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[Continued on next page]

(54) Title: TUMOR-SPECIFIC IFNA SECRETION BY CAR T-CELLS TO REPROGRAM THE SOLID TUMOR MICROENVIRONMENT

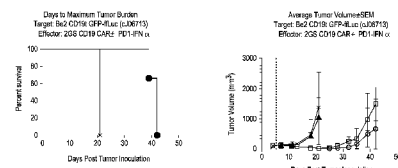


FIG. II

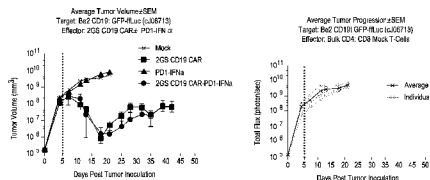


FIG. II CONT.

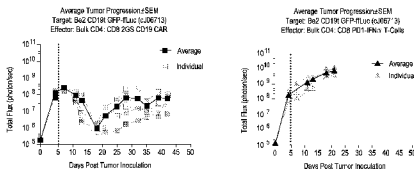


FIG. II CONT.

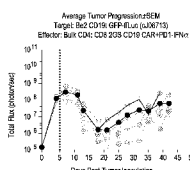


FIG. II CONT.

(57) Abstract: The present application relates to fusion proteins, chimeric antigen bearing cells expressing fusion proteins and compositions comprising chimeric antigen bearing cells expressing fusion proteins. The application further relates to methods of using the fusion proteins, cells and compositions for modulating an immune response.



MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
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TUMOR-SPECIFIC IFNA SECRETION BY CAR T-CELLS TO REPROGRAM THE SOLID TUMOR MICROENVIRONMENT

RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/278,648, filed January 14, 2016. The entire disclosure of the aforementioned application is expressly incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING, TABLE, OR COMPUTER PROGRAM LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 2017_01_10_SQ_LISTING_SCRI_109WO.TXT, created January 10, 2017, which is 25kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] Disclosed herein are compositions and methods relating to a dual-active, secretable fusion protein that can be used in chimeric antigen receptor (CAR) therapy for a subject. Additionally, methods for intrinsic production and secretion of a PD-1:IFN α 2a fusion protein in CAR T-cells to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment, are also provided. These methods and compositions can improve the therapeutic efficacy of CAR T-cell therapy targeted against solid tumors by providing regulatory inputs for multiple immune cell subsets found in the tumor microenvironment.

BACKGROUND OF THE INVENTION

[0004] The tumor microenvironment (TME) is the cellular environment in which the tumor exists, and can include surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules

and the extracellular matrix (ECM). The tumor and the surrounding microenvironment are closely related and interact constantly. Tumors influence the microenvironment by releasing extracellular signals, promoting tumor angiogenesis and inducing peripheral immune tolerance, while the immune cells in the microenvironment can affect the growth and evolution of cancerous cells.

[0005] Due to the TME, the solid tumors and the microenvironment created by the tumors impact infiltrating immune cells that are responsible for the elimination of the tumor and other cancerous type cells. Furthermore, the TME has been known to create a barrier that can also preclude CAR T-cell immunotherapies and other types of therapy for the treatment of cancerous tumors. As such, developments to decrease the effects of the TME and to improve the efficacy of immunotherapies are much needed.

[0006] The use of proteins, which illicit an immune response have been investigated for immunotherapy. Type I interferons (IFN) are a large subgroup of interferon proteins that have been known to regulate the activity of the immune system. The type I interferon IFN α 2a, can confer dynamic roles against multiple immune cell subsets. More specifically, IFN α 2a confers enhanced cytotoxicity in T and NK cells, promotes T-cell stimulatory cytokine production in macrophages and inhibits regulatory T cell function.

[0007] The protein, programmed cell death 1 protein (PD-1) and cluster of differentiation protein 279 (CD279) is encoded by the PDCD1 gene, and has been studied for its role in the immune system. PD-1 is a cell surface receptor belonging to the immunoglobulin superfamily and is expressed on T-cells and pro-B cells. PD-1 binds two ligands, PD-L1 and PD-L2.

[0008] PD-1 can function as an immune checkpoint, playing an important role in down regulating the immune system by preventing the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The inhibitory effect of PD-1 is accomplished through a dual mechanism of promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes while simultaneously reducing apoptosis in regulatory T-cells (suppressor T-cells).

[0009] Studies that incorporated the soluble, extracellular domain of PD-1 during therapy demonstrate that soluble PD-1 blocks the PD-1 pathway and modulates the antitumor immune response (Pan et al., Oneal Lett (2013); hereby expressly incorporated by reference in its entirety). Alternatively, IFN α treatment has been used for

clinical application (Kreutzer et al., Dtsch Dermatol Ges (2004); hereby expressly incorporated by reference in its entirety).

[0010] Fusion proteins, which incorporate separate moieties of different proteins, have also been previously described for therapy. For example, Trinh *et al* developed and described an anti-CD20-IFN α fusion protein used in a mouse model for treatment of lymphomas expressing CD20 (Trinh *et al* J Immunother (2013); incorporated by referenced in its entirety herein). Osborn *et al* describes pharmacokinetic studies, which were performed using a human serum albumin-IFN α fusion protein that was developed so as to improve the half-life of IFN α and decrease its dosing frequency (Osborn *et al* J Pharmacol Exp Ther (2002); incorporated by referenced in its entirety herein). Lee *et al* describes a cytoplasmic transduction peptide-IFN α fusion used to enhance conjugation capacity of cell membranes and antiviral activity (US20120134961 A1); hereby expressly incorporated by referenced in its entirety). Furthermore Morrison *et al.* describes the attachment of novel chimeric moieties to IFN α or IFN β (US8563692 B2; hereby expressly incorporated by reference in its entirety).

[0011] As the tumor environment is known to preclude known treatments, such as immunotherapy, approaches are needed to overcome the influence of the TME and methods for modulating the immune response during immunotherapies, such as CAR T-therapy, are also manifest.

SUMMARY OF THE INVENTION

[0012] In a first aspect, a nucleic acid encoding a fusion protein is provided. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA

GGACAGCAGCGCCGCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some
 alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID
 NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT

ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPA
 LLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA
 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSG
 TYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP
 RPAGQFQTLV; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
 of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
 P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
 E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
 R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
 A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
 F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
 alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
 such as glycines, or a number of amino acids, such as glycines, within a range defined by
 any two of the aforementioned numbers. In some alternatives, the amino acid spacer
 comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
 sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,

wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattctcctgatccca; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13).

[0013] In a second aspect, an expression vector comprising the nucleic acid of anyone of the alternatives described herein is provided. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO:1

(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC

TGGCCCAGATGCGGAAGATCAGCCTGTTTCAGCTGCCTGAAGGACCGGCACGA
 CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
 ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
 GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGCGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some
 alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID

NO:

3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In

some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and

comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
such as glycines, or a number of amino acids, such as glycines, within a range defined by
any two of the aforementioned numbers. In some alternatives, the amino acid spacer
comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a

sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA.

[0014] In a third aspect, a cell for fusion protein secretion is provided, wherein the cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. In some alternatives, the vector is RNA or DNA. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an

amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO:

(TGC GACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
TGGGAGGTTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the

nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific

binding domains, antigen specific scFvs, an extracellular domain, DARPins, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3).

In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 4).

In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5).

In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A

PKIQIKESLR AELRV TERRAEVPTAHPSPSPRPAGQFQTLV;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In

some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as *E. coli*. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a nucleic acid encoding a chimeric antigen receptor r. In some alternatives, the chimeric antigen receptor is specific for CD19.

[0015] In a fourth aspect, a method of making a chimeric antigen receptor bearing cell, wherein the chimeric antigen receptor bearing cell expresses a fusion protein. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1

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(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
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CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some
 alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID
 NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC

CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
such as glycines, or a number of amino acids, such as glycines, within a range defined by
any two of the aforementioned numbers. In some alternatives, the amino acid spacer
comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
(GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
wherein the 2A linker is between the fusion protein and a protein for co-expression. In

some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is DNA or RNA. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all or a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T

cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the chimeric antigen receptor is specific for CD19.

[0016] In a fifth aspect, a composition comprising the cells of anyone of the alternatives described herein, or the cells manufactured by the methods of anyone of the alternatives described herein, is provided. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian

cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCTGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCTACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCACCTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTCAGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCAACGGCCGGGACTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGTGCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGCCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In

some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further

comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the method of making the cell can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The expression vector can comprise the nucleic acid of anyone of the alternatives described

herein. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all or a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a nucleic acid encoding a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19.

[0017] In a sixth aspect, a fusion protein encoded by the nucleic acid of anyone of the alternatives provided herein, or the vector of anyone of the alternatives provided herein, is provided. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGGCCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA

ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
 GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some
 alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID
 NO:

3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG

GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
such as glycines, or a number of amino acids, such as glycines, within a range defined by
any two of the aforementioned numbers. In some alternatives, the amino acid spacer
comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
(GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some

alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattcctcctgatccca; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA.

[0018] In a seventh aspect, a method of manufacturing a fusion protein is provided. The method can comprise delivering the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein to a bacterial cell, mammalian cell or insect cell, growing the cell up in a culture, inducing expression of the fusion protein and purifying the fusion protein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second

sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid

sequence set forth in SEQ ID NO: 1
 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
 TGGCCCAGATGCGGAAGATCAGCCTGTTTCAGCTGCCTGAAGGACCGGCACGA
 CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
 ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
 GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the

nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2

(GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific

binding domains, antigen specific scFvs, an extracellular domain, DARPins, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In

some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form

of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A

PKIQIKESLR AELRV TERRAEVPTAHPSPSPRPAGQFQTLV;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In

some alternatives, the inducing comprises adding IPTG, anhydrotetracycline, L-arabinose or rhamnose to the culture. In some alternatives, the cell is *E. coli*. In some alternatives, the cell is an insect-cell.

[0019] In an eighth aspect, a method of secreting the fusion protein of anyone of the alternatives described herein is provided. The method can comprise delivering to a subject the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC

AGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some
 alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID
 NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPA
 LLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLA
 AFPEDRSQPGQDCRFRTQLPNGRDFHMSVVRARRND SG
 TYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP

R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1

signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattcctcctgatccca; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGTAAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. The composition can comprise the cells of anyone of the alternatives described herein, or the cells manufactured by the methods of anyone of the alternatives described herein. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T

helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the method of making the cell can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the method further comprises administering to the subject an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg

or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the cell further comprises a nucleic acid encoding a chimeric antigen receptor.

[0020] In a ninth aspect, a method of increasing T-cell activity comprising administering an effective amount of the cell of anyone of the alternatives described herein or the composition anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to a subject in need. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1

(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCACC
TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In

some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCTACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCACCTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTCAGCCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCAACGGCCGGGACTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGTGCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGCCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M

QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPA
 LLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA
 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSG
 TYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP
 RPA G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
 of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
 P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
 E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
 R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
 A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
 F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
 alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
 such as glycines, or a number of amino acids, such as glycines, within a range defined by
 any two of the aforementioned numbers. In some alternatives, the amino acid spacer
 comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
 sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
 wherein the 2A linker is between the fusion protein and a protein for co-expression. In
 some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some
 alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker
 and comprises a nucleic acid sequence set forth in SEQ ID NO: 10
 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG
 AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a
 protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth
 sequence encoding an IRES sequence. In some alternatives, the nucleic acid further
 comprises a seventh sequence encoding a marker. In some alternatives, the marker is

Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the composition can comprise the cells of anyone of the alternatives described herein, or the cells manufactured by the methods of anyone of the alternatives described herein. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives

described herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as *E. coli*. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the cell further comprises a nucleic acid encoding a chimeric antigen receptor. In some alternatives, the method of making the cell can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. In some alternatives, the expression vector is RNA or DNA. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺

T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives provided herein or the vector of anyone of the alternatives provided herein. In some alternatives, the administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day.

[0021] In a tenth aspect, a method of decreasing immunosuppression in a tumor microenvironment is provided. The method can comprise administering an effective amount of the cell of anyone of the alternatives provided herein or the composition of anyone of the alternatives provided herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives provided herein to a subject in need. In some alternatives, the chimeric antigen receptor is specific for CD19. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives,

the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA AGGTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.

In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In

some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form

of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and

comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some

alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
such as glycines, or a number of amino acids, such as glycines, within a range defined by

any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte

cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the cell further comprises a nucleic acid encoding a chimeric antigen receptor. In some alternatives, the composition can comprise the cells of anyone of the alternatives described herein, or the cells manufactured by the methods of anyone of the alternatives described herein. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the method of making the cell can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The expression vector can comprise the

nucleic acid of anyone of the alternatives described herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives provided herein or the vector of anyone of the alternatives provided herein. In some alternatives, the administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day.

[0022] In an eleventh aspect, a method of inhibiting, ameliorating, or treating cancer in a subject such as a human, that expresses a tumor antigen, is provided. The method can comprise administering an effective amount of the cell of anyone of the

alternatives provided herein or the composition anyone of the alternatives provided herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives provided herein to the subject. In some alternatives, the chimeric antigen receptor is specific for CD19. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO:

1

(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGCGGCG TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA CTTCCAGCGGATCACCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC AGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGA

AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPINs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some
 alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID
 NO: 3

(ATGCAGATCCCTCAGGCCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPA
 LLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA
 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSG
 TYLCGAISLAPKAQIKESLR AELRV TERRAEVPTAHPSPSP
 RPA GQFQTLV; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form

of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF

signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccca; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGTAAGG; SEQ ID NO: 13). In some alternatives, the vector comprises the nucleic acid of any one of the alternatives herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the cell further comprises a nucleic acid encoding a chimeric antigen receptor. In some alternatives, the composition can comprise the cells of anyone of the alternatives described herein, or the cells manufactured by the methods of anyone of the alternatives described herein. The cell can comprise the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as E. coli. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a

CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the method of making the cell can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The expression vector can comprise the nucleic acid of anyone of the alternatives described herein. In some alternatives, the vector is RNA or DNA. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives provided herein or the vector of anyone of the alternatives provided herein. In some alternatives, the administering is performed by delivering the

composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **Figure 1** shows a general schematic of PD-1:IFN α 2a fusion protein.

[0024] **Figure 2** shows a Western blot analysis of H9T-cell supernatant of cells transduced with a PD-1:IFN α 2a-T2A-Her2tG vector and selected using Her2tG.

[0025] **Figure 3A and 3B** shows PD-1-IFN α 2a binding to target receptors.

[0026] **Figure 4** shows PD-1-IFN α 2a signals in a Jurkat reporter cell line.

[0027] **Figure 5** shows a schematic of the assay setup to analyze potency of PD-1:IFN α 2a in reference to native IFN α .

[0028] **Figure 6A and 6B** shows the analysis of the reporter cells, which are analyzed for the presence of secreted embryonic alkaline phosphatase (SEAP).

[0029] **Figures 7A and 7B** show that primary CD4 and CD8 T-cells can co-express a CAR and PD1:IFN α . **(7A)** Primary CD4 and CD8 T-cells were isolated from PBMCs using CD4 or CD8 microbeads, respectively. The CD4 and CD8 T-cells were selected up to 99% and 92.3% purity, respectively. **(7B)** Purified CD4 or CD8 T-cells were stimulated with CD3/CD28 microbeads for three days and then transduced with lentivirus containing the following: CD19scFv-IgG4hinge-CD28tm/41BB-zeta-T2A-Her2tG (2nd generation short spacer CD19CAR) and/or EGFRt-T2A-PD1:IFN α 2a. The transduced CD4 and CD8 T-cells were then expanded (10-12 days) and subjected to purification using biotinylated Herceptin or Erbitux to select for Her2tG or EGFRt positive populations, respectively. The dual transduced CD4 or CD8 T-cells were subjected to a second round of purification 4 days later for the second selection marker (Her2tG first selection, EGFRt second selection). Results demonstrate that the dual transduced T-cells were >95% dual positive following selections.

[0030] **Figure 8** demonstrates that primary T-cells produce PD1:IFN α 2a. Selected T-cell populations from **Figures 7A and 7B** were subjected to either a standard rapid expansion protocol (REP) (REP – Mock or PD1:IFN α 2a alone expressing) or a Seitaro REP (CD19CAR or dual CD19CAR and PD1:IFN α 2a expressing). On the 14th day of the REP, CD4 or CD8 T-cells were stimulated for 24 hours at a 1:1 ratio CD3/CD28 bead:T-cell. Following the 24 hour incubation period, supernatant was collected and then subjected to an IFN α 2a specific Bioplex assay. Results demonstrate basal PD1:IFN α 2a levels under normal conditions, but elevated PD1:IFN α 2a levels are seen upon stimulation.

[0031] **Figure 9** shows that the expression and secretion of PD1:IFN α 2a does not interfere with effector cytokine production. Selected T-cell populations from **Figure 7A and 7B** were subjected to either a standard rapid expansion protocol (REP) (REP – Mock or PD1:IFN α 2a alone expressing) or a Seitaro REP (CD19CAR or dual CD19CAR and PD1:IFN α 2a expressing). On the 14th day of the REP, CD4 or CD8 T-cells were co-cultured with target cells at a 2:1 effector:target ratio. Following the 24 hour incubation period, supernatant was collected and then subjected to a Bioplex assay for IL2 and TNF α production. K562 parental and the negative control, had no active T-cell target so there is no cytokine production. K562 OKT3 and the positive control, demonstrated that all T-cells were able to produce similar levels of effector cytokine upon stimulation. K562 CD19 and Raji parental co-culture only induced T-cell cytokine production if the T-cells expressed the CD19CAR. The production of the PD1:IFN α 2a did not interfere with IL2 and TNF α effector cytokine production.

[0032] **Figure 10** shows that the expression and secretion of PD1:IFN α 2a does not interfere with effector phenotype or target specificity. As shown is the *in vitro* cytotoxicity of CD8⁺ T-cells by chromium release. The 4hr chromium release assay showed CD19-CAR T-cell specificity against CD19⁺ (K562 CD19 and Raji parental) and control target cells (negative control = K562 parental; positive control = K562 OKT3). CD8⁺ T-cells were co-cultured with target cells at a 30:1, 10:1, 3.3:1 or 1.1:1 ratio.

[0033] **Figure 11** shows the *in vivo* antitumor activity of CD19CAR- or CD19CAR and PD1-IFN α 2a expressing T-cells. 5e⁶ Be2 CD19t eGFP:ffluc neuroblastoma cells were subcutaneously injected into the left and right flanks of NOD-SCID IL-2Ry null mice. Five days later 30e⁶ T-cells at a 1:1 CD4:CD8 ratio were injected intravenously (i.v.). Subsequent tumor volume measurements by caliper were

performed as was imaging using D-luciferin and the IVIS imaging system. CD19CAR and PD1-IFN α 2a expressing T-cells were able to inhibit tumor growth at a similar rate to CD19CAR-alone expressing T-cells.

[0034] **Figure 12** shows that H9 T-cells are able to produce and secrete PD1:IFN α 2a variants. H9 T-cells were transduced with EGFRt-T2A-PD1:IFN α 2a vector or vector containing the A99L (Lazar-Molnar et al 2008 PNAS), G14-1 (Maute et al 2015 PNAS), or HAC-1 (Maute et al 2015 PNAS) mutant and then selected by EGFRt. Selected H9 cultures were washed in media to remove excess PD1:IFN α 2a in supernatant. Supernatant from the H9 cultures was then harvested 24 hours later and concentrated using an Amicon Ultra-4 Centrifugal Filter Unit (Millipore) and subjected to an IFN α specific Bioplex Pro Inflammation Panel assay to quantify the presence of soluble PD1:IFN α 2a in the supernatant in reference to an undiluted native IFN α (140000 pmol/mL or 140000nM). Data is represented as molecules/cell-hr from 1e6 cells. Results demonstrate that all variants of the PD1:IFN α 2a were produced at similar levels.

[0035] **Figure 13** shows that PD1:IFN α 2a variants display higher binding affinity to PDL1 than wild-type PD1:IFN α 2a. 1e6 PDL1-expressing U293T cells were harvested from culture and washed with PBS. Equal amounts of PD1:IFN α 2a, PD1(A99L):IFN α 2a, PD1(G14-1):IFN α 2a, or PD1(HAC-1):IFN α 2a as determined by Bioplex assay of culture supernatants was then added to the U293T cells for 20min. Following incubation, cells were washed twice in PBS and then stained with PE-conjugated anti-IFN α antibody for 20min. Cells were then washed twice in PBS and re-suspended in FACS fixative solution. Fixed cells were then run on the LSRFortessa for FACS analysis. Median fluorescence intensity differences between PD1:IFN α 2a and variants demonstrate a higher level of PDL1 binding for some of the PD1 mutants.

[0036] **Figure 14** shows that the fusion protein, PD1:IFN α 2a, can stimulate the regulatory signaling molecules that are produced by macrophages.

Definitions

[0037] 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.

[0038] As used herein, “a” or “an” may mean one or more than one.

[0039] “About” as used herein when referring to a measurable value is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value.

[0040] “Nucleic acid” or “nucleic acid molecule” refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogous of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded. In some alternatives, a nucleic acid sequence encoding a fusion protein is provided. In some alternatives, the nucleic acid is RNA or DNA.

[0041] “Coding for” or “encoding” are used herein, and refers to the 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 macromolecules such as a defined sequence of amino acids. Thus, a gene codes for a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system.

[0042] A “nucleic acid sequence coding for a polypeptide” includes all nucleotide sequences that are degenerate versions of each other and that code for the

same amino acid sequence. In some alternatives, a nucleic acid is provided, wherein the nucleic acid encodes a fusion protein. In the some alternatives described herein, the fusion protein comprises PD1 and IFN α 2a. The PD1 component of the PD1:IFN α 2a fusion protein can sequester the T-cell inhibitory interaction of PD1 with its ligand POL 1. These characteristics make the PD1 :IFN α 2a fusion protein a novel, molecular hub of antineoplastic function and an auspicious candidate to supplement solid tumor CAR therapy. The use of this protein can make CAR therapy more interactive against the immunosuppressive milieu found in solid tumors by eliciting stimulatory and suppressive functions to specific tumor-resident immune cell subsets. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression.

[0043] “Vector,” “Expression vector” or “construct” is a nucleic acid used to introduce heterologous nucleic acids into a cell that has regulatory elements to provide expression of the heterologous nucleic acids in the cell. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*.

[0044] As used herein, “fusion proteins” or “chimeric proteins” are proteins created through the joining of two or more genes that originally coded for separate proteins or portions of proteins. The fusion proteins can also be made up of specific protein domains from two or more separate proteins. Translation of this fusion gene can result in a single or multiple polypeptides with functional properties derived from each of the original proteins. Recombinant fusion proteins can be created artificially by recombinant DNA technology for use in biological research or therapeutics. Such methods for creating fusion proteins are known to those skilled in the art. Some fusion

proteins combine whole peptides and therefore can contain all domains, especially functional domains, of the original proteins. However, other fusion proteins, especially those that are non-naturally occurring, combine only portions of coding sequences and therefore do not maintain the original functions of the parental genes that formed them. In some alternatives, a fusion protein is provided, wherein the fusion protein comprises an interferon and a PD-1 protein.

[0045] Alternative to the fusion of IFN α 2a to PD1, IFN α 2a can also be fused to other tumor resident antigens or secretable factors known to inhibit immunotherapeutic approaches. Without being limiting examples can include the soluble TGF β R2, antigen specific scFvs or homeostatic cytokines. The alternatives described herein can also test the ability to use T cells as a delivery vehicle for secreted, immunoregulatory proteins.

[0046] In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines.

[0047] Many whole gene fusions can be fully functional, and can be used, for example to replace an original peptide which can be missing in a biological system. Some, however, experience interactions between the two proteins that modify their functions. Beyond these effects, some gene fusions may cause regulatory changes that can alter when and where these genes can act. For partial gene fusions, the shuffling of different active sites and binding domains have potential to result in new proteins with novel functions. In some alternatives, a nucleic acid encoding a fusion protein is provided. In some alternatives, the fusion protein comprises a peptide that can modulate an immune response, an amino acid spacer or linker, such as a plurality of glycines, also referred to throughout as a glycine spacer, or flexible domains from the lambda phage, and an interferon or a portion thereof. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the fusion protein comprises a protein or portion thereof that can modulate an immune response. In some alternatives, the protein is PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.

In some alternatives, the protein is PD-1, wherein PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1A.

[0048] Alternative to the fusion of an interferon to PD1, an interferon can be fused to another tumor resident antigen or secretable factor that is known to inhibit immunotherapeutic approaches. In some alternatives herein, fusion protein is provided. In some alternatives, the fusion protein comprises an interferon. In some alternatives, the fusion protein further comprises TGF β RII or a portion thereof. In some alternatives, the fusion protein further comprises an antibody, or a portion thereof. In some alternatives, the fusion protein further comprises an scFv. In some alternatives, the fusion protein further comprises a homeostatic cytokine. Homeostatic cytokines control the cells of the immune system during immune surveillance processes.

[0049] In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines.

[0050] “Programmed cell death protein 1”, also known as PD-1 and CD279 (cluster of differentiation 279), is a protein that in humans is encoded by the PDCD1 gene. PD-1 is a protein that functions as an immune checkpoint and plays a role in modulating the immune response, down regulating the immune system by preventing the activation of T cells to reduce autoimmunity and promote self-tolerance. PD-1 has an inhibitory effect of programming apoptosis in antigen specific T cells in the lymph nodes and simultaneously reducing apoptosis in regulatory T cells. PD-1 inhibitors can be used however to activate the immune system to attack tumors and can be used to treat some types of cancers. PD-1 has two ligands PD-L1 and PD-L2. Binding of PD-L1 to PD-1 allows the transmittal of an inhibitory signal, which reduces the proliferation of CD8⁺ T cells at lymph nodes. PD-L1 can also bind PD-1 on activated T cells, B cells and myeloid cells to modulate activation or inhibition. In some alternatives, a nucleic acid encoding a fusion protein is provided. The nucleic acid can have a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer or linker, such as a glycine spacer also referred to as a plurality of glycines, and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the first sequence comprises a nucleic acid

sequence	set	forth	in	SEQ	ID	NO:	3
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(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCCGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
 L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
 A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
 T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
 R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, a 3' terminus of the first
 sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the
 second sequence is adjacent to a 5' terminus of the third sequence. The first sequence,
 second sequence and third sequence can be in any order in the nucleic acid.

[0051] Mutated forms of PD-1 have also been described by Lazar-Molnar *et al.* (2008) and Maute *et al.* (2015) (Lázár-Molnár *et al.* Proc Natl Acad Sci U S A. 2008 Feb 19;105(7):2658-63, Lázár-Molnár *et al.* Proc Natl Acad Sci U S A. 2008 Jul 29;105(30):10483-8, and Proc Natl Acad Sci U S A. 2015 Nov 24;112(47); all hereby incorporated by reference in their entireties). Lázár-Molnár *et al.* describes a PD-1 with one point mutation, which has the amino acid sequence amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). Maute *et al.* describes a PD-1 with 11 point mutations, in which the PD-1 mutant has the amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H

R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M
S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R
A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, a
 nucleic acid encoding a fusion protein is provided. In some alternatives, the nucleic acid
 comprises a first sequence, wherein the first sequence encodes a protein that modulates an
 immune response, a second sequence, wherein the second sequence encodes a spacer or
 linker, such as a glycine spacer, and a third sequence, wherein the third sequence encodes
 an interferon. In some alternatives, the first sequence encodes a PD-1 protein, a PD-1
 mutant or a functional portion thereof. In some alternatives, the first sequence comprises
 a nucleic acid sequence set forth in SEQ ID NO: 3 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (**M**
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
 of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (**M**
Q I P Q A
P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (**M**
Q I P Q A P W P V V

WAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNA
 TFTCSFSNTSESFHVVWHRESPSGQTDTLAAFPEDRSQPG
 QDCRFRVTQLPNGRDFHMSVVRARRNDSGTYVCGVISLA
 PKIQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV;
 SEQ ID NO: 6). The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence.

[0052] The PD1 mutants described herein are affinity matured mutants created either by site-directed mutagenesis (the A99L mutant) or by directed evolution (HAC-V mutant). Both mutants display a greater ability to bind their ligand PD-L1 and may therefore elicit a better ability to inhibit negative PD1 signals inside the tumor microenvironment that can occur on therapeutic T cells by acting as a decoy receptor separate to PD1 found on the T cells. The A99L mutant confers a 3-fold increase in affinity and the HAC-V mutant confers a ~35000-fold increase in affinity to PDL1 relative to wild-type PD1. Mutated forms of protein can be manufactured by standard site directed mutagenesis protocols for mutating nucleic acids to encode a protein with point mutations and can be appreciated by those skilled in the art.

[0053] A "single-chain variable fragment," (scFv) is a fusion protein that can have variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to 25 amino acids. The short linker peptide can comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 amino acids or any number of amino acids in between a range defined by any two aforementioned values. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. The scFv can be specific for an antigen. "Antigen" or "Ag" as used herein refers to a molecule that provokes an immune response. This immune response can involve either antibody production, or the activation of specific immunologically-competent cells, or both. An antigen can be generated, synthesized, produced recombinantly or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid such, for example, blood, plasma or

ascites fluid. In some alternatives herein, a composition is provided, wherein the composition comprises cells manufactured by any one of the alternative methods herein. In some alternatives, the cells comprise a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an scFv that is specific for an antigen.

[0054] “Antigen specific binding domains” can include protein or protein domains that can specifically bind to an epitope on a protein at a low or high binding affinity (μM to mM binding capacity). In some alternatives, the fusion protein comprises a protein or portion thereof that can modulate an immune response. In some alternatives, the protein comprises an antigen specific binding domain.

[0055] In some alternatives, the fusion protein comprises PD-1. In some alternatives, the fusion protein further comprises IFN α 2a. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, α GD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression.

[0056] “Extracellular domains” are protein or receptor domains that protrude from the outer membrane of a cell organelle and a cell. Usually direct contacts between extracellular domains of membrane proteins with its specific ligand are required for receptor activation and cell signaling.

[0057] DARPins (designed Ankyrin repeat proteins) are genetically engineered antibody mimetic proteins typically exhibiting highly specific and high-affinity target protein binding. They are derived from natural ankyrin proteins and consist of at least three, usually four or five repeat motifs of these proteins. A DARPIn can have several clinical benefits by overcoming the limitation of conventional therapeutic approaches by enabling the targeting many disease pathways. DARPIn fusions are also beneficial because they can be made to resist aggregation. DARPins can also be designed to act as receptor agonists, antagonists, inverse agonists, enzyme inhibitors or a target protein binder.

[0058] In some alternatives, the fusion protein comprises a protein or portion thereof that can modulate an immune response. In some alternatives, the protein comprises a DARPIn. In some alternatives, the fusion protein comprises an interferon

fused to a DARPIn. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some of the alternative fusion proteins herein, the DARPIn is a receptor agonist, antagonist, inverse agonist, enzyme inhibitor or a target protein binder.

[0059] Secretion of a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of a fusion protein such as PD1-IFN α 2a, can be used against solid tumors that express targets where the tumor microenvironment plays a larger role in tumor progression. Without being limiting, examples of targets are EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example.

[0060] “Her2” as described herein, is the receptor tyrosine-protein kinase erbB-2, also known as CD340 (cluster of differentiation 340), proto-oncogene Neu, Erbb2 (rodent), or ERBB2. It is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family and has been known to play an important role in the development and progression of certain aggressive types of breast cancer. In some alternatives, the fusion protein comprises a protein or portion thereof that can modulate an immune response. In some alternatives, the fusion protein comprises a protein that binds Her2. In some alternatives, a cell is provided, wherein the cell comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for HER2.

[0061] “ROR1” or tyrosine-protein kinase transmembrane receptor ROR1, also known as neurotrophic tyrosine kinase, receptor-related 1 (NTRKR1), is an enzyme that in humans is encoded by the ROR1 gene. ROR1 is a member of the receptor tyrosine kinase-like orphan receptor (ROR) family and has been shown to be expressed on ovarian cancer stem cells. In some alternatives, the fusion protein comprises a protein or portion thereof that modulates an immune response. In some alternatives, the fusion protein comprises a protein that binds ROR1. In some alternatives, a cell is provided, wherein the cell comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for ROR1.

[0062] The “extracellular matrix,” (ECM) as described herein, comprises a collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells. The composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM. Without being limiting, proteins of the

ECM can include embryonal fibronectin, fibronectin, laminin and collagen. In some alternatives, the fusion protein comprises a protein or portion thereof that modulates an immune response. In some alternatives, the protein comprises an extracellular matrix protein. In some alternatives, the extracellular matrix protein is embryonal fibronectin, fibronectin, laminin or collagen

[0063] “Glycine spacer” or “glycine linker” can be used to separate one protein domain from another and can range in length from 2, 4, 6, 8, or 10 glycines or any number of glycines that are within a range between any two aforementioned values. A glycine spacer is an amino acid spacer and can be modified in order to increase the binding affinity of one or more binding portions in a fusion protein. Additionally, a glycine spacer may be modified in length in order to prevent interactions between different binding modalities of a fusion protein. In some alternatives described herein, the glycine spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 glycines or a number of glycines within a range defined by any two of the aforementioned numbers. In some alternatives, the glycine spacer comprises at least 3 glycines. In some alternatives, the glycine spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9).

[0064] In some alternatives, a glycine spacer is modified if during testing, there is reduced activity. The glycine spacer, for example, can be varied in length in order to facilitate proper protein folding and protein activity.

[0065] “Immune response” as described herein, are the processes of the immune system that are used to prevent or limit an infection or a disease upon recognition of a pathogen such as viruses or bacteria or the presence of abnormal cells such as cancer cells. Examples of an immune response can include but are not limited to inflammation, triggering of the innate immune system, activation of the complement system, triggering of cellular barriers, triggering of the natural killer cells and triggering of the adaptive immune system.

[0062] Inflammation is an immune response in which growth factors and cytotoxic factors are released. Cytokines and other chemicals recruit immune cells to the site of infection and promote healing of any damaged tissue following the removal of pathogens. In some alternatives, a fusion protein is provided, wherein the fusion protein comprises a peptide that modulates an immune response. In some alternatives, the fusion protein promotes an inflammatory response. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being

limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines.

[0066] “Interferons” are a group of signaling proteins that are produced and released by cells in response to pathogens, viruses, bacteria, parasites and signals from foreign cells and tumor cells in order to increase anti-viral defenses. In some alternatives described herein, a nucleic acid encoding a fusion protein is provided, wherein the fusion protein comprises an interferon or a portion thereof. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the sequence encoding the interferon comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGGCCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1).

[0067] The IFN- α proteins are produced by leukocytes and are mainly involved in innate immune response against viral infection. They are 13 subtypes: IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, and IFNA21. In some alternatives described herein, a nucleic acid encoding a fusion protein is provided, wherein the fusion protein comprises an interferon or a functional portion thereof. In some alternatives, the interferon is IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17 or IFNA21 or a functional portion thereof.

[0068] The IFN- β proteins are produced by fibroblasts. They have antiviral activity that is involved mainly in innate immune response. Two types of IFN- β have been described, IFN- β 1 (IFNB1) and IFN- β 3 (IFNB3). In some alternatives described herein, a nucleic acid encoding a fusion protein is provided, wherein the fusion protein

comprises an interferon or a functional portion thereof. In some alternatives, the interferon is IFN- β 1 (IFNB1) or IFN- β 3 (IFNB3) or a functional portion thereof.

[0069] A “promoter” is a region of DNA that initiates transcription of a specific gene. The promoters can be located near the transcription start site of a gene, on the same strand and upstream on the DNA (the 5’ region of the sense strand). The promoter can be a conditional, inducible or a constitutive promoter. The promoter can be specific for bacterial, mammalian or insect cell protein expression. In some alternatives, wherein a nucleic acid encoding a fusion protein is provided, the nucleic acid further comprises a promoter sequence. In some alternatives, the promoter is specific for bacterial, mammalian or insect cell protein expression. In some alternatives, the promoter is a conditional, inducible or a constitutive promoter.

[0070] “Conditional” or “Inducible” as used herein refers to a nucleic acid construct that includes a promoter that provides for gene expression in the presence of an inducer and does not substantially provide for gene expression in the absence of the inducer. Without being limiting, examples of inducible promoters for mammalian expression constructs include tetracycline, ecdysone, streptogramins, macrolides or doxycycline inducible promoters. Without being limiting, examples of inducible promoters for bacterial expression constructs include but are not limited to a T7 promoter, *lac* promoter, *trc* promoter, *tac* promoter, *tetA* promoter, *araBAD* promoter or a *rhaP_{BAD}* promoters. Without being limiting, insect-derived promoters include but are not limited to pB2 and polyhedrin promoters. In some alternatives, wherein a nucleic acid encoding a fusion protein is provided, the nucleic acid further comprises a promoter sequence. In some alternatives, the promoter is an inducible promoter. In some alternatives, the promoter is an inducible promoter for bacterial protein expression. In some alternatives, the inducible promoter for bacterial expression is a T7 promoter, *lac* promoter, *trc* promoter, *tac* promoter, *tetA* promoter, *araBAD* promoter or a *rhaP_{BAD}* promoter. In some alternatives, the promoter is an inducible promoter for expression in a mammalian system. In some alternatives, wherein the promoter is an inducible promoter for mammalian expression, the inducible promoter is a tetracycline, ecdysone, streptogramins, macrolides or doxycycline inducible promoter. In some alternatives, the promoter is for protein expression in an insect cell. In some alternatives, the promoter is a pB2 or a polyhedrin type promoter. In some alternatives, the bacterial promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives,

wherein the promoter is for mammalian cell protein expression, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, wherein the promoter is for mammalian cell protein expression, the promoter is regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system.

[0071] Systemic administration of IFN α 2a can cause adverse effects in normal tissue. In some alternatives, wherein the systemic administration of PD1:IFN α 2a fusion can cause adverse effects in normal tissue. If the PD1:IFN α 2a also causes off-target toxicities expression can be regulated by control under a promoter, such as a drug regulatable promoter or by CAR-dependent signaling (e.g. tamoxifen or NFAT regulated promoter systems).

[0072] “Constitutive” as used herein refer to the nucleic acid construct that includes a promoter that is constitutive, and thus provides for expression of a polypeptide that is continuously produced. In some alternatives, wherein a nucleic acid for expressing a fusion protein is provided, the nucleic acid further comprises a promoter for protein expression in a bacterial, mammalian or an insect cell. In some alternatives, the promoter is EF-1 Promoter (EF1P).

[0073] As described, the EF1P promoter is a constitutive promoter of human origin that can drive ectopic gene expression *in vivo* and *in vitro*. In some alternatives, an EF1P promoter drives the expression of the fusion protein. In some alternatives, the sequence encoding the promoter comprises the nucleic acid sequence set forth in SEQ ID NO: 2

(GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2).

[0074] Promoter systems can also be regulated by a drug. Without being limiting, a drug that can be used to regulate a promoter can include tamoxifen. “Tamoxifen,” as described herein, is an estrogen antagonist/partial agonist that is an FDA-approved and commercially available drug. It is taken orally and can be administered on a daily basis over an extended period of time. Tamoxifen has a proven safety record, favorable pharmacokinetic profile, excellent tissue distribution and a low

partition coefficient between the extracellular space and cytosol. Other drugs can be selected based on safety record, favorable pharmacokinetic profile, and excellent tissue distribution, a low partition coefficient between the extracellular space and cytosol, and/or low toxicities.

[0075] In some alternatives, the system employs a synthetic transcriptional regulator, which, in the presence of tamoxifen, binds a synthetic promoter upstream of a transgene to induce expression. The tamoxifen regulated transcription factor (“TamR-tf”, also designated “HEA3”) is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1 α) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF- κ B (p65). The mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids 282-595 of the TamR-tf and has a mutation at position 521. Further changes can be made to the transcriptional activator to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain to increase the affinity of the factor for estrogen analogs and altering one or more amino acids in the p65 transactivating domain.

[0076] In the absence of tamoxifen, TamR-tf is excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen binding active site and transgene expression is in the “OFF” state. Nanomolar concentrations of cytosolic tamoxifen actively out competes HSP90 for ER-LBD binding, resulting in TamR-tf translocation to the nucleus. Upon nuclear translocation, TamR-tf is readily available to bind its restricted synthetic promoter (e.g. 7xHBD/EF1 α p). In the presence of tamoxifen, binding of TamR-tf to 7xHBD/EF1 α p promoter induces the “ON” state of transgene expression. In some alternatives, this transcriptional regulator can be modified to provide for a varying level of control of transgene expression. Amino acid substitutions in the LBD of TamR-tf permit selective responsiveness to tamoxifen and its metabolites, where 4-hydroxy tamoxifen (4-OHT) is the most pharmacologically active metabolite, in regards to TamR-tf activity, while lacking interaction with endogenous estrogen. In some alternatives, a nucleic acid encoding a fusion protein is provided, wherein the nucleic acid comprises a promoter regulated by a drug. In some alternatives, the drug includes

tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof.

[0077] Tamoxifen, CAS RN: 10540-29-1, is also known as 2-(4-((1Z)-1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethyl-ethanamine, or (Z)-2-(para-(1,2-Diphenyl-1-butenyl)phenoxy)-N,N-dimethylamine (IUPAC), and has a molecular formula of $C_{26}H_{29}NO$, M.W. 371.52. Tamoxifen is a Selective Estrogen Receptor Modulator with tissue-specific activities. Tamoxifen acts as an anti-estrogen (inhibiting agent) agent in the mammary tissue, but as an estrogen (stimulating agent) in cholesterol metabolism, bone density, and cell proliferation in the endometrium. Tamoxifen is frequently administered orally as a pharmaceutically acceptable salt. For example, Tamoxifen citrate (RN 54965-24-1, M.W. 563.643) is indicated for treatment of metastatic breast cancer, and as an adjuvant for the treatment of breast cancer in women following mastectomy axillary dissection, and breast irradiation.

[0078] Metabolites of tamoxifen in rat, mouse and human breast cancer patients, including major metabolites N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494) and 4-hydroxytamoxifen (4-OHT) (RN 68392-35-8, M.W. 387.52, Afimoxifene), are disclosed in Robinson et al., Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab Dispos January 1991 19:36-43, which is incorporated by reference herein in its entirety. Additional cytochrome P-450 metabolites are disclosed in Crewe et al., 2002, including cis-4-hydroxytamoxifen (RN 174592, M.W. 387.52; Afimoxifene, E-isomer), and 4'-hydroxytamoxifen ((Z)-4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-1-phenylbut-1-en-2-yl)phenol). See Crewe et al., 2002, Metabolism of Tamoxifen by recombinant human cytochrome P-450 enzymes: Formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen, Drug Metab Dispos, 30(8): 869-874, FIG. 1, which is incorporated herein by reference.

[0079] Compounds with structural similarity to tamoxifen include, but are not limited to, cis-tamoxifen (RN 13002-65-8, M.W. 371.521), 4-methyltamoxifen (RN 73717-95-5, M.W. 385.548), N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494), (Z)-desethyl methyl tamoxifen (RN 15917-50-7, M.W. 357.494), (E)-desethyl methyl tamoxifen (RN 31750-45-5, M.W. 357.494), trans-4-hydroxytamoxifen (RN 68047-06-3, M.W. 387.52), Afimoxifene (RN 68392-35-8, M.W. 387.52, 4-hydroxytamoxifen), Afimoxifene, E-isomer (RN 174592-47-3, M.W. 387.52), 4-chlorotamoxifen (RN 77588-

46-6, M.W. 405.966), 4-fluorotamoxifen (RN 73617-96-6, M.W. 389.511), Toremifene (RN 89778-26-7, M.W. 405.966), desethyl tamoxifen (RN 19957-51-8, M.W. 343.47), (E)-desethyl tamoxifen (RN 97151-10-5, M.W. 343.47), (Z)-desethyl tamoxifen (RN 97151-11-6, M.W. 343.47), Miproxifene (RN 129612-87-9, M.W. 429.6), 2-(p-(beta-ethyl-alpha-phenylstyryl)phenoxy)triethylamine (RN 749-86-0, M.W. 399.575), Droloxifene (RN 82413-20-5, M.W. 387.52), 4-iodo-tamoxifen (RN 116057-68-2, M.W. 497.413), dihydrotamoxifen (RN 109640-20-2, M.W. 373.537), (E)-N,N-dimethyl-2-(4-(1-(2-methylphenyl)-2-phenyl-1-butenyl)phenoxy)ethanamine (RN 97150-96-4, M.W. 385.548), or 4-hydroxytoremifene (RN 110503-62-3, M.W. 421.965); and/or pharmaceutically acceptable salts and/or hydrates or solvates thereof.

[0080] For example, citrate salts of tamoxifen, or citrate salts of compounds with structural similarity to tamoxifen, include, but are not limited to tamoxifen citrate (RN 54965-24-1, M.W. 563.64), 2-(p-(1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethylethylamine citrate (RN 7244-97-5, 563.64), (E)-tamoxifen citrate (RN 76487-65-5, M.W. 563.64), Toremifene citrate (RN 89778-27-8, M.W. 598.088), Droloxifene citrate (RN 97752-20-0, M.W. 579.64), 2-(p-(1,2-bis(p-methoxyphenyl)-1-butenyl)phenoxy)triethylamine citrate (RN 42920-39-8, M.W. 651.748), 2-(4-(1,2-diphenylethenyl)phenoxy)-N,N-diethyl-ethanamine 2-hydroxy-1,2,3-propanetricarboxylate (RN 40297-42-5, M.W. 563.643), 2-(p-(alpha-phenylstyryl)phenoxy)triethylamine citrate (RN 102433-95-4, M.W. 563.64), 2-(p-(2-(p-methoxyphenyl)-1-phenyl-1-butenyl)phenoxy)triethylamine citrate (1:1) (RN 42824-34-0, M.W. 637.72), 2-(p-(1-(p-methoxyphenyl)-2-phenylpropenyl)phenoxy)triethylamine citrate (RN 13554-24-0, M.W. 607.696), 2-(p-(alpha-(p-methoxyphenyl)styryl)phenoxy)triethylamine citrate monohydrate (RN 13542-71-7, M.W. 593.669), 2-(p-(p-methoxy-alpha-phenylphenethyl) phenoxy)triethylamine citrate (RN 16421-72-0, M.W. 595.685), alpha-(p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-p-methoxy-alpha-phenylphenethyl alcohol citrate (1:1) (RN 35263-93-5, M.W. 639.737), 1-(p-(2-(diethylamino)ethoxy)phenyl)-2-(p-methoxyphenyl)-1-phenylethanol citrate (M.W. 611.68), alpha-p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-alpha-(p-hydroxyphenyl)-p-methoxyphenethyl alcohol citrate (RN 35263-96-8, M.W. 655.737), and/or 2-(p-(p-methoxy-alpha-methylphenethyl)phenoxy)-triethylamine citrate (RN 15624-34-7, M.W. 533.614).

[0081] In some alternatives, an affective amount of the drug for inducing expression is an amount that provides for an increase in transgene expression over uninduced and/or basal level of expression. In some alternatives, this amount can be readily determined by using known dosages and pharmacokinetic profile of the drug.

[0082] In some alternatives, the inducible promoter has a low level of basal activity. In some alternatives, wherein a lentiviral vector is used, the level of basal activity in uninduced cells is 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% or less, as compared to when cells are induced to express the gene. The level of basal activity can be determined by measuring the amount of the expression of the transgene (e.g. marker gene) in the absence of the inducer (e.g. drug) using flow cytometry. In some alternatives described herein a marker protein such as Her2tg or EGFRt is used for determination of expression.

[0083] In some alternatives, the inducible promoter provides for a high level of induced activity, as compared to uninduced or basal activity. In some alternatives, the level of activity in the induced state is 2, 4, 6, 8, or 10 fold or greater than the activity level in the uninduced state. In some alternatives, transgene expression under control of the inducible promoter is turned off in the absence of a transactivator in less than 10, 8, 6, 4, 2, or 1 days excluding 0 days.

[0084] In some alternatives, an inducible promoter is designed and/or modified to provide for a low level of basal activity, a high level of inducibility, and/or a short time for reversibility. In some alternatives, the inducible promoter is the 7xHBD/mE1b promoter. For example, in the 7xHBD/mE1b promoter, mutations can be made to enhance the binding of the transcriptional activator.

[0085] As described herein, the “NFAT regulated promoter system” is a promoter activated by NFAT. The NFAT family of transcription factors encompasses five proteins evolutionarily related to the Rel/NFκB family (Chytil and Verdine 1996; Graef et al. 2001b; incorporated by reference in its entirety herein). NFAT activates transcription of a large number of genes during an effective immune response (Rao et al. 1997; Kiani et al. 2000; Serfling et al. 2000; Macián et al. 2001; hereby expressly incorporated by reference in its entirety). In some alternatives, wherein a nucleic acid encoding a fusion protein is provided, the nucleic acid further comprises a promoter sequence. In some alternatives, the promoter is an inducible promoter. In some alternatives, the promoter is

induced by a member of the NFAT protein family. The promoter can be used to induce the protein and to control expression if the fusion protein causes off-target toxicities.

[0086] “2A linker” as described herein, is a self-cleaving sequence that can be used to separate each element of the nucleic acid, such as sequences encoding one protein domain from another. In some alternatives, wherein a nucleic acid encoding a fusion protein is provided, the nucleic acid further comprises a sequence encoding a 2A linker. In some alternatives, the 2A linker is a T2A linker, an E2A linker or a P2A linker. In some alternatives, the T2A linker is between the fusion protein and a protein for co-expression.

[0087] The “IRES” sequence (internal ribosome entry site), as described herein, is a nucleotide sequence that can allow translation initiation in the middle of a messenger RNA (mRNA) sequence as part of the greater process of protein synthesis. In eukaryotes, translation can be initiated only at the 5' end of the mRNA molecule, since 5' cap recognition is required for the assembly of the initiation complex. The location for these sites is often in the 5'UTR, but can occur in many different places in an mRNA.

[0088] The “E2A” sequence, as described herein, encodes transcription factors known to play a critical role in the regulation of lymphocyte development. E2A proteins are highly expressed in developing lymphoid cells and are required for the initiation of B cell development in the bone marrow. In some alternatives, wherein a nucleic acid encoding a fusion protein is provided, the nucleic acid further comprises an E2A sequence.

[0089] The “P2A” sequence, as described herein, is a self-cleaving 2A peptide. In some alternatives, wherein a nucleic acid encoding a fusion protein is provided, the nucleic acid further comprises a P2A sequence.

[0090] A “marker sequence,” as described herein, encodes a protein that is used for selecting or tracking a protein or cell that has a protein of interest. In the alternatives described herein, the fusion protein provided can comprise a marker sequence that can be selected in experiments, such as flow cytometry. In some alternatives, the marker is the protein Her2tG or EGFRt.

[0091] A “signal sequence” for secretion, can also be referred to as a “signal peptide.” The signal peptide can be used for secretion efficiency and in some systems it is recognized by a signal recognition particle which halts translation and directs the signal sequence to a SRP receptor for secretion. In some alternatives of the fusion proteins

provided herein, the fusion protein further comprises a signal sequence. In some alternatives, of the nucleic acid encoding a fusion protein, the nucleic acid comprises a sequence encoding a signal sequence. In some alternatives, the signaling sequence is a GMCSF signaling sequence, PD-1 signaling sequence or a CD19 signaling sequence. In some alternatives, the nucleic acid encoding the GMCSF signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattcctcctgatccca; SEQ ID NO: 11). In some alternatives, the nucleic acid encoding the PD-1 signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the nucleic acid encoding the CD19 signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13).

[0092] As described herein, the “tumor microenvironment” (TME) is the surrounding microenvironment that constantly interacts with tumor cells, which is conducive to allow cross-talk between tumor cells and its environment. A tumor microenvironment plays a role in disrupting the cancer immunity cycle and plays a critical role in multiple aspects of cancer progression. For example, the TME can decrease drug penetration, confer proliferative and anti-apoptotic advantages to surviving cells, facilitate resistance without causing genetic mutations and epigenetic changes, and collectively modify disease modality and distort clinical indices. Without being limiting, the tumor microenvironment can include the cellular environment of the tumor, surrounding blood vessels, immune cells, fibroblasts, bone marrow derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix. The tumor environment can include tumor cells or malignant cells that are aided and influenced by the tumor microenvironment to ensure growth and survival. The tumor microenvironment can also include tumor-infiltrating immune cells, such as lymphoid and myeloid cells, which can stimulate or inhibit the antitumor immune response and stromal cells such as tumor-associated fibroblasts and endothelial cells that contribute to the tumor’s structural integrity. Without being limiting, stromal cells include cells that make up tumor-associated blood vessels, such as endothelial cells and pericytes, which are cells that contribute to structural integrity (fibroblasts), as well as tumor-associated macrophages

(TAMs) and infiltrating immune cells including monocytes, neutrophils (PMN), dendritic cells (DCs), T and B cells, mast cells, and natural killer (NK) cells. The stromal cells make up the bulk of tumor cellularity while the dominating cell type in solid tumors is the macrophage. In some alternatives described herein, a fusion protein is provided wherein the fusion protein is used for administration into a tumor microenvironment.

[0093] “Chimeric receptor” or “chimeric antigen receptor,” as used herein refers to a synthetically designed receptor comprising a ligand binding domain of an antibody or other protein sequence that binds to a molecule associated with the disease or disorder and is linked via a spacer domain to one or more intracellular signaling domains of a T-cell or other receptors, such as a costimulatory domain. In some alternatives, a cell is manufactured wherein the cell comprises a nucleic acid encoding a fusion protein and wherein the cell comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain that is specific for a tumor surface molecule or protein. In some alternatives, the chimeric antigen receptor comprises an scFv that can recognize an epitope on a cancer cell. In some alternatives, the scFv is specific for CD19.

[0094] “CD19,” as described herein, is a protein encoded by the CD19 gene and resides on the surfaces of B-cells. CD19 has been implicated in several autoimmune diseases and can be useful as a treatment target. In some alternatives herein, a cell is provided, wherein the cell comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19.

[0095] “Macrophage inflammatory proteins,” (MIP) as described herein is a family of proteins that belong to the family of chemotactic cytokines known as chemokines. Humans have two major forms, MIP-1 α and MIP-1 β that are now officially named CCL3 and CCL4, respectively. MIP-1 α and MIP-1 β are produced by macrophages after they are stimulated with toxins, such as bacterial endotoxins. MIPs are crucial for immune responses towards infection and inflammation. MIPs can activate human granulocytes (neutrophils, eosinophils and basophils) which can lead to acute neutrophilic inflammation. They also induce the synthesis and release of other pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and TNF- α from fibroblasts and macrophages. In some alternatives herein, the fusion protein PD1:IFN α 2a stimulates the production of MIP-1a. In some alternatives herein, the fusion protein PD1:IFN α 2a stimulates the production of MIP-1b.

[0096] “CXCL9,” as described herein, is a small cytokine belonging to the CXC chemokine family that is also known as “Monokine induced by gamma interferon” (MIG). CXCL9 is a T-cell chemoattractant, and can be induced by IFN- γ . CXCL9 can elicit its chemotactic functions by interacting with the chemokine receptor CXCR3. In a recent study, CXCL9 was shown to induce chemotaxis and chemorepulsion through CXCR-3 mediated activation of melanoma cells (Amatschek *et al.*). In some alternatives herein, a fusion protein is provided, wherein the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a.

[0097] “CXCL10,” as described herein, is also known as “Interferon gamma-induced protein 10” (IP-10) or “small-inducible cytokine B10.” CXCL10 can be secreted by several cell types in response to IFN- γ . Without being limiting, these cell types can include monocytes, endothelial cells and fibroblasts, for example. CXCL10 has been attributed to several roles, such as chemoattraction for many cells, such as monocytes/macrophages, T cells, NK cells, and dendritic cells for example. Without being limiting, functions of CXCL10 can include promotion of T cell adhesion to endothelial cells, antitumor activity, and inhibition of bone marrow colony formation and angiogenesis, for example. CXCL10 elicits its effects by binding to the cell surface chemokine receptor CXCR3. In a recent study, CXCL10 was shown to inhibit the proliferation and metastasis in many tumors (Jiang *et al.*) In some alternatives herein, a fusion protein is provided, wherein the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a.

[0098] Co-stimulatory domain,” or “intracellular signaling domain” as the term is used herein refers to a signaling moiety that provides to T-cells a signal which, in addition to the primary signal provided by for instance the CD3 zeta chain of the TCR/CD3 complex, mediates a T-cell response, including, but not limited to, activation, proliferation, differentiation, cytokine secretion, and the like. A co-stimulatory domain can include all or a portion of, but is not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83. In some alternatives, a cell is manufactured, wherein the cell comprises a fusion protein for secretion and, wherein the cell further comprises a chimeric antigen receptor. In some alternatives, the

chimeric antigen receptor further comprises a co-stimulatory domain. In some alternatives, the co-stimulatory domain is an intracellular signaling domain that interacts with other intracellular mediators to mediate a cell response including activation, proliferation, differentiation and cytokine secretion, and the like. In some alternatives, all or a portion of one or more domains of a molecule (here the chimeric receptor molecule) provides for activation of a lymphocyte through the co-stimulatory domain. Co-stimulatory domains of such molecules mediate a signal by interacting with cellular mediators to result in proliferation, differentiation, activation and other effector functions. In some alternatives, such molecules include all or portions of CD28, CD3, or 4-1BB, or combinations thereof.

[0099] “Cytotoxic T lymphocyte” (CTL), as used herein, refers to a T lymphocyte that expresses CD8 on the surface thereof (e.g., a CD8⁺ T-cell). In some alternatives, such cells are preferably "memory" T-cells (T_M cells) that are antigen-experienced. In some alternatives, a cell for fusion protein secretion is provided. In some alternatives, the cell is a cytotoxic T lymphocyte. "Central memory" T-cell (or "T_{CM}") as used herein, refers to an antigen experienced CTL that expresses CD62L, CCR-7 and/or CD45RO on the surface thereof, and does not express or has decreased expression of CD45RA, as compared to naïve cells. In some alternatives, a cell for fusion protein secretion is provided. In some alternatives, the cell is a central memory T-cell (T_{CM}). In some alternatives, the central memory cells are positive for expression of CD62L, CCR7, CD28, CD127, CD45RO, and/or CD95, and may have decreased expression of CD45RA, as compared to naïve cells. "Effector memory" T-cell (or "T_{EM}") as used herein refers to an antigen experienced T-cell that does not express or has decreased expression of CD62L on the surface thereof, as compared to central memory cells, and does not express or has a decreased expression of CD45RA, as compared to naïve cell. In some alternatives, a cell for fusion protein secretion is provided. In some alternatives, the cell is an effector memory T-cell. In some alternatives, effector memory cells are negative for expression of CD62L and/or CCR7, as compared to naïve cells or central memory cells, and may have variable expression of CD28 and/or CD45RA.

[0100] “Naïve “ T-cells as used herein, refers to a non-antigen experienced T lymphocyte that expresses CD62L and/or CD45RA, and does not express CD45RO-, as compared to central or effector memory cells. In some alternatives, a cell for fusion protein secretion is provided. In some alternatives, the cell is a naïve T-cell. In some

alternatives, naïve CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naïve T-cells including CD62L, CCR7, CD28, CD127, and/or CD45RA.

[0101] “Effector “ “T_E” T-cells as used herein, refers to antigen experienced cytotoxic T lymphocyte cells that do not express or have decreased expression of CD62L, CCR7, and/or CD28, and are positive for granzyme B and/or perforin, as compared to central memory or naïve T-cells. In some alternatives, a cell for fusion protein secretion is provided. In some alternatives, the cell is an effector T-cell. In some alternatives, the cell does not express or have decreased expression of CD62L, CCR7, and/or CD28, and are positive for granzyme B and/or perforin, as compared to central memory or naïve T-cells.

[0102] “Epitope” as used herein, refers to a part of an antigen or molecule that is recognized by the immune system including antibodies, T-cells, and/or B-cells. Epitopes usually have at least 7 amino acids and can be a linear or a conformational epitope. In some alternatives, a cell expressing a fusion protein is provided wherein the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises an scFv that can recognize an epitope on a cancer cell. “Isolating,” or “purifying” when used to describe the various polypeptides or nucleic acids disclosed herein, refers to a polypeptide or nucleic acid that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide or nucleic acid is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide or nucleic acid, and can include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some alternatives, a method is provided wherein the method comprises delivering the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein to a bacterial cell, mammalian cell or insect cell, growing the cell up in a culture, inducing expression of the fusion protein and purifying the fusion protein for treatment.

[0103] **[0104]** “Ligand” as used herein refers to a substance that binds specifically to another substance to form a complex. Examples of ligands include epitopes on antigens, molecules that bind to receptors, substrates, inhibitors, hormones, and/or activators. “Ligand binding domain” as used herein refers to substance or portion of a substance that binds to a ligand. Examples of ligand binding domains include antigen

binding portions of antibodies, extracellular domains of receptors, and/or active sites of enzymes. In some alternatives, a cell is provided wherein the cell comprises a fusion protein for secretion. In some alternatives, the cell further comprises a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain. In some alternatives, the ligand binding domain is specific for a tumor expressed protein, PDL1 or IFNAR.

[0104] Percent (%) amino acid sequence identity" with respect to the chimeric receptor polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence for each of the ligand binding domain, spacer, transmembrane domain, and/or the lymphocyte activating domain, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, % amino acid sequence identity values generated using the WU-BLAST-2 computer program (Altschul et al., Methods in Enzymology, 266:460-480 (1996)) uses several search parameters, most of which are set to the default values. Those that are not set to default values (i.e., the adjustable parameters) are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T) =11 and scoring matrix=BLOSUM62. In some alternatives of the fusion protein, the fusion protein comprises a domain, wherein the domain comprises PD-1 or a mutated or truncated form of PD-1. In some alternatives, PD-1 or a mutated or truncated form of PD-1 comprises an amino acid sequence with 100%, 95%, 90%, 85%, or a percent sequence identity that is within a range defined by any two of the aforementioned percentages to a sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T

Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R
P A G Q F Q T L V; SEQ ID NO: 4).

[0105] "Chimeric receptor variant polynucleotide" or "chimeric receptor variant nucleic acid sequence" as used herein refers to a polypeptide-encoding nucleic acid molecule as defined below having at least 80%, 85%, 90%, or 95% nucleic acid sequence identity (or a percentage nucleic acid sequence identity within a range defined by any two of the aforementioned percentages) with the polynucleotide acid sequences provided herein, or a specifically derived fragment thereof, such as polynucleotide coding for an antigen binding domain, a polynucleotide encoding a spacer or linker domain, a polynucleotide coding for a transmembrane domain and/ or a polynucleotide coding for a lymphocyte stimulatory domain. Ordinarily, a chimeric receptor variant of polynucleotide or fragment thereof will have at least 80% nucleic acid sequence identity, more preferably at least 81% nucleic acid sequence identity, more preferably at least 82% nucleic acid sequence identity, more preferably at least 83% nucleic acid sequence identity, more preferably at least 84% nucleic acid sequence identity, more preferably at least 85% nucleic acid sequence identity, more preferably at least 86% nucleic acid sequence identity, more preferably at least 87% nucleic acid sequence identity, more preferably at least 88% nucleic acid sequence identity, more preferably at least 89% nucleic acid sequence identity, more preferably at least 90% nucleic acid sequence identity, more preferably at least 91% nucleic acid sequence identity, more preferably at least 92% nucleic acid sequence identity, more preferably at least 93% nucleic acid sequence identity, more preferably at least 94% nucleic acid sequence identity, more preferably at least 95% nucleic acid sequence identity, more preferably at least 96% nucleic acid sequence identity, more preferably at least 97% nucleic acid sequence identity, more preferably at least 98% nucleic acid sequence identity and yet more preferably at least 99% nucleic acid sequence identity with the nucleic acid sequence or a derived fragment thereof. Variants do not encompass the native nucleotide sequence.

[0106] "T-cells" or "T lymphocytes" as used herein can be from any mammalian, preferably primate, species, including monkeys, dogs, and humans. In some alternatives the T-cells are allogeneic (from the same species but different donor) as the recipient subject; in some alternatives the T-cells are autologous (the donor and the recipient are the same); in some alternatives the T-cells are syngeneic (the donor and the recipients are different but are identical twins).

[0107] In some alternatives, a nucleic acid encoding a fusion protein, wherein the nucleic acid comprises a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGGCCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGCGGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACCTGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the third sequence comprises a nucleic acid sequence that is 100%, 95%, 90% or 85% sequence identity to the sequence set forth in SEQ ID NO: 1 or is within a range defined by any two of the aforementioned percentages. In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the fourth sequence comprises a nucleic acid sequence that is about 100%, 95%, 90% or 85% sequence identity to the sequence set forth in SEQ ID NO: 2 or is within a range defined by any two of the aforementioned percentages. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence.

DETAILED DESCRIPTION

[0108] Provided herein are compositions comprising a secretable fusion protein for use in conjunction with chimeric antigen receptor (CAR) T-cell therapy, as well as, methods of using said compositions. Also provided are compositions comprising CAR bearing lymphocytes that can also express secretable fusion proteins. Uses of the alternatives described herein include the intrinsic production and secretion of a PD-1:IFN α 2a fusion protein in CAR T-cells to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. As such, the fusion protein and compositions provided herein improve the therapeutic efficacy of CAR therapy targeted against solid tumors by providing regulatory inputs for multiple immune cell subsets found in the tumor microenvironment. As shown in the alternatives described herein, the initial studies demonstrated that the PD-1:IFN α 2a fusion proteins are efficiently secreted by T-cells and each component of the fusion protein functionally binds to specifically targeted antibodies. Furthermore, *in vitro* assays demonstrate that the secreted PD-1:IFN α 2a protein leads to transcriptional activation of type I IFN elements in a reporter T cell line.

[0109] Alternative to the fusion of IFN α 2a to PD-1, IFN α 2a can also be fused to other tumor resident antigens or secretable factors known to limit and/or inhibit immunotherapeutic approaches. In the alternatives described herein, the cells provided were also tested for their ability to use T cells as a delivery vehicle for secreted, immunoregulatory proteins.

[0110] In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines.

[0111] Previously, studies that incorporated the soluble, extracellular domain of PD-1 during therapy demonstrated that soluble PD-1 blocks the PD-1 pathway and augments the antitumor immune response (Pan et al., Oneal Lett (2013)). Alternatively, IFN α treatment has been used for clinical application (Kreutzer et al., Dtsch Dermatol Ges (2004)). The fusion of both proteins and the secretion of the resultant protein by CAR T-cells have not been described.

Nucleic acids encoding the fusion protein

[0112] As described herein, a nucleic acid encoding a fusion protein is provided. The nucleic acid can have three sequences. The first sequence encodes a protein that modulates an immune response, the second sequence encodes a glycine spacer, and the third sequence encodes an interferon, wherein a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. The nucleic acid can also be either DNA or RNA.

[0113] Interferons are signaling proteins that are released by host cells in a response to pathogen invasion (e.g. virus, bacteria, parasites) as well as in response to tumor cells. In the alternatives herein, the fusion protein comprises an interferon or a portion thereof, which can be used to modulate the functions of the immune system. Thus the fusion protein can be used with other therapies to control diseases such as an autoimmune disorder, cancer and leukemias for example.

[0114] In some alternatives of the fusion protein, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence encoding the interferon comprises a sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1).

[0115] The nucleic acid encoding a fusion protein can further comprise a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. The promoter can be an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID

NO:

2

(GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. The promoter can thus be used to control the expression of the fusion protein, if there are off-target effects or toxicities from the fusion protein.

[0116] In some alternatives of the nucleic acid encoding the fusion protein, the nucleic acid comprises a first, second and a third sequence. The first sequence encodes a protein that modulates an immune response, the second sequence encodes an amino spacer or linker, such as a plurality of glycines or a glycine spacer, and the third sequence encodes an interferon, wherein a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, CD19, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCTACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCACCTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC

AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
 L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
 A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
 T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
 R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form
 of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A
 P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T
 E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D
 R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G
 A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q
 F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6).

[0117] In some alternatives of the nucleic acid, the amino acid spacer or linker
 comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of
 amino acids, such as glycine, within a range defined by any two of the aforementioned
 numbers. In some alternatives, the glycine spacer comprises at least 3 glycines. In some
 alternatives, the glycine spacer comprises a sequence set forth in SEQ ID NO:7 (GGGS;
 SEQ ID NO: 7), SEQ ID NO: 8 (GGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG;
 SEQ ID NO: 9). The glycine linker can also be adjusted to improve protein folding
 dynamics of the fusion protein, to increase binding affinities of the fusion protein, and/or
 to prevent aggregation of the fusion protein.

[0118] In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the fifth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a E2A linker or a P2A linker.

[0119] In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence, E2A sequence, or P2A sequence.

[0120] In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt.

[0121] In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13).

Vectors for expressing the fusion protein

[0122] A variety of vector combinations can be constructed to provide for efficient transduction and transgene expression. The vector comprises any one of the alternative nucleic acids provided herein. In some alternatives, the vector is a viral vector. In other alternatives, the vectors can include a combination of viral vectors and plasmid vectors. Other viral vectors include foamy virus, adenoviral vectors, retroviral vectors,

and lentiviral vectors. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*.

[0123] In some alternatives, a plasmid vector or a viral vector comprises the nucleic acid encoding a fusion protein. In some alternatives, the vector is RNA or DNA.

Cells and Compositions: T lymphocyte populations.

[0124] The compositions described herein provide for genetically modified cells with the vectors and/or constructs as described herein. In some alternatives, the cells are CD4⁺ and/or CD8⁺ T lymphocytes.

[0125] Provided herein are cells for fusion protein secretion, wherein the cell comprises the nucleic acid of anyone of the alternatives described herein or the expression vector anyone of the alternatives described herein. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte. In some alternatives, the cell is *E. coli*. In some alternatives, the cell is an insect cell capable of protein expression. In some alternatives, the cell is a lymphocyte.

[0126] In some alternatives, the cell is a CD4⁺ or CD8⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells.

[0127] In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells.

[0128] In some alternatives, the cell is a precursor T-cell.

[0129] In some alternatives, the cell is a stem cell.

[0130] In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a NK cell.

[0131] In some alternatives, the cell is a B cell.

[0132] In some alternatives, the cell is a neuronal stem cell.

[0133] In some alternatives, the cell further comprises a chimeric antigen receptor.

[0134] The lymphocytes (T lymphocytes) can be collected in accordance with known techniques and enriched or depleted by known techniques such as affinity binding to antibodies such as flow cytometry and/or immunomagnetic selection. After enrichment and/or depletion steps, *in vitro* expansion of the desired T lymphocytes can be carried out in accordance with known techniques or variations thereof that will be apparent to those skilled in the art. In some alternatives, the T cells are autologous T cells obtained from the patient.

[0135] For example, the desired T cell population or subpopulation can be expanded by adding an initial T lymphocyte population to a culture medium *in vitro*, and then adding to the culture medium feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). The non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some alternatives, the PBMC are irradiated with gamma rays in the range of 3000 to 3600 rads to prevent cell division. In some alternatives, the PBMC are irradiated with gamma rays of 3000, 3100, 3200, 3300, 3400, 3500 or 3600 rads or any value of rads between any two endpoints of any of the listed values to prevent cell division. The order of addition of the T cells and feeder cells to the culture media can be reversed if desired. The culture can typically be incubated under conditions of temperature and the like that are suitable for the growth of T lymphocytes. For the growth of human T lymphocytes, for example, the temperature will generally be at least 25 degrees Celsius, preferably at least 30 degrees, more preferably 37 degrees. In some alternatives, the temperature for the growth of human T lymphocytes is 22, 24, 26, 28, 30, 32, 34, 36, 37 degrees Celsius or any other temperature between any two endpoints of any of the listed values.

[0136] After isolation of T lymphocytes both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after expansion.

[0137] CD8⁺ cells can be obtained by using standard methods. In some alternatives, CD8⁺ cells are further sorted into naïve, central memory, and effector

memory cells by identifying cell surface antigens that are associated with each of those types of CD8⁺ cells. In some alternatives, memory T cells are present in both CD62L⁺ and CD62L⁻ subsets of CD8⁺ peripheral blood lymphocytes. PBMC are sorted into CD62L⁻CD8⁺ and CD62L⁺CD8⁺ fractions after staining with anti-CD8 and anti-CD62L antibodies. In some alternatives, the expression of phenotypic markers of central memory T_{CM} include CD45RO, CD62L, CCR7, CD28, CD3, and/or CD127 and are negative or low for granzyme B. In some alternatives, central memory T cells are CD45RO⁺, CD62L⁺, and/or CD8⁺ T cells. In some alternatives, effector T_E are negative for CD62L, CCR7, CD28, and/or CD127, and positive for granzyme B and/or perforin. In some alternatives, naïve CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including CD62L, CCR7, CD28, CD3, CD127, and/or CD45RA.

[0138] CD4⁺ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4⁺ lymphocytes can be obtained by standard methods. In some alternatives, naïve CD4⁺ T lymphocytes are CD45RO⁻, CD45RA⁺, CD62L⁺, and/or CD4⁺ T cells. In some alternatives, central memory CD4⁺ cells are CD62L⁺ and/or CD45RO⁺. In some alternatives, effector CD4⁺ cells are CD62L⁻ and/or CD45RO⁻.

[0139] Whether a cell or cell population is positive for a particular cell surface marker can be determined by flow cytometry using staining with a specific antibody for the surface marker and an isotype matched control antibody. A cell population negative for a marker refers to the absence of significant staining of the cell population with the specific antibody above the isotype control, positive refers to uniform staining of the cell population above the isotype control. In some alternatives, a decrease in expression of one or markers refers to loss of 1 log₁₀ in the mean fluorescence intensity and/or decrease of percentage of cells that exhibit the marker of at least 20% of the cells, 25% of the cells, 30% of the cells, 35% of the cells, 40% of the cells, 45% of the cells, 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, and 100% of the cells or any % between any two of the aforementioned percentages when compared to a reference cell population. In some alternatives, a cell population positive for one or markers refers to a percentage of cells that exhibit the marker of at least 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells,

80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, or 100% of the cells or any % between any two of the aforementioned percentages when compared to a reference cell population.

[0140] Whether a cell or cell population is positive for a particular cell surface marker can be determined by flow cytometry using staining with a specific antibody for the surface marker and an isotype matched control antibody. A cell population negative for a marker refers to the absence of significant staining of the cell population with the specific antibody above the isotype control, positive refers to uniform staining of the cell population above the isotype control. In some alternatives, a decrease in expression of one or markers refers to loss of 1 log₁₀ in the mean fluorescence intensity and/or decrease of percentage of cells that exhibit the marker of at least 20% of the cells, 25% of the cells, 30% of the cells, 35% of the cells, 40% of the cells, 45% of the cells, 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, and 100% of the cells or any % between any two of the aforementioned percentages when compared to a reference cell population. In some alternatives, an increase refers to an increase in mean fluorescence intensity and/or to an increase in the number of cells in a cell population that are positive for one or a given marker, such as a population in which s refers to a percentage of cells that exhibit the marker of at least 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, or 100% of the cells or any % between any two of the aforementioned percentages exhibit the marker, e.g., when compared to a reference cell population.

[0141] In some alternatives, populations of CD4⁺ and CD8⁺ that are antigen specific can be obtained by stimulating naïve or antigen specific T lymphocytes with antigen. Naïve T cells can also be used. Any number of antigens from tumor cells can be utilized as targets to modulate T cell responses. In some alternatives, the compositions comprising the cells are useful in the treatment of a disease or disorder including a solid tumor, hematologic malignancy, breast cancer or melanoma.

Method of making a chimeric antigen receptor bearing cell that expresses a fusion protein

[0142] In the alternatives described herein, it can be desired to introduce a nucleic acid or vector into a host cell such as a lymphocyte to be used in immunotherapy. The nucleic acid or vector of any one of the alternatives provided herein can be used for introduction into the host cell for immunotherapy. For example, the introduced gene or genes can improve the efficacy of therapy by enhancing the cytotoxicity in T and NK cells, promote T-cell stimulatory cytokine production in macrophages and inhibit regulatory T cell function. Furthermore, the fusion protein can also sequester the T-cell inhibitory interaction of PD-1 with the ligand PDL-1. The use of a fusion protein comprising a PD-1 and a IFN α 2a component can make CAR T-cell therapy more interactive against the immunosuppressive milieu found in solid tumors by modulating stimulatory and suppressive functions to specific tumor resident immune cell subsets. Preparation of these cells can be carried out in accordance with known techniques that will be apparent to those skilled in the art based upon the present disclosure.

[0143] In some alternatives, a method of making a chimeric antigen receptor bearing cell is provided, wherein the chimeric antigen receptor bearing cell expresses a fusion protein. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the ligand binding domain is specific for CD19. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central

memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives the cell is an NK cell.

Compositions

[0144] Provided herein are compositions that comprise a genetically modified cell preparation as set forth in this disclosure. In some alternatives, the cells comprise the nucleic acids encoding a fusion protein, as described in the alternatives herein. In some alternatives, the compositions comprises CD4⁺ T cells that have a chimeric antigen receptor comprising an extracellular antibody variable domain specific for a ligand associated with the disease or disorder, a spacer region, a transmembrane domain, and an intracellular signaling domain of a T cell receptor or other receptors. In other alternatives, the composition further comprises a chimeric receptor modified tumor-specific CD8⁺ cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8⁺ T cells that have a chimeric receptor comprising an extracellular single chain antibody specific for a ligand associated with the disease or disorder, a spacer region, a transmembrane domain, and an intracellular signaling domain of a T cell receptor. In some alternatives, the chimeric antigen receptor is under the control of a drug inducible promoter as described herein. In some alternatives, the chimeric receptor modified T cell population of the disclosure persist *in vivo* for at least 3 days or longer. In an alternative, each of these populations can be combined with one another or other cell types to provide a composition.

[0145] In some alternatives, the cells of the composition are CD4⁺ cells. The CD4⁺ cell can be T helper lymphocyte cells, naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, or bulk CD4⁺ T cells. In some alternatives, the CD4⁺ helper lymphocyte cell is a naïve CD4⁺ T cell, wherein the naïve CD4⁺ T cell comprises a CD45RO⁻, CD45RA⁺, and/or is a CD62L⁺ CD4⁺ T cell.

[0146] In some alternatives, the cells of the composition are CD8⁺ cells. The CD8⁺ cell can be a T cytotoxic lymphocyte cell, a naïve CD8⁺ T cell, central memory CD8⁺ T cell, effector memory CD8⁺ T cell and/or bulk CD8⁺ T cell. In some alternatives, the CD8⁺ cytotoxic T lymphocyte cell is a central memory T cell, wherein

the central memory T cell comprises a CD45RO⁺, CD62L⁺, and/or CD8⁺ T cell. In yet other alternatives, the CD8⁺ cytotoxic T lymphocyte cell is a central memory T cell and the CD4⁺ helper T lymphocyte cell is a naïve or central memory CD4⁺ T cell.

[0147] In some alternatives, the compositions comprise T cell precursors. In some alternatives, the compositions comprise hematopoietic stem cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

[0148] In some compositions, the cells are NK cells.

[0149] In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor.

Fusion proteins for administration to a subject

[0150] As provided herein, are fusion proteins for treatment that can be manufactured by the methods described herein. The fusion proteins can comprise a protein that can modulate an immune response, an amino acid spacer, preferably a plurality of glycines, and an interferon. In some alternatives of the fusion protein the protein domain of the interferon is from IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives of the fusion protein, the protein that can modulate an immune response is PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPin, affinity peptides,

extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the glycine spacer comprises at least 3 glycines. In some alternatives, the glycine spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the fusion protein further comprises a marker. In some alternatives, the marker is Her2tG or EGFRt.

Methods for manufacturing a fusion protein

[0151] As described herein are methods for manufacturing a fusion protein. The method can comprise delivering the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein to a bacterial cell, mammalian cell or insect cell, growing the cell up in a culture, inducing expression of the fusion protein and purifying the fusion protein for treatment.

[0152] In some alternatives, the inducing comprises adding IPTG, anhydrotetracycline, L-arabinose or rhamnose to the culture. In some alternatives, the cell is *E. coli*. In some alternatives, the cell is an insect-cell.

[0153] Purifying a protein from a mammalian cell, bacterial cell or insect cell is known to those skilled in the art. Without being limiting, purification can involve lysing the cell, and collecting the cell lysate. The lysate can then be subjected to precipitation protocols and differential solubilization to obtain the protein of interest. Proteins can then be separated out of the lysate by ion exchange chromatography, hydrophobic interaction chromatography and/or size exclusion chromatography. Such methods are known to those skilled in the art.

Methods of secreting the fusion protein in a subject

[0154] As described herein are methods of secreting the fusion protein in a subject in need. The method can comprise delivering to a subject the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein. In some alternatives, the method further comprises administering to the subject an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the subject has cancer. In some alternatives the cancer comprises adrenal cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, Castleman disease, cervical cancer, colon cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, Hodgkin disease, Kaposi Sarcoma, kidney cancer, Laryngeal and hypopharyngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, multiple myeloma, malignant mesothelioma, myelodysplastic syndrome,

nasopharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer or uterine sarcoma. In some alternatives, the subject is selected or identified for cancer treatment. Such selection or identification can be made by clinical or diagnostic evaluation and confirmation of the presence of a cancer.

Methods of increasing T-cell activity

[0155] Described herein, are also methods of increasing T-cell activity. The method can comprise administering an effective amount of the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to a subject in need. In some alternatives, administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the subject has cancer. In some alternatives the cancer comprises adrenal cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, Castleman disease, cervical cancer, colon cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, Hodgkin disease, Kaposi Sarcoma, kidney cancer, Laryngeal and hypopharyngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, multiple myeloma, malignant mesothelioma, myelodysplastic syndrome, nasopharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer or uterine sarcoma. In some alternatives, the subject is selected or identified for cancer

treatment. Such selection or identification can be made by clinical or diagnostic evaluation and confirmation of the presence of a cancer.

Method of decreasing immunosuppression in a tumor microenvironment

[0156] In some alternatives, a method of decreasing immunosuppression in a tumor microenvironment is provided. The method can comprise administering an effective amount of the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to a subject in need. In some alternatives, administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the subject has cancer. In some alternatives the cancer comprises adrenal cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, Castleman disease, cervical cancer, colon cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, Hodgkin disease, Kaposi Sarcoma, kidney cancer, Laryngeal and hypopharyngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, multiple myeloma, malignant mesothelioma, myelodysplastic syndrome, nasopharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer or uterine sarcoma. In some alternatives, the subject is selected or identified for cancer treatment. Such selection or identification can be made by clinical or diagnostic evaluation and confirmation of the presence of a cancer.

Methods of treating , inhibiting, treating, or ameliorating cancer in a subject, such as a human

[0157] In some alternatives, a method of inhibiting, treating, or ameliorating cancer in a subject, such as a human expressing a tumor antigen is provided. The method can comprise administering an effective amount of the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to the patient. In some alternatives, administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives the cancer comprises adrenal cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, Castleman disease, cervical cancer, colon cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, Hodgkin disease, Kaposi Sarcoma, kidney cancer, Laryngeal and hypopharyngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, multiple myeloma, malignant mesothelioma, myelodysplastic syndrome, nasopharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pituitary tumors, prostate cancer, retinoblastoma, skin cancer, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer or uterine sarcoma. In some alternatives, the cancer is breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer or lymphoma. In some alternatives, the subject is selected or identified for cancer treatment. Such selection or identification can be made by clinical or diagnostic evaluation and confirmation of the presence of a cancer.

The PD-1:IFN α 2a fusion protein

[0158] As described herein, the nucleic acid encoding the fusion protein comprises a EF1p sequence, a sequence for PD1ECD, a sequence encoding a G3 spacer, a sequence encoding IFN α 2a, a T2A sequence, a GMCSFR signal sequence, and a sequence encoding a Her2tG marker as shown in **Figure 1**. H9T cells transformed with the PD1:IFN α 2a-T2AHer2tG vector and selected using Her2tG. The cell culture supernatant was concentrated and subjected to anti-2A and anti-PD1 western blot Results demonstrate that H9 T cells efficiently secrete the PD1:IFN α 2a fusion and the secreted protein can be recognized by anti-PD1 antibodies (**Figure 2**).

[0159] The fusion protein was then tested for binding onto target receptors. Concentrated supernatant from PD1-IFN α 2a transduced H9 cells was added to target U293T cells that overexpress PD-L1, the ligand to PD1, or Jurkat cells that express IFN α R, the receptor to IFN α 2a. Following incubation of supernatant with target cells, the cells were washed and then stained with an antibody specific to IFN α (U293T cells) or PD1 (Jurkat cells). U293T cells do not express IFN α R and Jurkat cells do not express PD-L1. Results demonstrate that the two components of PD1-IFN α 2a can bind to its respective ligand (PD-L1 with the PD1 component) and receptor (IFN α R with the IFN α 2a component) (**Figures 3A and 3B**).

[0160] In order to determine whether the fusion protein modulates immune signaling, concentrated supernatant from PD1-IFN α 2a transduced H9 cells or parental H9s was added to the Jurkat reporter cell line that has a secreted embryonic alkaline phosphatase (SEAP) reporter gene under control of a interferon regulatory factor (IRF). The supernatant was incubated with the reporter cell line for 24hr and the levels of IRF-induced SEAP was assessed from the culture supernatant using QUANTI-Blue. The schematic showing the test is shown in **Figure 5**.

[0161] As shown in **Figure 5**, an assay was set up to analyze potency of PD1:IFN α 2a in reference to native IFN α . In the first step, H9 T cells were transduced with the PD1:IFN α 2a and were then cultured for 24 hours and then the supernatant was harvested. Second, the harvested supernatant was concentrated using an Amicon Ultra-4 Centrifugal Filter Unit (Millipore) and subjected to an IFN α specific Bioplex Pro Inflammation Panel assay to quantify the presence of soluble PD1:IFN α 2a in the supernatant (8.1pmol/mL or 8.1nM) in reference to an undiluted native IFN α (140000 pmol/mL or 140000nM). As shown in the third step, based on the prior bioplex results, a known concentration range of native IFN α (0-140000nM) and a 1:20 dilution of PD1:

IFN α 2a was added to an IFN α responsive Jurkat reporter cell line for 24-hr. Results demonstrate an increase in IRF-induced SEAP from PD1-IFN α 2a supernatant relative to parental supernatant (**Figure 4 and 6**).

[0162] As shown in **Figure 6A**, following a 24-hr incubation period, supernatant from the Jurkat reporter cells were analyzed for the presence of secreted embryonic alkaline phosphatase (SEAP) as demonstrated by absorbance (x-axis). According to the manufacturer, the native concentrated IFN α has an activity of 6.48e7U/ml and its dilutions are represented on the y-axis. In **Figure 6B**, a table is shown representing relative concentrations and activity levels of native IFN α and the PD1:IFN α 2a supernatant. The PD1:IFN α 2a supernatant was diluted 1:20 in assay, resulting in an activity of 96.1U/ml as represented by the large green square in **Figure 6 A (at 100U/mL)**. Accounting for the 1:20 dilution, the stock PD1:IFN α 2a has an activity of 1921U/ml. The U/ml was divided by pmol/ml to calculate the U/pmol of both the native IFN α and the PD1:IFN α 2a containing supernatant. Results demonstrate that the PD1:IFN α 2a is around 50% as potent as native IFN α for inducing SEAP activity in the Jurkat reporter cell line.

Activity of the PD-1-IFN α 2a protein

[0163] While it is known that PD-1-IFN α 2a is recognized by PD-1 and IFN α 2a specific antibodies (flow cytometric and bead array analysis), studies to demonstrate complete activity relative to its individual counterparts can be tested. In some alternatives, if reduced activity is demonstrated, variable glycine-based spacer lengths between PD-1 and IFN α 2a can be introduced to better facilitate proper protein folding and protein activity. Furthermore, the systemic administration of IFN α 2a can cause adverse effects in normal tissue. If the PD-1:IFN α 2a also causes off-target toxicities expression can be regulated by control under a drug regulatable promoter or by CAR-dependent signaling (e.g. tamoxifen or NFAT regulated promoter systems).

Examining the effects of PD1:IFN α 2a on primed or stimulated Monocytes

[0164] PD1:IFN α 2a containing H9 supernatant stimulates regulatory signaling molecules produced by macrophages, as shown in **Figure 14**. Macrophages were M1-differentiated via treatment with IFN γ and LPS. The M1-differentiated cells were then cultured for 24hrs in the presence of varying concentrations of PD1:IFN α 2a supernatant. Following the 24hr culture, the supernatant was harvested and subjected to a luminex bead-based assay for multiple macrophage specific secretory proteins. Macrophage inflammatory proteins (MIPs) stimulate pro-inflammatory cytokine release from fibroblasts and other macrophages. Chemokine (CXC motif) ligands 9 and 10 are chemo-attractants of T-cells, NK cells, macrophages and dendritic cells. CXCL10 has been shown to prevent angiogenesis and promote the generation of tumor-specific T-cells.

Examining the effects of PD1:IFN α 2a on primed or stimulated Monocytes

[0165] Secreted fusion proteins offer an attractive means of engineering cellular interplay with the advantage of reaching a diverse cell population. Type I IFN drives anti-tumor activity in virtually all immune cell subsets, including increased cytotoxicity in T and NK cells and increased inflammatory cytokine production in macrophages. To examine the cytokine response of macrophages to the PD1:IFN α 2a fusion protein an assay is developed to culture primed (GM-CSF cultured) or classically activated/stimulated (GM-CSF, LPS and IFN γ cultured) monocytes, driven towards an M1-polarized macrophage phenotype, in the presence or absence of PD1:IFN α 2a.

Following a 24-hr culture period, supernatant will be harvested and analyzed by Bioplex assay. Included in this assay is an antibody panel able to specifically identify and quantify levels of inflammatory cytokine (such as IL12, TNF α , IL4, etc.) located in the supernatant. Results will inform the role PD1:IFN α 2a plays on the cytokine profile produced by M1-polarized macrophages and advise how these macrophages may positively affect other immune cell subsets such as T and NK cells.

CD4 and CD8 T-cells can co-express a CAR and PD1:IFN α 2a.

[0166] In order to test whether primary CD4 and CD8 T-cells can co-express a CAR and PD1:IFN α 2a, Primary CD4 and CD8 T-cells were isolated from PBMCs using CD4 or CD8 microbeads, respectively. The CD4 and CD8 T-cells were selected up to 99% and 92.3% purity, respectively (**Figure 7A**). The purified CD4 or CD8 T-cells were then stimulated with CD3/CD28 microbeads for three days and then transduced with lentivirus containing the following: CD19scFv-IgG4hinge-CD28tm/41BB-zeta-T2A-Her2tG (2nd generation short spacer CD19CAR) and/or EGFRt-T2A-PD1:IFN α 2a. The transduced CD4 and CD8 T-cells were then expanded (10-12 days) and subjected to purification using biotinylated Herceptin or Erbitux to select for Her2tG or EGFRt positive populations, respectively. The dual transduced CD4 or CD8 T-cells were subjected to a second round of purification 4 days later for the second selection marker (Her2tG first selection, EGFRt second selection). Results as shown in **Figure 7B** demonstrated that the dual transduced T-cells were >95% dual positive following the selection methods.

[0167] The EGFRt-T2A-PD1:IFN α 2a fusion protein was encoded by the nucleic acid sequence set forth in SEQ ID NO: 14 (ATGCTT CTCCTGGTGA CAAGCCTTCT GCTCTGTGAG TTACCACACC CAGCATTCTT CCTGATCCCA CGCAAAGTGT GTAACGGAAT AGGTATTGGT GAATTAAAG ACTCACTCTC CATAAATGCT ACGAATATTA AACACTTCAA AAAGTGCACC TCCATCAGTG GCGATCTCCA CATCCTGCCG GTGGCATTTA GGGGTGACTC CTTACACAT ACTCCTCCTC TGGATCCACA GGAAGTGGAT ATTCTGAAAA CCGTAAAGGA AATCACAGGG TTTTGTGCTGA TTCAGGCTTG GCCTGAAAAC AGGACGGACC TCCATGCCTT TGAGAACCTA GAAATCATAC GCGGCAGGAC CAAGCAACAT GGTCAGTTTT CTCTTGCAGT CGTCAGCCTG AACATAACAT CCTTGGGATT ACGCTCCCTC AAGGAGATAA GTGATGGAGA TGTGATAATT TCAGGAAACA

AAAATTTGTG CTATGCAAAT ACAATAAACT GGAAAAAACT GTTTGGGACC
TCCGGTCAGA AAACCAAAAT TATAAGCAAC AGAGGTGAAA ACAGCTGCAA
GGCCACAGGC CAGGTCTGCC ATGCCTTGTG CTCCCCGAG GGCTGCTGGG
GCCCCGAGCC CAGGGACTGC GTCTCTTGCC GGAATGTCAG CCGAGGCAGG
GAATGCGTGG ACAAGTGCAA CCTTCTGGAG GGTGAGCCAA GGGAGTTTGT
GGAGAACTCT GAGTGCATAC AGTGCCACCC AGAGTGCCTG CCTCAGGCCA
TGAACATCAC CTGCACAGGA CGGGGACCAG ACAACTGTAT CCAGTGTGCC
CACTACATTG ACGGCCCCCA CTGCGTCAAG ACCTGCCCGG CAGGAGTCAT
GGGAGAAAAC AACACCCTGG TCTGGAAGTA CGCAGACGCC GGCCATGTGT
GCCACCTGTG CCATCCAAAC TGCACCTACG GATGCACTGG GCCAGGTCTT
GAAGGCTGTC CAACGAATGG GCCTAAGATC CCGTCCATCG CCACTGGGAT
GGTGGGGGCC CTCCTCTTGC TGCTGGTGGT GGCCCTGGGG ATCGGCCTCT
TCATGGGCGG CGGAGAGGGC AGAGGAAGTC TTCTAACATG CCGTGACGTG
GAGGAGAATC CCGGCCCTAG GATGCAGATC CCTCAGGCCC CTTGGCCTGT
CGTGTGGGCT GTGCTGCAGC TGGGATGGCG GCCTGGCTGG TTTCTGGACA
GCCCCGACAG ACCCTGGAAC CCCCCTACAT TTTCCCCTGC CCTGCTGGTC
GTGACCGAGG GCGACAATGC CACCTTCACC TGTAGCTTCA GCAACACCAG
CGAGAGCTTC GTGCTGAACT GGTACAGAAT GAGCCCCAGC AACCAGACCG
ACAAGCTGGC CGCCTTCCCC GAGGATAGAT CTCAGCCCGG CCAGGACTGC
CGGTTTCAGAG TGACCCAGCT GCCCAACGGC CGGGACTTCC ACATGTCTGT
CGTGCGGGCC AGACGGAACG ACAGCGGCAC ATATCTGTGC GGCGCCATCA
GCCTGGCCCC CAAGCTCCAG ATCAAAGAGA GCCTGAGAGC CGAGCTGAGA
GTGACCGAGA GAAGGGCCGA AGTGCCTACC GCCCACCCTA GCCCATCTCC
AAGACCTGCC GGCCAGTTCC AGACACTCGT GGGCGGAGGA TGCGACCTGC
CTCAGACACA CAGCCTGGGC AGCAGACGGA CCCTGATGCT GCTGGCCCAG
ATGCGGAAGA TCAGCCTGTT CAGCTGCCTG AAGGACCGGC ACGACTTCGG
CTTCCCTCAG GAAGAGTTCG GCAACCAGTT TCAGAAGGCC GAGACAATCC
CCGTGCTGCA CGAGATGATC CAGCAGATCT TCAACCTGTT CTCCACCAAG
GACAGCAGCG CCGCCTGGGA CGAGACACTG CTGGACAAGT TCTACACCGA
GCTGTACCAG CAGCTGAATG ACCTGGAAGC CTGCGTGATC CAGGGCGTGG
GCGTGACAGA GACACCCCTG ATGAAGGAAG ATAGCATCCT GGCCGTGCGC
AAGTACTTCC AGCGGATCAC CCTGTACCTG AAAGAGAAGA AGTACAGCCC
CTGCGCCTGG GAGGTCGTGC GCGCCGAGAT CATGAGAAGC TTCAGCCTGA

GCACCAACCT GCAGGAAAGC CTGCGGAGCA AAGAATAA; SEQ ID NO: 14).
 EGFRt-T2A-PD1:IFN α 2a fusion protein comprises an amino acid sequence as set forth in
 SEQ ID NO: 15 (M L L L V T S L L L C E L P H P A F L L I P R K V
 C N G I G I G E F K D S L S I N A T N I K H F K N C T S I S G
 D L H I L P V A F R G D S F T H T P P L D P Q E L D I L K T
 V K E I T G F L L I Q A W P E N R T D L H A F E N L E I I R
 G R T K Q H G Q F S L A V V S L N I T S L G L R S L K E I S
 D G D V I I S G N K N L C Y A N T I N W K K L F G T S G Q K
 T K I I S N R G E N S C K A T G Q V C H A L C S P E G C W G
 P E P R D C V S C R N V S R G R E C V D K C N L L E G E P
 R E F V E N S E C I Q C H P E C L P Q A M N I T C T G R G P
 D N C I Q C A H Y I D G P H C V K T C P A G V M G E N N T
 L V W K Y A D A G H V C H L C H P N C T Y G C T G P G L E
 G C P T N G P K I P S I A T G M V G A L L L L L V V A L G I
 G L F M G G G E G R G S L L T C G D V E E N P G P R M Q I
 P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W
 N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S
 F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q
 D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T
 Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E
 V P T A H P S P S P R P A G Q F Q T L V G G G C D L P Q T H
 S L G S R R T L M L L A Q M R K I S L F S C L K D R H D F G
 F P Q E E F G N Q F Q K A E T I P V L H E M I Q Q I F N L F
 S T K D S S A A W D E T L L D K F Y T E L Y Q Q L N D L E A
 C V I Q G V G V T E T P L M K E D S I L A V R K Y F Q R I T
 L Y L K E K K Y S P C A W E V V R A E I M R S F S L S T N L
 Q E S L R S K E; SEQ ID NO: 15)

[0168] The CD4 and CD8 cells were then examined to determine whether the primary T-cells can produce the PD1: IFN α 2a. The selected T-cell populations from **Figure 7** were subjected to either a standard rapid expansion protocol (REP) (REP – Mock or PD1:IFN α 2a alone expressing) or a Seitaro REP (CD19CAR or dual CD19CAR and PD1:IFN α 2a expressing). On the 14th day of REP, CD4 or CD8 T-cells were stimulated for 24 hours at a 1:1 ratio CD3/CD28 bead:T-cell. Following the 24 hour

incubation period, the supernatant was collected and then subjected to an IFN α 2a specific Bioplex assay. Results demonstrate basal PD1:IFN α 2a levels under normal conditions, but elevated PD1:IFN α 2a levels were seen upon stimulation (**Figure 8**).

[0169] The expression and secretion of PD1:IFN α 2a was then evaluated to determine if the expression and secretion of PD1:IFN α 2a would interfere with effector cytokine production. Selected T-cell populations from **Figure 7** were subjected to either a standard rapid expansion protocol (REP) (REP – Mock or PD1:IFN α 2a alone expressing) or a Seitaro REP (CD19CAR or dual CD19CAR and PD1:IFN α 2a expressing). On the 14th day of REP, CD4 or CD8 T-cells were co-cultured with target cells at a 2:1 effector:target ratio. Following the 24 hour incubation period, supernatant was collected and then subjected to a Bioplex assay for IL2 and TNF α production. The K562 parental and negative control had no active T-cell target so there is no cytokine production. The K562 OKT3, positive control, demonstrates that all T-cells were able to produce similar levels of effector cytokine upon stimulation. K562 CD19 and Raji parental co-culture only induced T-cell cytokine production if the T-cells expressed the CD19CAR. The production of the PD1:IFN α 2a did not interfere with IL2 and TNF α effector cytokine production as shown in **Figure 9**.

[0170] The expression and secretion of PD1:IFN α 2a was also examined to determine if expression and secretion of PD1:IFN α 2a interferes with effector phenotype or target specificity. An *in vitro* cytotoxicity of CD8⁺ T-cells by chromium release was performed. A 4 hour chromium release assay was performed that showed CD19-CAR T-cell specificity against CD19⁺ (K562 CD19 and Raji parental) and control target cells (negative control = K562 parental ; positive control = K562 OKT3). CD8⁺ T-cells were co-cultured with target cells at a 30:1, 10:1, 3.3:1 or 1.1:1 ratio. As shown, in **Figure 10**, the expression and secretion of PD1:IFN α 2a does not interfere with effector phenotype or target specificity.

The *in vivo* antitumor activity of CD19CAR- or CD19CAR and PD1-IFN α 2a expressing T-cells

[0171] 5e⁶ Be2 CD19t eGFP:ffluc neuroblastoma cells were subcutaneously injected into the left and right flanks of NOD-SCID IL-2Ry null mice. Five days later 30e⁶ T-cells at a 1:1 CD4:CD8 ratio were injected intravenously (i.v.) into the mice. Subsequent tumor volume measurements by caliper were performed as was imaged using D-luciferin and the IVIS imaging system. As shown in **Figure 11**, CD19CAR and PD1-

IFN α 2a expressing T-cells were able to inhibit tumor growth at a similar rate to CD19CAR-alone expressing T-cells. As such, the alternatives as described herein can be used to inhibit tumor growth.

[0172] In some alternatives cells can be used in the treatment of subjects in need, wherein the subject has a cancer. In some alternatives, the cancer is a solid tumor. In some alternatives, the cells and compositions can be used to inhibit tumor growth.

Production and secretion of PD1:IFN α 2a variants

[0173] H9 T-cells were shown to be able to produce and secrete PD1:IFN α 2a variants. H9 T-cells were transduced with EGFRt-T2A-PD1:IFN α 2a vector or vector containing the A99L (Lazar-Molnar et al 2008 PNAS), G14-1 (Maute et al 2015 PNAS), or HAC-1(Maute et al 2015 PNAS) mutant and then selected by EGFRt. Selected H9 cultures were washed in media to remove excess PD1:IFN α 2a in supernatant. Supernatant from the H9 cultures was then harvested 24 hours later and concentrated using an Amicon Ultra-4 Centrifugal Filter Unit (Millipore) and subjected to an IFN α specific Bioplex Pro Inflammation Panel assay to quantify the presence of soluble PD1:IFN α 2a in the supernatant in reference to an undiluted native IFN α (140000 pmol/mL or 140000nM). Data is represented as molecules/cell-hr from 1e⁶ cells. Results demonstrate that all variants of the PD1:IFN α 2a were produced at similar levels (**Figure 12**). As shown in the experiments, cells can be produced to have high levels of fusion protein expression.

[0174] As described in the alternatives herein, the EGFRt-T2A-PD1(G14-1):IFN α 2a is encoded by the nucleic acid sequence set forth in SEQ ID NO: 17 (ATGCTTCTC CTGGTGACAA GCCTTCTGCT CTGTGAGTTA CCACACCCAG CATTCCTCCT GATCCCACGC AAAGTGTGTA ACGGAATAGG TATTGGTGAA TTAAAGACT CACTCTCCAT AAATGCTACG AATATTAAAC ACTTCAAAAA CTGCACCTCC ATCAGTGGCG ATCTCCACAT CCTGCCGGTG GCATTTAGGG GTGACTCCTT CACACATACT CCTCCTCTGG ATCCACAGGA ACTGGATATT CTGAAAACCG TAAAGGAAAT CACAGGGTTT TTGCTGATTC AGGCTTGGCC TGAAAACAGG ACGGACCTCC ATGCCTTTGA GAACCTAGAA ATCATACGCG GCAGGACCAA GCAACATGGT CAGTTTTCTC TTGCAGTCGT CAGCCTGAAC ATAACATCCT TGGGATTACG CTCCCTCAAG GAGATAAGTG ATGGAGATGT GATAATTTCA GGAAACAAAA ATTTGTGCTA TGCAAATACA ATAACTGGA AAAAAGTGT TGGGACCTCC GGTCAGAAAA CAAAATTAT AAGCAACAGA

GGTGAAAACA GCTGCAAGGC CACAGGCCAG GTCTGCCATG CCTTGTGCTC
CCCCGAGGGC TGCTGGGGCC CGGAGCCCAG GGA CTGCGTC TCTTGCCGA
ATGTCAGCCG AGGCAGGGAA TCGTGGACA AGTGCAACCT TCTGGAGGGT
GAGCCAAGGG AGTTTGTGGA GAACTCTGAG TGCATACAGT GCCACCCAGA
GTGCCTGCCT CAGGCCATGA ACATCACCTG CACAGGACGG GGACCAGACA
ACTGTATCCA GTGTGCCCAC TACATTGACG GCCCCCACTG CGTCAAGACC
TGCCCGGCAG GAGTCATGGG AGAAAACAAC ACCCTGGTCT GGAAGTACGC
AGACGCCGGC CATGTGTGCC ACCTGTGCCA TCCAAACTGC ACCTACGGAT
GCACTGGGCC AGGTCTTGAA GGCTGTCCAA CGAATGGGCC TAAGATCCCC
TCCATCGCCA CTGGGATGGT GGGGGCCCTC CTCTTGCTGC TGGTGGTGGC
CCTGGGGATC GGCCTCTTCA TGGGCGGCGG AGAGGGCAGA GGAAGTCTTC
TAACATGCGG TGACGTGGAG GAGAATCCCG GCCCTAGGAT GCAGATCCCT
CAGGCCCCTT GGCCTGTCGT GTGGGCTGTG CTGCAGCTGG GATGGCGGCC
TGGCTGGTTT CTGGACAGCC CCGACAGACC CTGGAACCCC CCTACATTTT
CCCCTGCCCT GCTGGTCGTG ACCGAGGGCG ACAATGCCAC CTTACCTGT
AGCTTCAGCA ACACCAGCGA GAGCTTCAGA CTGGTGTGGC ATAGAGAAAG
CCCCGGCTAC GAGACCGACA CTCTGGCCAG CTTCCCCGAG GATAGATCTA
CCCCCCTGCC TGA CTGCCGG TTCAGAGTGA CCCAGCTGCC CAACGGCCGG
GACTTCCACA TGTCTGTCGT GCGGGCCAGA CGGAACGACA GCGGCACATA
TGTTTGCGGC GCCATCGCCT TCCACCCCGT GATTCAGATC AAAGAGAGCC
TGAGAGCCGA GCTGAGAGTG ACCGAGAGAA GGGCCGAAGT GCCTACCGCC
CACCCTAGCC CATCTCCAAG ACCTGCCGGC CAGTTCCAGA CACTCGTGGG
CGGAGGATGC GACCTGCCTC AGACACACAG CCTGGGCAGC AGACGGACCC
TGATGCTGCT GGCCAGATG CGGAAGATCA GCCTGTTCAG CTGCCTGAAG
GACCGGCACG ACTTCGGCTT CCCTCAGGAA GAGTTCGGCA ACCAGTTTCA
GAAGGCCGAG ACAATCCCCG TGCTGCACGA GATGATCCAG CAGATCTTCA
ACCTGTTCTC CACCAAGGAC AGCAGCGCCG CCTGGGACGA GAACTGCTG
GACAAGTTCT ACACCGAGCT GTACCAGCAG CTGAATGACC TGGAAGCCTG
CGTGATCCAG GCGTGGGCG TGACAGAGAC ACCCCTGATG AAGGAAGATA
GCATCCTGGC CGTGCGCAAG TACTTCCAGC GGATCACCTT GTACCTGAAA
GAGAAGAAGT ACAGCCCCTG CGCCTGGGAG GTCGTGCGCG CCGAGATCAT
GAGAAGCTTC AGCCTGAGCA CCAACCTGCA GGAAAGCCTG CGGAGCAAAG
AATAA; SEQ ID NO: 17).

Binding affinity of PD1:IFN α 2a variants

[0175] PD1:IFN α 2a variants were evaluated for their binding affinity in comparison to the wild-type PD1:IFN α 2a. $1e^6$ PDL1-expressing U293T cells were harvested from culture and washed with PBS. Equal amounts of PD1:IFN α 2a, PD1(A99L):IFN α 2a, PD1(G14-1):IFN α 2a, or PD1(HAC-1):IFN α 2a as determined by Bioplex assay of culture supernatants were then added to the U293T cells for 20 minutes. Following incubation, cells were washed twice in PBS and then stained with PE-conjugated anti-IFN α antibody for 20 minutes. Cells were then washed twice in PBS and re-suspended in FACS fixative solution. Fixed cells were then run on the LSRFortessa for FACS (Fluorescence activated cell sorting) analysis. Median fluorescence intensity differences between PD1:IFN α 2a and variants demonstrate a higher level of PDL1 binding for some of the PD1 mutants. As shown in **Figure 13**, PD1:IFN α 2a variants display higher binding affinity to PDL1 than wild-type PD1:IFN α 2a.

[0176] In the alternatives herein, the PD1(HAC-1):IFN α 2a is encoded by the nucleic acid sequence set forth in SEQ ID NO: 16 (ATGCTTC TCCTGGTGAC AAGCCTTCTG CTCTGTGAGT TACCACACCC AGCATTCCTC CTGATCCCAC GCAAAGTGTG TAACGGAATA GGTATTGGTG AATTAAAGA CTCACTCTCC ATAAATGCTA CGAATATTAA ACACTTCAAA AACTGCACCT CCATCAGTGG CGATCTCCAC ATCCTGCCGG TGGCATTAG GGGTGACTCC TTCACACATA CTCCTCCTCT GGATCCACAG GAACTGGATA TTCTGAAAAC CGTAAAGGAA ATCACAGGGT TTTTGCTGAT TCAGGCTTGG CCTGAAAACA GGACGGACCT CCATGCCTTT GAGAACCTAG AAATCATACG CGGCAGGACC AAGCAACATG GTCAGTTTTT TCTTGCAGTC GTCAGCCTGA ACATAACATC CTTGGGATTA CGCTCCCTCA AGGAGATAAG TGATGGAGAT GTGATAATTT CAGGAAACAA AAATTTGTGC TATGCAAATA CAATAAACTG GAAAAAACTG TTTGGGACCT CCGGTCAGAA AACCAAAATT ATAAGCAACA GAGGTGAAAA CAGCTGCAAG GCCACAGGCC AGGTCTGCCA TGCCTTGTGC TCCCCGAGG GCTGCTGGGG CCCGGAGCCC AGGGACTGCG TCTCTTGCCG GAATGTCAGC CGAGGCAGGG AATGCGTGGA CAAGTGCAAC CTTCTGGAGG GTGAGCCAAG GGAGTTTGTG GAGAACTCTG AGTGCATACA GTGCCACCCA GAGTGCCTGC CTCAGGCCAT GAACATCACC TGCACAGGAC GGGGACCAGA CAACTGTATC CAGTGTGCCC ACTACATTGA CGGCCCCCAC TCGTCAAGA CCTGCCCGGC AGGAGTCATG

GGAGAAAACA ACACCCTGGT CTGGAAGTAC GCAGACGCCG GCCATGTGTG
 CCACCTGTGC CATCCAAACT GCACCTACGG ATGCACTGGG CCAGGTCTTG
 AAGGCTGTCC AACGAATGGG CCTAAGATCC CGTCCATCGC CACTGGGATG
 GTGGGGGCCC TCCTCTTGCT GCTGGTGGTG GCCCTGGGGA TCGGCCTCTT
 CATGGGCGGC GGAGAGGGCA GAGGAAGTCT TCTAACATGC GGTGACGTGG
 AGGAGAATCC CGGCCCTAGG ATGCAGATCC CTCAGGCCCC TTGGCCTGTC
 GTGTGGGCTG TGCTGCAGCT GGGATGGCGG CCTGGCTGGT TTCTGGACAG
 CCCCACAGA CCCTGGAACC CCCCTACATT TTCCCCTGCC CTGCTGGTCG
 TGACCGAGGG CGACAATGCC ACCTTCACCT GTAGCTTCAG CAACACCAGC
 GAGAGCTTCC ATGTTATTTG GCATAGAGAA AGCCCCAGCG GTCAGACCGA
 CACTCTGGCC GCCTTCCCCG AGGATAGATC TCAGCCCGGC CAGGACTGCC
 GGTCAGAGT GACCCAGCTG CCCAACGGCC GGGACTTCCA CATGTCTGTC
 GTGCGGGCCA GACGGAACGA CAGCGGCACA TATGTTTGCG GCGTTATCAG
 CCTGGCCCCC AAGATTCAGA TCAAAGAGAG CCTGAGAGCC GAGCTGAGAG
 TGACCGAGAG AAGGGCCGAA GTGCCTACCG CCCACCCTAG CCCATCTCCA
 AGACCTGCCG GCCAGTTCCA GACACTCGTG GGCGGAGGAT GCGACCTGCC
 TCAGACACAC AGCCTGGGCA GCAGACGGAC CCTGATGCTG CTGGCCCAGA
 TGCGGAAGAT CAGCCTGTTC AGCTGCCTGA AGGACCGGCA CGACTTCGGC
 TTCCCTCAGG AAGAGTTCGG CAACCAGTTT CAGAAGGCCG AGACAATCCC
 CGTGCTGCAC GAGATGATCC AGCAGATCTT CAACCTGTTC TCCACCAAGG
 ACAGCAGCGC CGCCTGGGAC GAGACACTGC TGGACAAGTT CTACACCGAG
 CTGTACCAGC AGCTGAATGA CCTGGAAGCC TCGTGATCC AGGGCGTGGG
 CGTGACAGAG ACACCCCTGA TGAAGGAAGA TAGCATCCTG GCCGTGCGCA
 AGTACTTCCA GCGGATCACC CTGTACCTGA AAGAGAAGAA GTACAGCCCC
 TGCGCCTGGG AGGTCGTGCG CGCCGAGATC ATGAGAAGCT TCAGCCTGAG
 CACCAACCTG CAGGAAAGCC TGCGGAGCAA AGAATAA; SEQ ID NO: 16).

More alternatives

[0177] In some alternatives, a nucleic acid encoding a fusion protein is provided. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes an amino acid spacer, such as a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second

sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGGCCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated

promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPin, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In

some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G

QDCRFRTVQLPNGRDFHMSVVRARRNDSTGYVCGVISLA
PKIQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the fusion protein stimulates the

production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0178] In some alternatives, a vector is provided. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes an amino acid spacer such as a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1

(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGCTGCGCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGCGGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACCTGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1).

In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence

comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCTACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCACCTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTCAGCCCCGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGTGCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGCCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A

A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
 T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
 R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
 PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
 W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
 G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
 S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
 I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
 Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
 alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids
 (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any
 two of the aforementioned numbers. In some alternatives, the amino acid spacer
 comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
 sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
 wherein the 2A linker is between the fusion protein and a protein for co-expression. In
 some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some
 alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker
 and comprises a nucleic acid sequence set forth in SEQ ID NO: 10
 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG
 AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a
 protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth
 sequence encoding an IRES sequence. In some alternatives, the nucleic acid further
 comprises a seventh sequence encoding a marker. In some alternatives, the marker is
 Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth
 sequence, wherein the eighth sequence encodes a signal for protein secretion. In some

alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGTAAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine

is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0179] In some alternatives, a cell for fusion protein secretion is provided. The cell can comprise a nucleic acid of any one of the alternatives described herein or the expression vector of any one of the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes an amino acid spacer such as a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1

(TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGCTGCGCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGCGGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACCTGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGA

AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In
 some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ
 ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
 L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
 A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
 T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
 R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of

PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF

signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGTAAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell. In some alternatives, the cell is a bacterial cell, such as *E. coli*. In some alternatives, the cell is an insect cell for protein expression. In some alternatives, the cell is CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell or NK cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the cell further comprises a chimeric antigen receptor. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid

tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0180] In some alternatives, method of making a chimeric antigen receptor bearing cell is provided. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer, (e.g., glycine spacer), and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1

(TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA

GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCTGTACCTGAAAGAGAAGAAGTACAGCCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In
 some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ
 ID NO: 3
 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT

ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
 L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
 A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
 T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
 R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
 PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
 W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
 G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
 S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
 I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
 Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
 alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids,
 such as glycines, or a number of amino acids, such as glycines, within a range defined by
 any two of the aforementioned numbers. In some alternatives, the amino acid spacer
 comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
 sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,

wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattctcctgatccca; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a

length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all or a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines.

[0181] In some alternatives, a composition is provided. In some alternatives, the composition comprises the cells of anyone of the alternatives described herein or the cells manufactured by the methods of anyone of the alternatives described herein. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein

the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes an amino acid spacer such as a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some

alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPins, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ

ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In

some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and

comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is

encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the chimeric antigen receptor is specific for CD19. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid

tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0182] In some alternatives, a fusion protein is provided. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives described herein or the vector of anyone of the alternatives described herein. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes an amino acid spacer such as a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ

ID	NO:	1
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(TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
 TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
 CTTGCGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
 ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
 GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA

CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In
 some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ
 ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC

AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
 CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
 L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
 A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
 T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
 R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
 PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
 W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
 G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
 S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
 I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
 Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
 alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids
 (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any
 two of the aforementioned numbers. In some alternatives, the amino acid spacer
 comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
 sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
 wherein the 2A linker is between the fusion protein and a protein for co-expression. In
 some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some
 alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker

and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or a DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1,

B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0183] In some alternatives, a method of manufacturing a fusion protein for treatment is provided, wherein the method comprises delivering the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein to a bacterial cell, mammalian cell or insect cell, growing the cell up in a culture, inducing expression of the fusion protein and purifying the fusion protein for treatment. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes an amino acid spacer such as a glycine spacer and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG

TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In
 some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ
 ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG

AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids
(e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any
two of the aforementioned numbers. In some alternatives, the amino acid spacer
comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
(GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
wherein the 2A linker is between the fusion protein and a protein for co-expression. In
some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some

alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, inducing comprises adding IPTG, anhydrotetracycline, L-arabinose or rhamnose to the culture. In some alternatives, the cell is *E. coli*. In some alternatives, the cell is an insect-cell. In some alternatives, the fusion protein can be scFv based and be used to target

cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0184] In some alternatives, a method of secreting the fusion protein of anyone of the alternatives, is provided, wherein the method comprises delivering to a subject the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein. In some alternatives, the composition comprises the cells of anyone of the alternatives described herein or the cells manufactured by the methods of anyone of the alternatives described herein. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID

NO: 1

(TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA

ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
 GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In
 some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ
 ID NO: 3
 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG

GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids
(e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any
two of the aforementioned numbers. In some alternatives, the amino acid spacer
comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
(GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some

alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the

chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives the chimeric antigen receptor is specific for CD19. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. In some alternatives, the method further comprises administering to the subject an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a

larger role in tumor progression. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0185] In some alternatives, a method of increasing T-cell activity is provided, wherein the method comprises administering an effective amount of the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to a subject in need. In some alternatives, the composition comprises the cells of anyone of the alternatives described herein or the cells manufactured by the methods of anyone of the alternatives described herein. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is specific for CD19. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGGCCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA

CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
 ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
 GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
 CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
 TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
 CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
 TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the
 nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a
 promoter. In some alternatives, the promoter is a mammalian promoter for mammalian
 cell protein expression or a bacterial promoter for bacterial cell protein expression. In
 some alternatives, the promoter is an inducible promoter or a constitutive promoter. In
 some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence
 comprises a nucleic acid sequence set forth in SEQ ID NO: 2
 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
 AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
 AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
 TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
 CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
 alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
 promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
 is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some
 alternatives, the promoter is regulated by a drug. In some alternatives, the drug is
 tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a
 CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated
 promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific
 binding domains, antigen specific scFvs, an extracellular domain, DARPins, affinity
 peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific
 extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix
 protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen.
 In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In
 some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ
 ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC AGCCCCGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8

(GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group

consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives described herein or the vector of anyone of the alternatives described herein. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ

ID	NO:	1
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(TGCGACCTGCCTCAGACACAGCCTGGGCAGCAGACGGACCCTGATGCTGCTGCTGCGCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCGTGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC

TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPINs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA

CTTCCACATGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA
 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSG
 TYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP
 RPAGQFQTLV; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
 PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
 W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
 G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
 S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
 I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
 Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
 alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids
 (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any
 two of the aforementioned numbers. In some alternatives, the amino acid spacer
 comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
 sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
 wherein the 2A linker is between the fusion protein and a protein for co-expression. In
 some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some
 alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker
 and comprises a nucleic acid sequence set forth in SEQ ID NO: 10

(GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some

alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, α GD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0186] In some alternatives, a method of decreasing immunosuppression in a tumor microenvironment comprising administering an effective amount of the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to a subject in need. In some alternatives, the chimeric antigen receptor is specific for CD19. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an

amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1

(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1).

In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2
(GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
AGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGA
AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is

tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPINs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA
CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3).

In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V

WAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNA
 TFTCSFSNTSESFHVVWHRESPSGQTDTLAAFPEDRSQPG
 QDCRFRVTQLPNGRDFHMSVVRARRNDSGTYVCGVISLA
 PKIQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV;

SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattctcctgatccca; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13

(ATGCCCCCTCCCAGACTTCTCTTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives described herein or the vector of anyone of the alternatives described herein. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence,

wherein the second sequence encodes a spacer, such as an amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ

ID NO: 1
(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
CTTCAGCGGATCACCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the

nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2
(GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC
AGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGA
AGGTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT
TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT
CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some
alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA
promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter
is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some

alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPins, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGA CTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT GCGGCGCCATCAGCCTGGCCCCCAAGGCCCAGATCAAAGAGAGCCTGAGAGC CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and

comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V; SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids (e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any two of the aforementioned numbers. In some alternatives, the amino acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression. In some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence

comprises a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCCAGACTTCTCTTTTCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In

some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

[0187] In some alternatives, a method of treating patient having a cancer expressing a tumor antigen is provided. The method can comprise administering an effective amount of the cell of anyone of the alternatives described herein or the composition of anyone of the alternatives described herein, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of anyone of the alternatives described herein to the patient. In some alternatives, the chimeric antigen receptor is specific for CD19. The method can comprise delivering to a cell, the nucleic acid of anyone of the alternatives described herein or the expression vector of anyone of the alternatives described herein and delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. The first sequence, second sequence and third sequence can be in any order in the nucleic acid. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC

TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
 TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCGCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG
 GATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCT
 ACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCAC
 CTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATG
 AGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTC
 AGCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCAACGGCCGGGA

CTTCCACATGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT
 GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
 CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
 CCATCTCCAAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3). In
 some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
 QIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA
 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSG
 TYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSP
 RPAGQFQTLV; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
 PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
 W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
 G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
 S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
 I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
 Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
 comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
 W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
 T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
 Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
 P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
 SEQ ID NO: 6). In some alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7,
 8, 9 or 10 amino acids (e.g., glycines) or a number of amino acids (e.g., glycines) within a
 range defined by any two of the aforementioned numbers. In some alternatives, the amino
 acid spacer comprises at least 3 glycines. In some alternatives, the amino acid spacer
 comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
 (GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
 alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
 wherein the 2A linker is between the fusion protein and a protein for co-expression. In
 some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some
 alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker
 and comprises a nucleic acid sequence set forth in SEQ ID NO: 10
 (GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG

AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain. In some alternatives, the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group

consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof. In some alternatives, the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR. In some alternatives, the cell is a CD8⁺ or a CD4⁺ cell. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells. In some alternatives, the cell is a precursor T-cell. In some alternatives, the cell is a stem cell. In some alternatives, the cell is a hematopoietic stem cell. In some alternatives, the cell is a B cell. In some alternatives, the cell is a neuronal stem cell. The fusion protein can be encoded by the nucleic acid of anyone of the alternatives described herein or the vector of anyone of the alternatives described herein. The vector can comprise the nucleic acid of anyone or more the alternatives described herein. The nucleic acid can comprise a first sequence, wherein the first sequence encodes a protein that modulates an immune response, a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer (e.g., a plurality of glycines) and a third sequence, wherein the third sequence encodes an interferon. In some alternatives, a 3' terminus of the first sequence is adjacent to a 5' terminus of the second sequence and a 3' terminus of the second sequence is adjacent to a 5' terminus of the third sequence. In some alternatives, the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω . In some alternatives, the interferon is IFN α 2a. In some alternatives, the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO:

1

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(TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGATGCTGC
TGGCCCAGATGCGGAAGATCAGCCTGTTCAGCTGCCTGAAGGACCGGCACGA
CTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCAGAAGGCCGAGACA
ATCCCCGTGCTGCACGAGATGATCCAGCAGATCTTCAACCTGTTCTCCACCAA
GGACAGCAGCGCCGCCTGGGACGAGACACTGCTGGACAAGTTCTACACCGAG
CTGTACCAGCAGCTGAATGACCTGGAAGCCTGCGTGATCCAGGGCGTGGGCG
TGACAGAGACACCCCTGATGAAGGAAGATAGCATCCTGGCCGTGCGCAAGTA
CTTCCAGCGGATCACCCCTGTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCC
TGGGAGGTCGTGCGCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACC
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TGCAGGAAAGCCTGCGGAGCAAAGAA; SEQ ID NO: 1). In some alternatives, the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter. In some alternatives, the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression. In some alternatives, the promoter is an inducible promoter or a constitutive promoter. In some alternatives, the promoter is EF1P. In some alternatives, the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2 (GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTG; SEQ ID NO: 2). In some alternatives, the promoter is a T7 promoter, lac promoter, trc promoter, tac promoter, tetA promoter, araBAD promoter or a rhaPBAD promoter. In some alternatives, the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose. In some alternatives, the promoter is regulated by a drug. In some alternatives, the drug is tamoxifen. In some alternatives, the promoter is a regulatable promoter, regulated by a CAR-dependent signal. In some alternatives, the promoter is an NFAT regulated promoter system. In some alternatives, the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPs, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein. In some alternatives, the extracellular protein is fibronectin, laminin or collagen. In some alternatives, PD-1 is a wild type PD-1 or a mutated or truncated form of PD-1. In some alternatives, the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3

(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGGGATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGGAACCCCCCTACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACAATGCCACCTTCACCTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCGACAAGCTGGCCGCCTTCCCCGAGGATAGATCTCAGCCCCGGCCAGGACTGCCGGTTCAGAGTGACCCAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTGTCGTGCGGGCCAGACGGAACGACAGCGGCACATATCTGT

GCGGCGCCATCAGCCTGGCCCCCAAGGCCAGATCAAAGAGAGCCTGAGAGC
CGAGCTGAGAGTGACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGC
CCATCTCCAAGACCTGCCGGCCAGTTCAGACACTCGTG; SEQ ID NO: 3). In
some alternatives, PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M
Q I P Q A P W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A
L L V V T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G
T Y L C G A I S L A P K A Q I K E S L R A E L R V T E R R A E V P T A H P S P S P
R P A G Q F Q T L V; SEQ ID NO: 4). In some alternatives, PD-1 is a mutated form of
PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (M Q I P Q A P
W P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E
G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A A F P E D R
S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y L C G A
I S L A P K L Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F
Q T L V; SEQ ID NO: 5). In some alternatives, PD-1 is a mutated form of PD-1 and
comprises an amino acid sequence set forth in SEQ ID NO: 6 (M Q I P Q A P W P V V
W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V T E G D N A
T F T C S F S N T S E S F H V V W H R E S P S G Q T D T L A A F P E D R S Q P G
Q D C R F R V T Q L P N G R D F H M S V V R A R R N D S G T Y V C G V I S L A
P K I Q I K E S L R A E L R V T E R R A E V P T A H P S P S P R P A G Q F Q T L V;
SEQ ID NO: 6). In some alternatives, the nucleic acid is a DNA or an RNA. In some
alternatives, the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids
(e.g., glycines) or a number of amino acids (e.g., glycines) within a range defined by any
two of the aforementioned numbers. In some alternatives, the amino acid spacer
comprises at least 3 glycines. In some alternatives, the amino acid spacer comprises a
sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8
(GGGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9). In some
alternatives, the nucleic acid further comprises a fifth sequence encoding a 2A linker,
wherein the 2A linker is between the fusion protein and a protein for co-expression. In
some alternatives, the 2A linker is a T2A linker, a P2A linker or an E2A linker. In some
alternatives, the fifth sequence comprises a nucleic acid sequence encoding a T2A linker
and comprises a nucleic acid sequence set forth in SEQ ID NO: 10
(GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAG

AATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding a protein for co-expression. In some alternatives, the nucleic acid further comprises a sixth sequence encoding an IRES sequence. In some alternatives, the nucleic acid further comprises a seventh sequence encoding a marker. In some alternatives, the marker is Her2tG or EGFRt. In some alternatives, the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion. In some alternatives, PD-1 further comprises a signaling domain for secretion. In some alternatives, the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence. In some alternatives, the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11 (ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATT CCTCCTGATCCCA; SEQ ID NO: 11). In some alternatives, the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12 (ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAGCTGG GATGGCGG; SEQ ID NO: 12). In some alternatives, the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (ATGCCCCCTCCAGACTTCTCTTTTCCTCCTCTTCCTGACGCCGATGGAAGT AAGG; SEQ ID NO: 13). In some alternatives, the vector is RNA or DNA. In some alternatives, the vector is a viral vector. In some alternatives, the vector is for expression of a protein in a bacterial, mammalian or insect system. Vectors include but are not limited to plasmid, minicircles, yeast, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, or viral genomes. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the vector is for protein expression in a bacterial system such as *E. coli*. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors. In some alternatives, the vector is for protein expression in a bacterial system, such as *E. coli*. In some alternatives, the administering is performed by delivering the composition to a tumor site. In some alternatives, the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers. In some alternatives, the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9

mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers. In some alternatives, the administering is performed 1, 2 or 3 times a day. In some alternatives, the fusion protein can be scFv based and be used to target cytokines. Without being limiting, the cytokines for targeting can be IL4, IL6 or IL10. Targeting these cytokines would help to limit the immunosuppressive effects of the cytokines. Secretion of the PD-1:IFN α 2a fusion protein in CAR T-cells can be used to support T-cell activity, promote inflammatory cytokine production and decrease immunosuppression within the solid tumor microenvironment. The use of PD1-IFN α 2a will have most use against solid tumors that express targets such as EGFRvIII, Her2, L1CAM, oaGD2, GD2, ROR1, B7H3, IL13Ra2 and/or EphA2, for example, where the tumor microenvironment plays a larger role in tumor progression. In some alternatives, the fusion protein stimulates the production of stimulates the production of MIP-1a. In some alternatives herein, the fusion protein stimulates the production of MIP-1b. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL9. In some alternatives, the fusion protein comprises PD1. In some alternatives, the fusion protein comprises IFN α 2a. In some alternatives, the fusion protein stimulates the production of a cytokine. In some alternatives, the cytokine is CXCL10.

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a fusion protein, the nucleic acid comprising:
 - a first sequence, wherein the first sequence encodes a protein that modulates an immune response;
 - a second sequence, wherein the second sequence encodes a spacer, such as an amino acid spacer e.g., a plurality of glycines; and
 - a third sequence, wherein the third sequence encodes an interferon.
2. The nucleic acid of Claim 1, wherein the interferon is IFN- α , IFN α 2a, IFN- β , IFN- ϵ , IFN- κ or IFN- ω .
3. The nucleic acid of Claim 1 or 2, wherein the interferon is IFN α 2a.
4. The nucleic acid of anyone of Claims 1-3, wherein the third sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 1
 (TGCGACCTGCCTCAGACACACAGCCTGGGCAGCAGACGGACCCTGAT
 GCTGCTGGCCCAGATGCGGAAGATCAGCCTGTTTCAGCTGCCTGAAGGA
 CCGGCACGACTTCGGCTTCCCTCAGGAAGAGTTCGGCAACCAGTTTCA
 GAAGGCCGAGACAATCCCCGTGCTGCACGAGATGATCCAGCAGATCTT
 CAACCTGTTCTCCACCAAGGACAGCAGCGCCGCCTGGGACGAGACACT
 GCTGGACAAGTTCTACACCGAGCTGTACCAGCAGCTGAATGACCTGGA
 AGCCTGCGTGATCCAGGGCGTGGGCGTGACAGAGACACCCCTGATGAA
 GGAAGATAGCATCCTGGCCGTGCGCAAGTACTTCCAGCGGATCACCCCT
 GTACCTGAAAGAGAAGAAGTACAGCCCCTGCGCCTGGGAGGTCGTGC
 GCGCCGAGATCATGAGAAGCTTCAGCCTGAGCACCAACCTGCAGGAA
 AGCCTGCGGAGCAAAGAA; SEQ ID NO: 1).
5. The nucleic acid of anyone of Claims 1-4, wherein the nucleic acid further comprises a fourth sequence, wherein the fourth sequence encodes a promoter.
6. The nucleic acid of Claim 5, wherein the promoter is a mammalian promoter for mammalian cell protein expression or a bacterial promoter for bacterial cell protein expression.

7. The nucleic acid of Claim 5 or 6, wherein the promoter is an inducible promoter or a constitutive promoter.

8. The nucleic acid of anyone of Claims 5-7, wherein the promoter is EF1P.

9. The nucleic acid of anyone of Claims 5-8, wherein the fourth sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 2

(GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCG
CCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGG
TGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGT
ACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTG
CAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGAAC
ACAGCTG; SEQ ID NO: 2).

10. The nucleic acid of anyone of Claims 5-7, wherein the promoter is a T7 promoter, *lac* promoter, *trc* promoter, *tac* promoter, *tetA* promoter, *araBAD* promoter or a *rhaP_{BAD}* promoter.

11. The nucleic acid of Claim 10, wherein the promoter is inducible by IPTG, anhydrotetracycline, L-arabinose or rhamnose.

12. The nucleic acid of anyone of Claims 5-7, wherein the promoter is regulated by a drug.

13. The nucleic acid of Claim 12, wherein the drug is tamoxifen.

14. The nucleic acid of anyone of Claims 5-7, wherein the promoter is a regulatable promoter, regulated by a CAR-dependent signal.

15. The nucleic acid of Claim 14, wherein the promoter is an NFAT regulated promoter system.

16. The nucleic acid anyone of Claims 1-15, wherein the first sequence encodes PD-1, antigen specific binding domains, antigen specific scFvs, an extracellular domain, DARPins, affinity peptides, extracellular proteins expressed by tumor cells, peptides, tumor specific extracellular proteins, Her2, ROR1, embryonal fibronectin or an extracellular matrix protein.

17. The nucleic acid of Claim 16, wherein the extracellular protein is fibronectin, laminin or collagen.

18. The nucleic acid of anyone of Claims 16 or 17, wherein PD-1 is a wild type PD-1 or a mutated form of PD-1.

19. The nucleic acid of anyone of Claims 1-18, wherein the first sequence comprises a nucleic acid sequence set forth in SEQ ID NO: 3
(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAG
CTGGGATGGCGGCCTGGCTGGTTTCTGGACAGCCCCGACAGACCCTGG
AACCCCCCTACATTTTCCCCTGCCCTGCTGGTCGTGACCGAGGGCGACA
ATGCCACCTTCACCTGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGC
TGAAGTGGTACAGAATGAGCCCCAGCAACCAGACCGACAAGCTGGCC
GCCTTCCCCGAGGATAGATCTCAGCCCGGCCAGGACTGCCGGTTCAGA
GTGACCCAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTGTCGTGCGG
GCCAGACGGAACGACAGCGGCACATATCTGTGCGGCGCCATCAGCCTG
GCCCCCAAGGCCCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGT
GACCGAGAGAAGGGCCGAAGTGCCTACCGCCCACCCTAGCCCATCTCC
AAGACCTGCCGGCCAGTTCCAGACACTCGTG; SEQ ID NO: 3).

20. The nucleic acid sequence of anyone of Claims 16-18, wherein PD-1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (M Q I P Q A P W
P V V W A V L Q L G W R P G W F L D S P D R P W N P P T F S P A L L V V
T E G D N A T F T C S F S N T S E S F V L N W Y R M S P S N Q T D K L A
A F P E D R S Q P G Q D C R F R V T Q L P N G R D F H M S V V R A R R N

DSGTYLCGAISLAPKAQIKESLRAELRV TERRAEVPT
AHPSPSPRPAGQFQTLV; SEQ ID NO: 4).

21. The nucleic acid of anyone of Claims 16-18, wherein the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 5 (MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKLQIKESLRAELRV TERRAEVPTAHPSPSPRPAGQFQTLV; SEQ ID NO: 5).

22. The nucleic acid of anyone of Claims 16-18, wherein the PD-1 is a mutated form of PD-1 and comprises an amino acid sequence set forth in SEQ ID NO: 6 (MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFHVVWHR E SPSGQTDTLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYVCGVISLAPKIQIKESLRAELRV TERRAEVPTAHPSPSPRPAGQFQTLV; SEQ ID NO: 6).

23. The nucleic acid of anyone of Claims 1-22, wherein the nucleic acid is DNA or RNA.

24. The nucleic acid of anyone of Claims 1-23, wherein the amino acid spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, such as glycines, or a number of amino acids, such as glycines, within a range defined by any two of the aforementioned numbers.

25. The nucleic acid of Claim 24, wherein the amino acid spacer comprises at least 3 glycines.

26. The nucleic acid of Claim 25, wherein the amino acid spacer comprises a sequence set forth in SEQ ID NO: 7 (GGGS; SEQ ID NO: 7), SEQ ID NO: 8 (GGSGGG; SEQ ID NO: 8) or SEQ ID NO: 9 (GGG; SEQ ID NO: 9).

27. The nucleic acid of anyone of Claims 1-26, wherein the nucleic acid further comprises a fifth sequence encoding a 2A linker, wherein the 2A linker is between the fusion protein and a protein for co-expression.

28. The nucleic acid of Claim 27, wherein the 2A linker is a T2A linker, a P2A linker or an E2A linker.

29. The nucleic acid of Claim 27 or 28, wherein the fifth sequence comprises a nucleic acid sequence encoding a T2A linker and comprises a nucleic acid sequence set forth in SEQ ID NO: 10
(GGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGA
GGAGAATCCCGGCCCTAGG; SEQ ID NO: 10) and further comprises a sequence encoding the protein for co-expression.

30. The nucleic acid of anyone of Claims 1-29, wherein the nucleic acid further comprises a sixth sequence encoding an IRES sequence.

31. The nucleic acid of anyone of Claims 1-30, wherein the nucleic acid further comprises a seventh sequence encoding a marker.

32. The nucleic acid of Claim 31, wherein the marker is Her2tG or EGFRt.

33. The nucleic acid of anyone of Claims 1-32, wherein the nucleic acid further comprises an eighth sequence, wherein the eighth sequence encodes a signal for protein secretion.

34. The nucleic acid of anyone of Claims 16-33, wherein PD-1 further comprises a signaling domain for secretion.

35. The nucleic acid of Claim 33 or 34, wherein the signal for protein secretion is a GMCSF signaling sequence, PD-1 signaling sequence or CD19 signaling sequence.

36. The nucleic acid of Claim 35, wherein the GMCSF signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 11
(ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCA
GCATTCTCCTGATCCCA; SEQ ID NO: 11).

37. The nucleic acid of anyone of Claims 33-35, wherein the PD-1 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12
(ATGCAGATCCCTCAGGCCCTTGGCCTGTCGTGTGGGCTGTGCTGCAG
CTGGGATGGCGG; SEQ ID NO: 12).

38. The nucleic acid of Claim 35, wherein the CD19 signaling sequence is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13
(ATGCCCCCTCCCAGACTTCTCTTTTTCCTCCTCTTCCTGACGCCGATGG
AAGTAAGG; SEQ ID NO: 13).

39. An expression vector comprising the nucleic acid of anyone or more of Claims 1-38.

40. The vector of Claim 39, wherein the vector is RNA or DNA.

41. A cell for fusion protein secretion, the cell comprising the nucleic acid of anyone of claims 1-38 or the expression vector of Claim 39 or 40.

42. The cell of Claim 41, wherein the cell is a bacterial cell or a mammalian cell, such as a lymphocyte or NK cell.

43. The cell of Claim 41 or 42, wherein the cell is a bacterial cell, such as *E. coli*.

44. The cell of Claim 41, wherein the cell is an insect cell for protein expression.

45. The cell of Claim 41 or 42, wherein the cell is CD8⁺ or a CD4⁺ cell.

46. The cell of Claim 41, 42 or 45, wherein the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells.

47. The cell of Claim 41, 42 or 45, wherein the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells.

48. The cell of Claim 41, 42 or 45, wherein the cell is a precursor T-cell.

49. The cell of Claim 41, 42 or 45, wherein the cell is a stem cell.

50. The cell of Claim 41, 42, 45 or 48, wherein the cell is a hematopoietic stem cell or NK cell.

51. The cell of Claim 41 or 42, wherein the cell is a B cell.

52. The cell of Claim 41, 42, 45 or 48, wherein the cell is a neuronal stem cell.

53. The cell of anyone of Claims 41, 42 or 45-52, wherein the cell further comprises a nucleic acid encoding a chimeric antigen receptor and a chimeric antigen receptor.

54. The cell of Claim 53, wherein the chimeric antigen receptor is specific for CD19.

55. A method of making a chimeric antigen receptor bearing cell, wherein the chimeric antigen receptor bearing cell expresses a fusion protein, the method comprising:

delivering to a cell, the nucleic acid of anyone of Claims 1-38 or the expression vector of Claim 39 or 40; and

delivering to the cell, a second nucleic acid, wherein the second nucleic acid encodes a chimeric antigen receptor.

56. The method of Claim 55, wherein the chimeric antigen receptor comprises a ligand binding domain, a polypeptide spacer of a length, wherein the length is optimized, a transmembrane domain, and a signaling domain.

57. The method of Claim 56, wherein the polypeptide spacer is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in length or a length within a range defined by any two of the aforementioned lengths.

58. The method of anyone of Claims 56-57, wherein the signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof.

59. The method of anyone of Claims 56-58, wherein the ligand binding domain is specific for tumor expressed protein, PDL1 or IFNAR.

60. The method of anyone of Claims 55-59, wherein the cell is a CD8⁺ or a CD4⁺ cell.

61. The method of anyone of Claims 55-60, wherein the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, central memory CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells.

62. The method of anyone of Claims 56-60, wherein the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, central memory CD4⁺ T-cells, effector memory CD4⁺ T-cells, and bulk CD4⁺ T-cells.

63. The method of anyone of Claims 55-60, wherein the cell is a precursor T-cell.

64. The method of anyone of Claims 55-60, wherein the cell is a stem cell.
65. The method of anyone of Claims 55-60 or 64, wherein the cell is a hematopoietic stem cell.
66. The method of anyone of Claims 55-60, wherein the cell is a B cell.
67. The method of anyone of Claims 55-60 or 64, wherein the cell is a neuronal stem cell.
68. The method of anyone of Claims 55-67, wherein the chimeric antigen receptor is specific for CD19.
69. A composition comprising the cells of anyone of claims 41-54 or the cells manufactured by the methods of anyone of Claims 55-68.
70. A fusion protein encoded by the nucleic acid of anyone of Claims 1-38 or the vector of Claim 39 or 40.
71. A method of manufacturing a fusion protein, the method comprising:
- delivering the nucleic acid of anyone of Claims 1-38 or the expression vector of Claim 39 or 40 to a bacterial cell, mammalian cell or insect cell;
 - growing the cell up in a culture;
 - inducing expression of the fusion protein; and
 - purifying the fusion protein.
72. The method of Claim 71, wherein the inducing comprises adding IPTG, anhydrotetracycline, L-arabinose or rhamnose to the culture.
73. The method of anyone of Claims 71-72, wherein the cell is *E. coli*.
74. The method of anyone of Claims 71-72, wherein the cell is an insect-cell.

75. A method of secreting the fusion protein of Claim 70, the method comprising
delivering to a subject the cell of anyone of Claims 41, 42 or 45-54 or the composition of Claim 69.

76. The method of Claim 75, further comprising administering to the subject an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof.

77. The method of Claim 76, wherein the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers.

78. The method of Claim 76 or 77, wherein the administering is performed 1, 2 or 3 times a day.

79. A method of increasing T-cell activity comprising administering an effective amount of the cell of Claims 41, 42 or 45-54 or the composition of Claim 69, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of Claim 70 to a subject in need.

80. A method of decreasing immunosuppression in a tumor microenvironment comprising administering an effective amount of the cell of anyone of Claims 41, 42 or 45-54 or the composition of Claim 69, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of Claim 70 to a subject in need.

81. A method of inhibiting, ameliorating, or treating cancer in a subject such as a human, that expresses a tumor antigen comprising administering an effective amount of the cell of anyone of Claims 41, 42 or 45-54 or the composition of

Claim 69, wherein the cells of the composition express a chimeric antigen receptor that comprises an antigen binding domain that binds to a tumor antigen expressed on a cancer cell, or the fusion protein of Claim 70 to the subject.

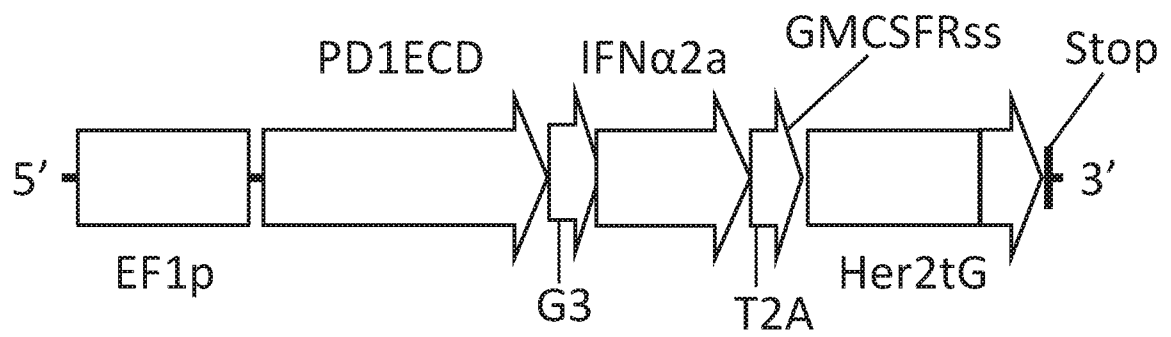
82. The method of anyone of Claims 79-81, wherein the administering is performed by delivering the composition to a tumor site.

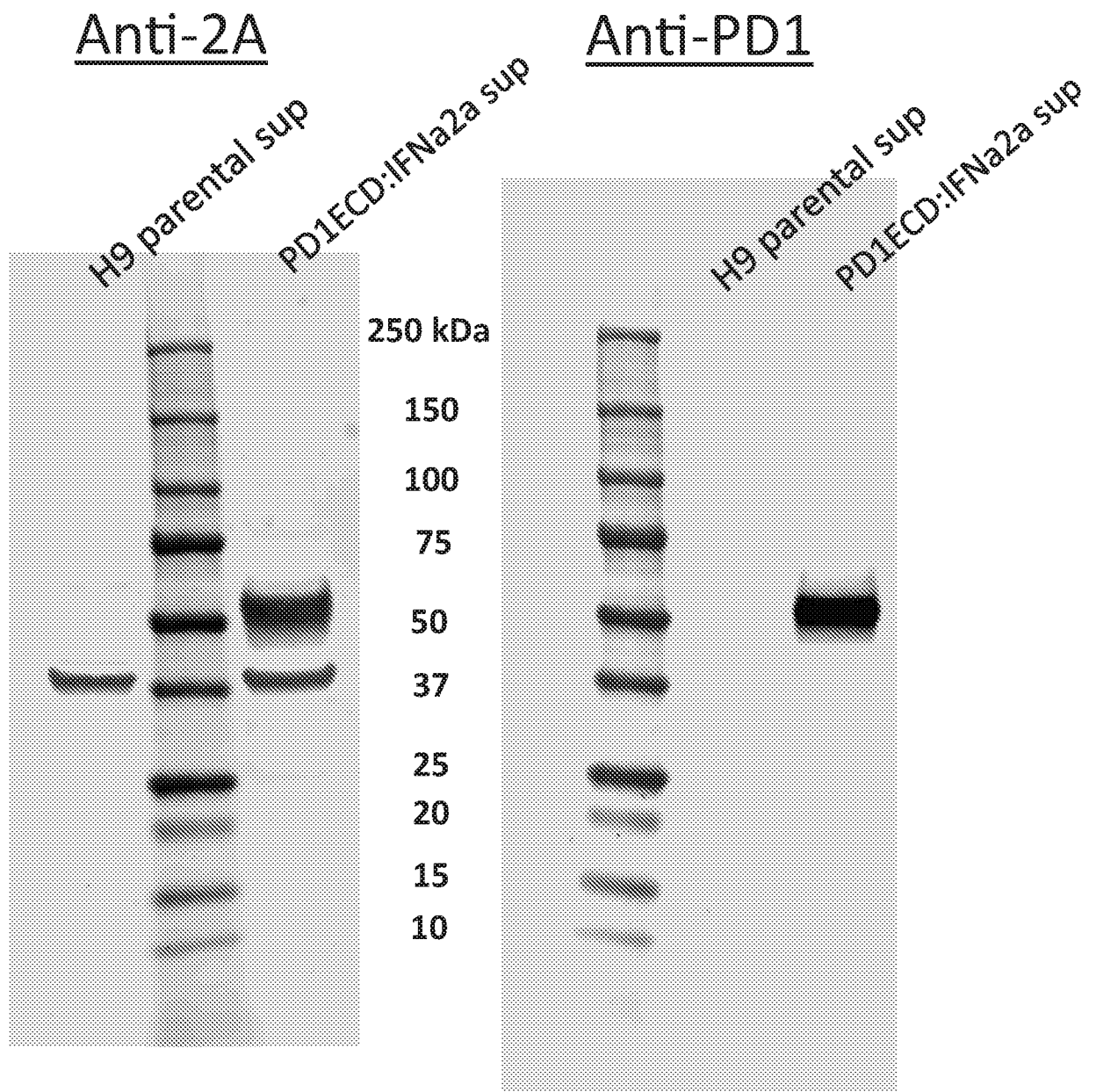
83. The method of anyone of Claims 79-82, wherein the method further comprises monitoring the cells of the composition by detection of Her2tG or EGFRt markers.

84. The method of anyone of Claims 79-83, wherein the method further comprises administering an effective amount of tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof.

85. The method of Claim 84, wherein the effective amount is 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg or 20 mg or any amount within a range defined by any two of the aforementioned numbers.

86. The method of Claim 84 or 85, wherein the administering is performed 1, 2 or 3 times a day.

*FIG. 1*

*FIG. 2*

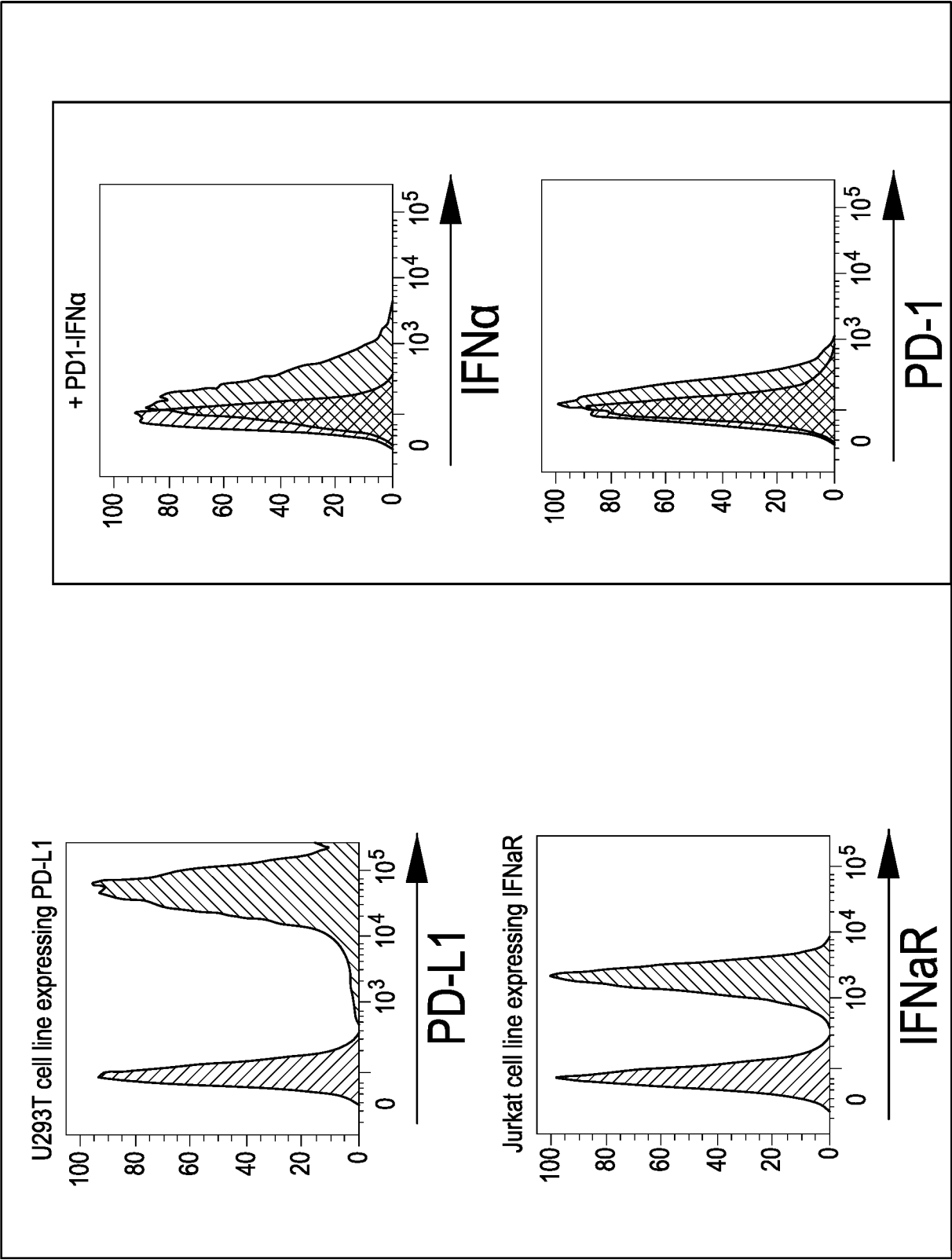


FIG. 3A

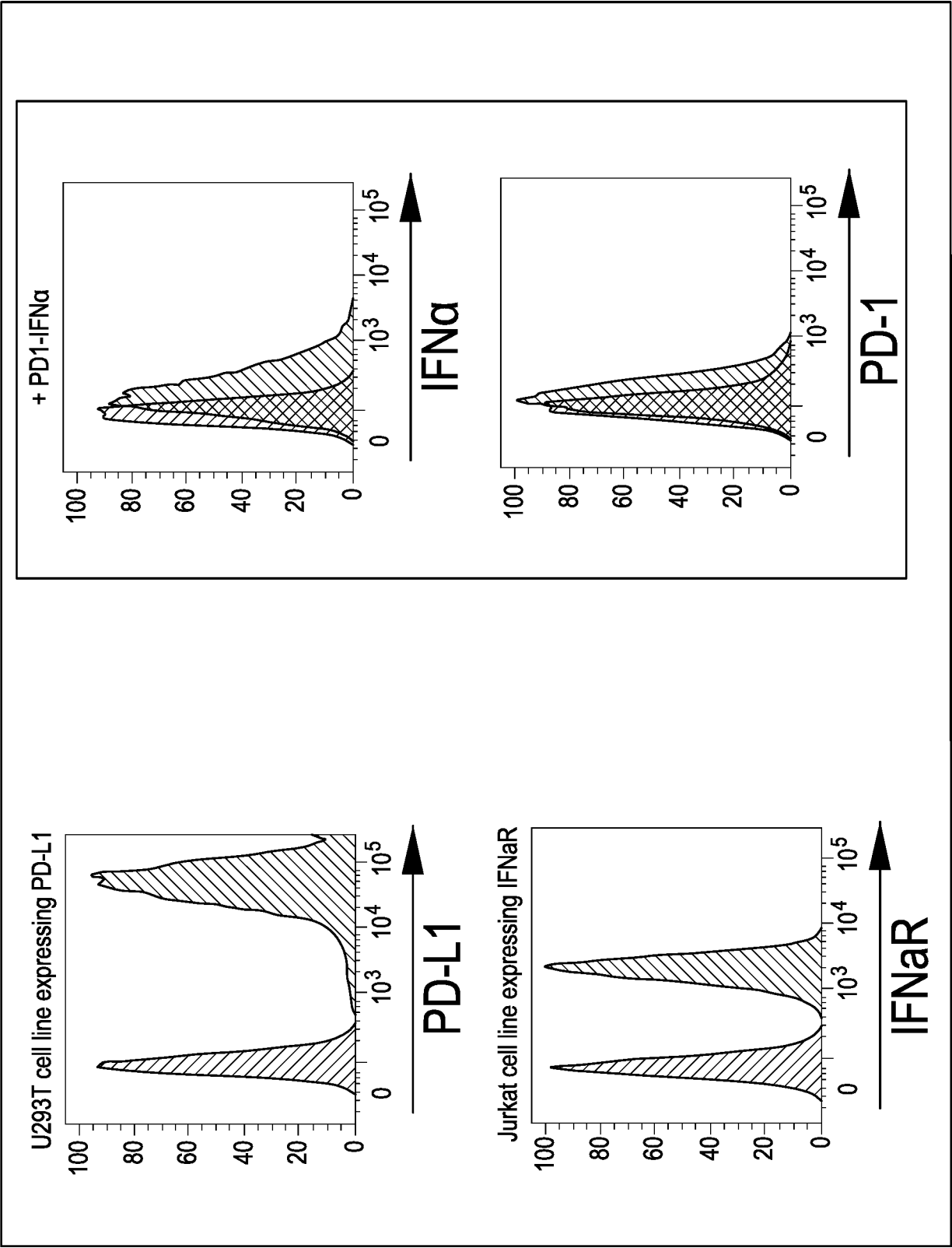
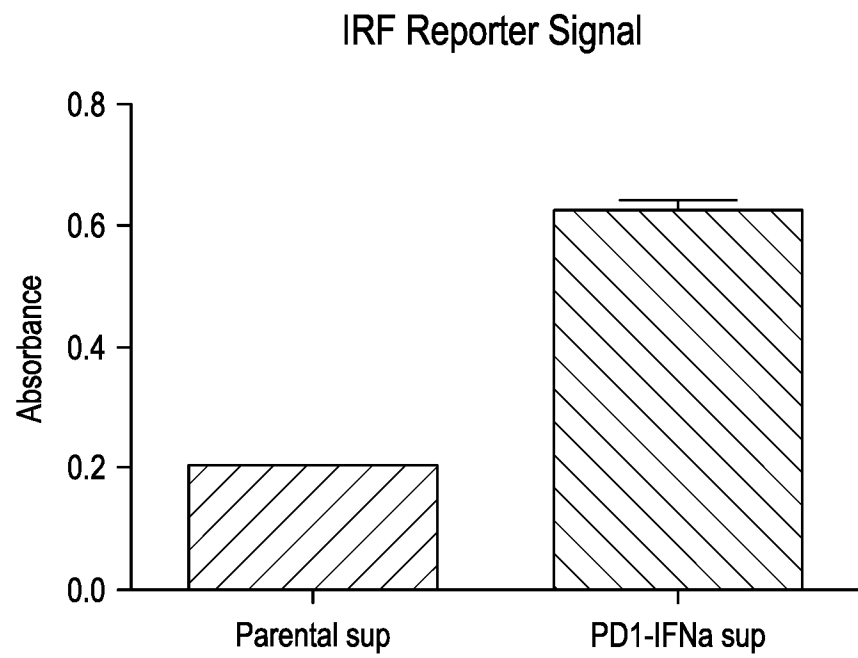
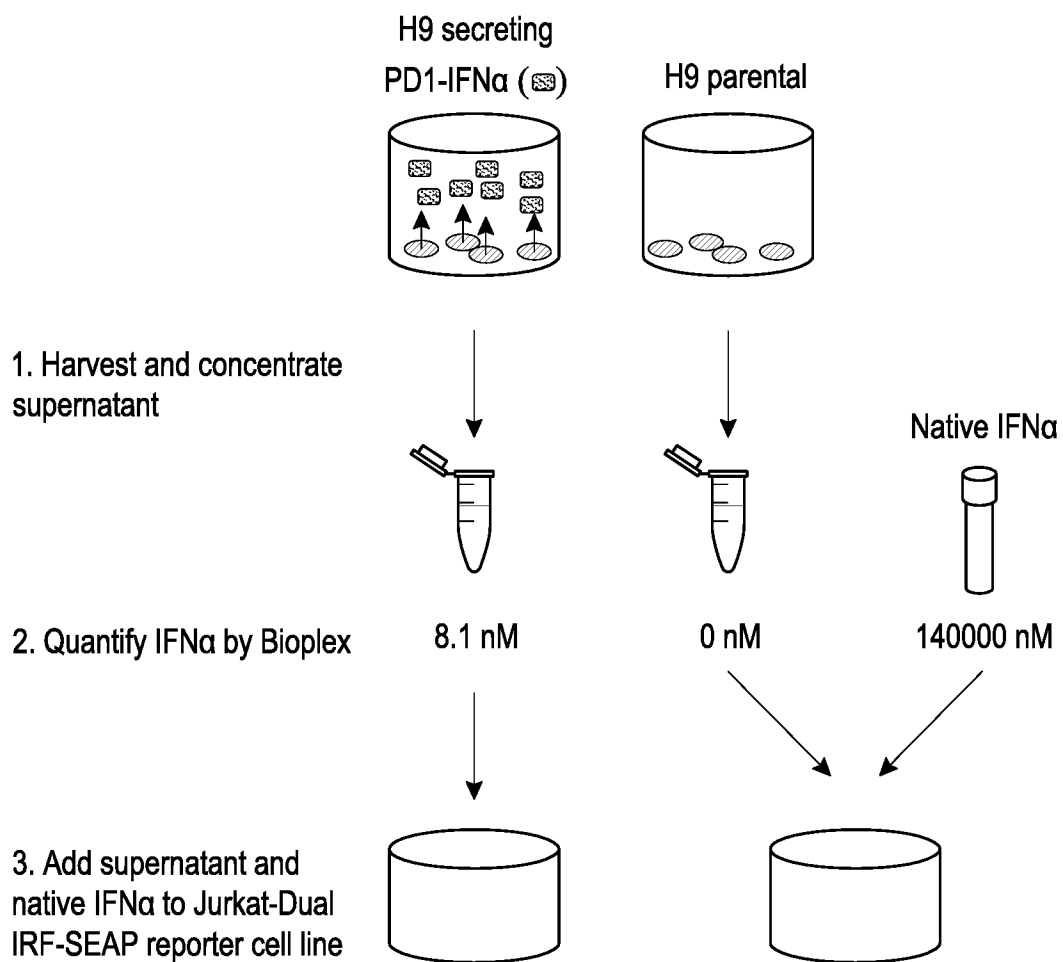
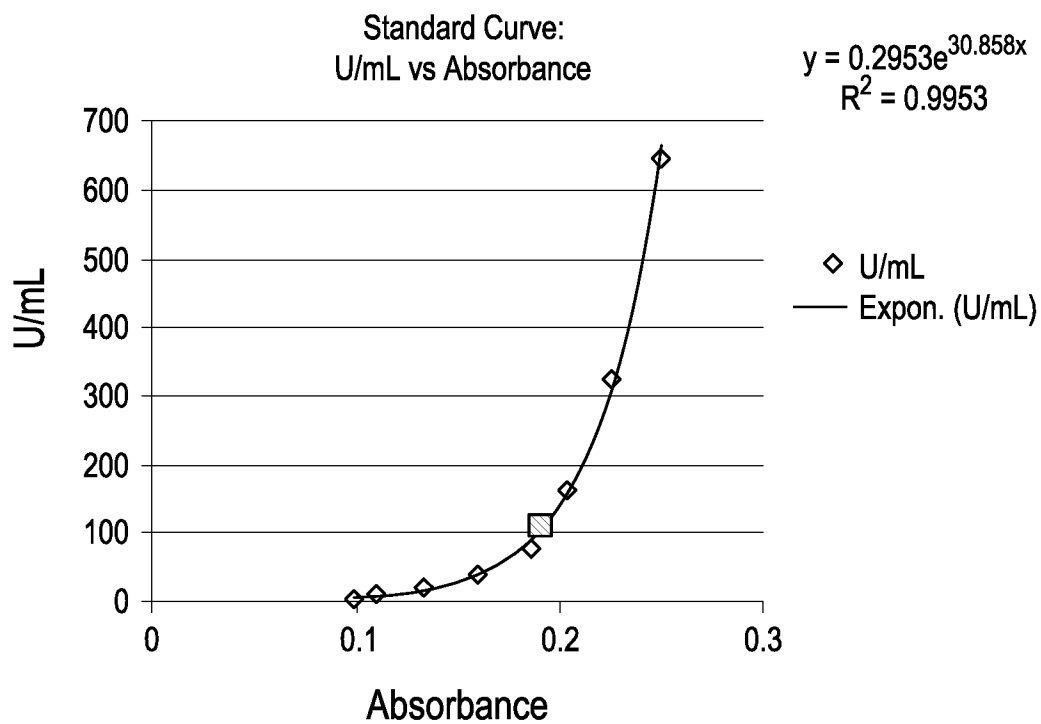


FIG. 3B

**FIG. 4**

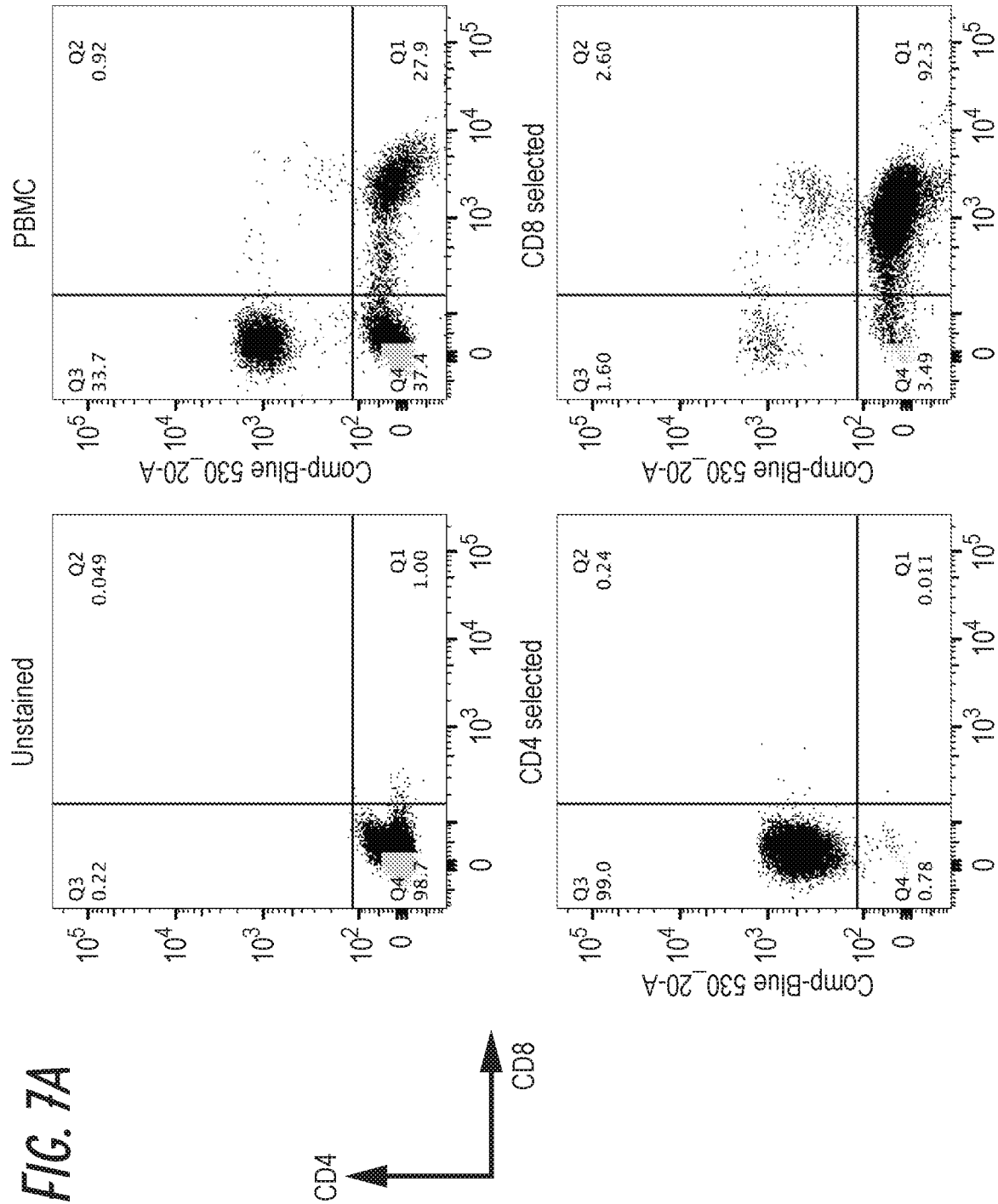
**FIG. 5**

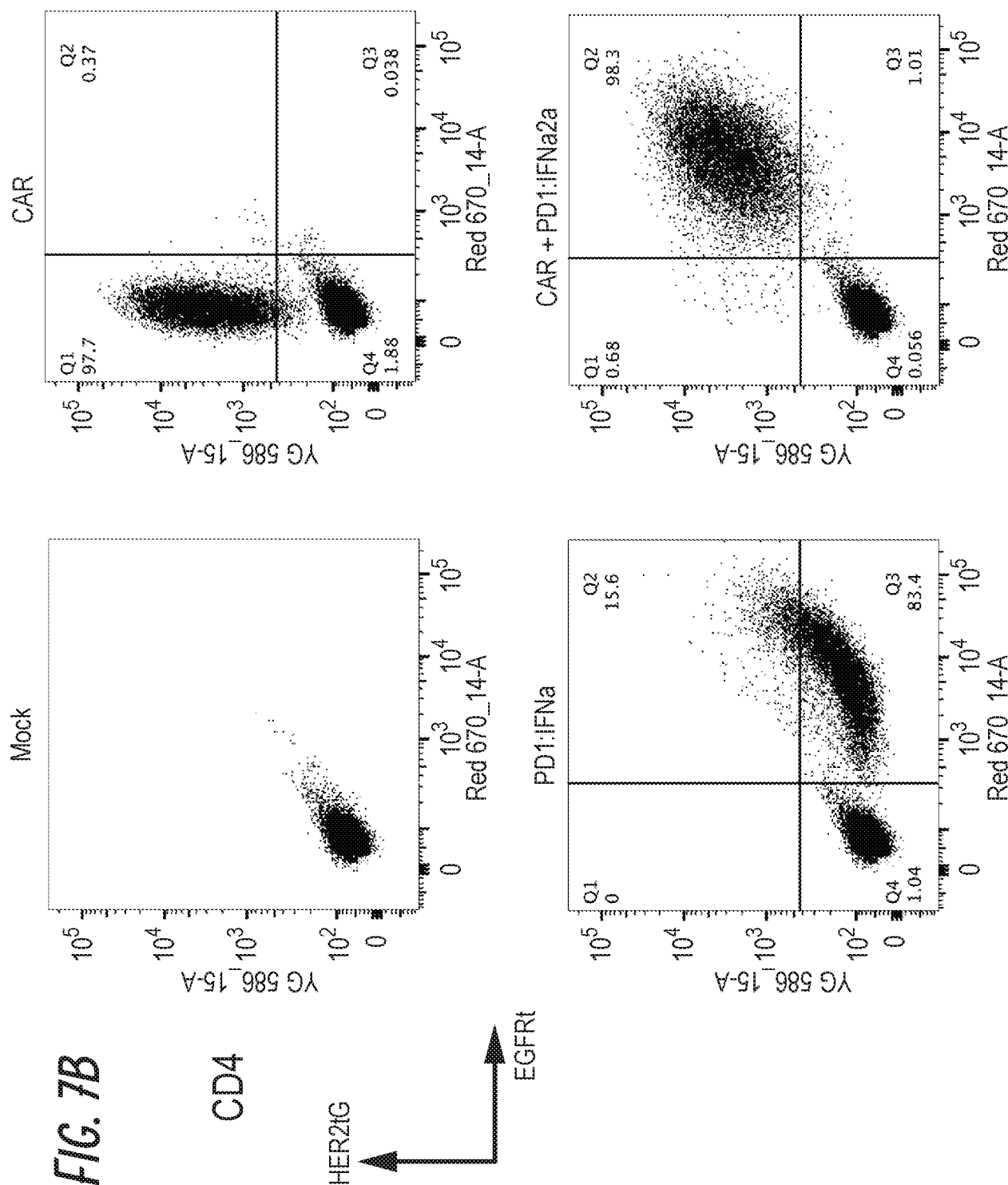
**FIG. 6A**

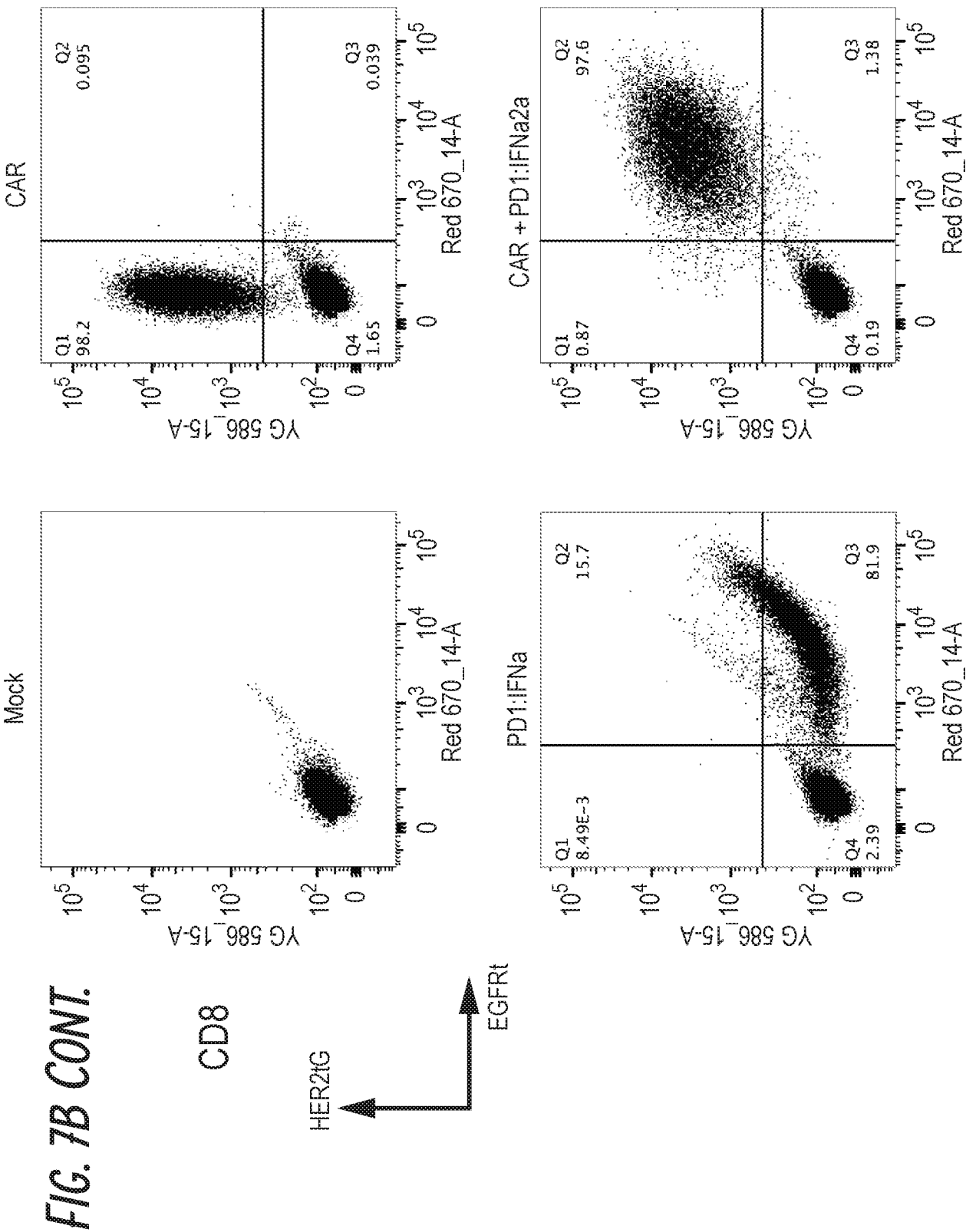
	Native IFN α	PD1-IFN α
pmol/mL	1.40E+05	8.1
U/mL	6.48E+07	1921
U/pmol	463	327

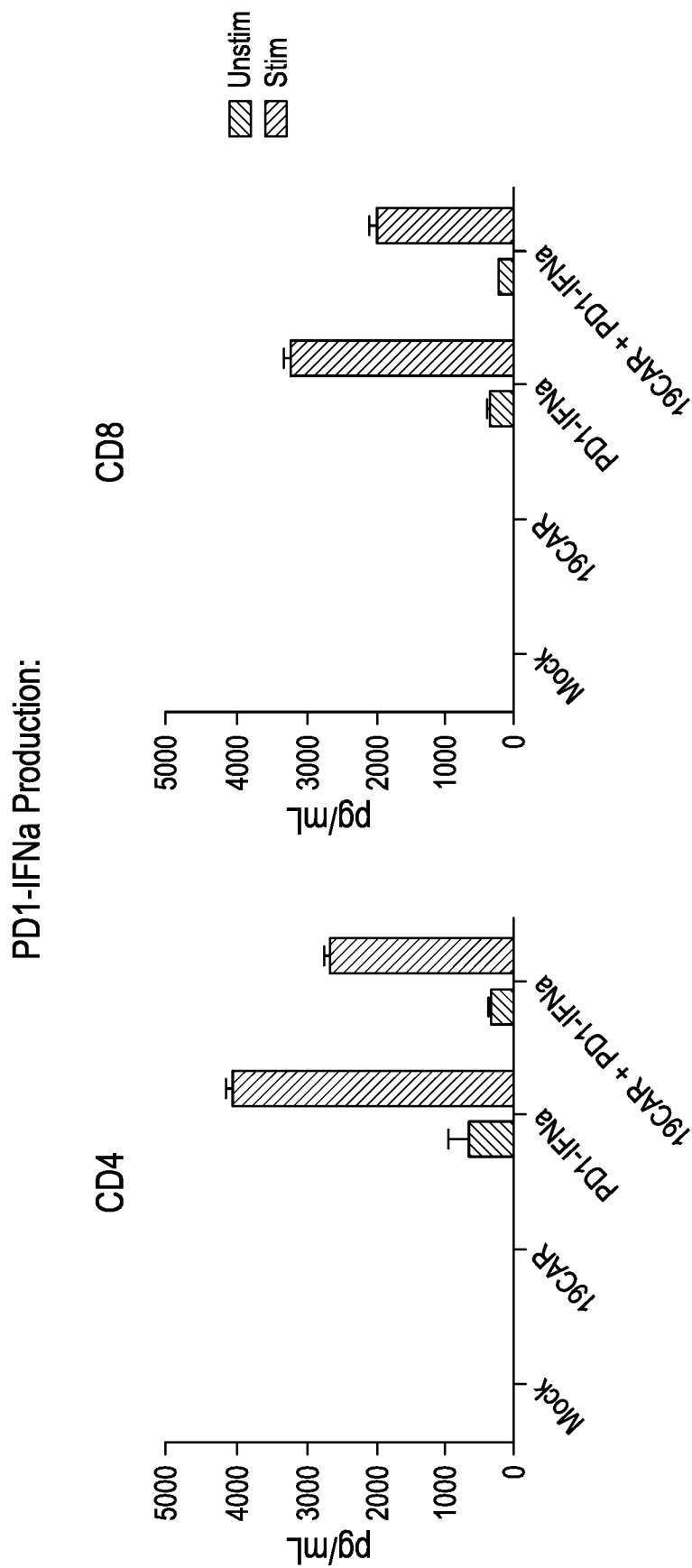
Result: PD1-IFN α is about 50%
as potent as native IFN α

FIG. 6B



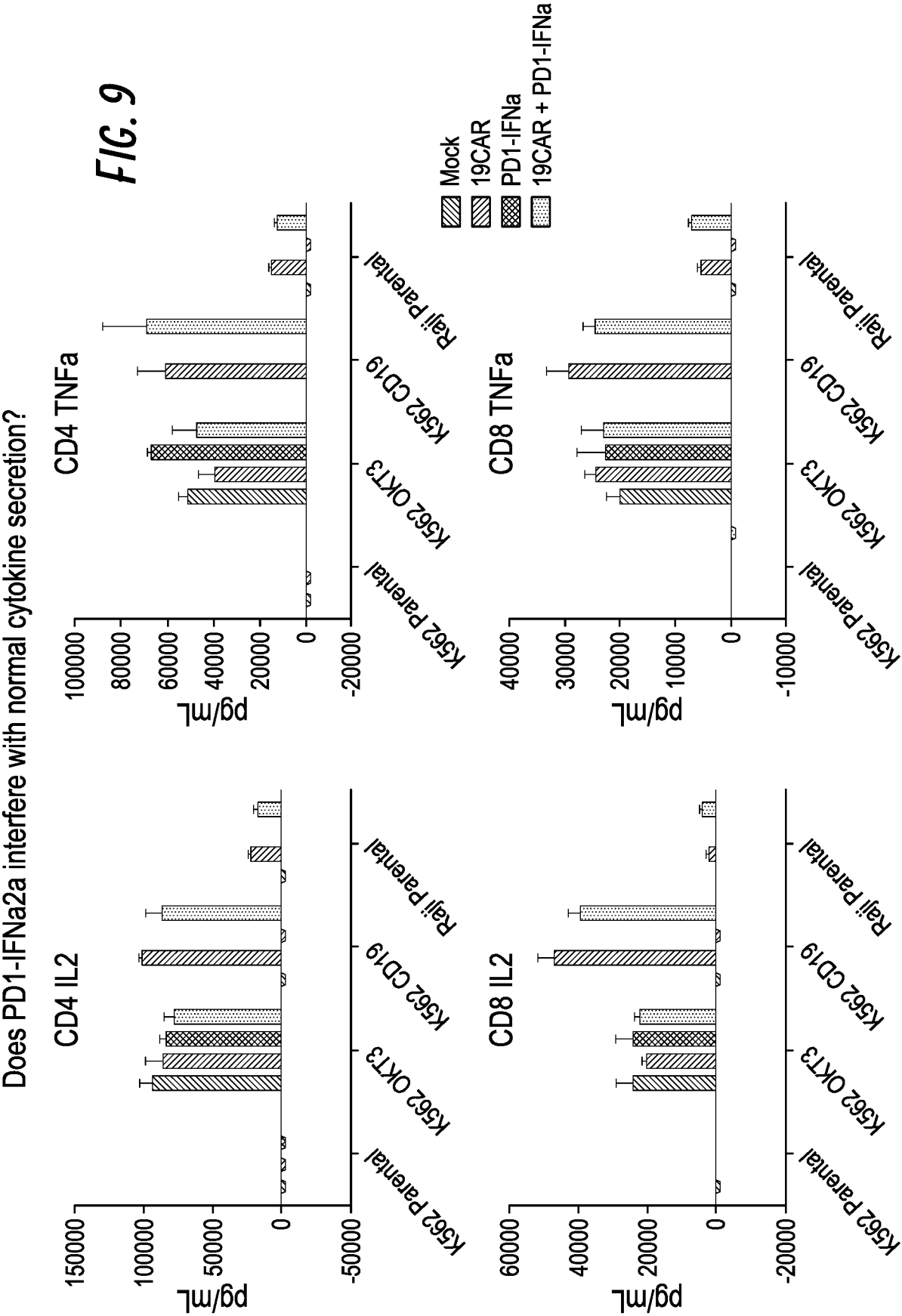


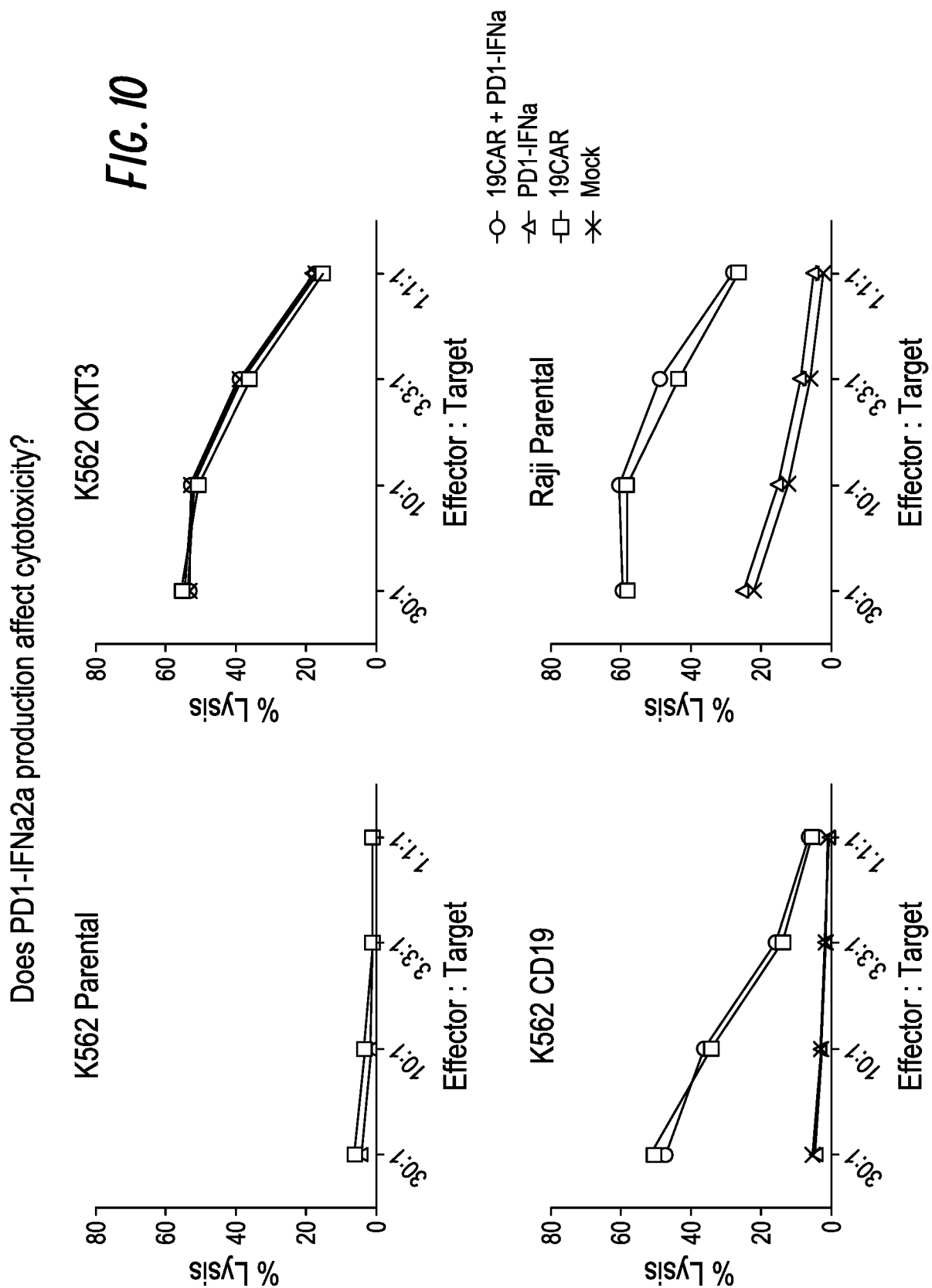




S₁R₁D₁₄, 1e6 cells/mL, 24 hrs
Stimmed with 1:1 bead:cell

FIG. 8





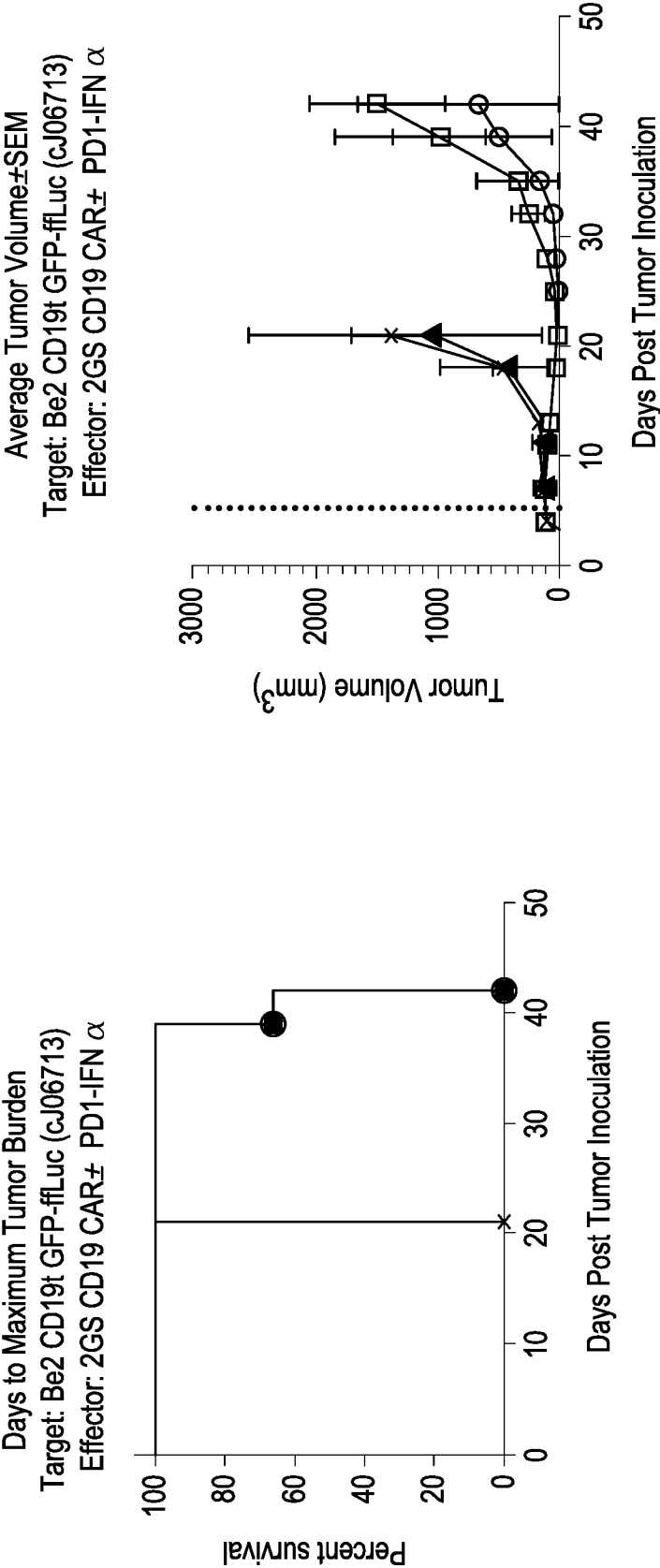


FIG. 11

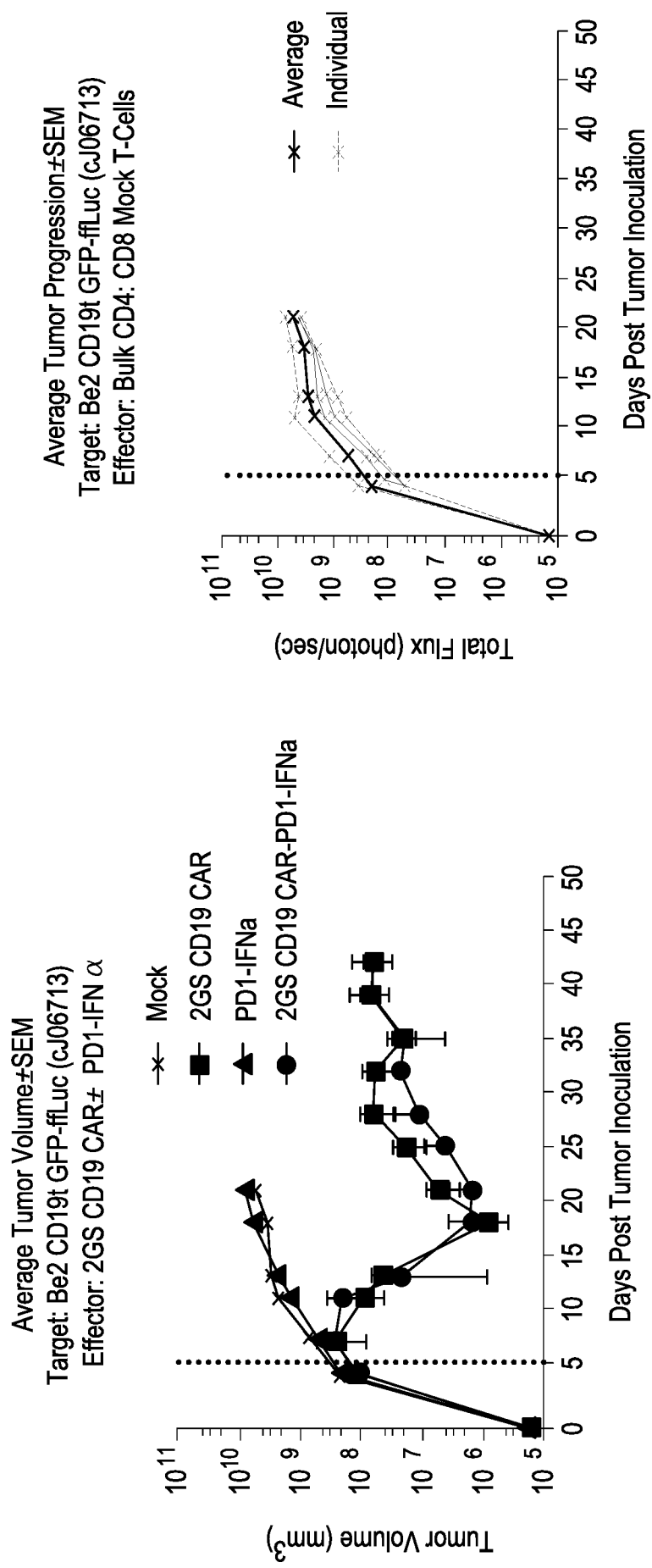


FIG. 11 CONT.

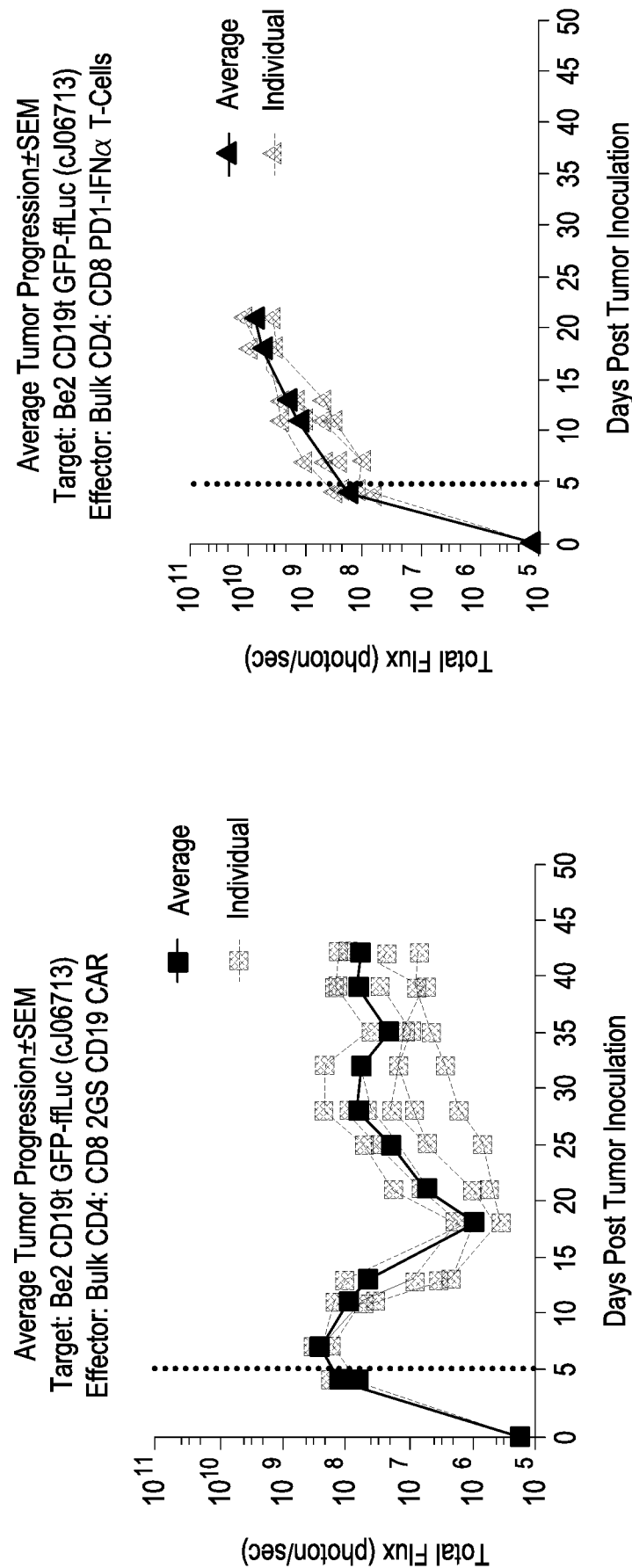


FIG. 11 CONT.

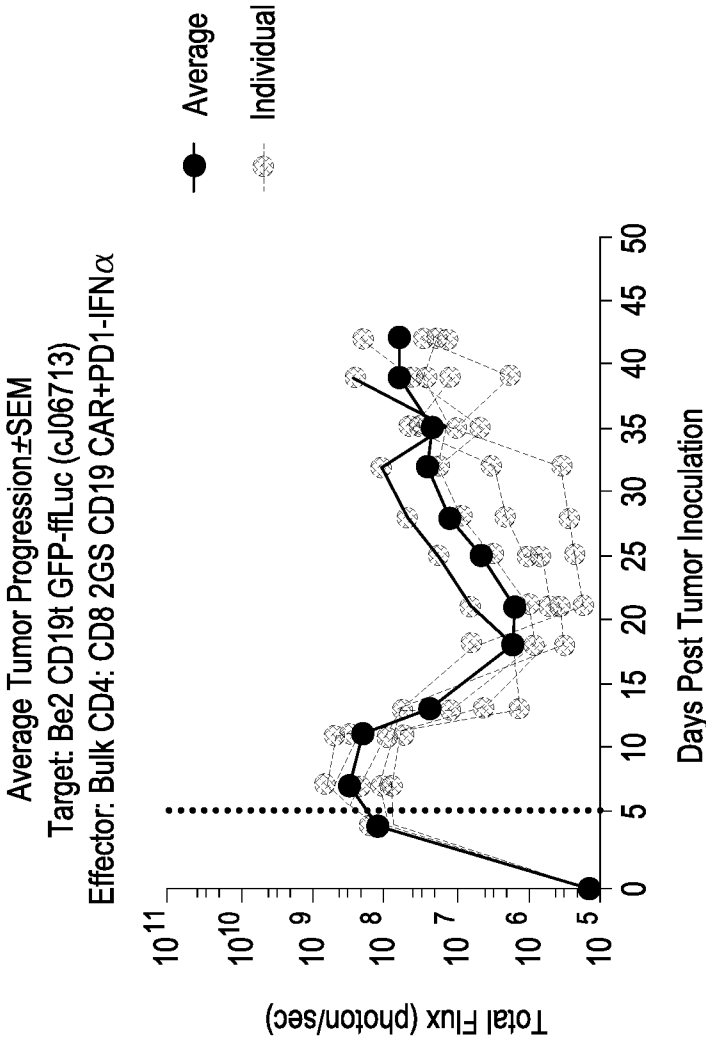
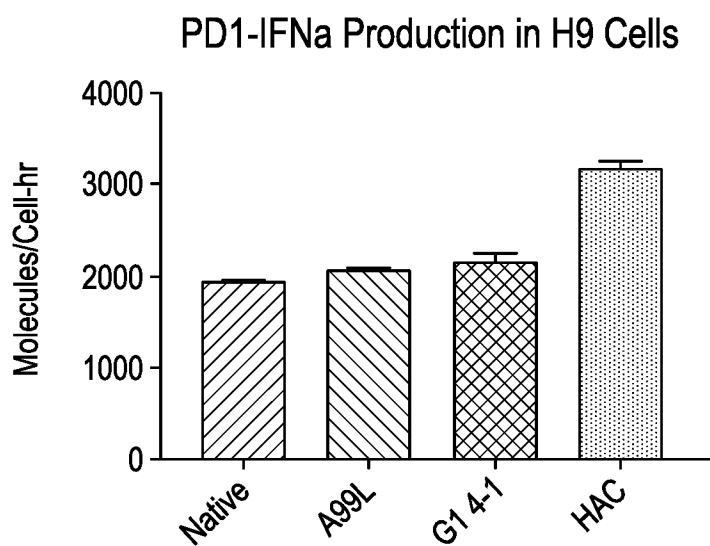


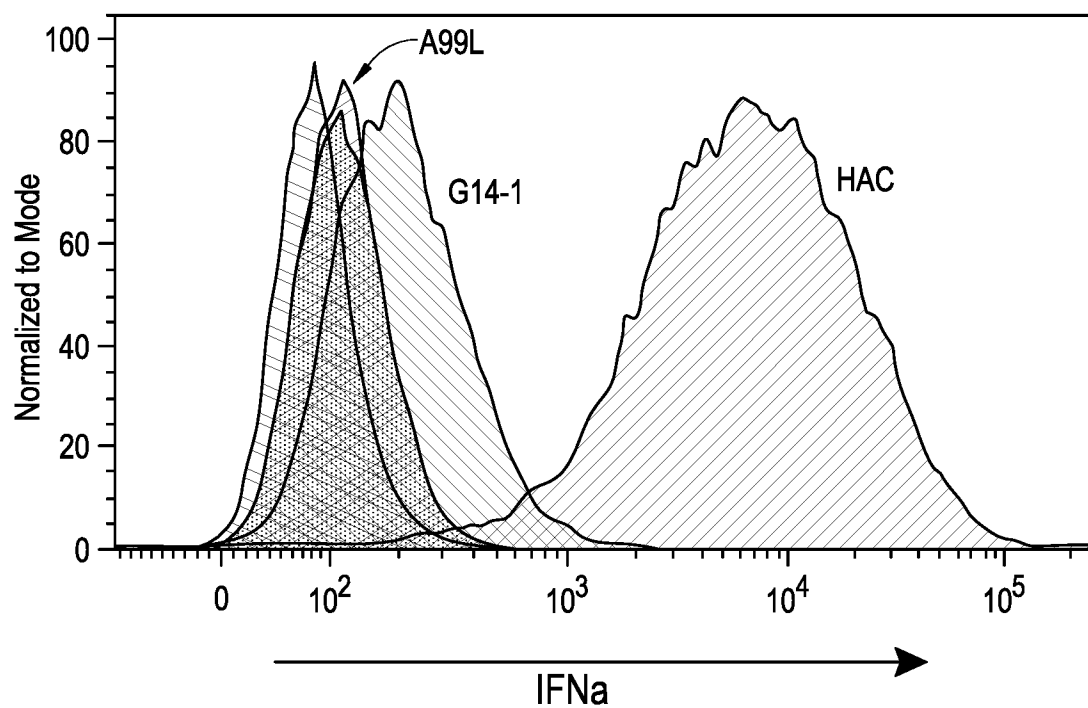
FIG. 11 CONT.

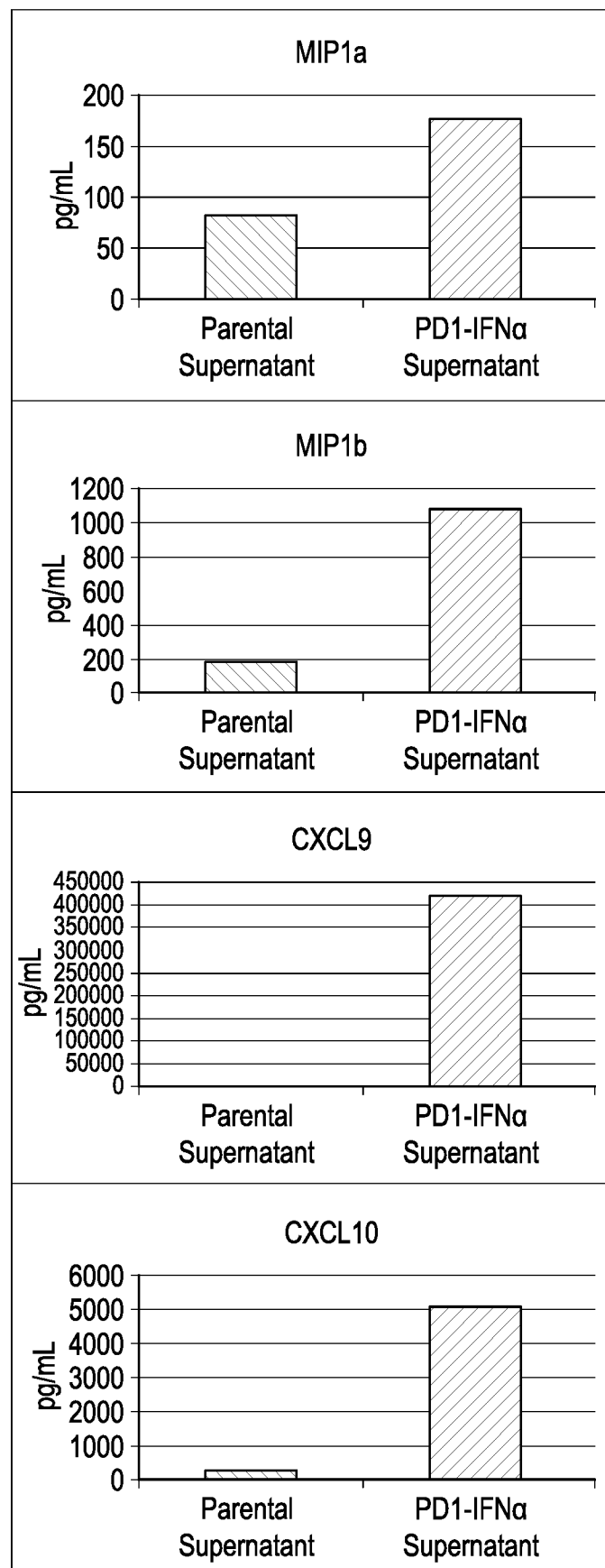
Versions produced:

Variant	K_D (pM)
Native	3,880,000
A99L	1,290,000
G14-1	7,780
HAC	107

**FIG. 12**

	Variant	K_D (pM)
	Native	3,880,000
	A99L	1,290,000
	G14-1	7,780
	HAC	107

**FIG. 13**

**FIG. 14**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/012858

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/21; C07K 14/555; C07K 14/56; C12N 15/62 (2017.01)

CPC - A61K 38/21; A61K 38/212; C07K 14/555; C07K 14/56; C12N 15/62 (2017.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/85.4; 424/85.7; 424/185.1; 424/192.1; 435/69.51; 530/351; 530/391.9 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XUAN et al. "Targeted delivery of interferon-alpha via fusion to anti-CD20 results in potent antitumor activity against B-cell lymphoma," Blood, 04 February 2010 (04.02.2010), Vol. 115, No. 14, Pgs. 2864-2871. entire document	1, 3
—		—
Y	US 2011/0008283 A1 (ARTYMIUK et al) 13 January 2011 (13.01.2011) entire document	2
Y	US 2010/0172868 A1 (MORRISON et al) 08 July 2010 (08.07.2010) entire document	2
A	US 2010/0172868 A1 (MORRISON et al) 08 July 2010 (08.07.2010) entire document	1-3
A	WO 2004/074486 A2 (MERCK PATENT GMBH et al) 02 September 2004 (02.09.2004) entire document	1-3
A	US 7,888,475 B2 (PADGETT et al) 15 February 2011 (15.02.2011) entire document	1-3
A	US 2003/0017550 A1 (PANG) 23 January 2003 (23.01.2003) entire document	1-3

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

08 March 2017

Date of mailing of the international search report

07 APR 2017

Name and mailing address of the ISA/US

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Blaine R. Copenhaver

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/012858

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-86
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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