse CAR T cells. This approach induced significant tumor regression in mice engrafted with highly immunosuppressive PDA tumors. In contrast, multiple dosing of CAR T cells failed to suppress tumor growth. Ad-mTNFα-mIL2 increased both CAR T cell and host T cell infiltration to the tumor and altered host tumor immune status with M1 polarization of macrophages and increased dendritic cell maturation. These findings indicate that combining cytokine armed-oncolytic adenovirus to enhance the efficacy of CAR T cells is a promising approach to overcome the

immunosuppressive TME by inducing both CAR-dependent and CAR-independent host immunity for the treatment of PDA.

EQUIVALENTS

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific aspects, it is apparent that other aspects and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such aspects and equivalent variations.

What is claimed is:

1. A composition comprising a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell") for use in the treatment of a subject having a disease associated with expression of mesothelin, e.g., a subject having a cancer, wherein:

the subject has received, is receiving, or is about to receive:

(i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

2. A method of treating a subject having a disease associated with expression of mesothelin, e.g., a method of treating a subject having a cancer, comprising administering to the subject:

a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein:

the subject has received, is receiving, or is about to receive:

(i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

3. The composition of claim 1 or method of claim 2, wherein the subject has received the virus (or the first virus and the second virus).

4. A composition comprising:

(i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule, for use in the treatment of a subject having a disease associated with expression of mesothelin, e.g., a subject having a cancer, wherein:

the subject has received, is receiving, or is about to receive a cell (e.g., a population of cells) that expresses a CAR molecule that binds to mesothelin ("mesothelin CAR-expressing cell").

5. A method of treating a subject having a disease associated with expression of mesothelin, e.g., a method of treating a subject having a cancer, comprising administering to the subject:

(i) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(ii) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(iii) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(iv) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule, for use in the treatment of a subject having a disease associated with expression of mesothelin, e.g., a subject having a cancer, wherein:

the subject has received, is receiving, or is about to receive a cell (e.g., a population of cells) that expresses a CAR molecule that binds to mesothelin ("mesothelin CAR-expressing cell").

6. The composition of claim 4 or method of claim 5, wherein the subject is about to receive the mesothelin CAR-expressing cell.

7. The composition or method of any one of claims 1-6, wherein the subject has a cancer, wherein the cancer exhibits or is identified as exhibiting heterogeneous expression of mesothelin, e.g., wherein less than 90%, 80%, 70%, 60%, or 50% of cancer cells express or are identified as expressing mesothelin.

8. The composition or method of any one of claims 1-7, wherein the virus, the first virus, and/or the second virus are non-oncolytic virus.

9. The composition or method of any one of claims 1-8, wherein the virus comprising the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule, the first virus comprising the nucleic acid molecule encoding the TNFα molecule, and/or the second virus comprising the nucleic acid molecule encoding the IL-2 molecule, have one, two, or all of the following properties:

(i) mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, activates the mesothelin CAR-expressing cell, e.g., at a level at least about 20, 50, 100, 150, or 200% higher than, e.g., mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, infected with an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of an activation marker (e.g., CD69), e.g., as assessed using methods described in Example 1 with respect to FIG. 1B or 1C,

(ii) mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, increases proliferation of the mesothelin CAR-expressing cell, e.g., at a level at least about 20, 50, 100, 150, or 200% higher than, e.g., mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, infected with an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 1D or 1E, or

(iii) the lytic activity of the mesothelin CAR-expressing cell against mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, e.g., BxPC-3 cells, infected with the virus, or the first and/or second virus, is increased by at least about 1, 2, 3, 4, or 5-fold, e.g., compared to the lytic activity of the mesothelin CAR-expressing cell against otherwise similar mesothelin-expressing target cells, e.g., mesothelin-expressing tumor cells, that are not infected with the virus, or the first and/or second virus, e.g., as assessed using methods described in Example 1 with respect to FIG. 1A.

10. The composition or method of any one of claims 1-9, wherein the administration of the mesothelin CAR-expressing cell and (a) the virus comprising the nucleic acid molecule encoding the TNFα

molecule and/or the IL-2 molecule, or (b) the first virus comprising the nucleic acid molecule encoding the TNFα molecule, and the second virus comprising the nucleic acid molecule encoding the IL-2 molecule,

results in one or more (2, 3, 4, 5, 6, 7, 8, 9, or all) of the following properties:

(i) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, produces a reduction in tumor load, e.g., a reduction of at least about 0.5, 1, 2, 5, 10, or 200-fold, e.g., about 15, 20, 25, 30, 35, 40, or 45 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2B, 2C, 2D, or 6B.

(ii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases survival of the subject, e.g., by at least about 2, 5, 10, 20, 50, or 100-fold, e.g., about 20, 40, 60, 80, or 100 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2E,

(iii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, reduces tumor metastasis, e.g., tumor metastasis to the lung, by at least about 20, 40, 60, or 80%, e.g., about 20, 50, 100, or 150 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2F,

(iv) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases expression of a cytokine molecule, e.g., IFN-γ, by the mesothelin CAR-expressing cell, by at least about 1, 2, 3, or 4-fold, e.g., about 5, 10, 15, or 20 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise

similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 3E,

(v) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases infiltration of the mesothelin CAR-expressing cell into a tumor, e.g., by at least about 5, 10, 25, 50, 75, 100, 125, 150, 175, or 200-fold, e.g., about 2, 4, 6, 8, 10, 12, 13, 14, 16, 18, 20, 30, 40, or 50 days after the administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 4A, 4B, or 6C,

(vi) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases infiltration of endogenous T cells, e.g., CD4+ and/or CD8+ T cells, into a tumor, e.g., by at least about 1, 2, 5, 10, 20, 30, or 50-fold, e.g., about 15, 20, 25, 30, or 35 days after the administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 3A, 3B, 9B, or 6D,

(vii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, activates tumor infiltrating lymphocytes (TILs), e.g., by at least about 20, 30, 40, or 50%, e.g., about 10, 20, 30, or 40 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of an activation marker, e.g., as measured by expression of CD69 and/or CD25, e.g., as assessed using methods described in Example 1 with respect to FIG. 3D or 9C,

(viii) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases M1 polarization of macrophages, e.g., by at least about 20, 30, 40, or 50%, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to

administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of CD80 and/or CD86 on macrophages, e.g., as assessed using methods described in Example 1 with respect to FIG. 6E,

(ix) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases maturation of dendritic cells, e.g., maturation of CD11c+ dendritic cells, e.g., by at least about 20, 30, 40, or 50%, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as measured by expression of CD80 and/or CD86 on dendritic cells, e.g., as assessed using methods described in Example 1 with respect to FIG. 10E, or

(x) administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in a subject having a cancer, e.g., a pancreatic cancer, increases the level of a chemokine in the subject, e.g., an immune-cell attractive chemokine, e.g., a TNF-α inducible chemokine, e.g., one, two, or all of: monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 10 (CXCL-10) and RANTES, by at least about 5, 10, 25, 50, 75, 100, 125, 150, 175, or 200-fold, e.g., about 1, 2, 3, 5, or 10 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 6F or 10F.

11. The composition or method of any one of claims 1-10, wherein the virus (or the first virus and the second virus) is administered prior to the administration of the mesothelin CAR-expressing cell, e.g., about 1, 2, 3, 4, or 5 days prior to the administration of the mesothelin CAR-expressing cell.

12. The composition or method of any one of claims 1, 2, 4, 5, or 7-10, wherein the virus (or the first virus and the second virus) is administered after the administration of the mesothelin CAR-expressing cell, e.g., about 1, 2, 3, 4, or 5 days after the administration of the mesothelin CAR-expressing cell.

13. The composition or method of any one of claims 1-12, wherein the first virus and the second virus are administered simultaneously.

14. The composition or method of any one of claims 1-13, wherein (i) the mesothelin CAR-expressing cell and (ii) the virus (or the first virus and the second virus) is administered for a first treatment interval, wherein the first treatment interval comprises a single dose of the mesothelin CAR-expressing cell, and a single dose of the virus (or the first virus and the second virus).

15. The composition or method of claim 14, wherein the first treatment interval is initiated upon administration of the single dose of the virus (or the first virus and the second virus), and completed upon administration of the single dose of the mesothelin CAR-expressing cell.

16. The composition or method of claim 14 or 15, wherein the single dose of the mesothelin CAR-expressing cell is administered, e.g., about 1, 2, 3, 4, or 5 days after the administration of the single dose of the virus (or the first virus and the second virus).

17. The composition or method of any one of claims 14-16, wherein the first treatment interval is repeated, e.g., one or more times, e.g., 1, 2, 3, 4, or 5 more times.

18. The composition or method of any one of claims 14-17, wherein the first treatment interval is followed by one or more, e.g., 1, 2, 3, 4, or 5, subsequent treatment intervals.

19. The composition or method of claim 18, wherein the one or more subsequent treatment intervals are different from the first treatment interval.

20. The composition or method of any one of claims 1-19, wherein the virus (or the first virus and the second virus) is administered systemically or locally.

21. The composition or method of claim 20, wherein the virus (or the first virus and the second virus) is administered locally, optionally wherein the subject has a cancer and the virus (or the first virus and the second virus) is administered intratumorally.

22. The composition or method of any one of claims 1-21, wherein the mesothelin CAR-expressing cell is administered intravenously.

23. The composition or method of any one of claims 1-22, wherein the first virus and the second virus are the same virus.

24. The composition or method of any one of claims 1-22, wherein the first virus and the second virus are different viruses.

25. The composition or method of any one of claims 1-22, wherein the virus, the first virus, and/or the second virus are chosen from adenovirus, herpes simplex virus, retrovirus, parvovirus, vaccinia virus, sinbis virus, influenza virus, or RNA virus (e.g., reovirus, newcastle disease virus (NDV), measles virus, or vesicular stomatitis virus (VSV)).

26. The composition or method of any one of claims 1-7 or 9-25, wherein the virus, the first virus, and/or the second virus are oncolytic virus, e.g., oncolytic adenovirus, oncolytic adeno-associated virus, oncolytic Herpes Simplex Virus (HSV), oncolytic parvovirus, oncolytic retrovirus, oncolytic lentivirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza virus, oncolytic reovirus, oncolytic Newcastle disease virus (NDV), oncolytic measles virus, oncolytic vesicular stomatitis virus (VSV), oncolytic poliovirus, oncolytic poxvirus, oncolytic Seneca Valley virus, oncolytic coxsackievirus, oncolytic enterovirus, oncolytic myxoma virus, or oncolytic maraba virus, optionally wherein the subject has cancer cells expressing desmoglein-2 (DSG-2).

27. The composition or method of any one of claims 1-26, wherein:

the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are encapsulated in a single viral particle,

the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are encapsulated in a single viral particle, or

the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are encapsulated in a single viral particle.

28. The composition or method of any one of claims 1-27, wherein:

(i) the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule,

(ii) the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule,

(iii) the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule, or

(iv) the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on a single nucleic acid molecule, e.g., a single DNA molecule or a single mRNA molecule.

29. The composition or method of any one of claims 1-28, wherein:

(i) the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site,

(ii) the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site,

(iii) the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site, or

(iv) the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are separated by a nucleic acid molecule encoding a self-cleavage site, e.g., a 2A site, or an internal ribosomal entry site.

30. The composition or method of any one of claims 1-27, wherein:

(i) the nucleic acid molecule encoding the TNFα molecule and/or the IL-2 molecule comprises a sequence encoding the TNFα molecule and a sequence encoding the IL-2 molecule, wherein the sequence encoding the TNFα molecule and the sequence encoding the IL-2 molecule are disposed on separate nucleic acid molecules,

(ii) the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on separate nucleic acid molecules,

(iii) the nucleic acid molecule encoding the TNFα molecule and the nucleic acid molecule encoding the IL-7 molecule are disposed on separate nucleic acid molecules, or

(iv) the nucleic acid molecule encoding the IL-7 molecule and the nucleic acid molecule encoding the IL-2 molecule are disposed on separate nucleic acid molecules.

31. The composition or method of any one of claims 1-30, wherein the CAR molecule that binds to mesothelin comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2.

32. The composition or method of claim 31, wherein the mesothelin binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 according to the HC CDR amino acid sequences in Table 4 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and a LC CDR1, a LC CDR2, and a LC CDR3 according to the LC CDR amino acid sequences in Table 5 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

33. The composition or method of claim 31 or 32, wherein the mesothelin binding domain comprises:

(i) a heavy chain variable region (VH) of any mesothelin binding domain listed in Table 2 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

(ii) a light chain variable region (VL) of any mesothelin binding domain listed in Table 2 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

34. The composition or method of any one of claims 31-33, wherein the mesothelin binding domain comprises:

(i) an scFv of any mesothelin binding domain listed in Table 2 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), or

(ii) an amino acid sequence chosen from: SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 275, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, or SEQ ID NO: 62 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

35. The composition or method of any one of claims 1-34, wherein the CAR molecule that binds to mesothelin comprises:

(i) a CAR sequence listed in Table 2 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1) (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), or

(ii) an amino acid sequence chosen from: SEQ ID NO: 67; SEQ ID NO: 73, SEQ ID NO: 278, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, or SEQ ID NO: 86 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1) (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

36. The composition or method of any one of claims 1-35, wherein the CAR molecule that binds to mesothelin comprises:

(i) HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NOs: 138, 156, and 179, respectively; and LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NOs: 203, 227, and 251, respectively,

(ii) the amino acid sequence of SEQ ID NO: 43, or

(iii) the amino acid sequence of SEQ ID NO: 67 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1).

37. The composition or method of any one of claims 1-35, wherein the CAR molecule that binds to mesothelin comprises:

(i) HC CDR1, HC CDR2, and HC CDR3 of SEQ ID NOs: 144, 162, and 185, respectively; and LC CDR1, LC CDR2, and LC CDR3 of SEQ ID NOs: 209, 233, and 257, respectively,

(ii) the amino acid sequence of SEQ ID NO: 49, or

(iii) the amino acid sequence of SEQ ID NO: 73 with or without the signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1).

38. The composition or method of any one of claims 1-37, wherein the CAR molecule that binds to mesothelin comprises a transmembrane domain, optionally wherein the transmembrane domain comprises a transmembrane domain from a protein chosen from the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 or CD154, optionally wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

39. The composition or method of any one of claims 1-38, wherein the CAR molecule that binds to mesothelin comprises a mesothelin binding domain and a transmembrane domain, wherein the mesothelin binding domain is connected to the transmembrane domain by a hinge region, optionally wherein the hinge region comprises an amino acid sequence chosen from SEQ ID NO: 2, 3, or 4 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

40. The composition or method of any one of claims 1-39, wherein the CAR molecule that binds to mesothelin comprises a primary signaling domain, optionally wherein the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), FcεRI, DAP10, DAP12, or CD66d, optionally wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 9 or 10 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

41. The composition or method of any one of claims 1-40, wherein the CAR molecule that binds to mesothelin comprises a costimulatory signaling domain, optionally wherein the costimulatory signaling domain comprises a functional signaling domain derived from a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signalling lymphocytic

activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, or a ligand that specifically binds with CD83, optionally wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 7 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

42. The composition or method of any one of claims 1-41, wherein the CAR molecule that binds to mesothelin comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta.

43. The composition or method of any one of claims 1-42, wherein the CAR molecule that binds to mesothelin comprises the amino acid sequence of SEQ ID NO: 7 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions) and the amino acid sequence of SEQ ID NO: 9 or 10 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), optionally wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10.

44. The composition or method of any one of claims 1-43, wherein the mesothelin CAR-expressing cell is a cell comprising a nucleic acid molecule encoding the CAR molecule, optionally wherein the nucleic acid molecule encoding the CAR molecule is an RNA molecule, e.g., an in vitro transcribed RNA molecule.

45. The composition or method of any one of claims 1-44, wherein the mesothelin CAR-expressing cell is a cell comprising a vector comprising a nucleic acid molecule encoding the CAR molecule, optionally wherein the vector is a lentiviral vector.

46. The composition or method of any one of claims 1-45, wherein the mesothelin CAR-expressing cell is a T cell (e.g., an autologous or allogeneic T cell) or an NK cell (e.g., an autologous or allogeneic NK cell).

47. The composition or method of claim 1-46, wherein the subject has a cancer, optionally wherein the subject has a solid tumor.

48. The composition or method of claim 47, wherein the cancer is chosen from one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma , breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof.

49. The composition or method of claim 47, wherein the cancer is chosen from chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary

cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

50. The composition or method of claim 47, wherein the cancer exhibits or is identified as exhibiting heterogeneous expression of mesothelin, e.g., wherein less than 90%, 80%, 70%, 60%, or 50% of cancer cells express or are identified as expressing mesothelin.

51. The composition or method of any one of claims 1-50, further comprising administering an additional therapeutic agent, e.g., an anti-cancer agent.

52. The composition or method of any one of claims 1-51, wherein the subject is a mammal, e.g., a human.

53. The composition or method of any one of claims 1-52, wherein the cell is a T cell or NK cell.

54. The composition or method of claim 53, wherein the cell is autologous to the subject.

55. The composition or method of claim 53, wherein the cell is allogeneic to the subject.

56. A combination comprising:

(i) a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2; and

(ii) (a) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(b) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(c) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule,

for use in treating a disease associated with mesothelin expression, e.g., a cancer, in a subject.

57. A composition (*e.g.*, one or more compositions or dosage forms), comprising:

(i) a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds to mesothelin ("mesothelin CAR-expressing cell"), wherein the CAR molecule comprises a mesothelin binding domain comprising a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any mesothelin heavy chain binding domain amino acid sequence listed in Table 2; and a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any mesothelin light chain binding domain amino acid sequence listed in Table 2; and

(ii) (a) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(b) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(c) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule.

58. A method of treating a subject having a metastatic cancer, comprising administering to the subject:

(i) a cell (e.g., a population of cells) that expresses a CAR molecule that binds to mesothelin ("mesothelin CAR-expressing cell"); and

(ii) (a) a virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule;

(b) a first virus comprising a nucleic acid molecule encoding a TNFα molecule, and a second virus comprising a nucleic acid molecule encoding an IL-2 molecule;

(c) a virus comprising a nucleic acid molecule encoding an IL-7 molecule, optionally wherein the virus further comprises a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule; or

(d) a first virus comprising a nucleic acid molecule encoding an IL-7 molecule and a second virus comprising a nucleic acid molecule encoding a TNFα molecule and/or an IL-2 molecule, optionally wherein:

administration of the mesothelin CAR-expressing cell and the virus (or the first and second viruses) in the subject having a metastatic cancer (e.g., a metastatic pancreatic cancer) reduces tumor metastasis (e.g., tumor metastasis to the lung), e.g., by at least about 20, 40, 60, or 80%, e.g., about 20, 50, 100, or 150 days after administration of the virus (or the first and second viruses), e.g., compared to administration of the mesothelin CAR-expressing cell without the virus (or without the first or second virus), or compared to administration of the mesothelin CAR-expressing cell and an otherwise similar virus that does not comprise the nucleic acid molecule encoding the TNFα molecule or the IL-2 molecule, e.g., as assessed using methods described in Example 1 with respect to FIG. 2F.

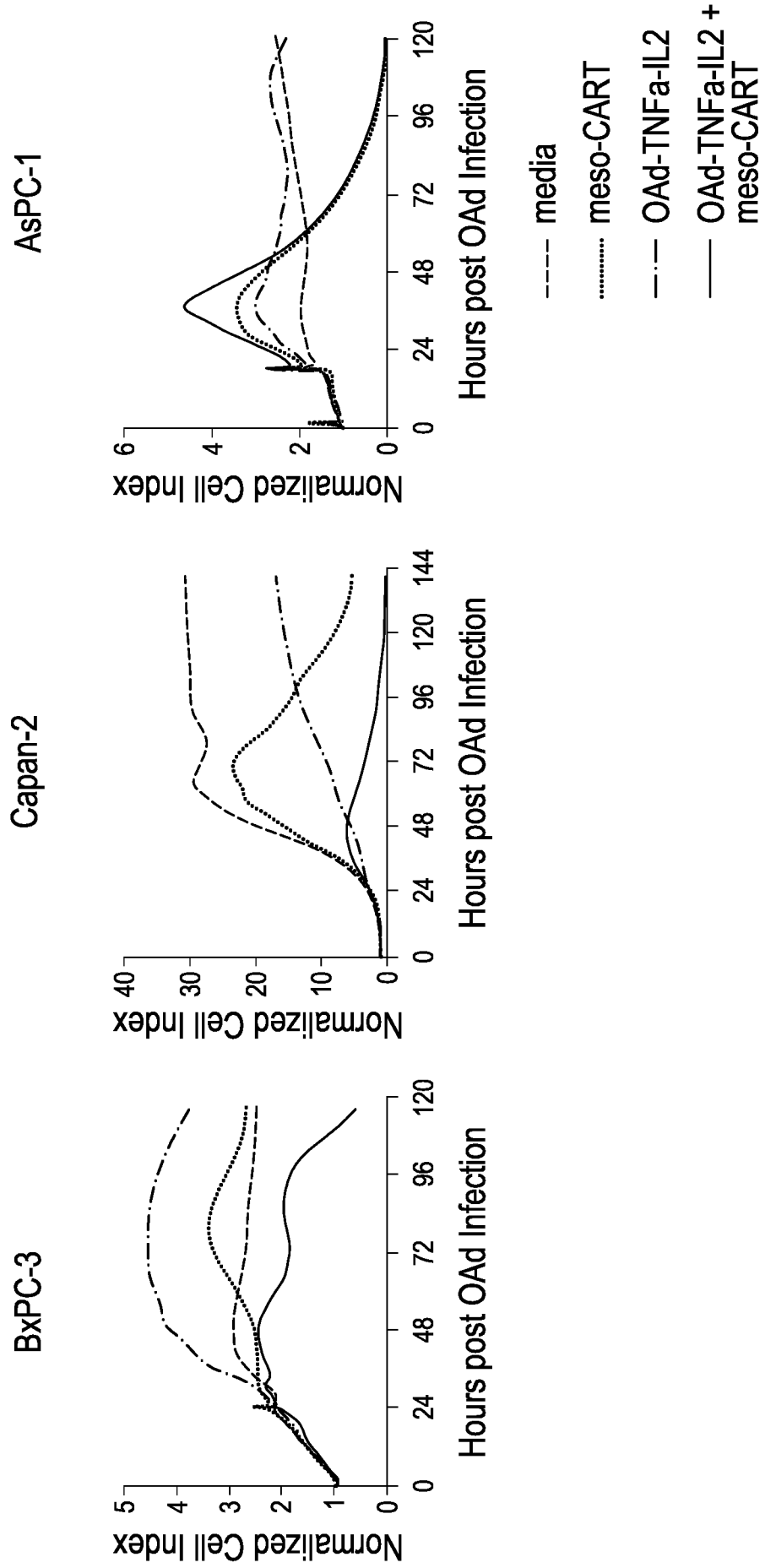
AsPC-1
Capan-2
BxPC-3
Normalized Cell Index
Hours post OAd Infection
media
meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART

FIG. 1A

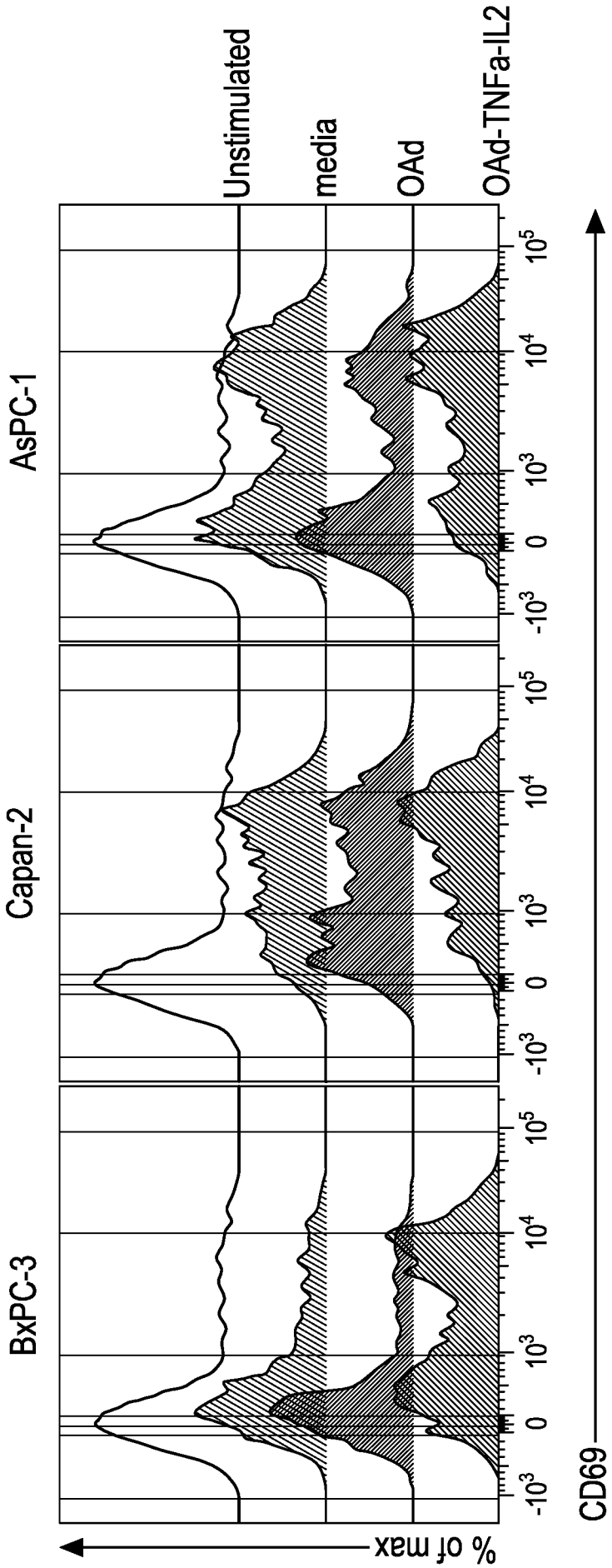
Unstimulated
media
OAd
OAd-TNFa-IL2
AsPC-1
Capan-2
BxPC-3
% of max
CD69
-10^3
0
10^3
10^4
10^5

FIG. 1B

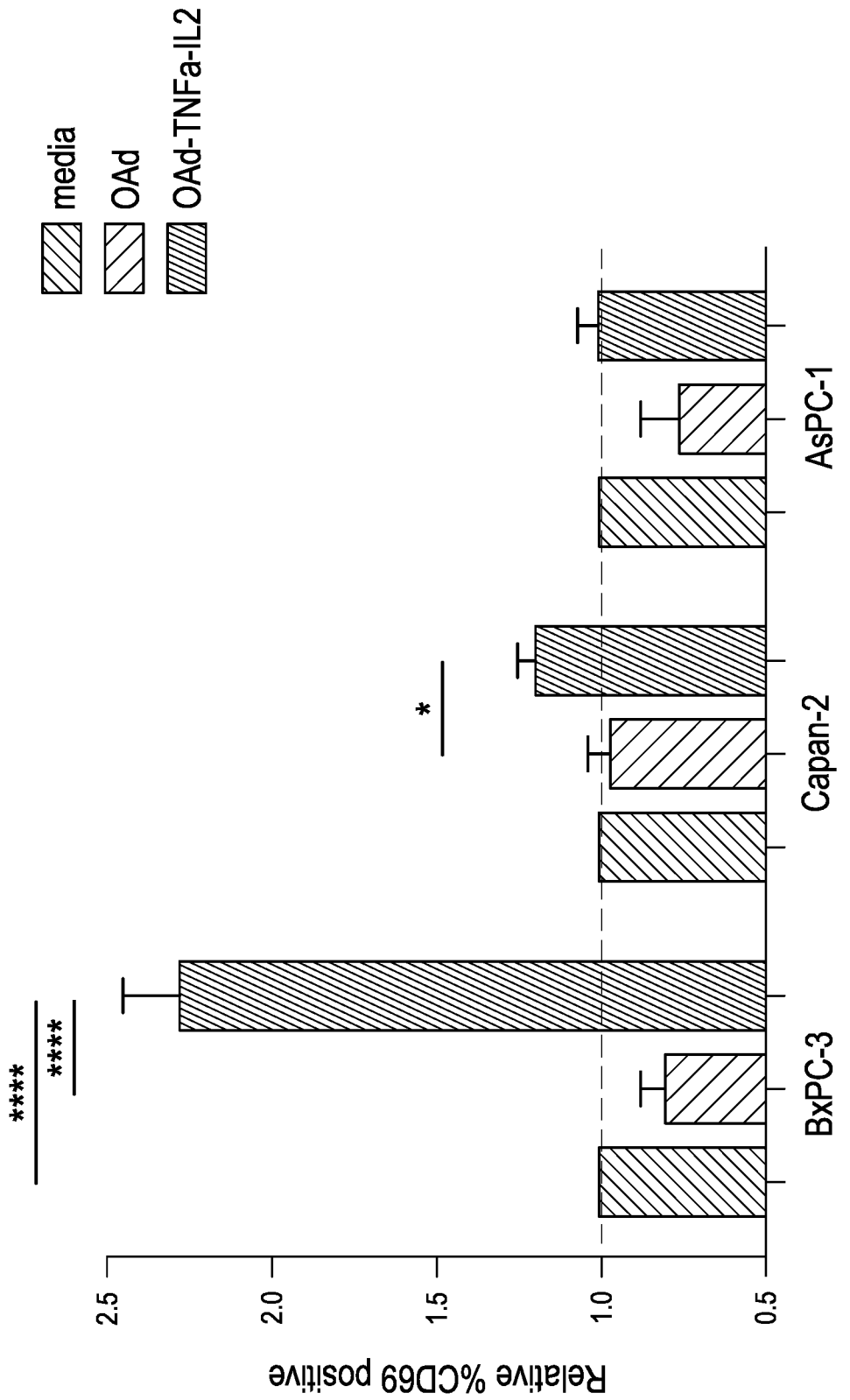
media
OAd
OAd-TNFa-IL2
Relative %CD69 positive
2.5
2.0
1.5
1.0
0.5
****
****
*
BxPC-3
Capan-2
AsPC-1

FIG. 1C

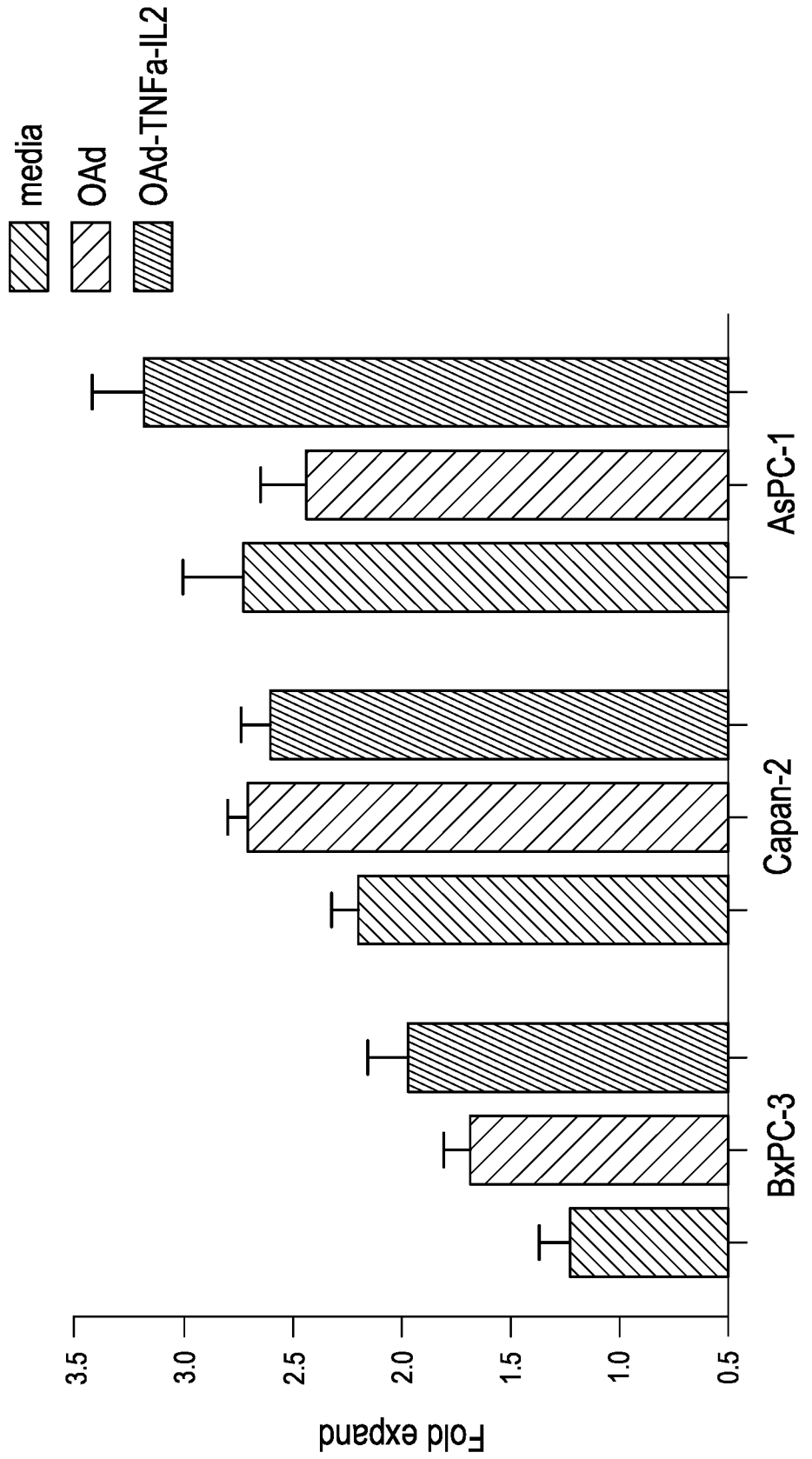
media
OAd
OAd-TNFa-IL2
Fold expand
3.5
3.0
2.5
2.0
1.5
1.0
0.5
BxPC-3
Capan-2
AsPC-1

FIG. 1D

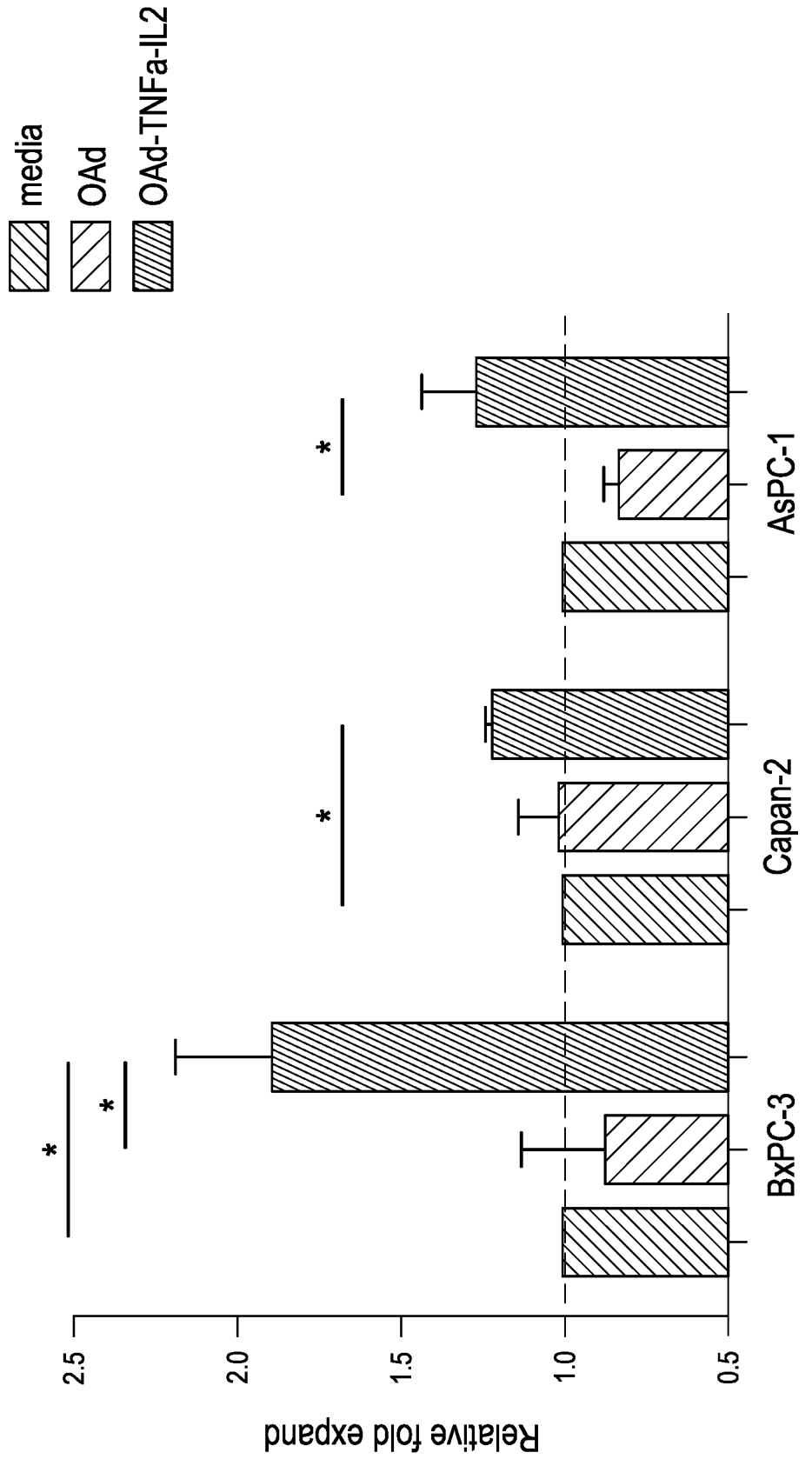
media
OAd
OAd-TNFa-IL2
Relative fold expand
2.5
2.0
1.5
1.0
0.5
BxPC-3
Capan-2
AsPC-1
*
*
*
*

FIG. 1E

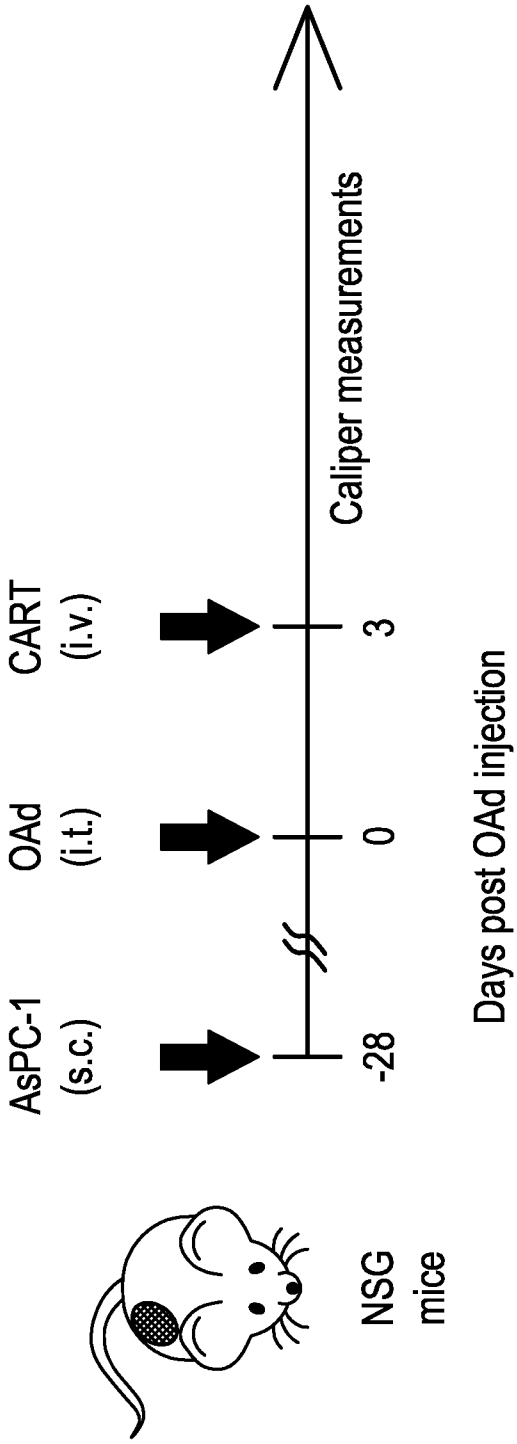
AsPC-1
(s.c.)
OAd
(i.t.)
CART
(i.v.)
-28
0
3
Days post OAd injection
Caliper measurements
NSG
mice

FIG. 2A

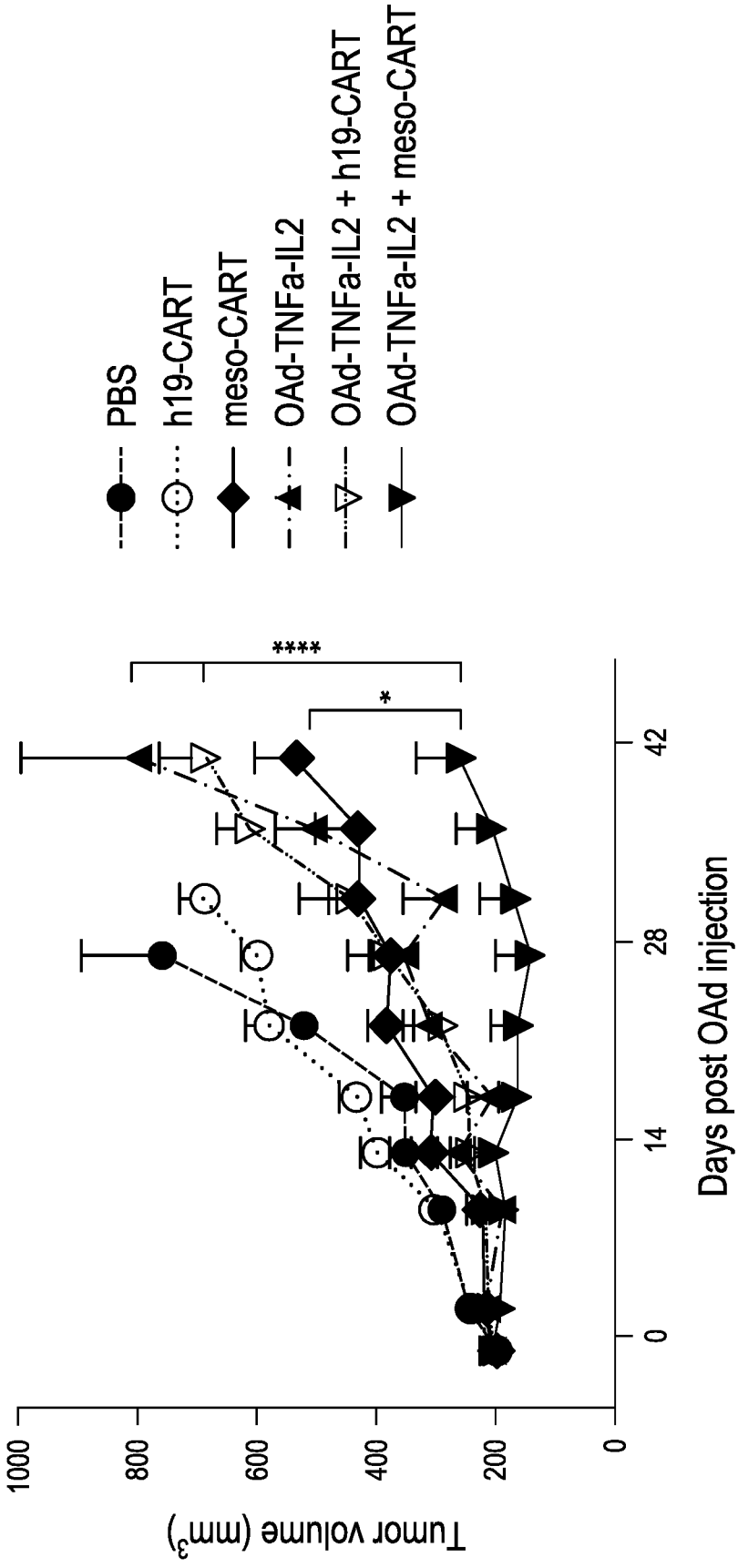
PBS
h19-CART
meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + h19-CART
OAd-TNFa-IL2 + meso-CART
****
*
Tumor volume (mm3)
0
200
400
600
800
1000
0
14
28
42
Days post OAd injection

FIG. 2B

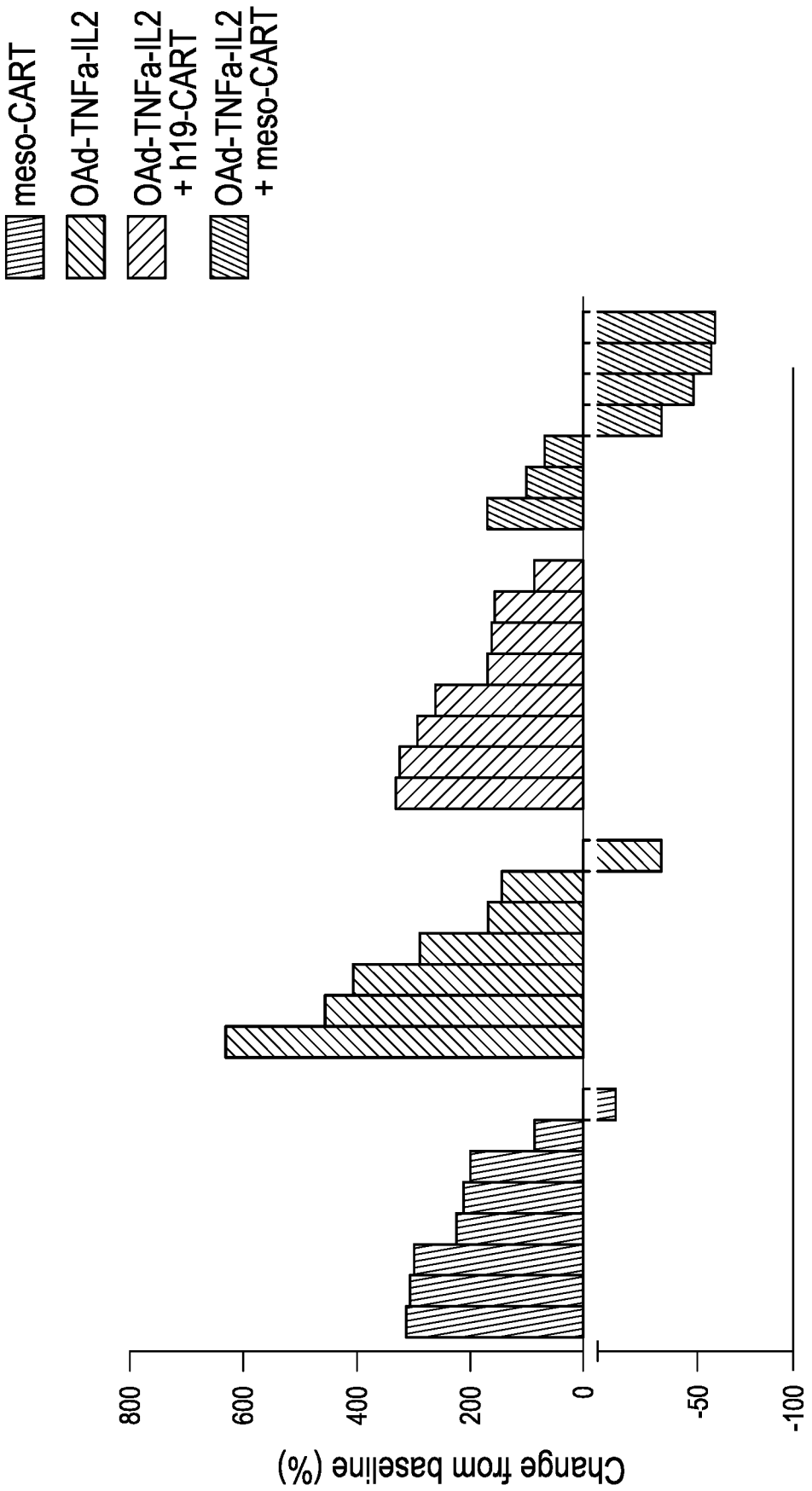
meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + h19-CART
OAd-TNFa-IL2 + meso-CART
Change from baseline (%)
800
600
400
200
0
-50
-100

FIG. 2C

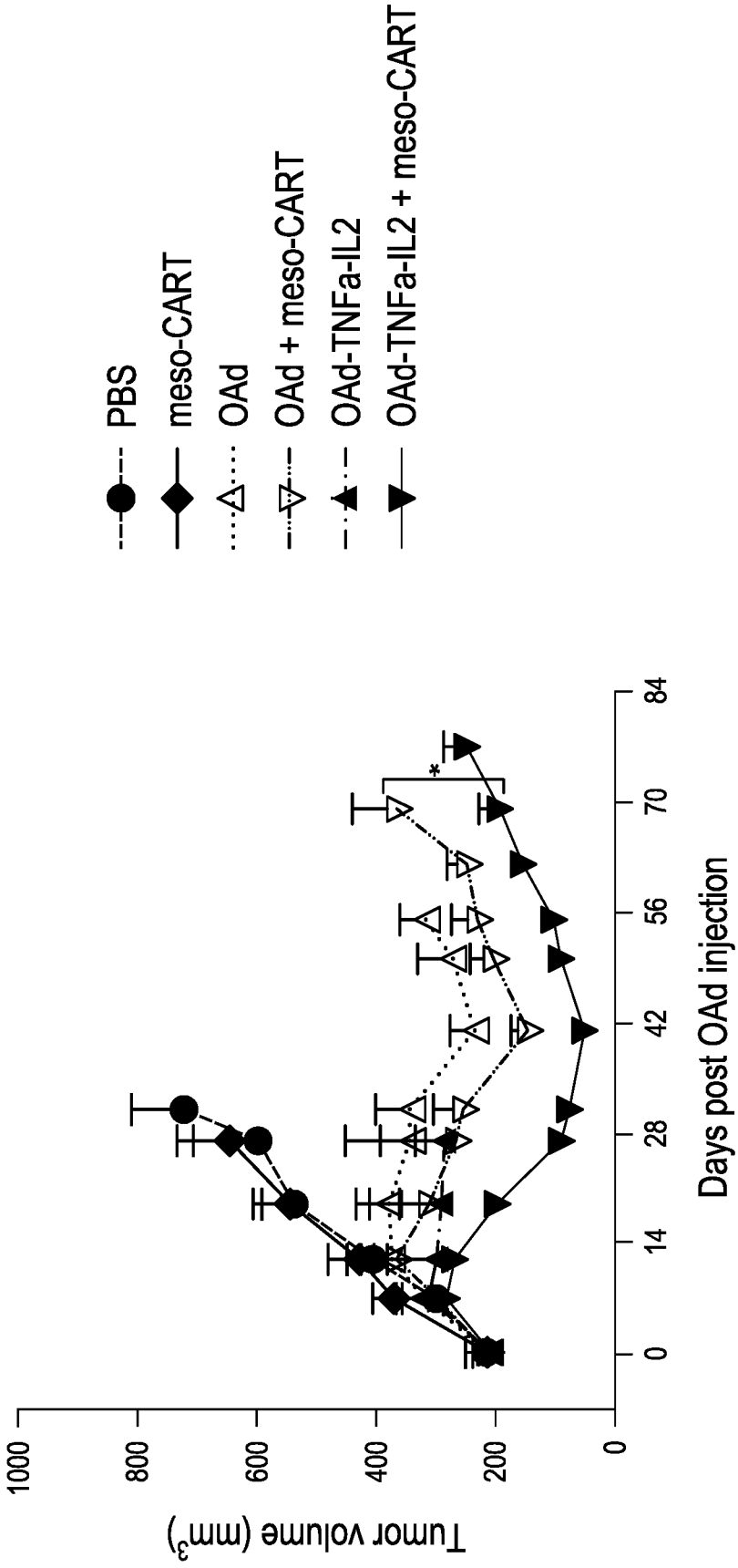
PBS
meso-CART
OAd
OAd + meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART
Tumor volume (mm3)
0
200
400
600
800
1000
0
14
28
42
56
70
84
Days post OAd injection
*
*

FIG. 2D

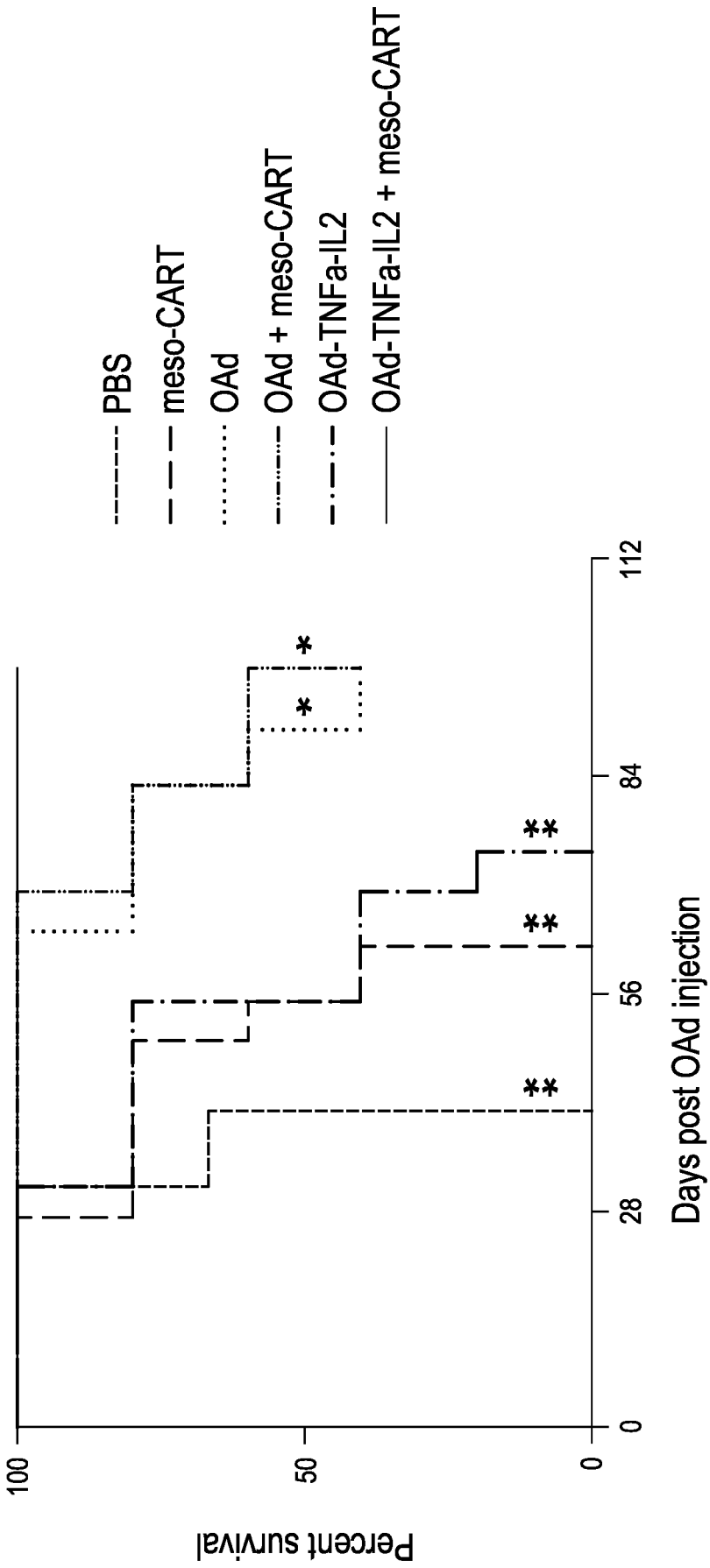
PBS
meso-CART
OAd
OAd + meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART
Percent survival
100
50
0
0
28
56
84
112
Days post OAd injection
*
*
**
**
**

FIG. 2E

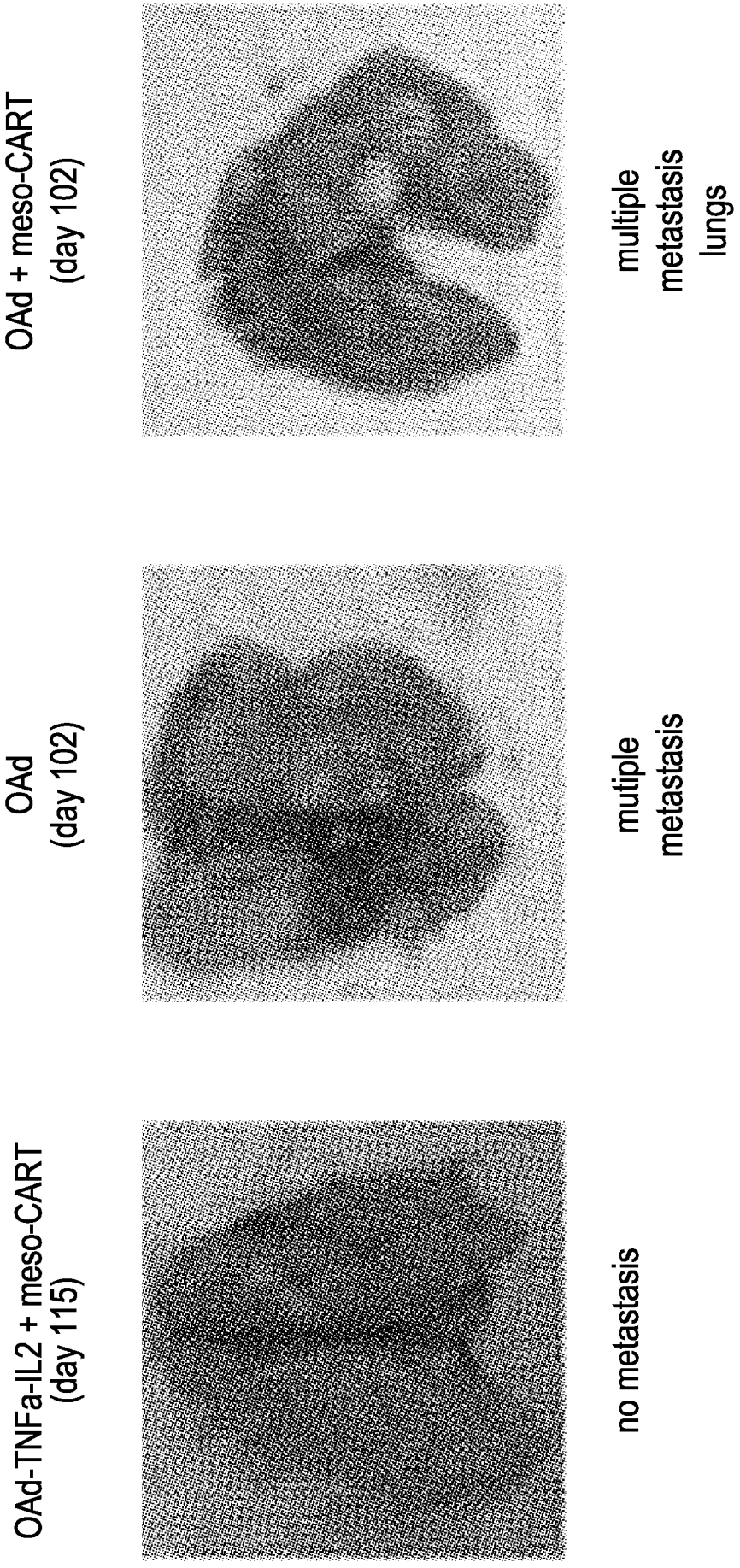
OAd + meso-CART
(day 102)
multiple
metastasis
lungs
OAd
(day 102)
mutiple
metastasis
OAd-TNFa-IL2 + meso-CART
(day 115)
no metastasis

FIG. 2F

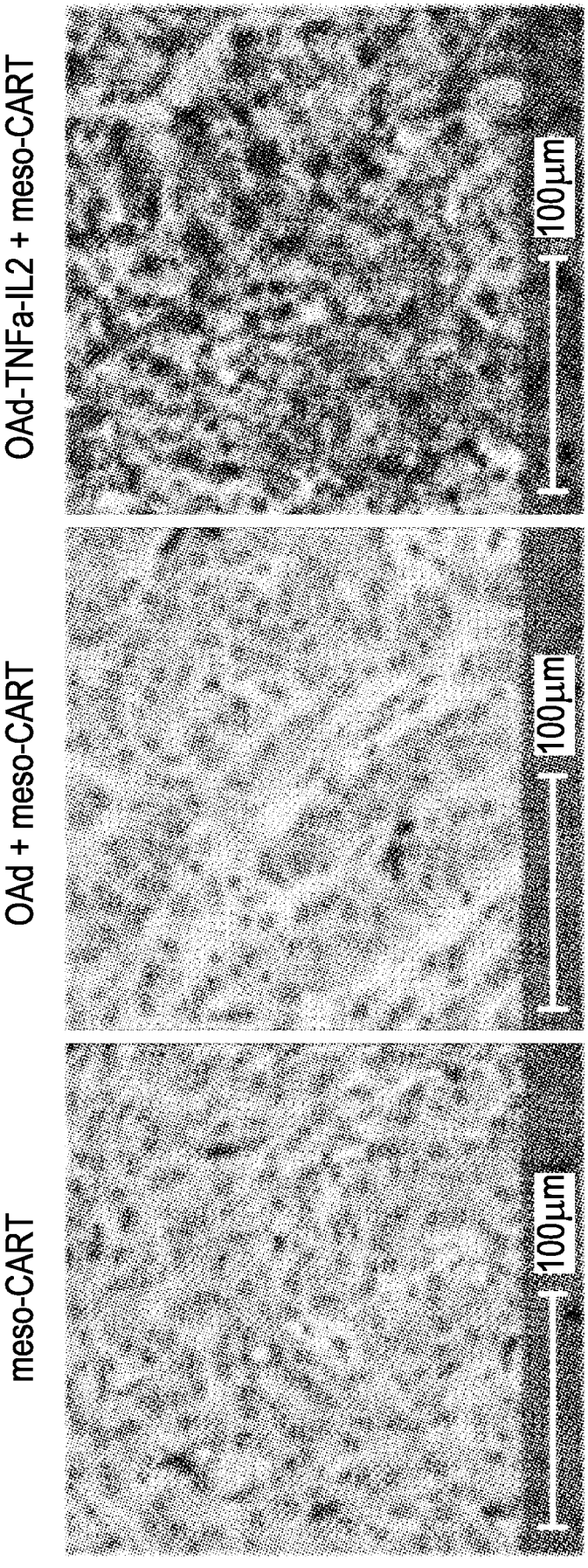
OAd-TNFa-IL2 + meso-CART
OAd + meso-CART
meso-CART
100µm
100µm
100µm
CD8

FIG. 3A

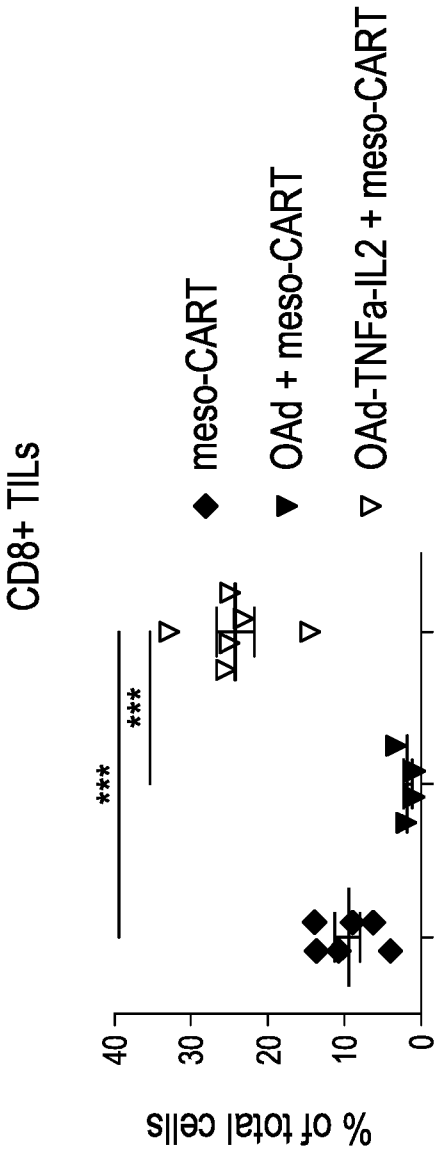
CD8+ TILs
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART
***
***
% of total cells
0
10
20
30
40

FIG. 3B

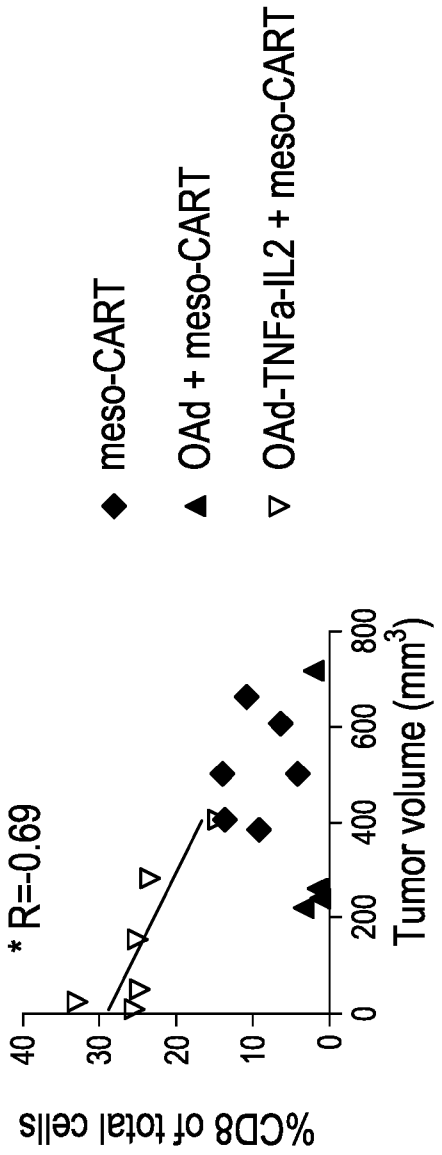
CD8+ TILs v.s. tumor volume
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART
* R=-0.69
%CD8 of total cells
Tumor volume (mm3)
0
10
20
30
40
0
200
400
600
800

FIG. 3C

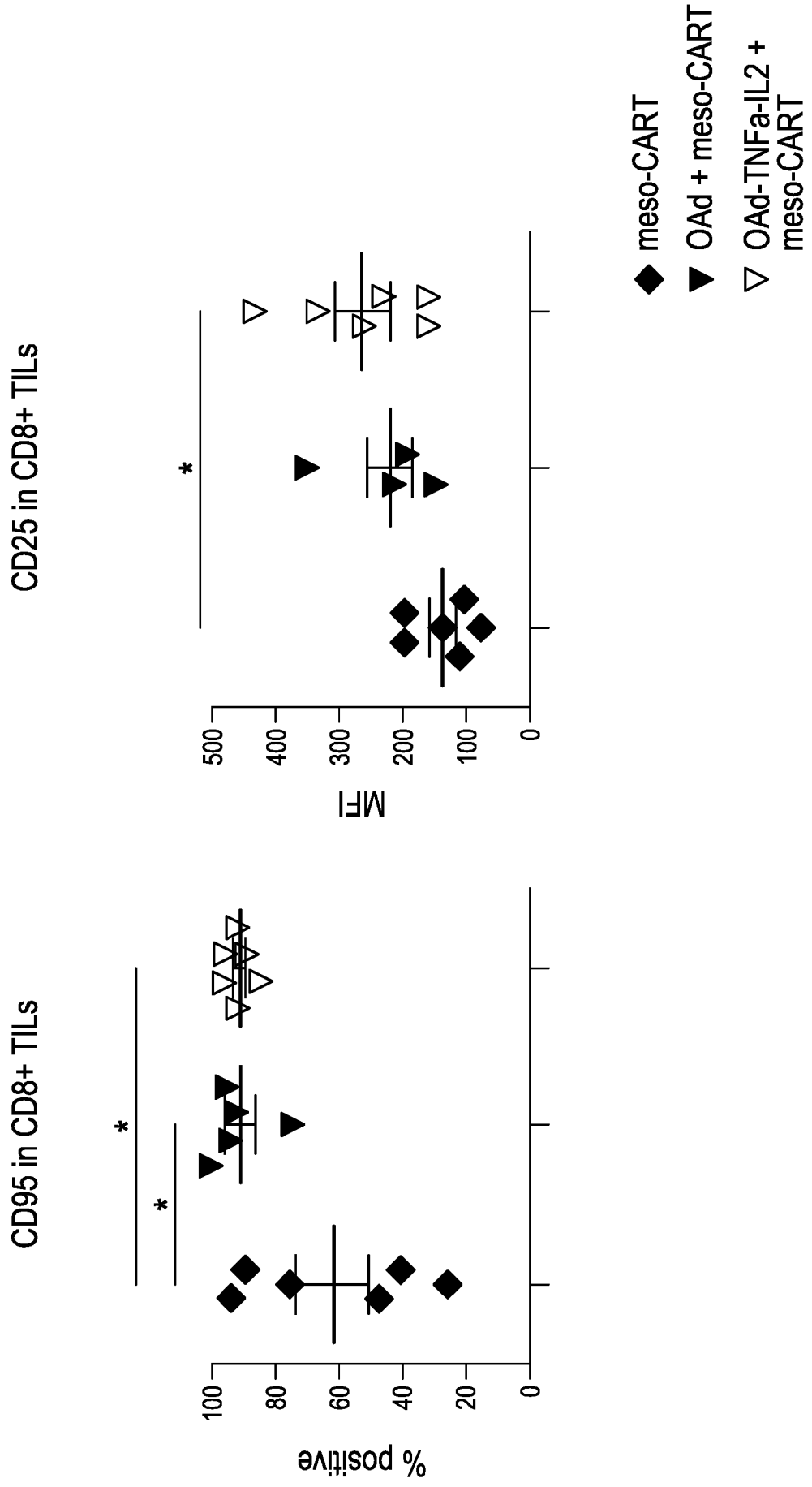
CD95 in CD8+ TILs
% positive
100
80
60
40
20
0
*
*
CD25 in CD8+ TILs
MFI
500
400
300
200
100
0
*
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 3D

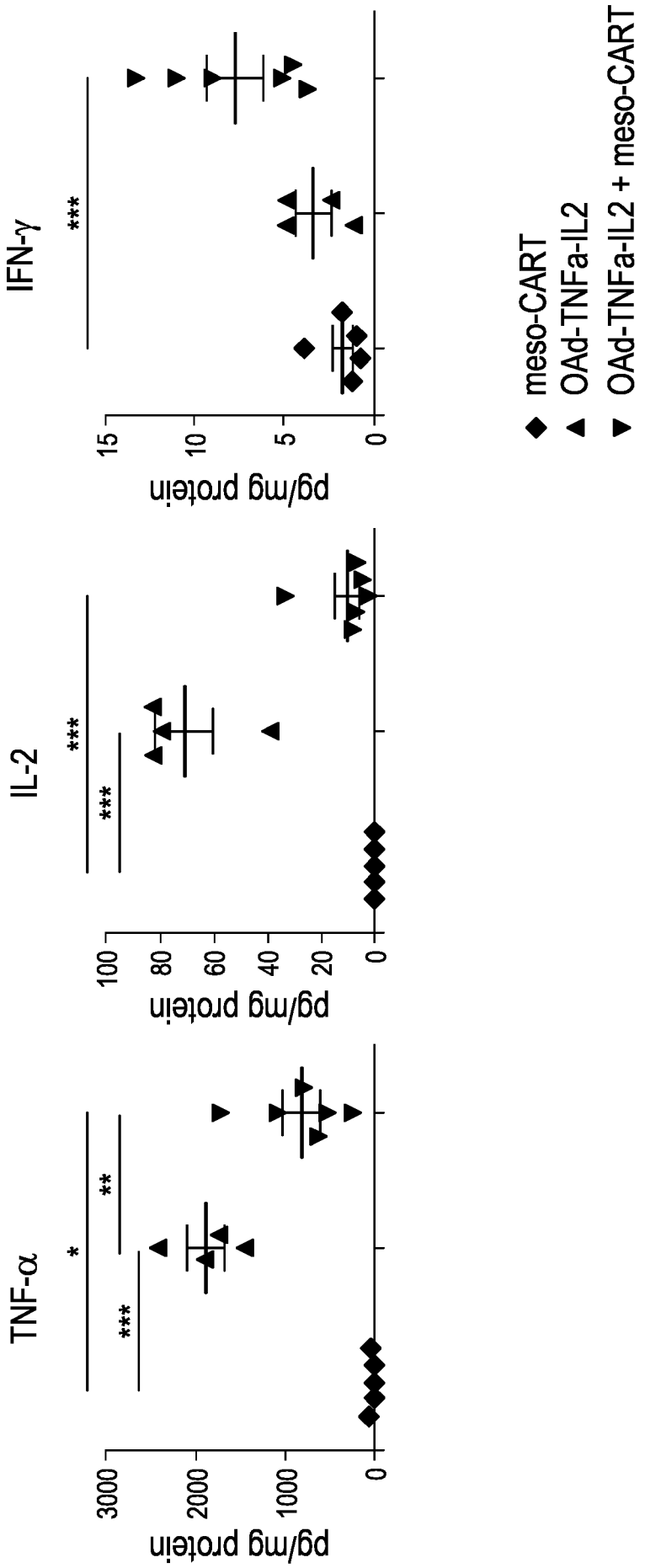
TNF-α
IL-2
IFN-γ
pg/mg protein
3000
2000
1000
0
100
80
60
40
20
15
10
5
*
**
***
meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART

FIG. 3E

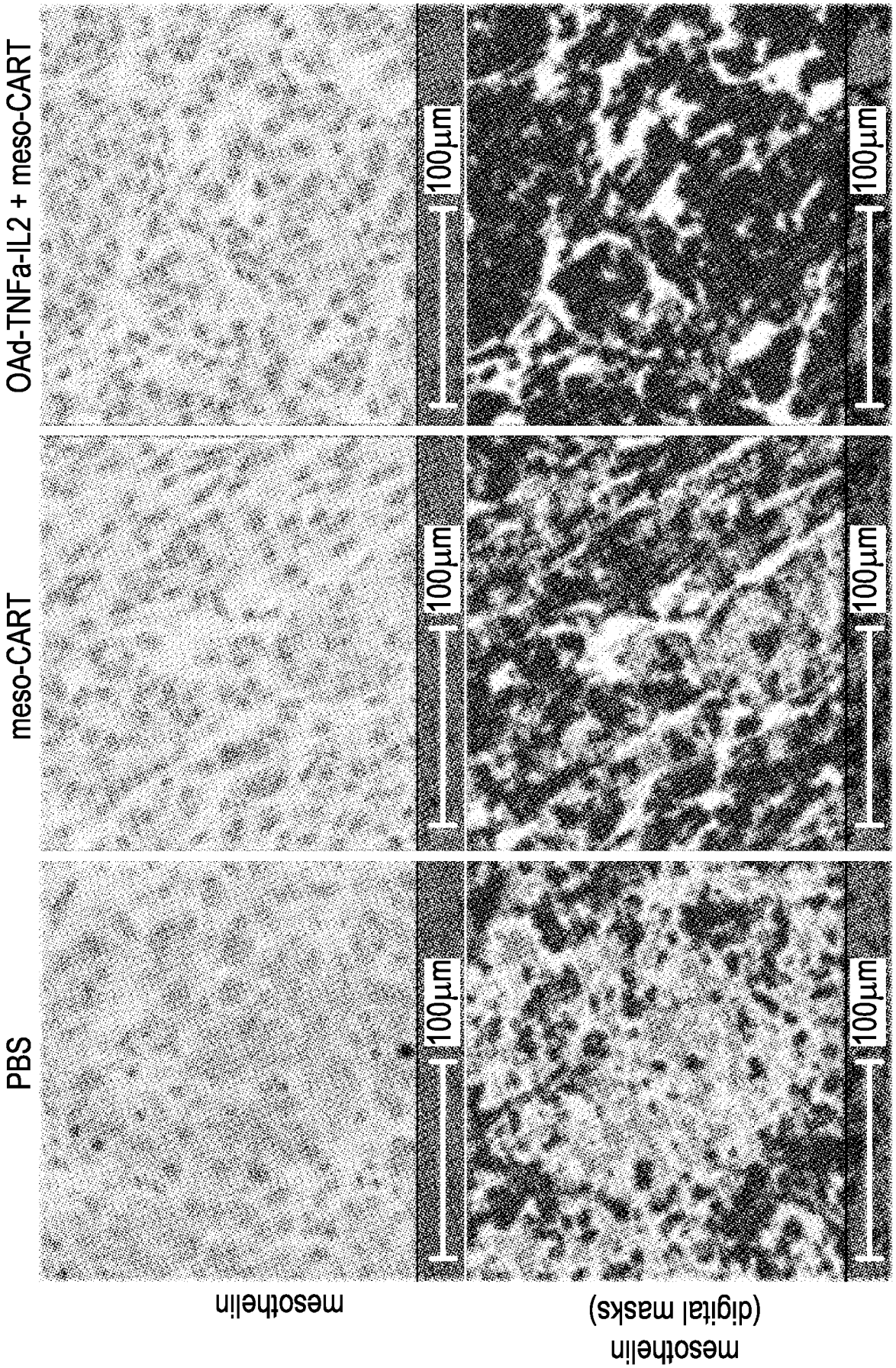
PBS
meso-CART
OAd-TNFa-IL2 + meso-CART
mesothelin
mesothelin
(digital masks)
100µm

FIG. 3F

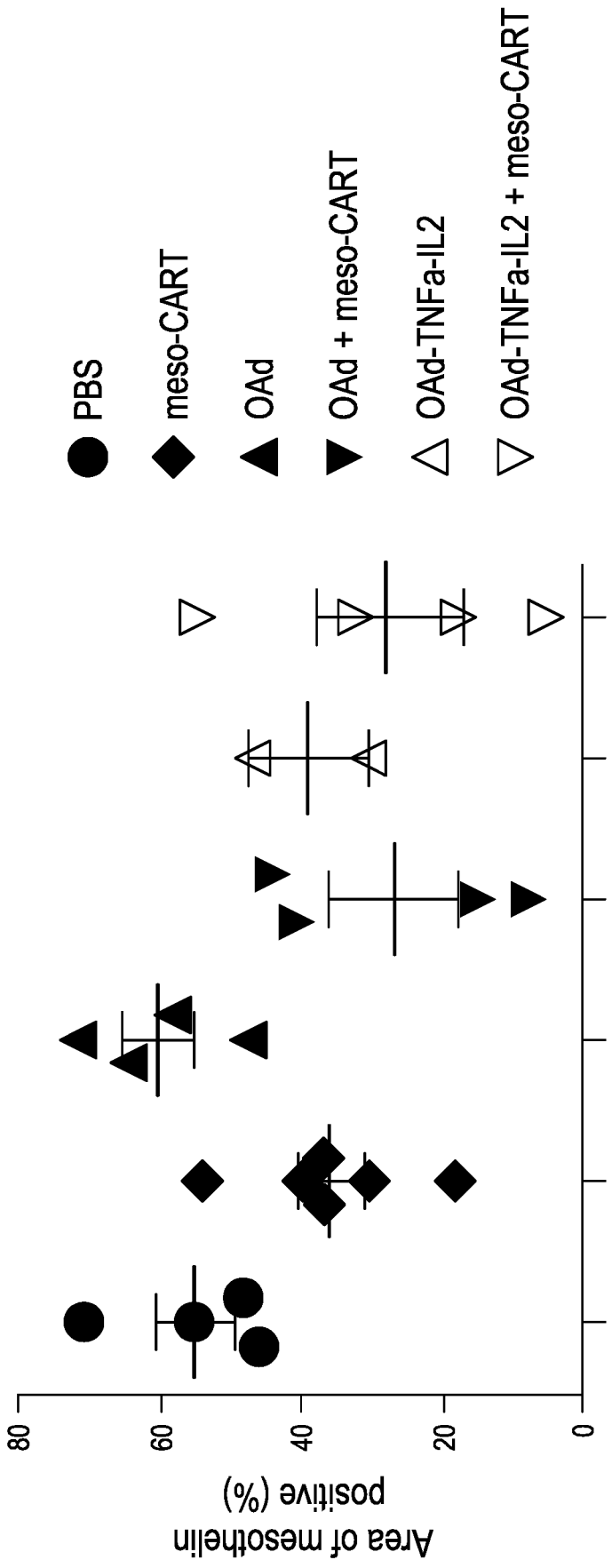
PBS
meso-CART
OAd
OAd + meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART
Area of mesothelin positive (%)
0
20
40
60
80

FIG. 3G

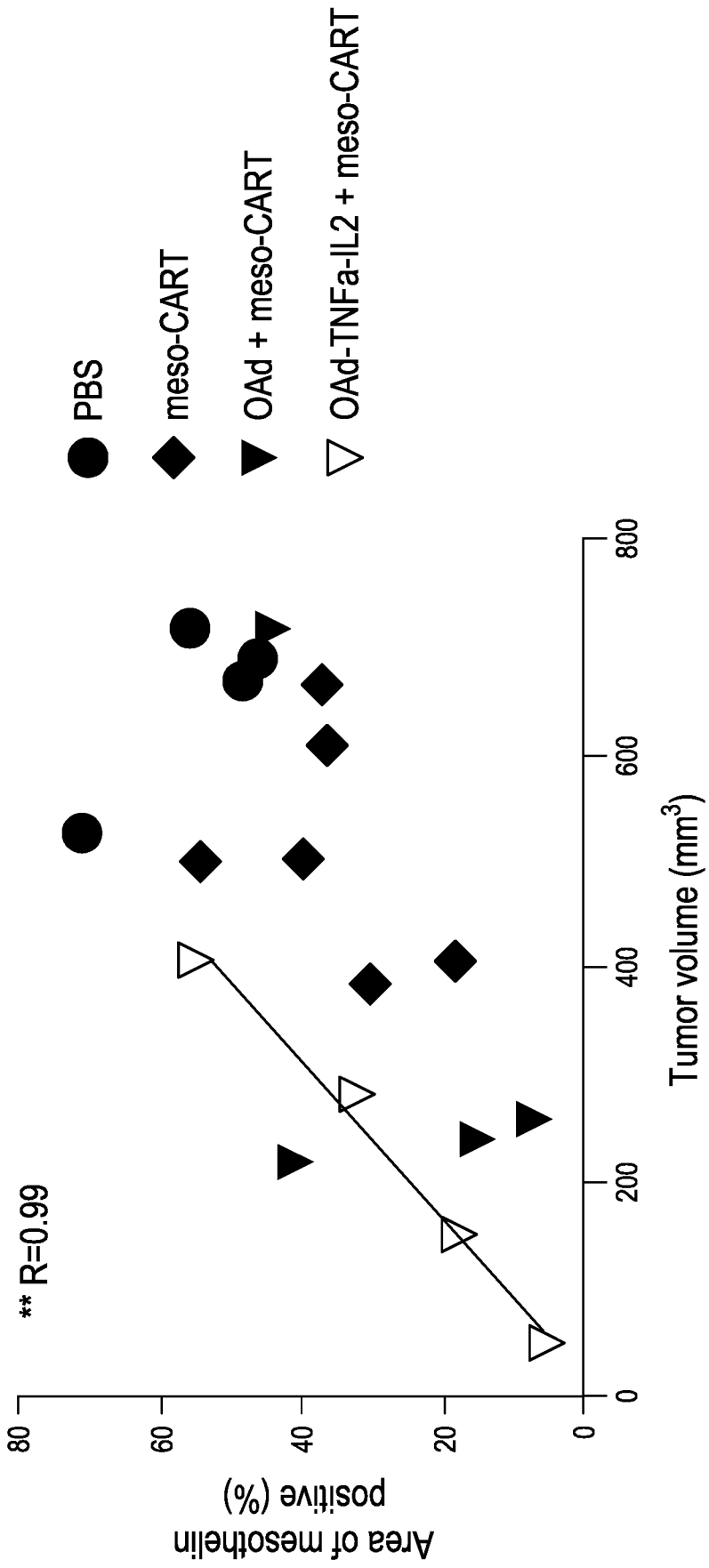
PBS
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART
** R=0.99
Area of mesothelin positive (%)
0
20
40
60
80
0
200
400
600
800
Tumor volume (mm3)

FIG. 3H

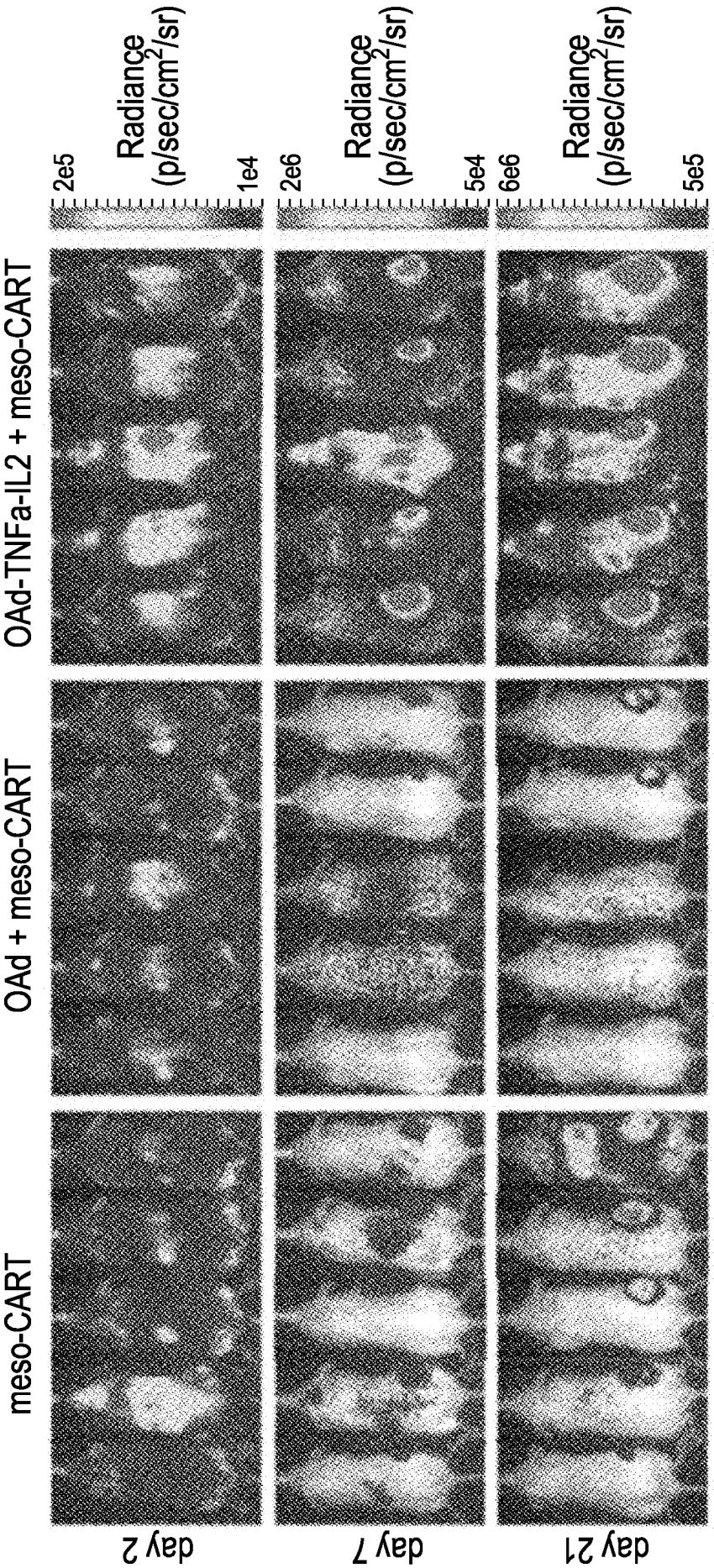
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART
Radiance (p/sec/cm²/sr)
2e5
1e4
2e6
5e4
6e6
5e5
day 2
day 7
day 21

FIG. 4A

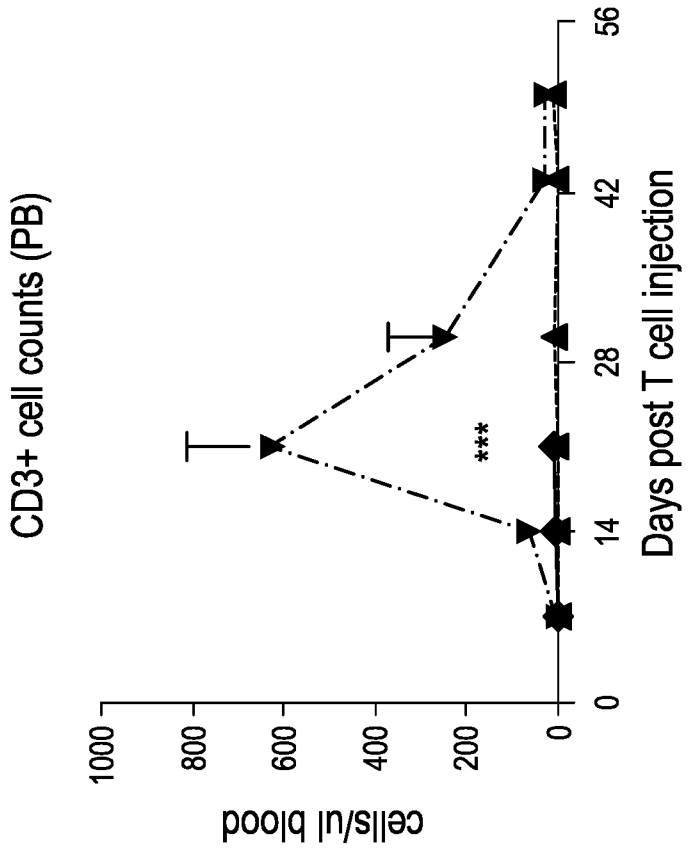
CD3+ cell counts (PB)
cells/ul blood
1000
800
600
400
200
0
***
0
14
28
42
56
Days post T cell injection
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 4C

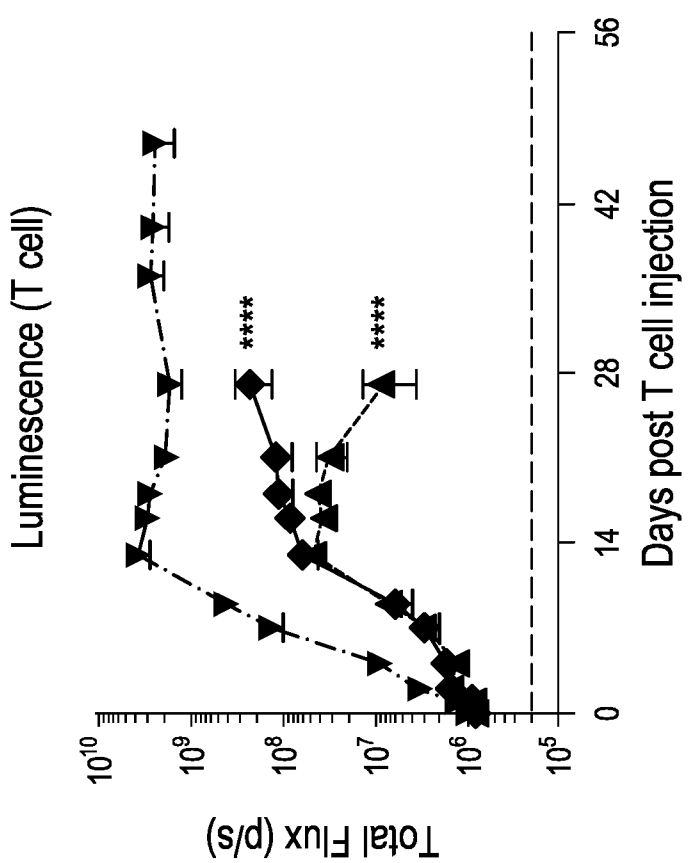
Luminescence (T cell)
Total Flux (p/s)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
****
****
0
14
28
42
56
Days post T cell injection
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 4B

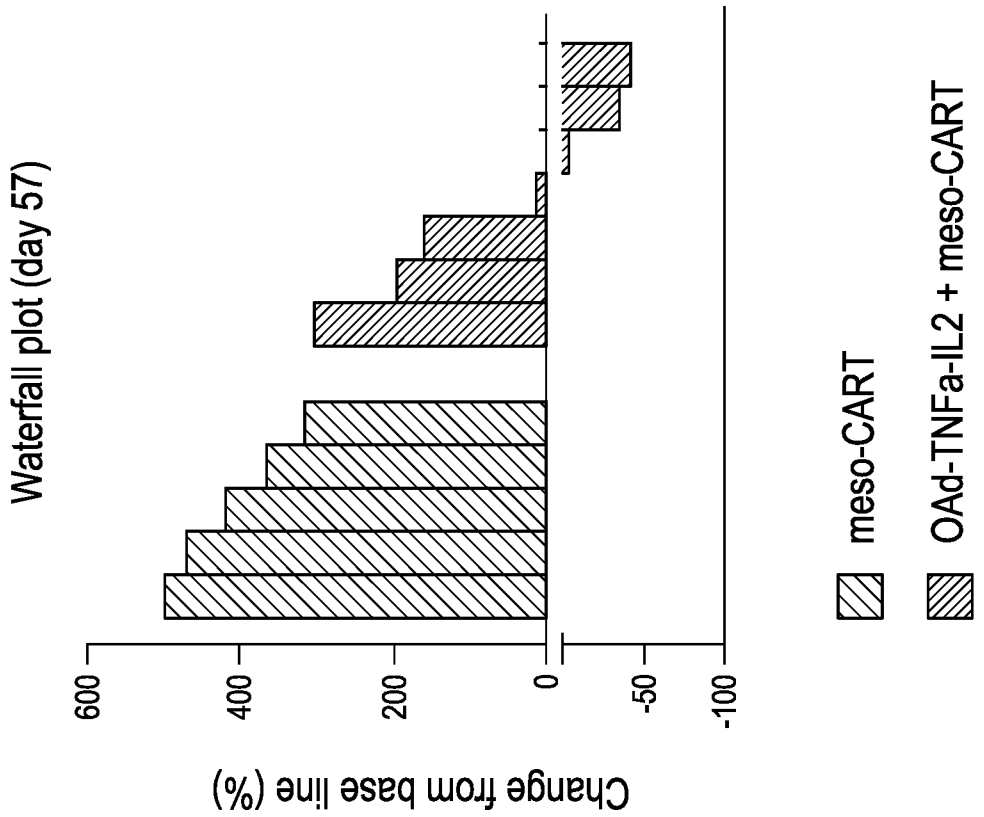
Waterfall plot (day 57)
Change from base line (%)
600
400
200
0
-50
-100
meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 5B

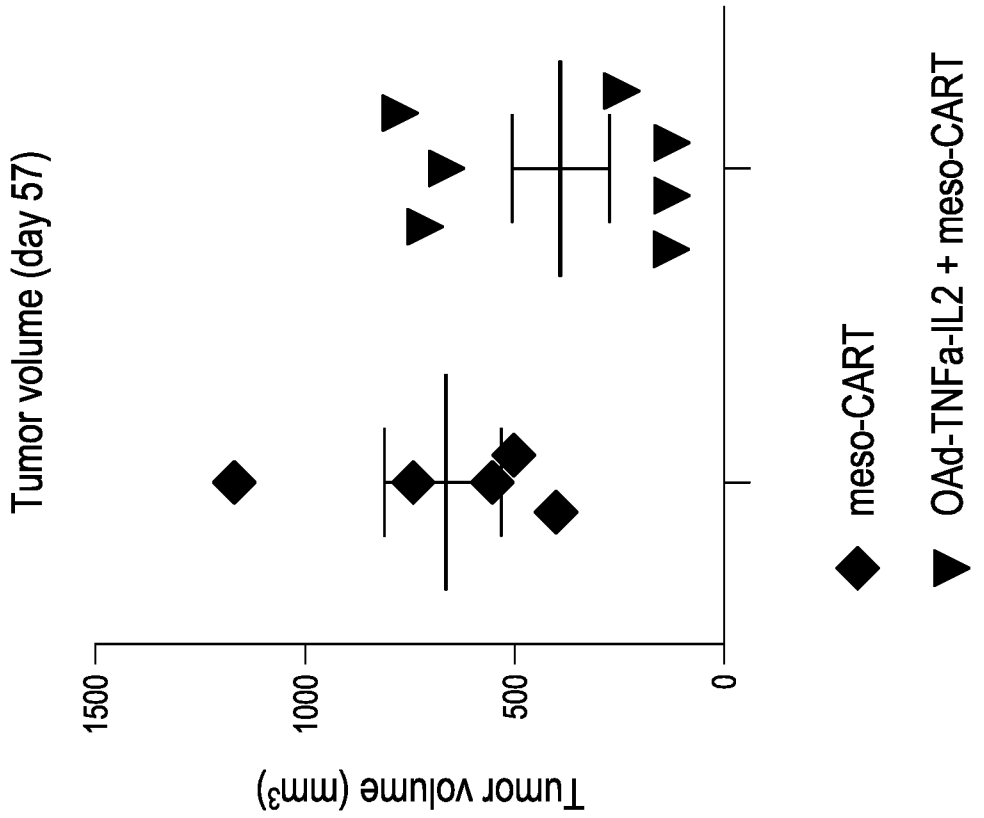
Tumor volume (day 57)
Tumor volume (mm3)
1500
1000
500
0
meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 5A

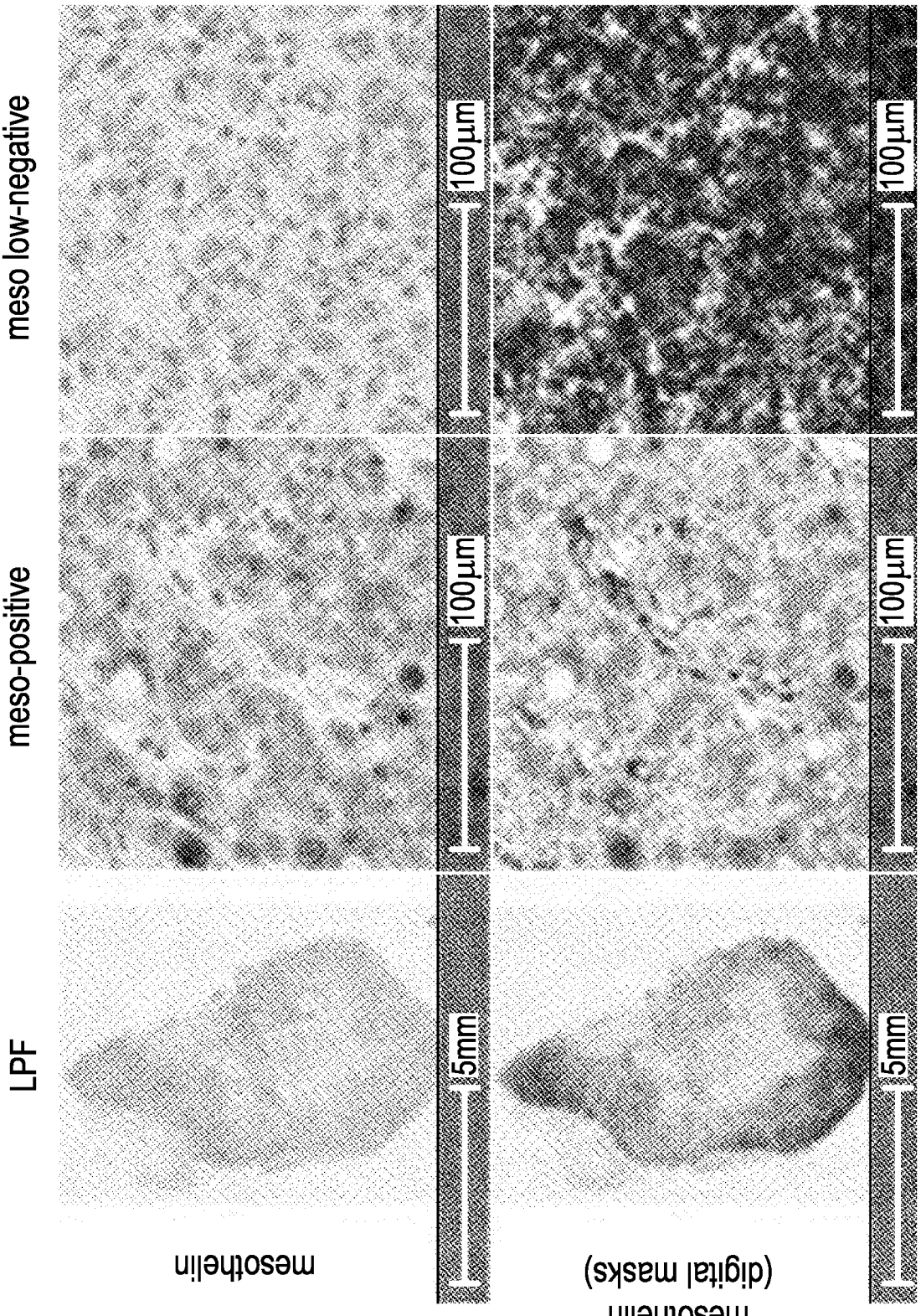
LPF
meso-positive
meso low-negative
mesothelin
mesothelin
(digital masks)
5mm
100µm
100µm
100µm
5mm
100µm
100µm

FIG. 5C

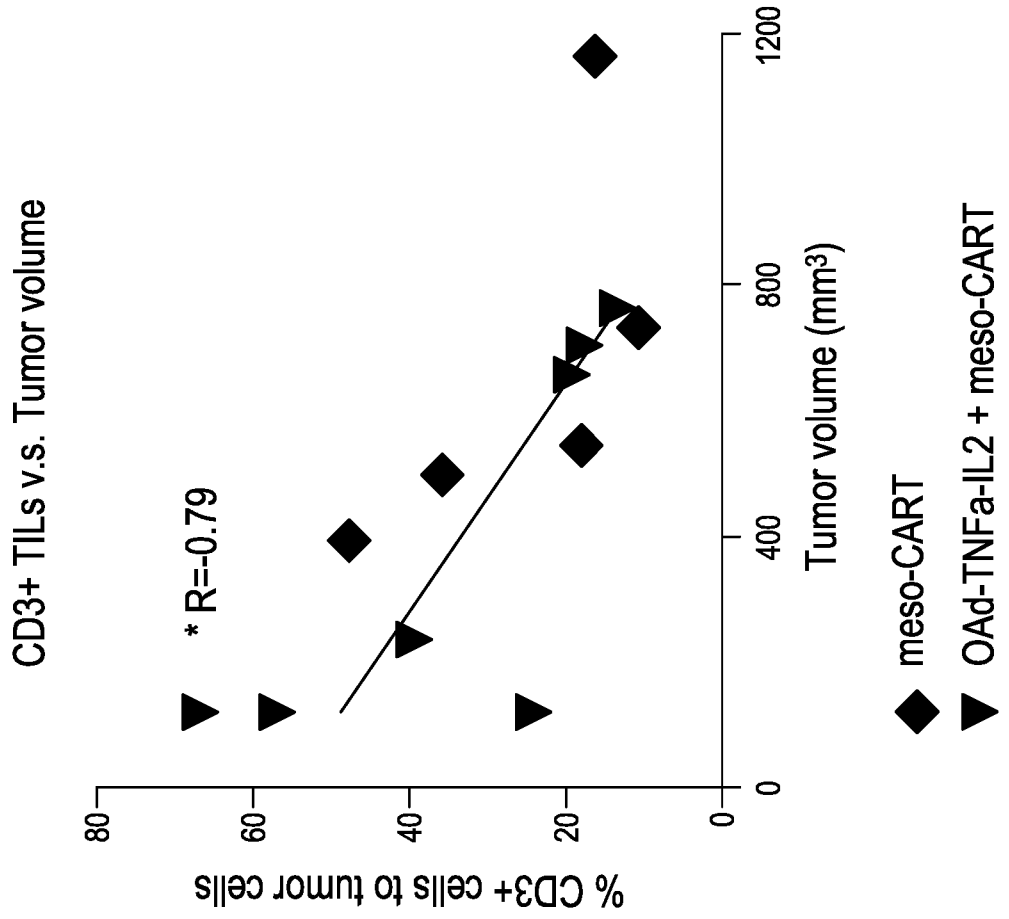
Mesothelin (%) v.s. Tumor volume
Area of mesothelin positive (%)
0
20
40
60
80
n.s.
0
400
800
1200
Tumor volume (mm3)
meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 5D

CD3+ TILs v.s. Tumor volume
% CD3+ cells to tumor cells
0
20
40
60
80
* R=-0.79
0
400
800
1200
Tumor volume (mm3)
meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 5E

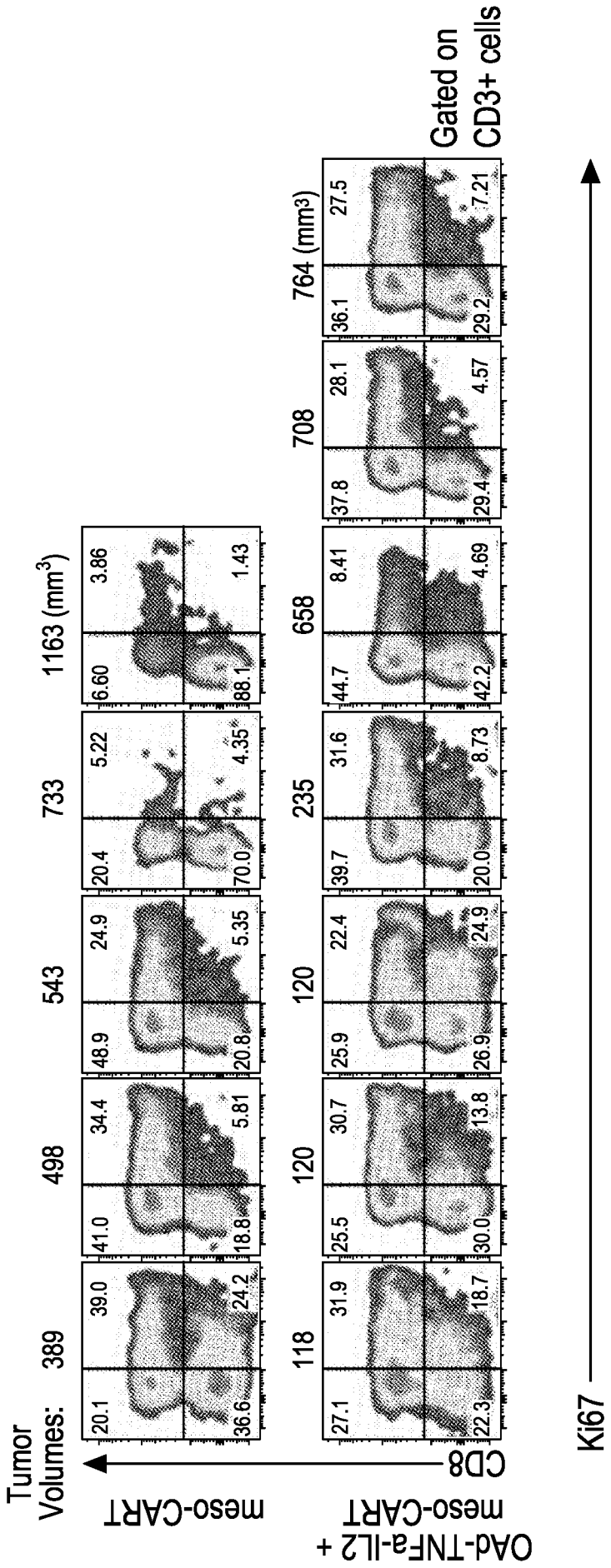
Tumor Volumes:
389
498
543
733
1163 (mm3)
118
120
120
235
658
708
764 (mm3)
meso-CART
OAd-TNFa-IL2 + meso-CART
CD8
Ki67
Gated on CD3+ cells

FIG. 5F

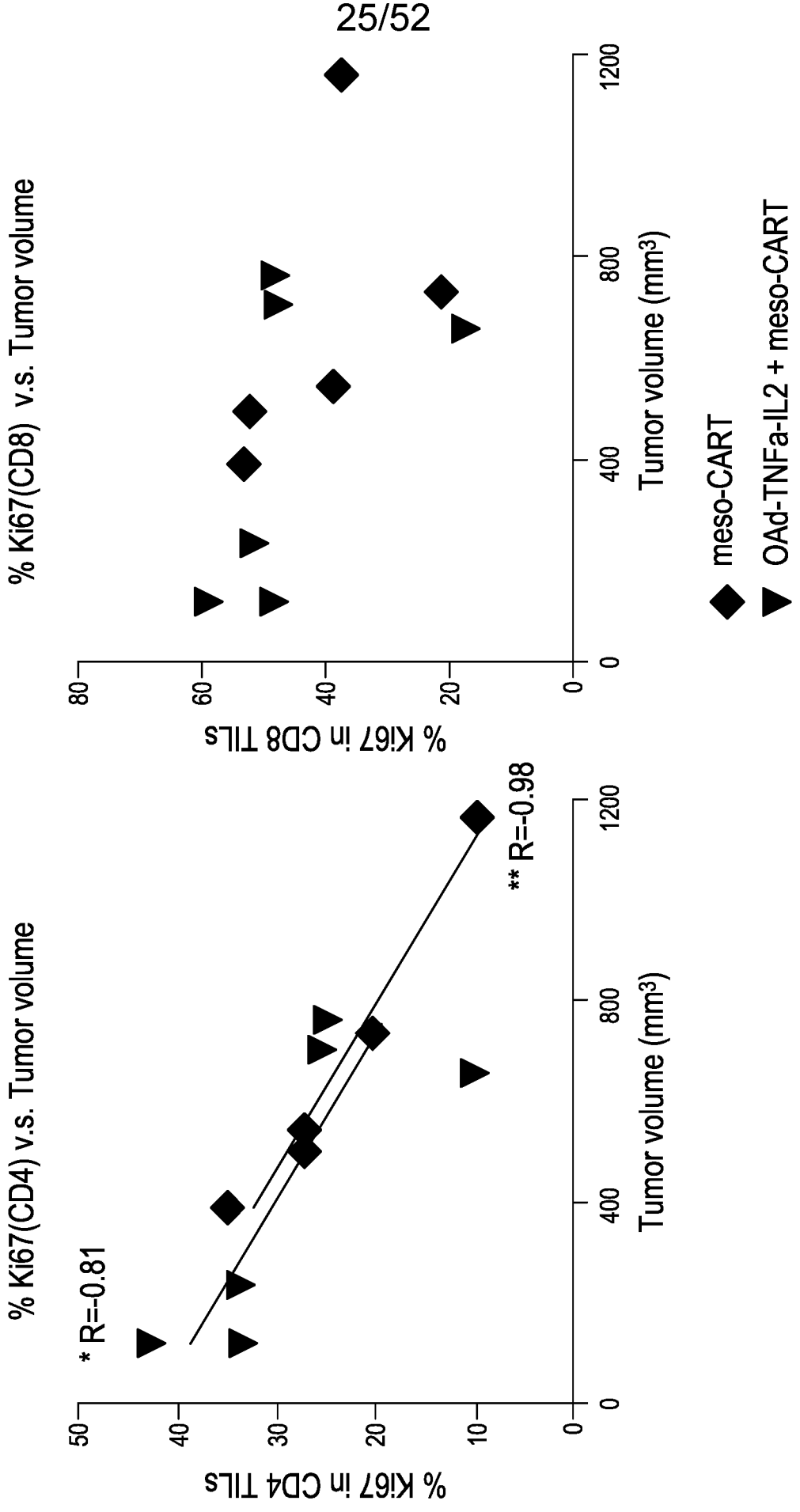
% Ki67(CD8) v.s. Tumor volume
% Ki67 in CD8 TILs
Tumor volume (mm3)
meso-CART
OAd-TNFa-IL2 + meso-CART
% Ki67(CD4) v.s. Tumor volume
* R=-0.81
** R=-0.98
% Ki67 in CD4 TILs
Tumor volume (mm3)

FIG. 5G

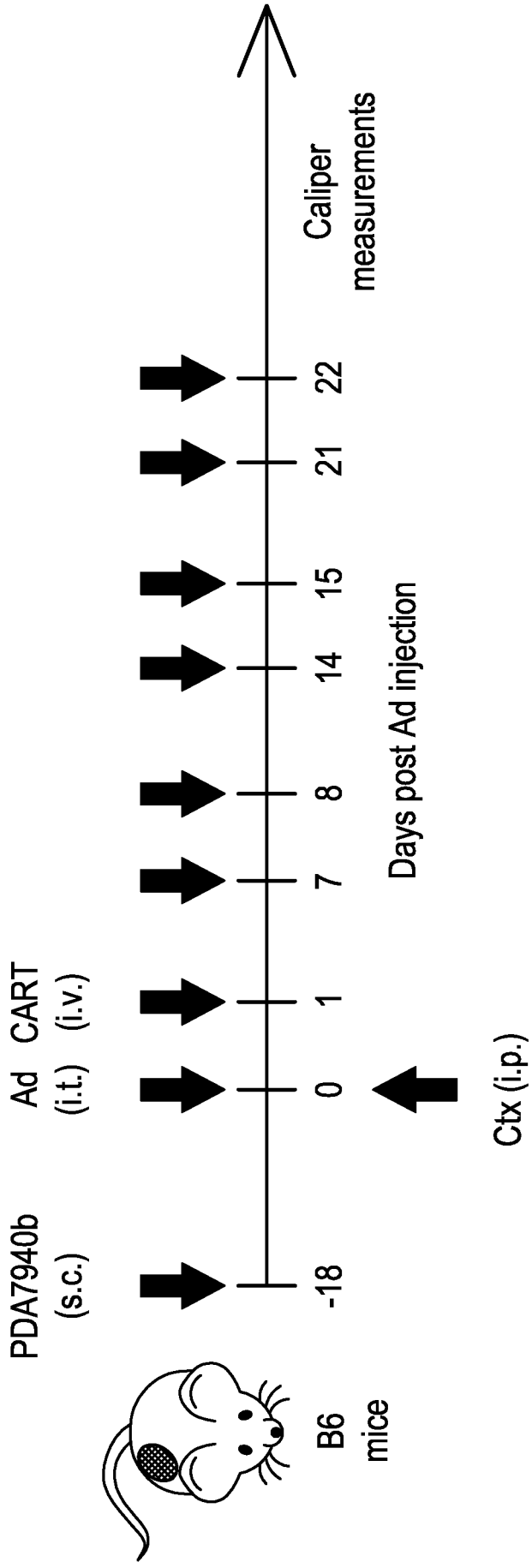
PDA7940b (s.c.)
Ad (i.t.)
CART (i.v.)
Ctx (i.p.)
B6 mice
-18
0
1
7
8
14
15
21
22
Days post Ad injection
Caliper measurements

FIG. 6A

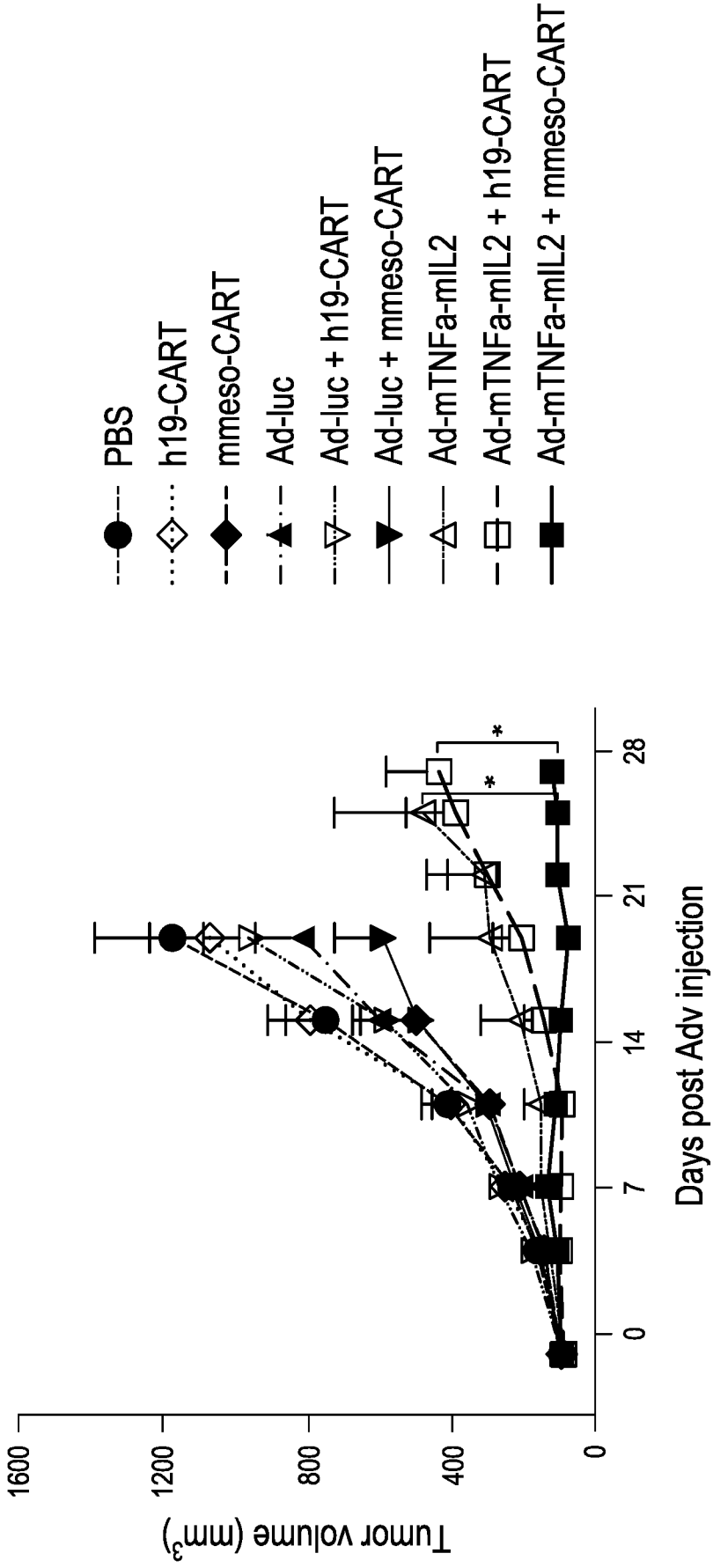
PBS
h19-CART
mmeso-CART
Ad-luc
Ad-luc + h19-CART
Ad-luc + mmeso-CART
Ad-mTNFa-mIL2
Ad-mTNFa-mIL2 + h19-CART
Ad-mTNFa-mIL2 + mmeso-CART
Tumor volume (mm3)
1600
1200
800
400
0
0
7
14
21
28
Days post Adv injection
*
*

FIG. 6B

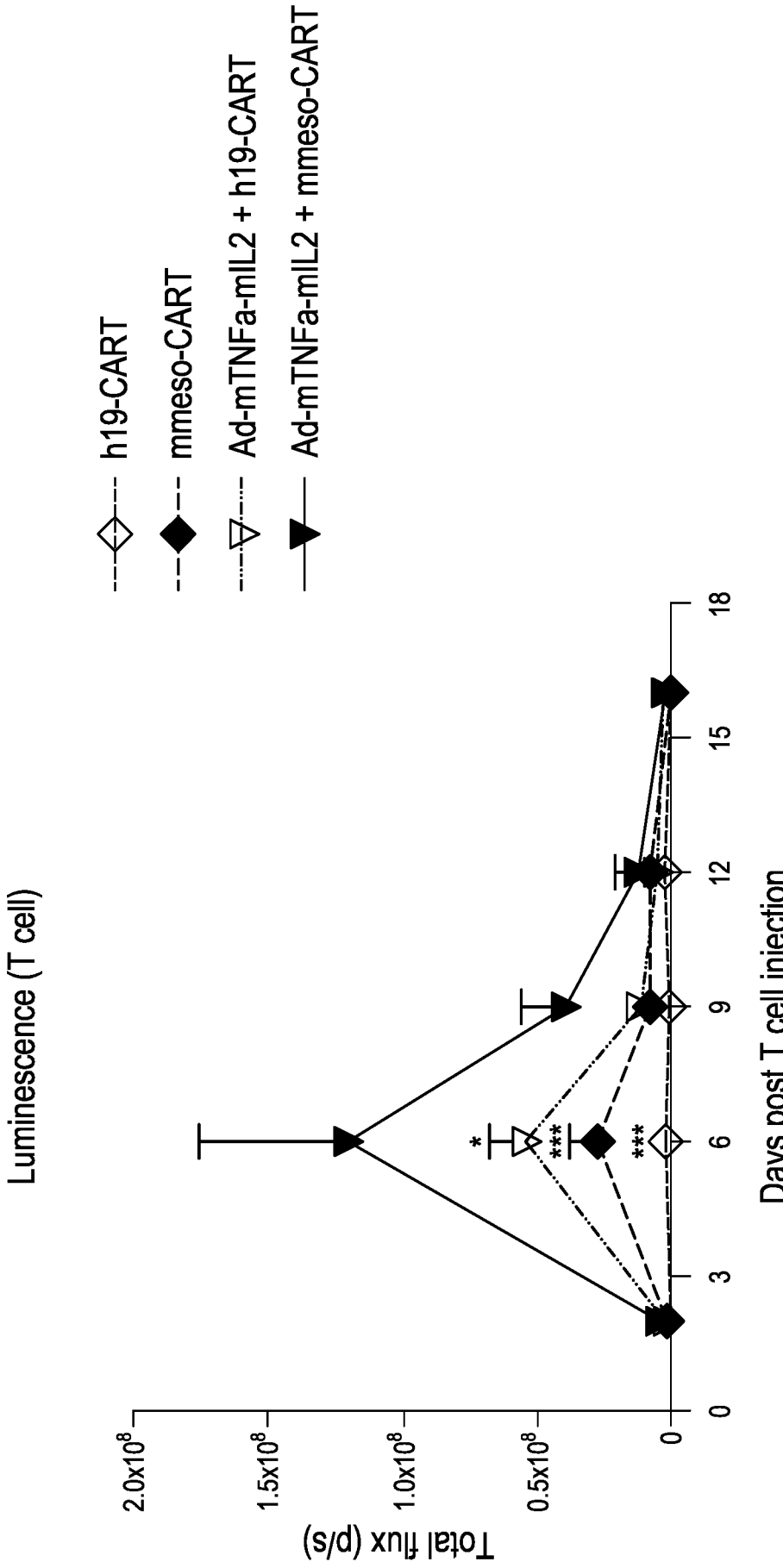
Luminescence (T cell)
h19-CART
mmeso-CART
Ad-mTNFa-mIL2 + h19-CART
Ad-mTNFa-mIL2 + mmeso-CART
Total flux (p/s)
2.0x10^8
1.5x10^8
1.0x10^8
0.5x10^8
0
0
3
6
9
12
15
18
Days post T cell injection
*
***
***

FIG. 6C

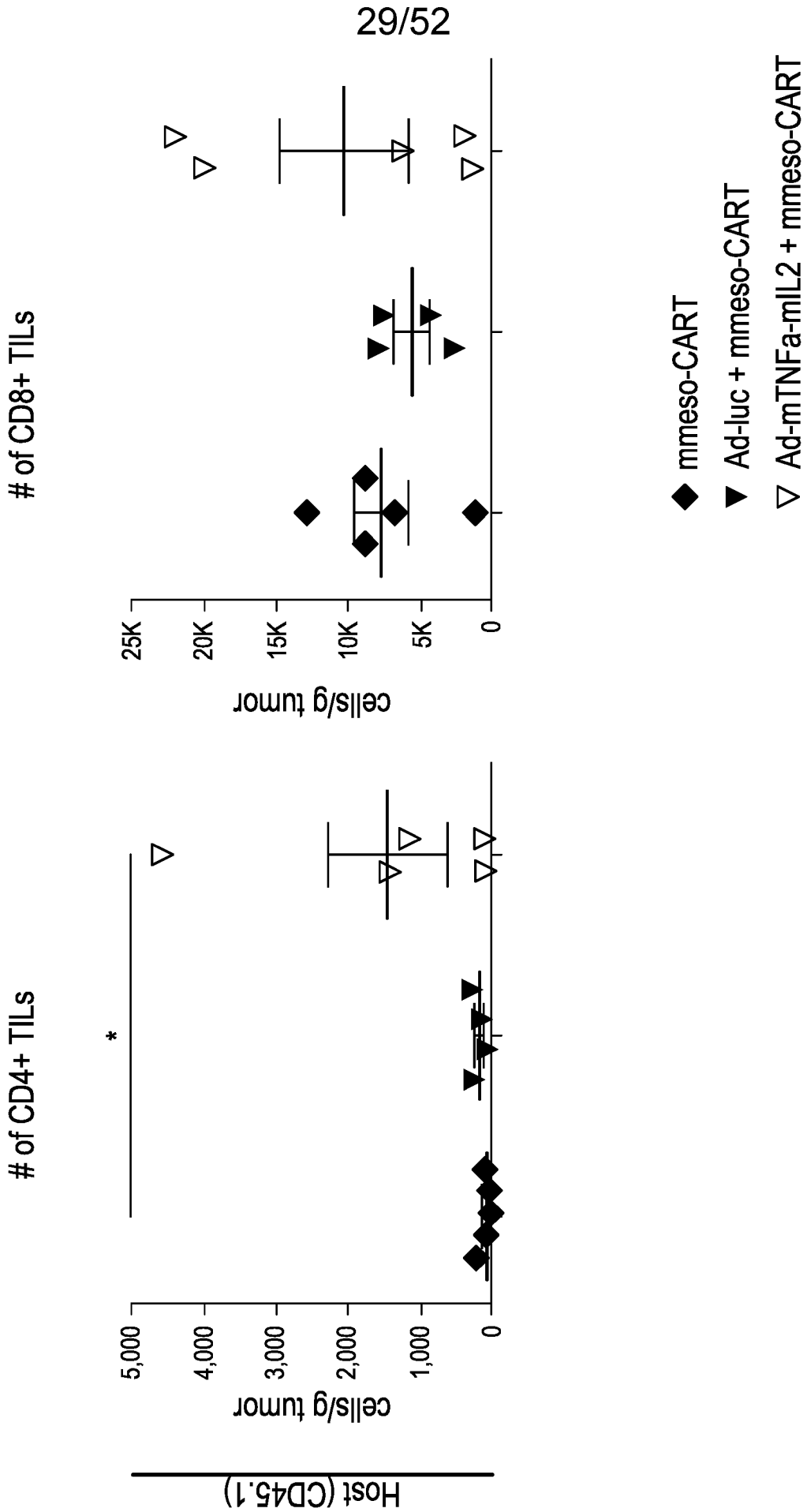
Host (CD45.1)
cells/g tumor
5,000
4,000
3,000
2,000
1,000
0
# of CD4+ TILs
*
cells/g tumor
25K
20K
15K
10K
5K
0
# of CD8+ TILs
mmeso-CART
Ad-luc + mmeso-CART
Ad-mTNFa-mIL2 + mmeso-CART

FIG. 6D (part 1)

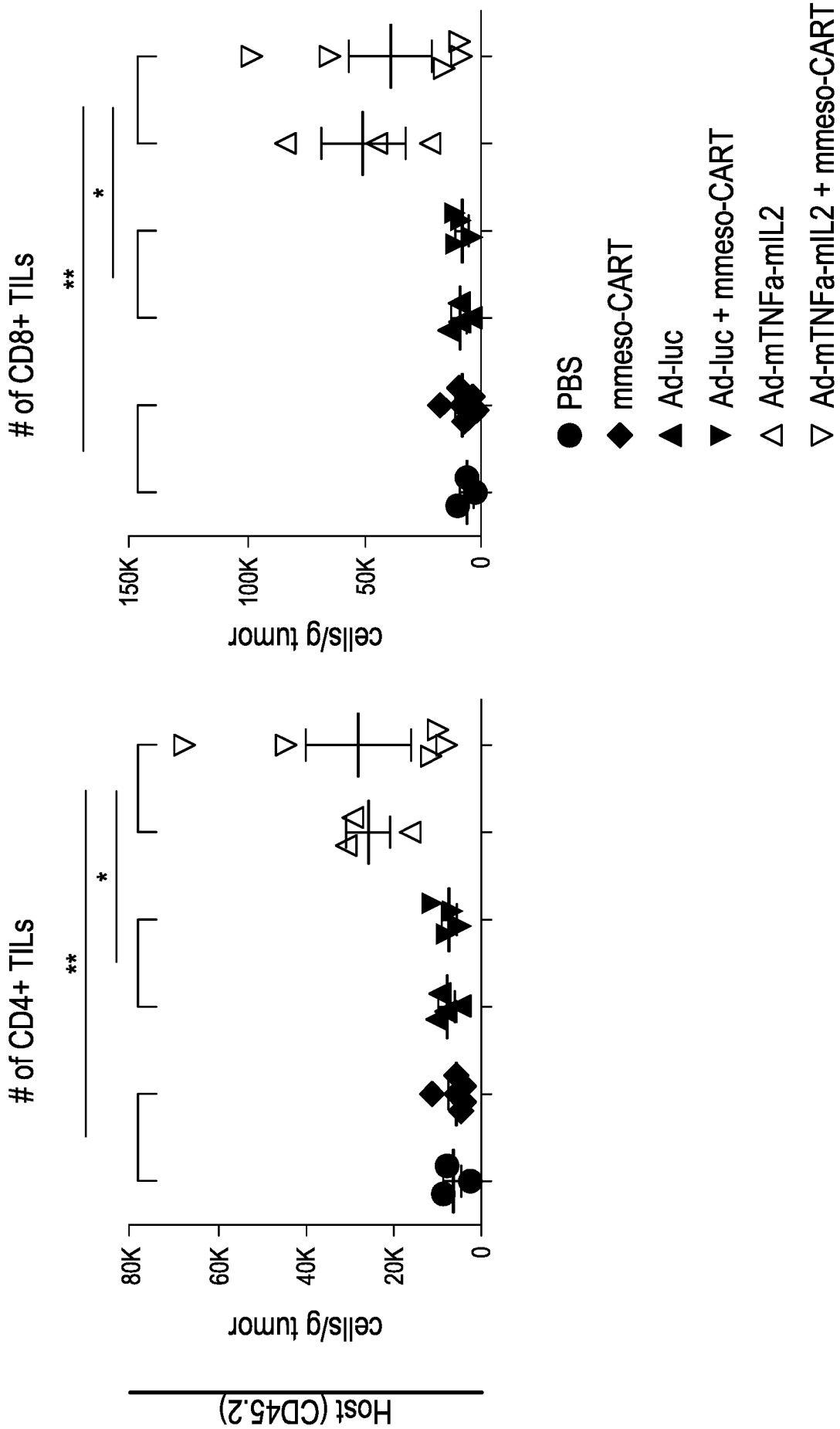
Host (CD45.2)
# of CD4+ TILs
cells/g tumor
80K
60K
40K
20K
0
**
*
# of CD8+ TILs
cells/g tumor
150K
100K
50K
0
**
*
PBS
mmeso-CART
Ad-luc
Ad-luc + mmeso-CART
Ad-mTNFa-mIL2
Ad-mTNFa-mIL2 + mmeso-CART

FIG. 6D (part 2)

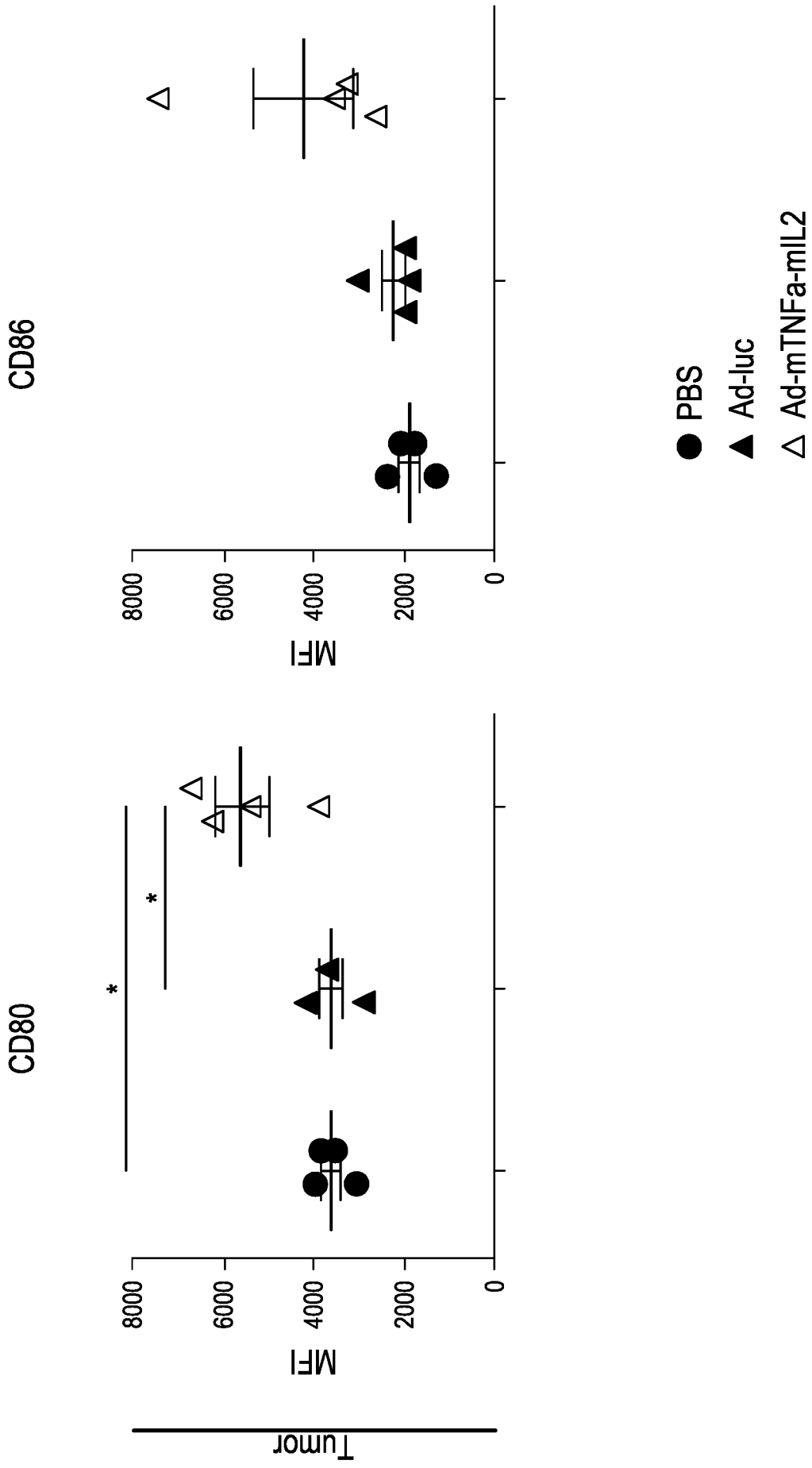
CD80
CD86
MFI
8000
6000
4000
2000
0
Tumor
PBS
Ad-luc
Ad-mTNFa-mIL2
*
*

FIG. 6E (part 1)

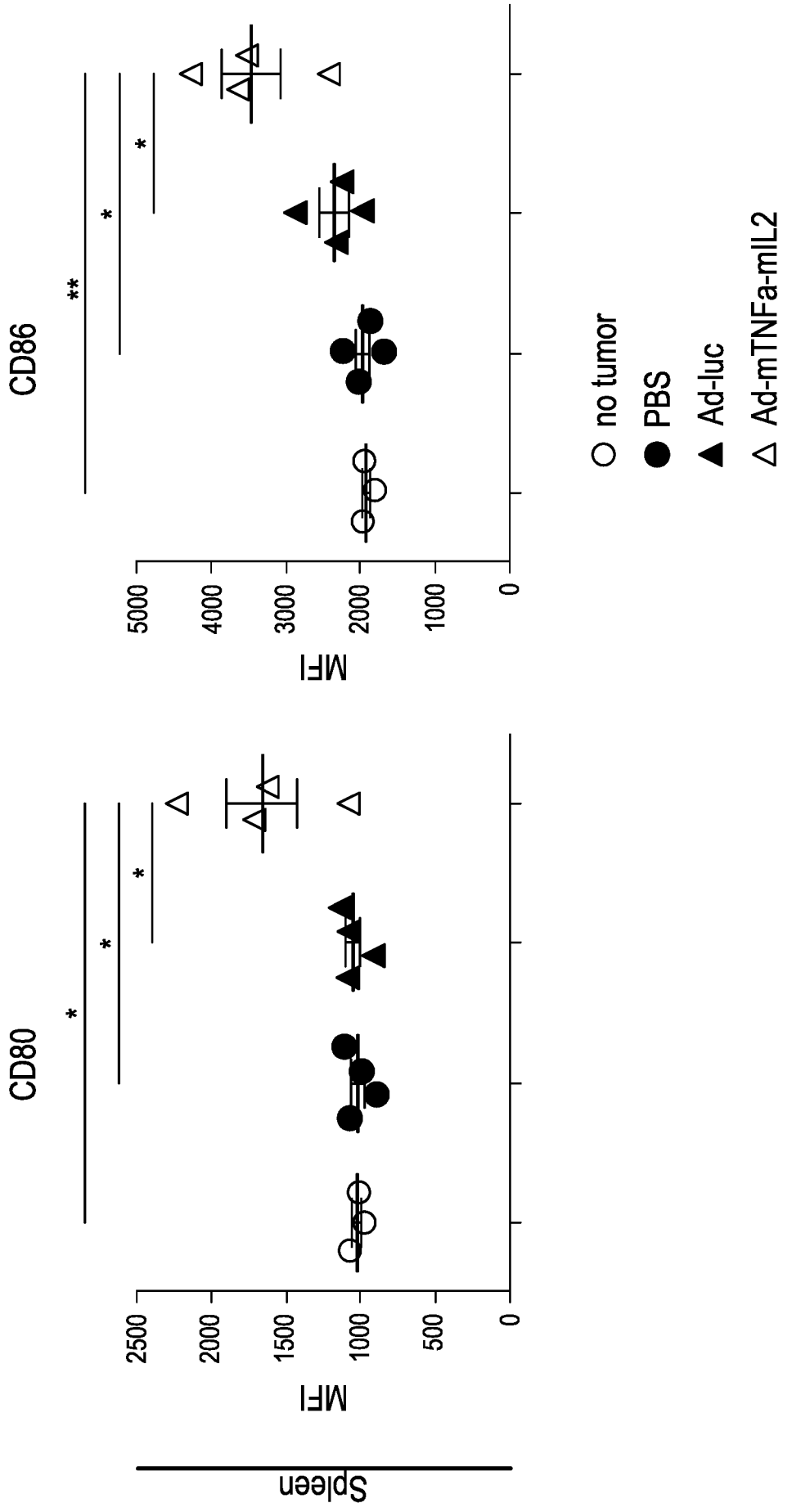
Spleen
CD80
CD86
MFI
2500
2000
1500
1000
500
0
5000
4000
3000
2000
1000
0
*
*
*
**
no tumor
PBS
Ad-luc
Ad-mTNFa-mIL2

FIG. 6E (part 2)

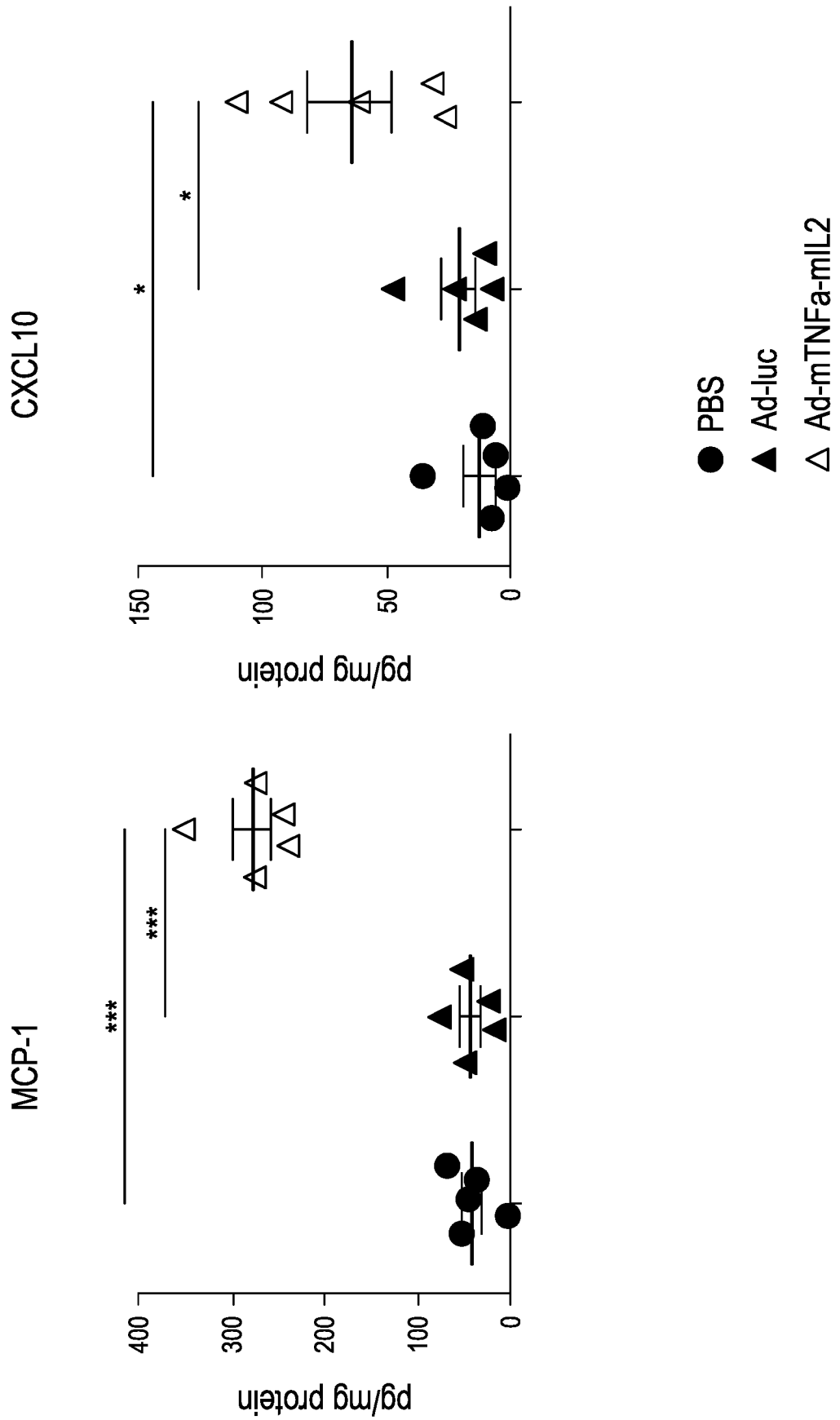
CXCL10
MCP-1
pg/mg protein
150
100
50
0
400
300
200
100
0
*
***
PBS
Ad-luc
Ad-mTNFa-mIL2

FIG. 6F

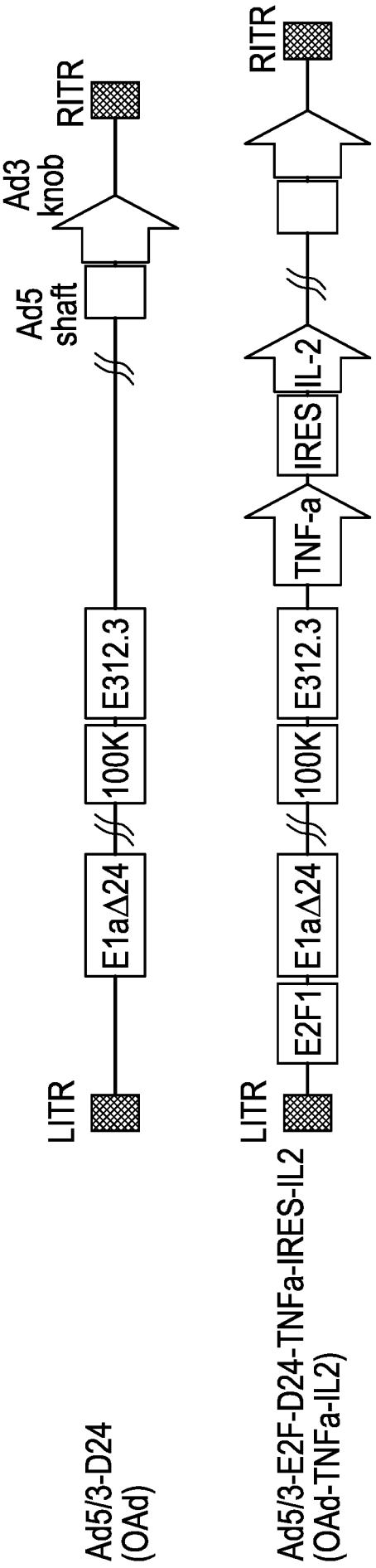
LITR
E1aΔ24
100K
E312.3
Ad5
shaft
Ad3
knob
RITR
Ad5/3-D24
(OAd)
LITR
E2F1
E1aΔ24
100K
E312.3
TNF-a
IRES
IL-2
RITR
Ad5/3-E2F-D24-TNFa-IRES-IL2
(OAd-TNFa-IL2)

FIG. 7A

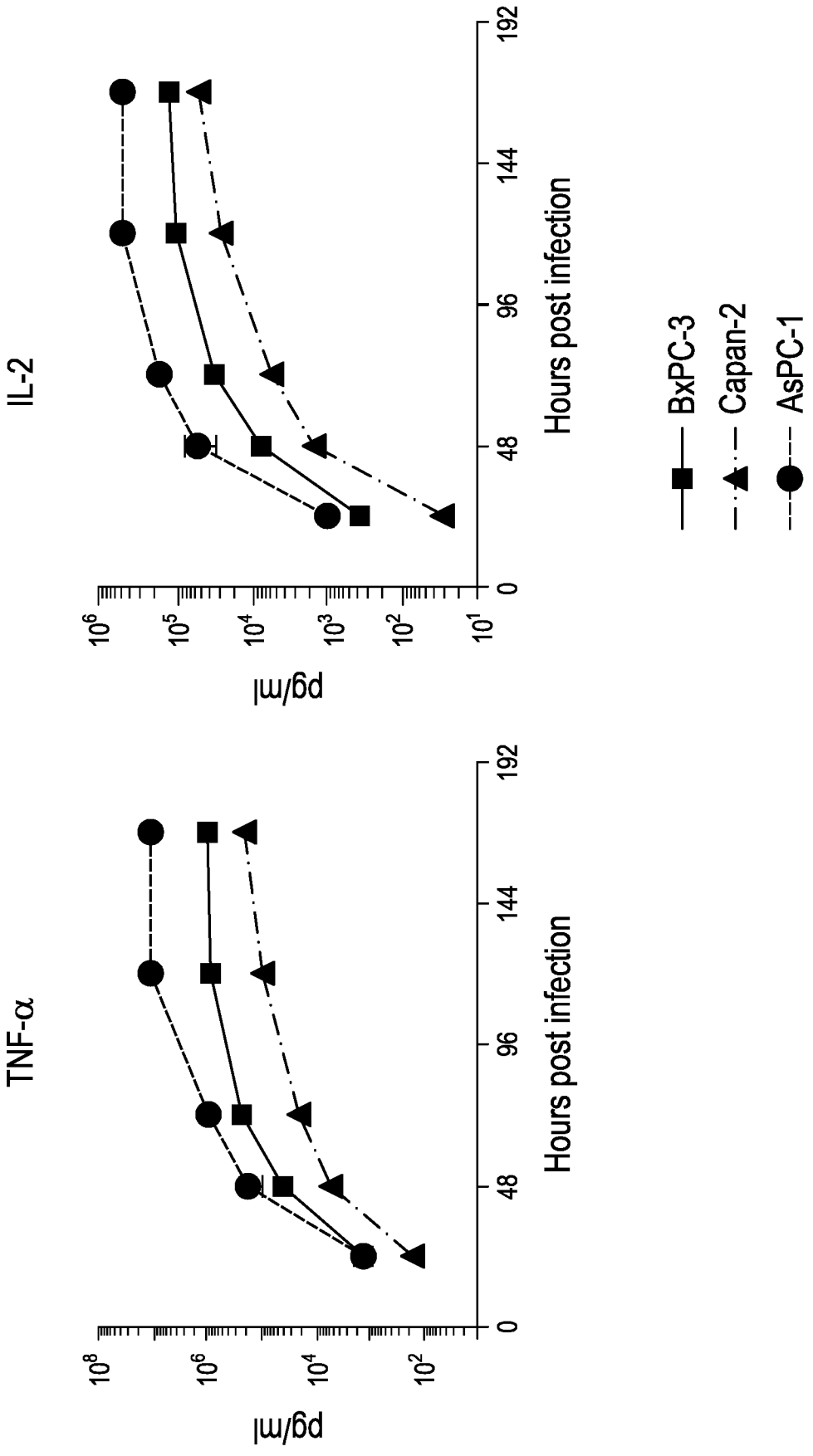
IL-2
pg/ml
10^6
10^5
10^4
10^3
10^2
10^1
0
48
96
144
192
Hours post infection
TNF-α
pg/ml
10^8
10^6
10^4
10^2
0
48
96
144
192
Hours post infection
BxPC-3
Capan-2
AsPC-1

FIG. 7B

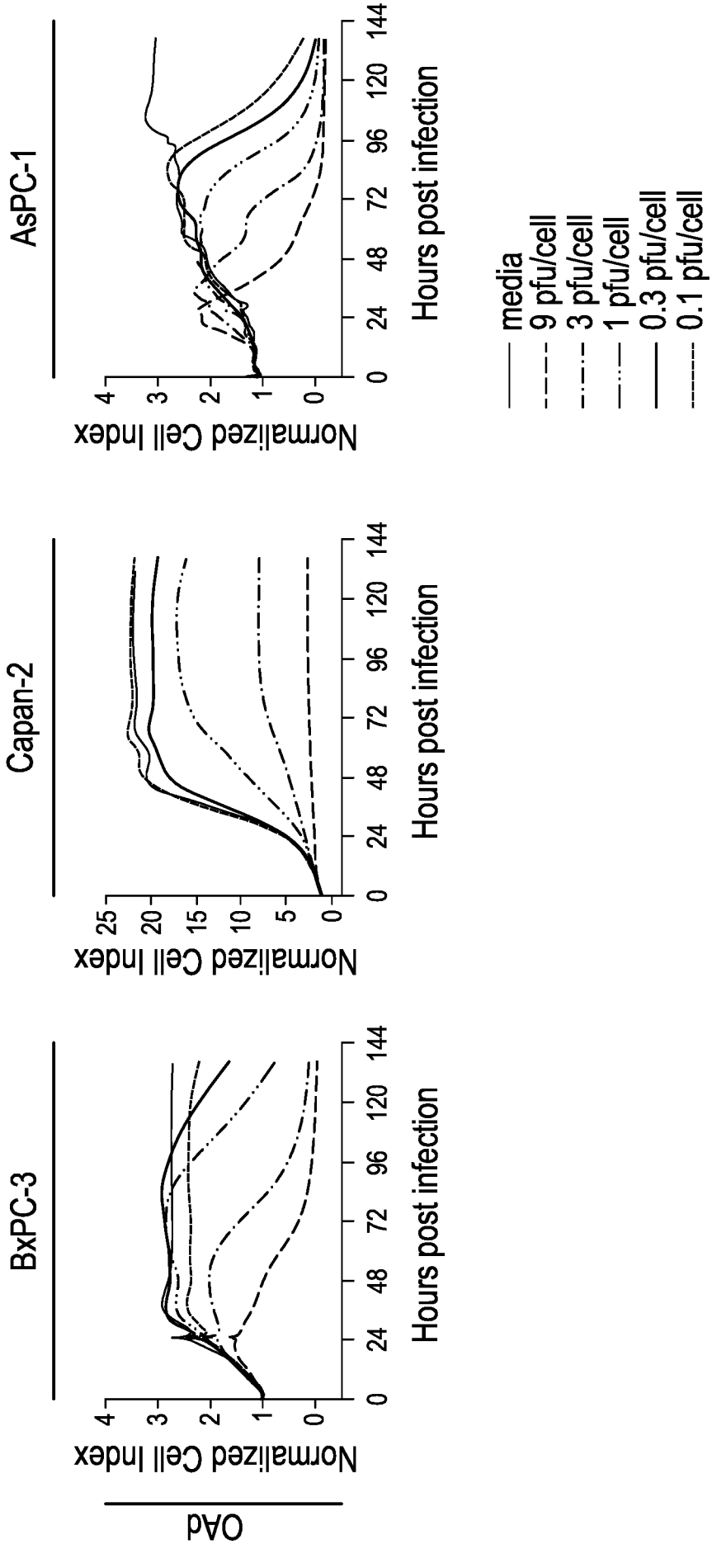
OAd
BxPC-3
Capan-2
AsPC-1
Normalized Cell Index
Hours post infection
0
24
48
72
96
120
144
media
9 pfu/cell
3 pfu/cell
1 pfu/cell
0.3 pfu/cell
0.1 pfu/cell

FIG. 7C (part 1)

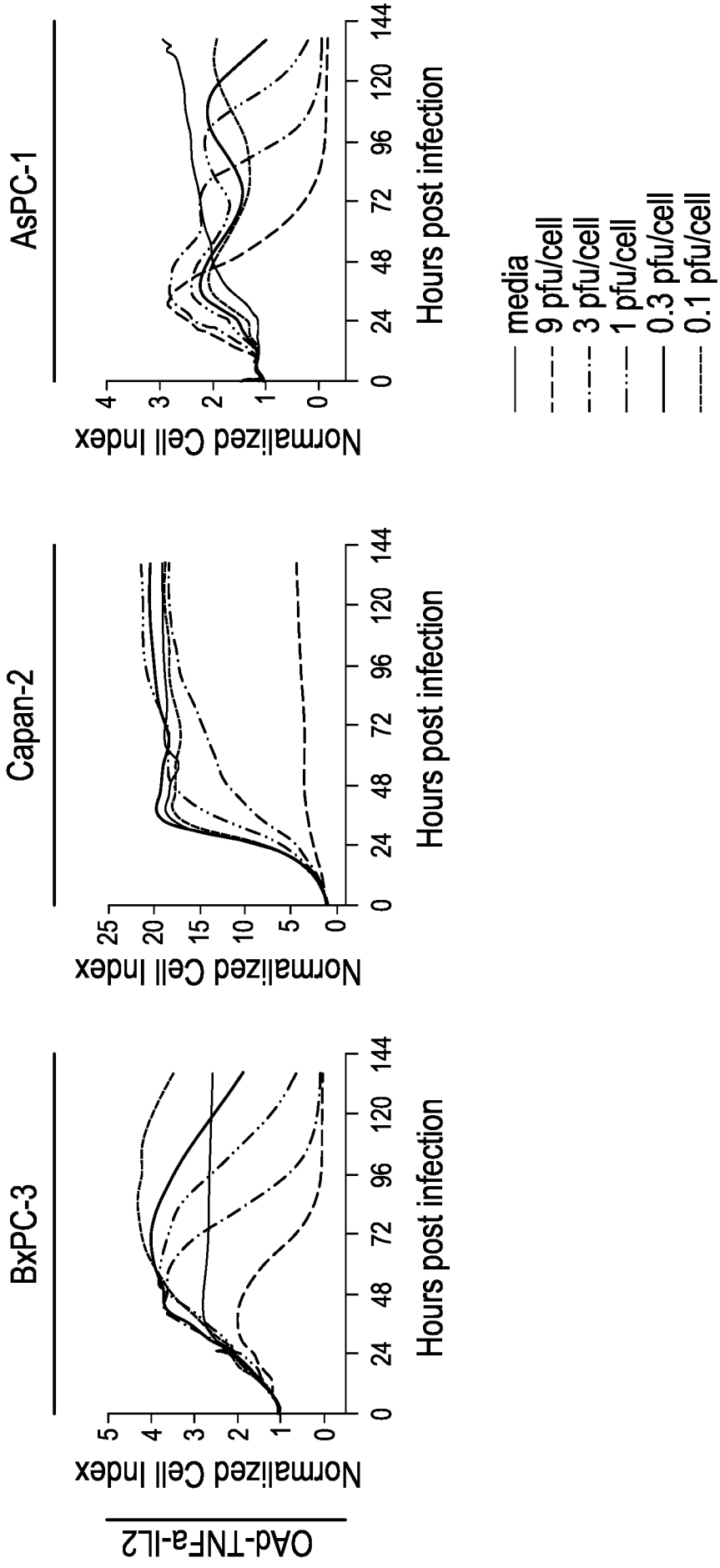
OAd-TNFa-IL2
BxPC-3
Capan-2
AsPC-1
Normalized Cell Index
Hours post infection
0
24
48
72
96
120
144
media
9 pfu/cell
3 pfu/cell
1 pfu/cell
0.3 pfu/cell
0.1 pfu/cell

FIG. 7C (part 2)

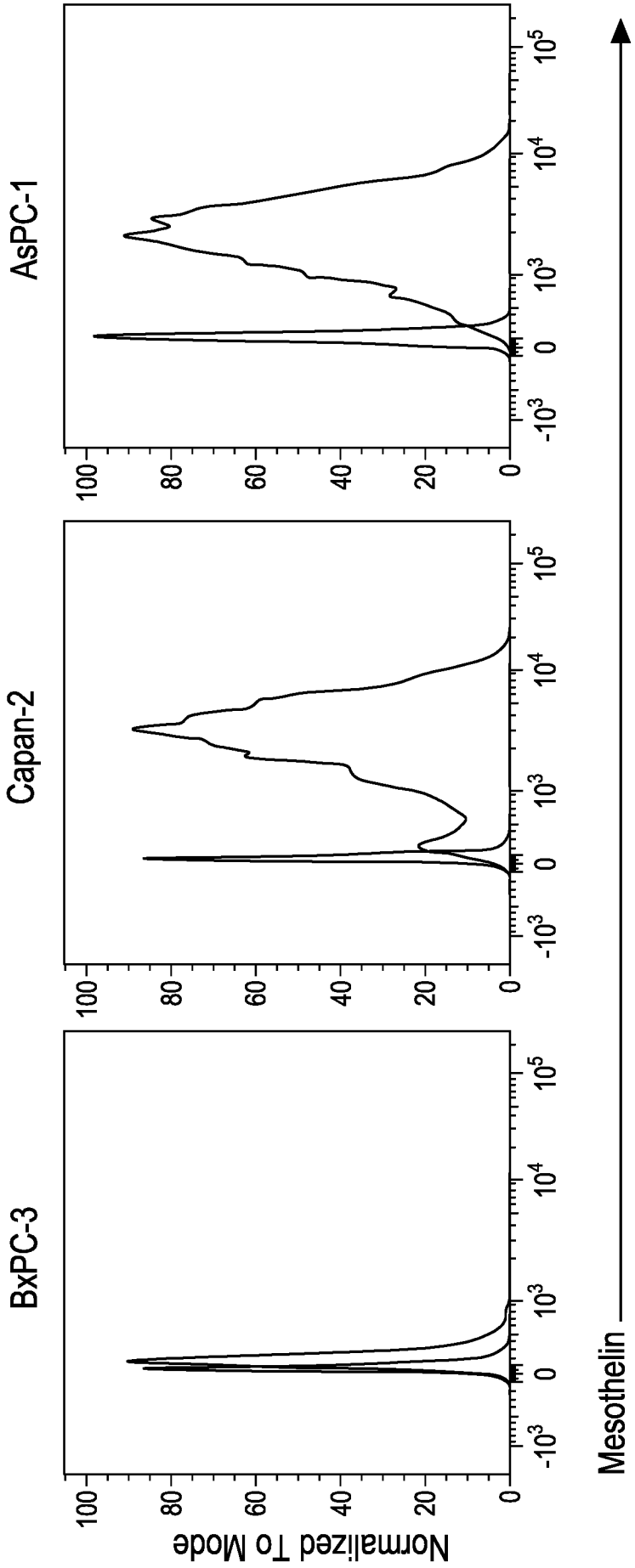
AsPC-1
Capan-2
BxPC-3
Normalized To Mode
Mesothelin
0
20
40
60
80
100
-10^3
0
10^3
10^4
10^5

FIG. 7D

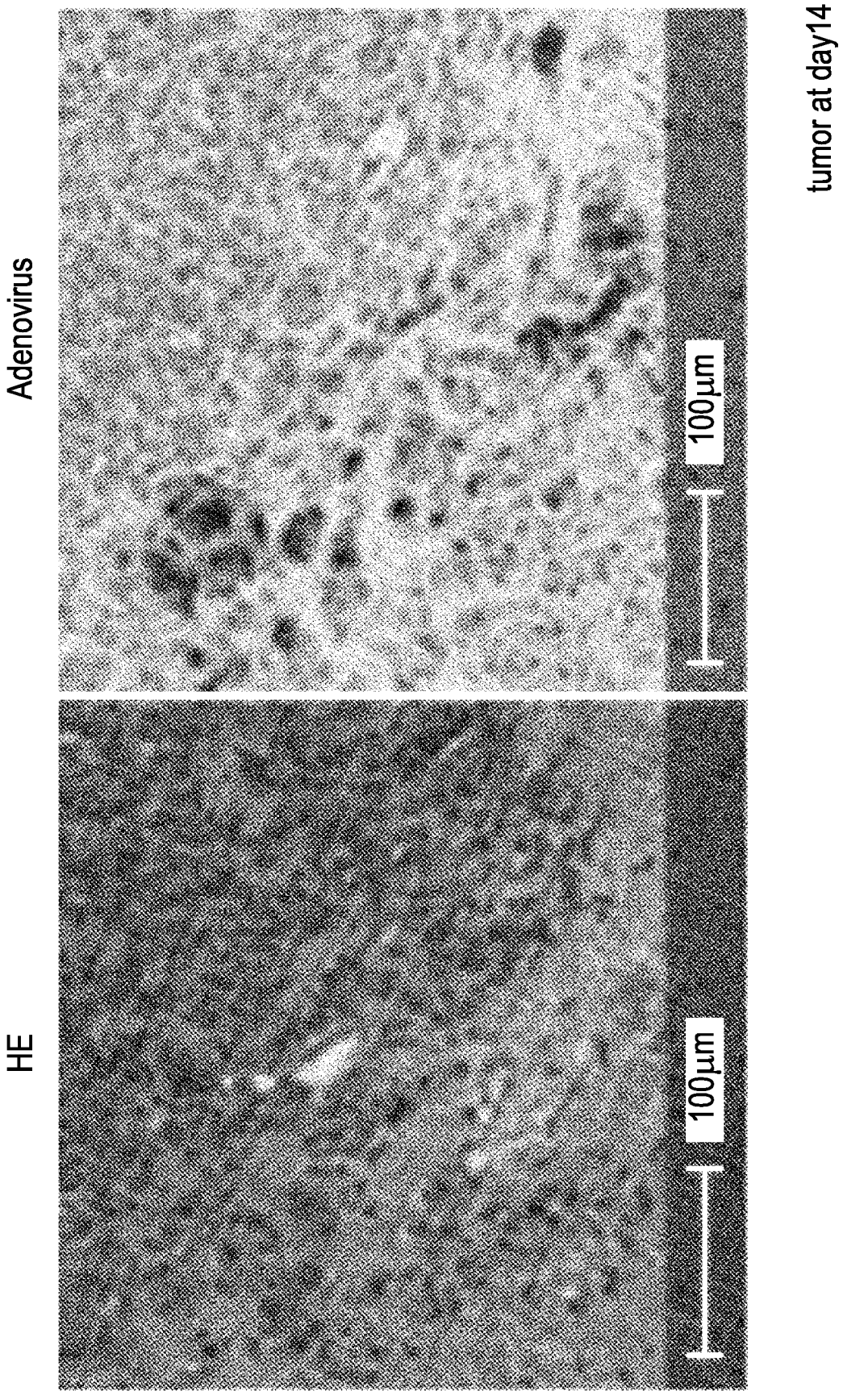

FIG. 8

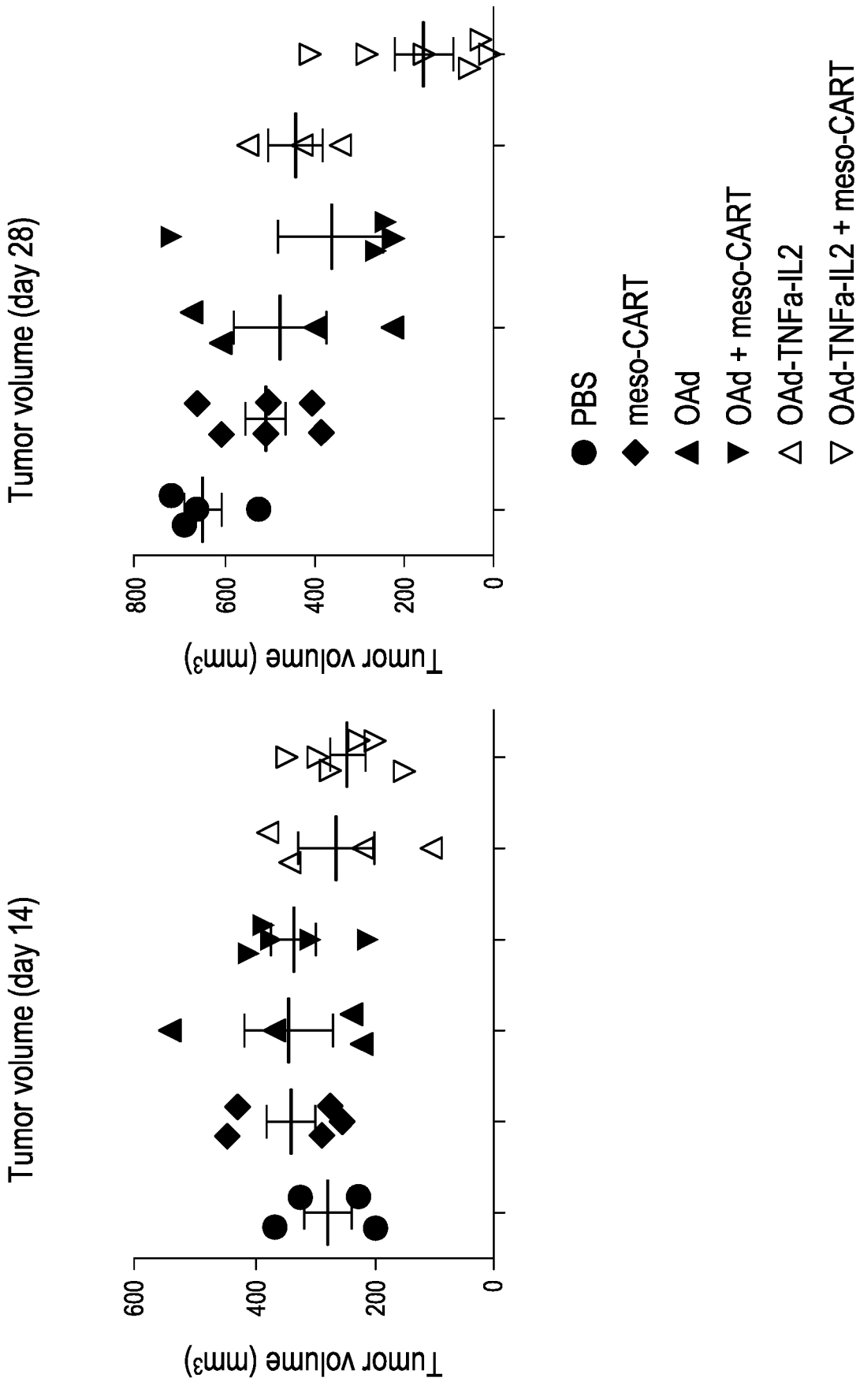
Tumor volume (day 14)
Tumor volume (day 28)
Tumor volume (mm3)
0
200
400
600
800
PBS
meso-CART
OAd
OAd + meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART

FIG. 9A

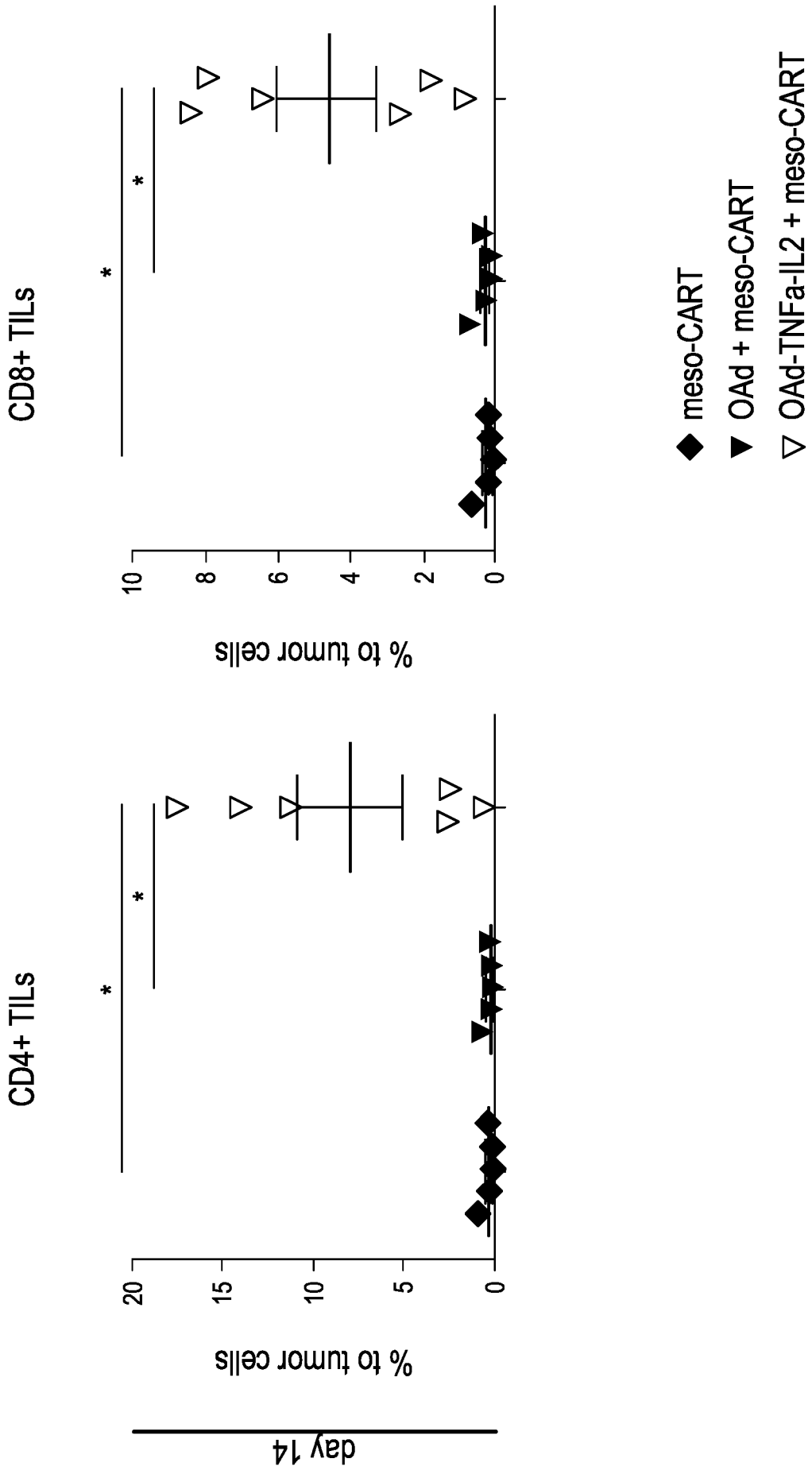
CD4+ TILs
CD8+ TILs
% to tumor cells
day 14
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 9B (part 1)

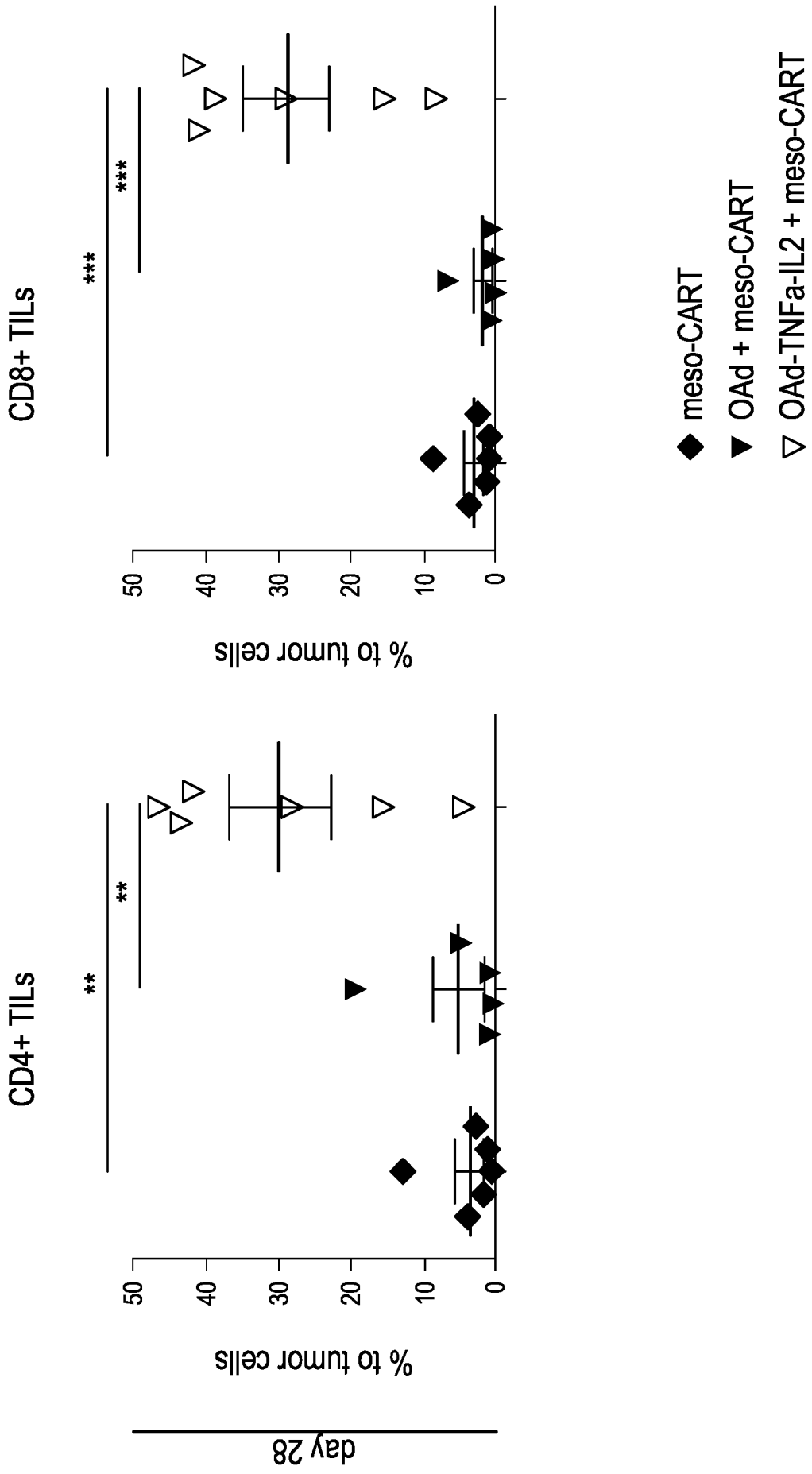
CD4+ TILs
CD8+ TILs
% to tumor cells
day 28
**
***
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 9B (part 2)

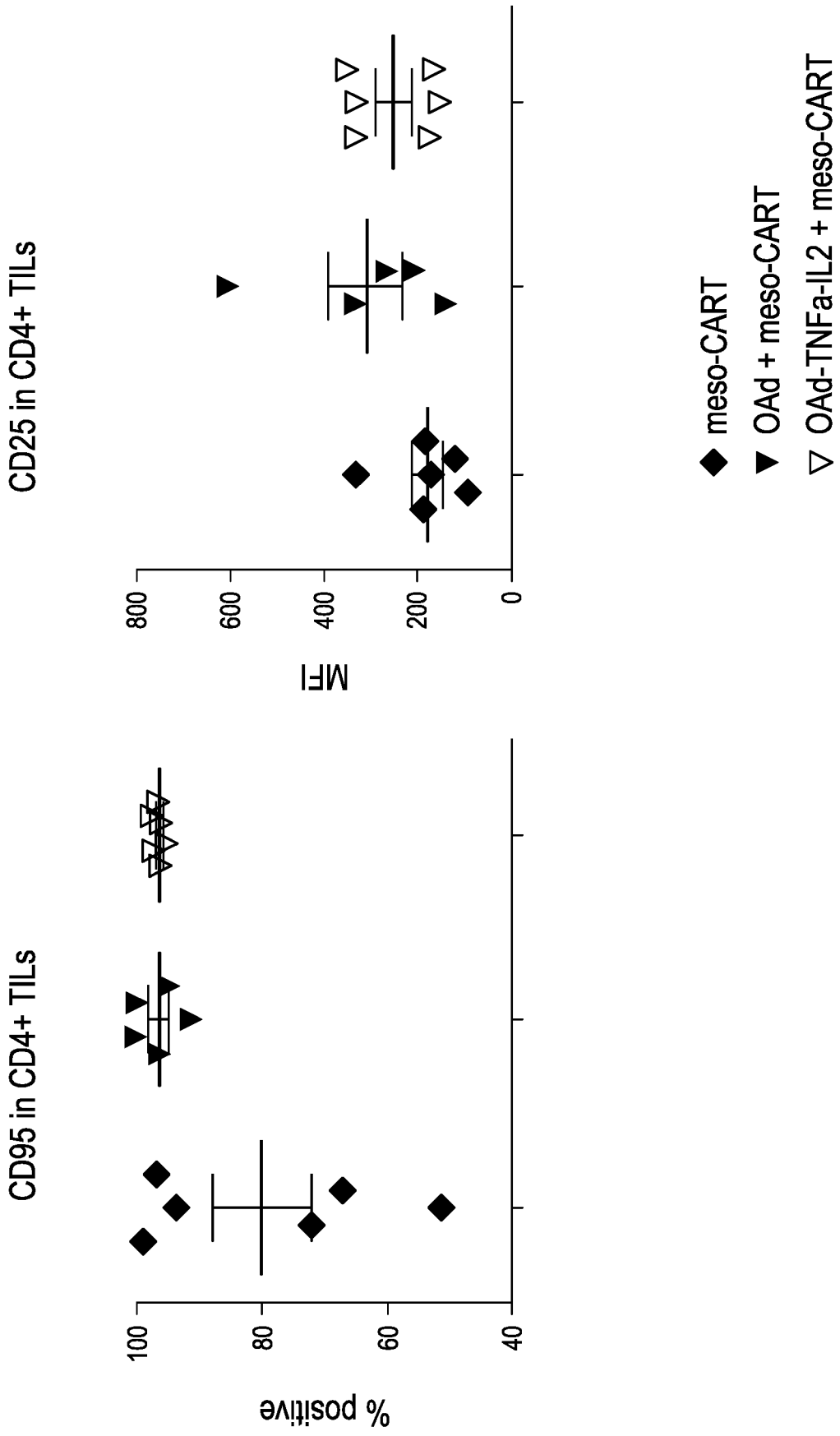
CD25 in CD4+ TILs
MFI
800
600
400
200
0
CD95 in CD4+ TILs
% positive
100
80
60
40
meso-CART
OAd + meso-CART
OAd-TNFa-IL2 + meso-CART

FIG. 9C

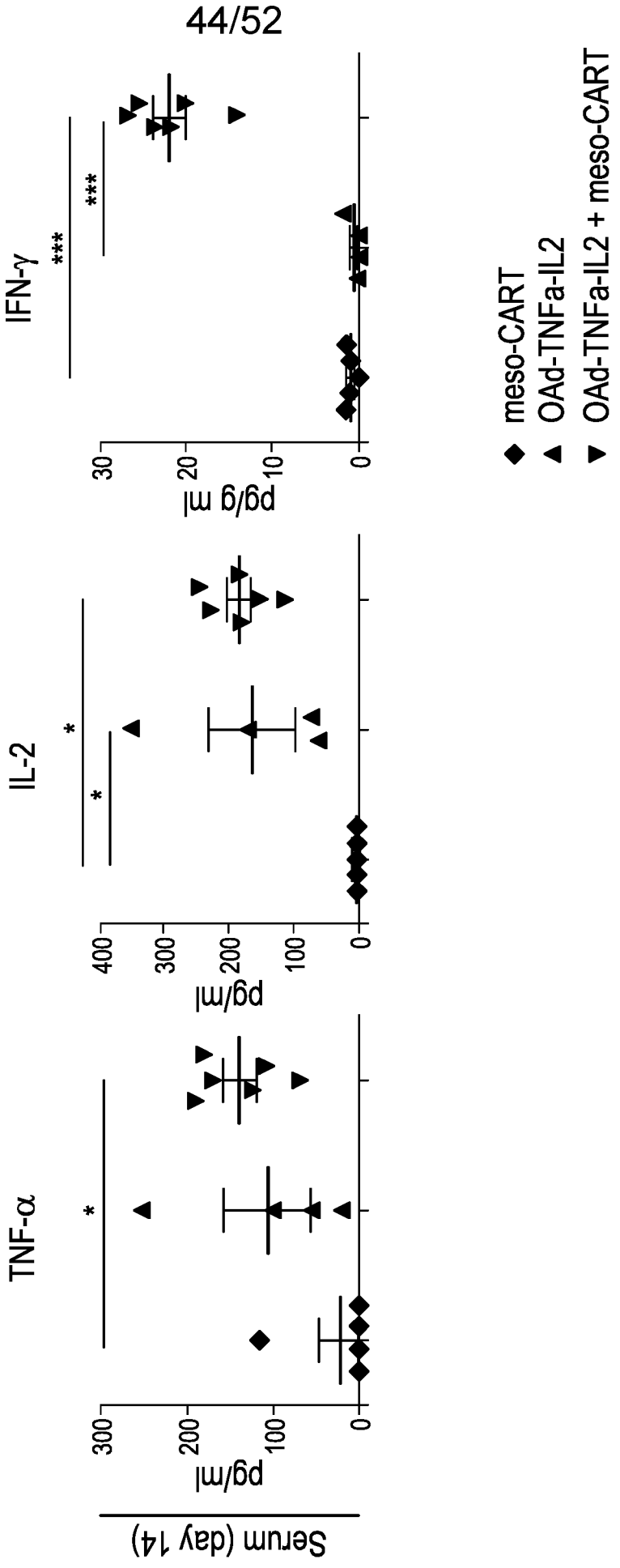
Serum (day 14)
TNF-α
IL-2
IFN-γ
pg/ml
pg/g ml
0
100
200
300
400
10
20
30
*
***
meso-CART
OAd-TNFa-IL2
OAd-TNFa-IL2 + meso-CART

FIG. 9D

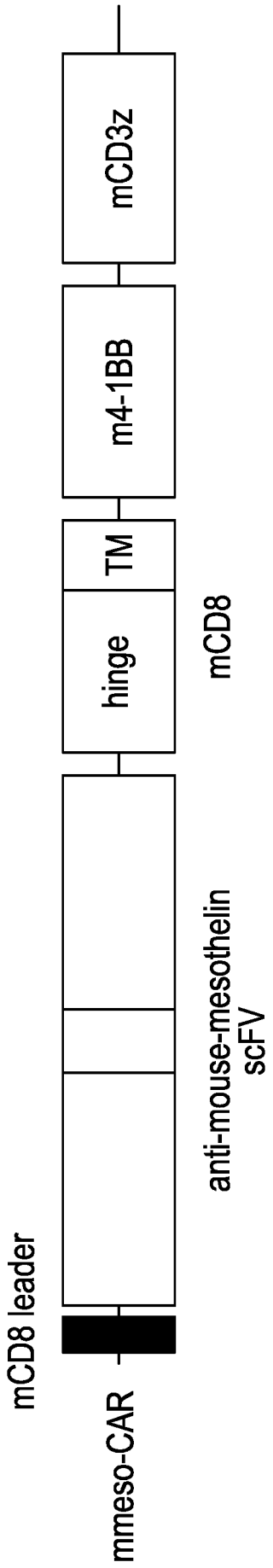
mCD3z
m4-1BB
TM
hinge
mCD8
anti-mouse-mesothelin scFV
mCD8 leader
mmeso-CAR

FIG. 10A

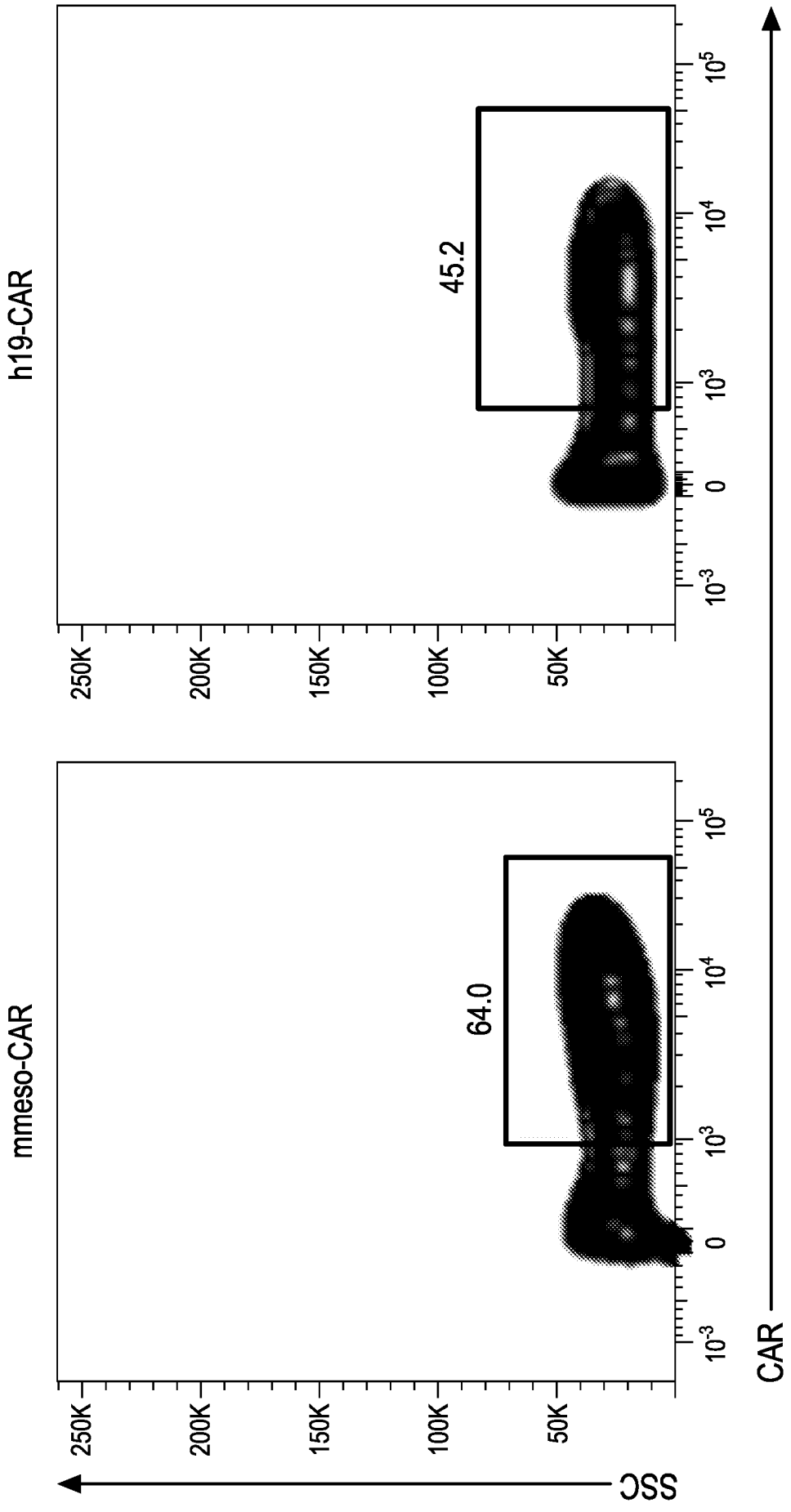
h19-CAR
45.2
mmeso-CAR
64.0
SSC
CAR
250K
200K
150K
100K
50K
10^{-3}
0
10^{3}
10^{4}
10^{5}

FIG. 10B

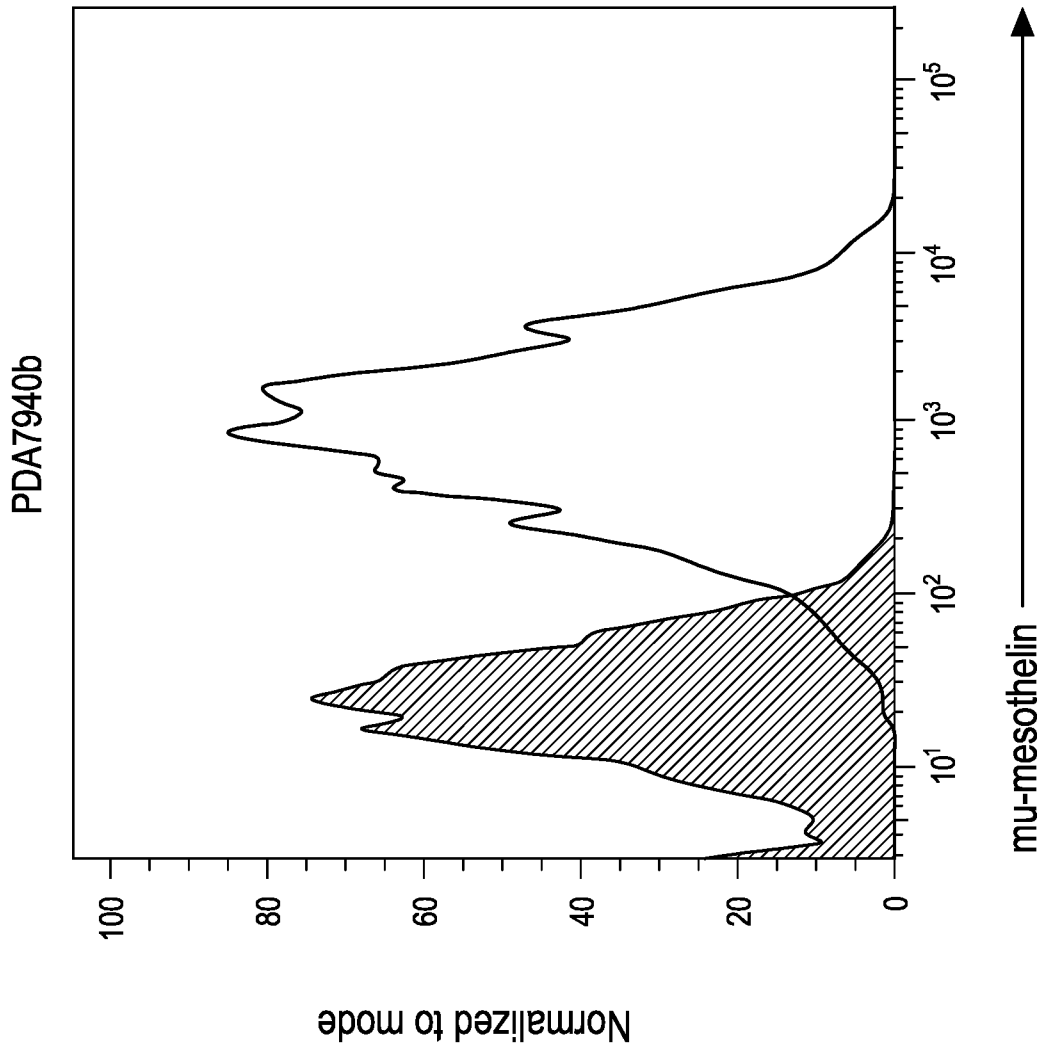

FIG. 10C (part 1)

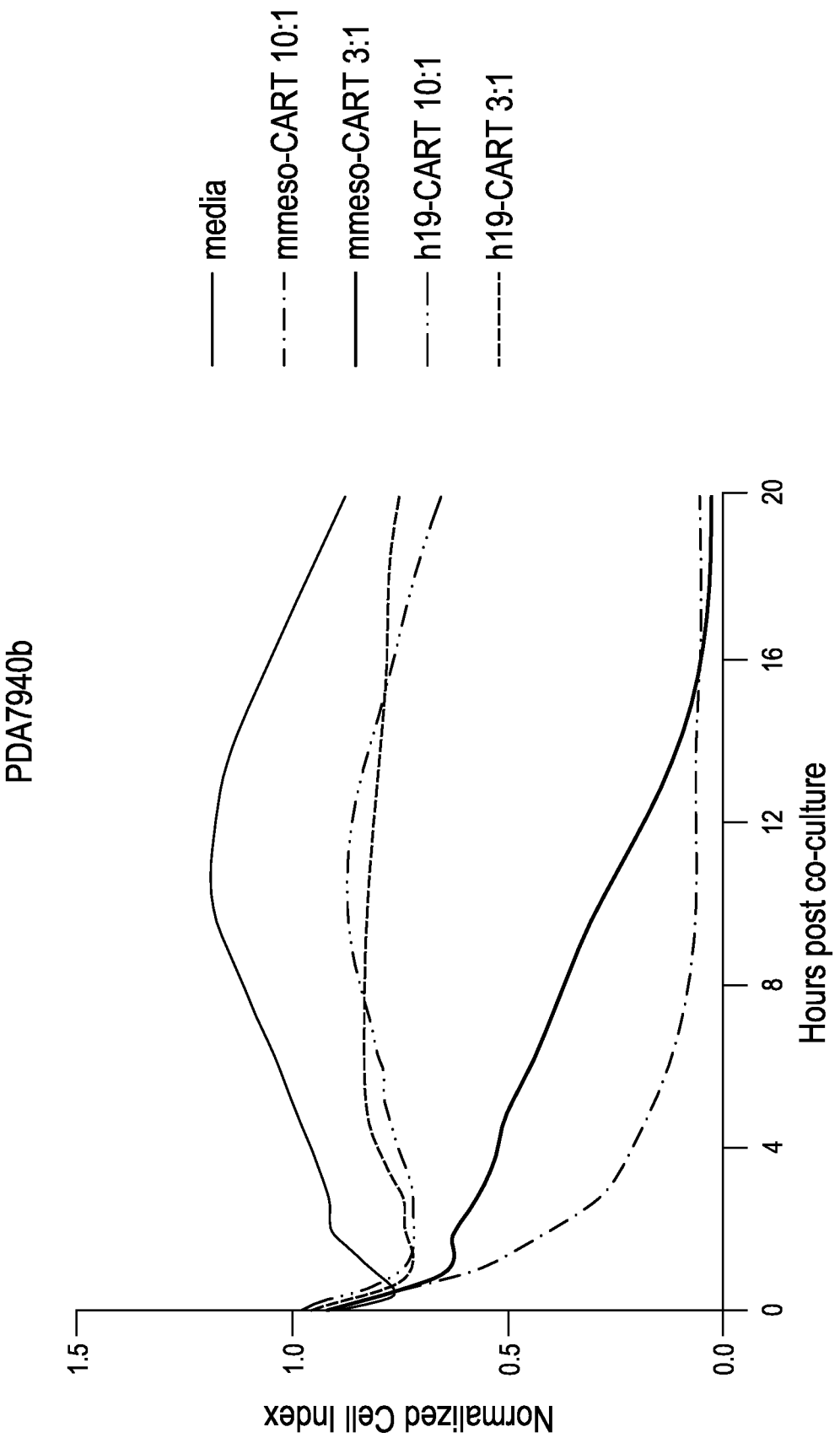

FIG. 10C (part 2)

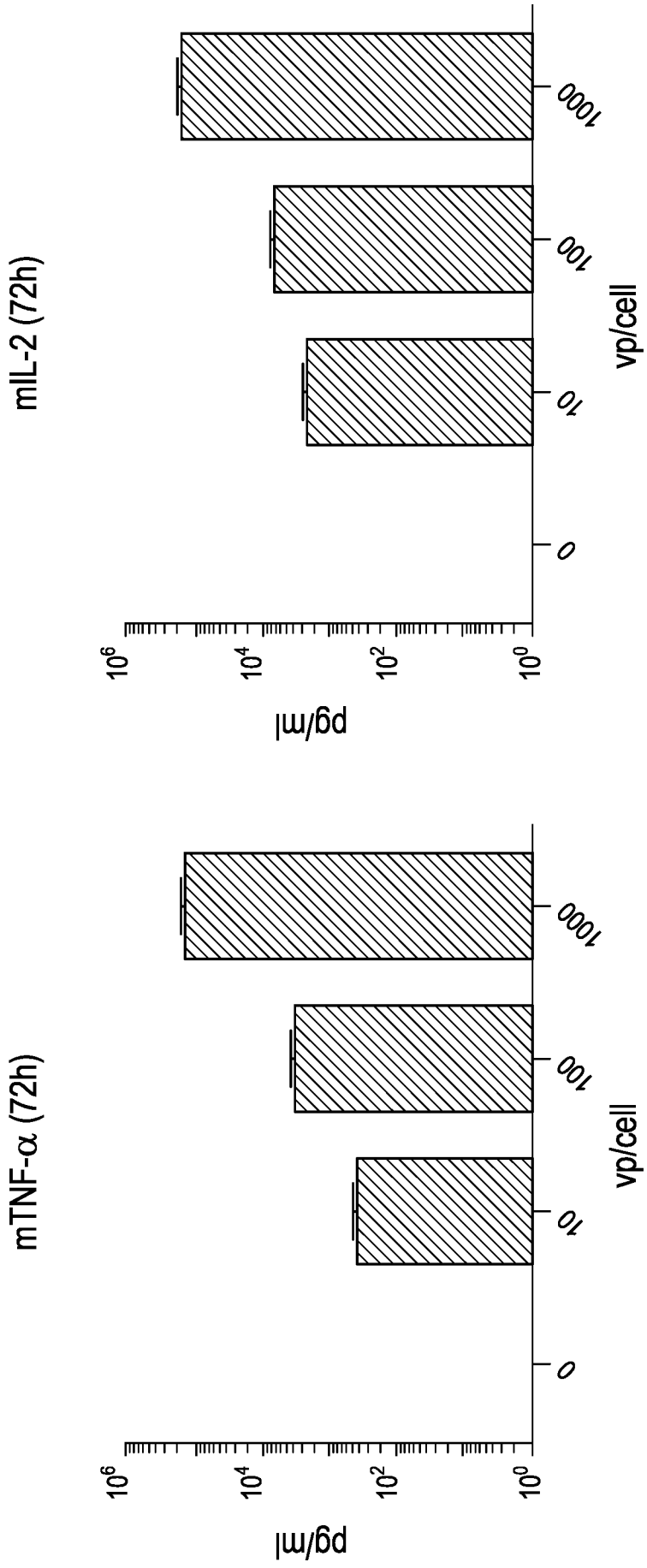
mIL-2 (72h)
pg/ml
10^6
10^4
10^2
10^0
0
10
100
1000
vp/cell
mTNF-α (72h)
pg/ml
10^6
10^4
10^2
10^0
0
10
100
1000
vp/cell

FIG. 10D

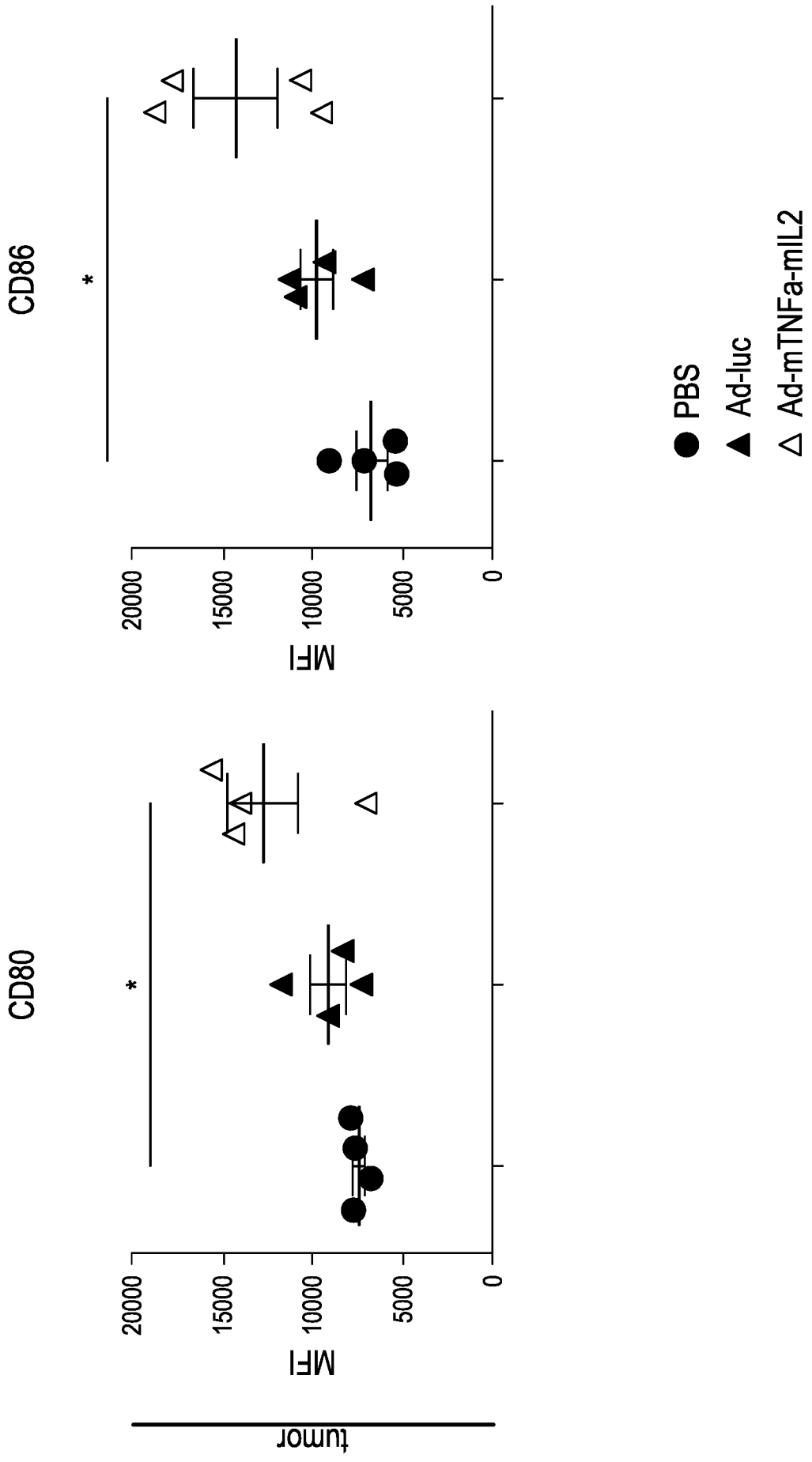

FIG. 10E (part 1)

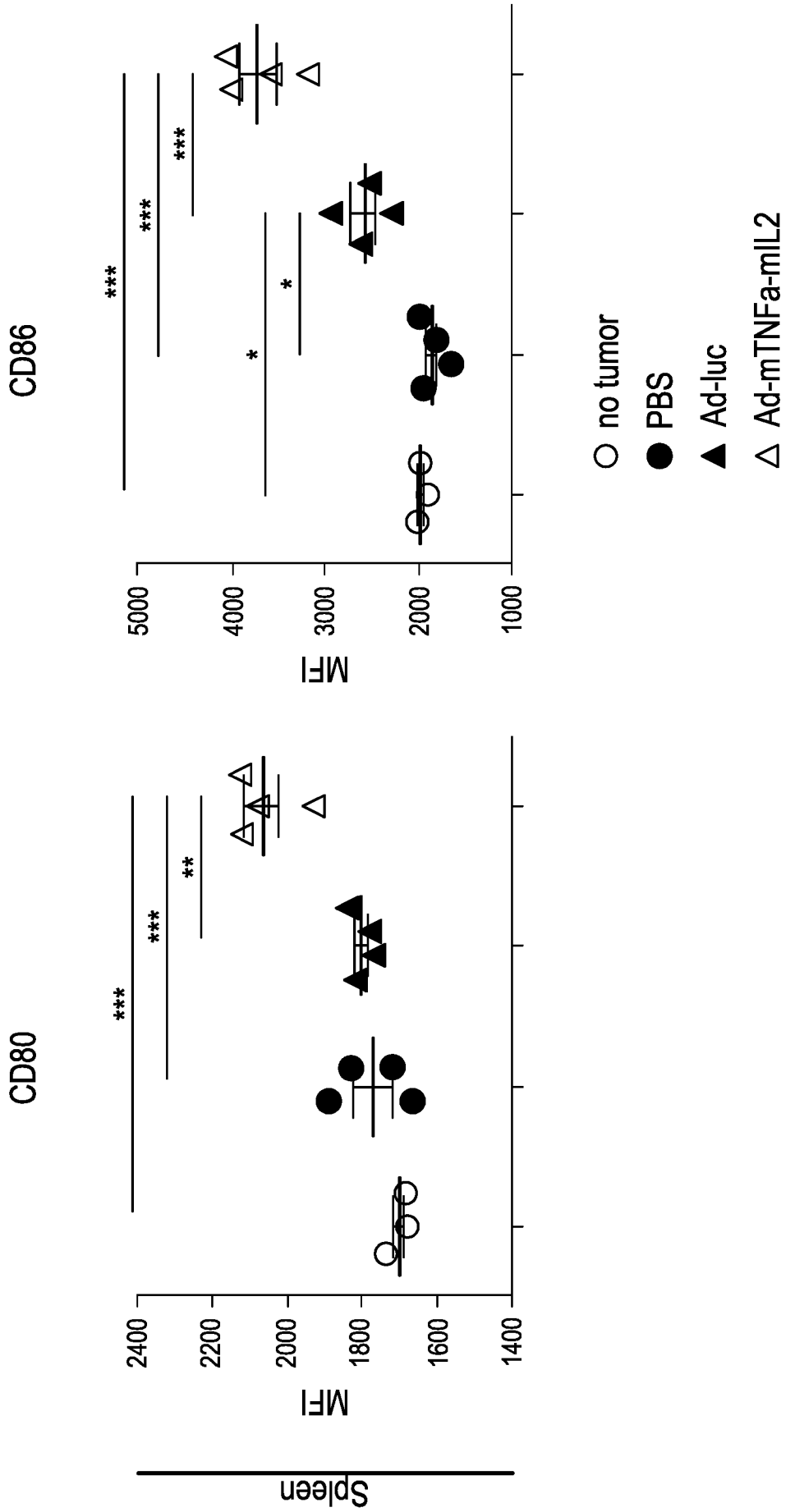

FIG. 10E (part 2)

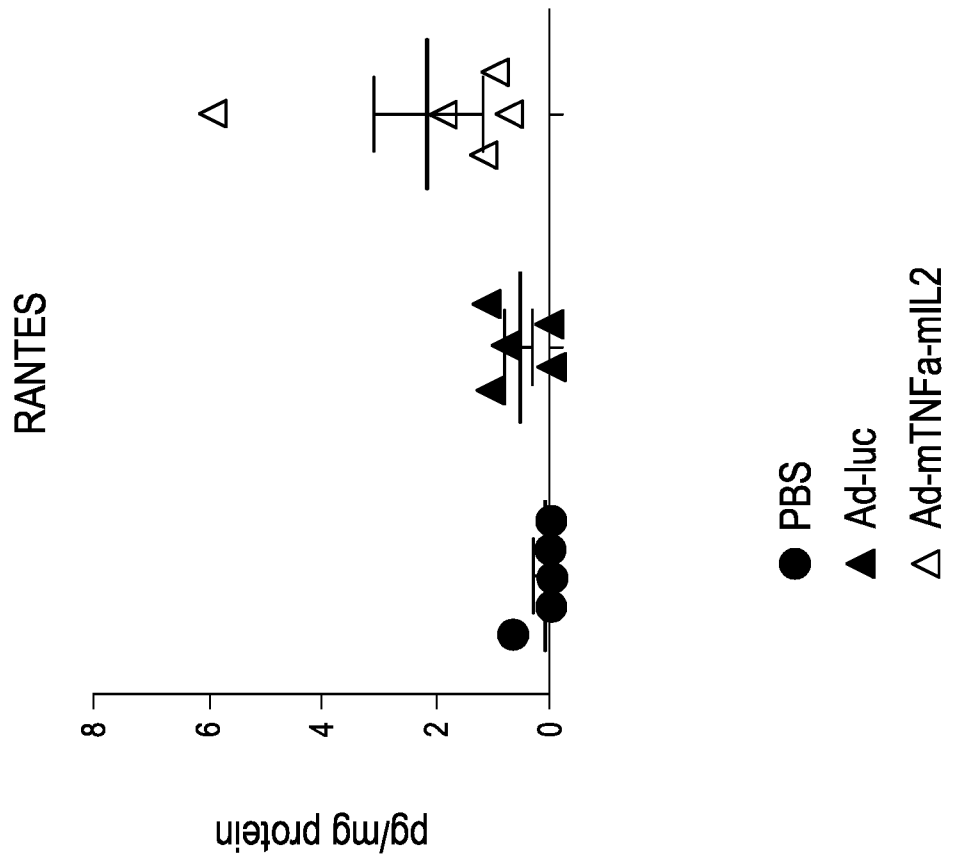

FIG. 10F