(54) Title: CHIMERIC ANTIGEN RECEPTORS (CAR) WITH INTRINSICALLY DISORDERED REGIONS AND METHODS OF USE THEREOF

(57) Abstract: Compositions and methods for improved Adoptive Cell Therapy (ACT) are provided. Compositions of CAR-T cells including CAR modified by fusion with one or more copies of a polypeptide including from between 50 and 500 amino acids derived from intrinsically disordered regions (IDRs) of proteins are described. CAR-IDR-T cells have improved condensation of CAR and T cell activation, and overall enhanced anti-tumor efficacy as compared with CAR-T cells lacking the IDR domain(s). In preferred embodiments, CAR-IDR fusion peptides include an IDR domain of any of SEQ ID NOs.1-6. The compositions and methods provide enhanced CAR-T cell therapy for cancer as well as other disease and disorders. Also disclosed are genetically modified cells and pharmaceutical compositions and methods of use thereof for treating subjects having diseases or disorders.

CHIMERIC ANTIGEN RECEPTORS (CAR) WITH INTRINSICALLY DISORDERED REGIONS AND METHODS OF USE THEREOF

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Application No. 63/480,488, filed January 18, 2023, and U.S.S.N. 63/481,737 filed January 26, 2023, each of which is hereby incorporated by

10   reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under GM138299 awarded by the National Institutes of Health. The government has certain

15   rights in the invention.

REFERENCE TO SEQUENCE LISTING

The Sequence Listing XML submitted as a file named "YU_8604_PCT_ST26.xml" and having a size of 42,656 bytes is hereby incorporated by reference pursuant to 37 C.F.R. § 1.834(c)(1).

20   ### FIELD OF THE INVENTION

The invention is generally in the field of immunotherapy, and more particularly to improved chimeric antigen receptor (CAR) T cells with enhanced anti-tumor efficacy, and methods of making and using thereof.

BACKGROUND OF THE INVENTION

25   Genetically engineered T cells represent a versatile and effective platform for cancer therapy. T cells equipped with Chimeric Antigen Receptors (CAR-Ts) have been tested in a variety of cancers and have achieved unprecedented success in treating blood malignancy (Ghobadi, A. C Curr Res Transl Med 66, 43-49, (2018); Lee, D. W. *et al.*, Lancet 385,

30   517-528, (2015); Porter, D. L., *et al.*, N Engl J Med 365, 725-733, (2011); Maldini, C. R., Ellis, G. I. & Riley, J. L. Nat Rev Immunol 18, 605-616, (2018)). As of 2022, there are six FDA approved CAR-T therapies for certain types of leukemia, lymphoma, and myeloma. These achievements have stimulated enthusiasm for developing new CAR-Ts targeting an

expanding pool of cancerous, infectious, autoimmune, and fibrotic diseases (Aghajanian et al., 2019; Amor et al., 2020; Elinav et al., 2008; Ellebrecht et al., 2016; Hale et al., 2017; Leibman et al., 2017).

However, CAR T therapy is still limited by several major obstacles, including the risk of cytokine release syndrome, neurotoxicity, and limited signaling efficiency. Though derived from the T cell receptor (TCR), CARs typically display a much lower antigen sensitivity than TCR: a few hundred or more antigen molecules are required to activate a CAR T cell (Gudipati et al., 2020; Watanabe, K. *et al.*, J Immunol 194, 911-920, (2015); Majzner, R. G. *et al.*, Cancer Discov, doi:10.1158/2159-8290.CD-19-0945 (2020)) whereas a single peptide-loaded MHC molecule is sufficient to trigger the activation of a normal T cell (Irvine, D. J., Purbhoo, M. A., Krogsgaard, M. & Davis, M. M. Nature 419, 845-849, (2002); Huang, J. et al. Immunity 39, 846-857, (2013)). This low antigen sensitivity not only limits CAR-T's target to high antigen-expressing cancers, but also brought a challenge on maintaining sustained CAR-T activity against tumors. Since high antigen-expressing cancers downregulate their antigen levels to escape CAR-T's attacking, CAR T-treated patients experience an up to 60% of relapse, which is mostly associated with antigen loss (Park, J. H. et al. N Engl J Med 378, 449-459, (2018); Maude, S. L. et al. N Engl J Med 378, 439-448, (2018); Majzner, R. G. & Mackall, C. L. Cancer Discov 8, 1219-1226, (2018)). Therefore, there is a critical need to develop CAR T cells that can recognize low antigen-expressing cancer cells.

Mechanistically, the cause for low antigen sensitivity of CARs remains unclear. Because the binding of antigen to CAR is much tighter (with a Kd of nM to pM) than the binding of pMHC to TCR (with a Kd of μM). Therefore, the inability of CAR-Ts to be activated by low antigen-expressing cells more likely results from the signal processing mechanism downstream CAR rather than the binding of CAR to antigen.

It is an object of the invention to provide CAR T cells with enhanced antigen sensitivity and sustained CAR-T activity against tumors.

It is also an object of the invention to provide enhanced CAR T cells having improved anti-tumor efficacy and methods thereof for treating proliferative diseases.

SUMMARY OF THE INVENTION

5   Compositions and methods for improving the efficiency of CAR-T cells through engineering CARs for enhanced condensation of CARs at the cell surface and/or subsequent T cell activation have been developed. The disclosed compositions and methods are especially applicable to development of enhanced chimeric antigen receptor engineered T cell

10   therapy (CAR-T).

        Polypeptides including an amino acid sequence of an intrinsically disordered region (IDR) of a protein and an amino acid sequence that is heterologous to IDR are provided. Typically, the IDR includes between 50 and 500 amino acids. Polypeptides including an amino acid sequence

15   including at least 70% and less than 100% sequence identity to any one of SEQ ID NOs:1-6 or functional fragment thereof; and a heterologous amino acid sequence that is heterologous to IDR are also described. In some embodiments, the polypeptide further includes the amino acid sequence of SEQ ID NO:1, or a functional fragment or variant thereof. In some

20   embodiments, the polypeptide includes the amino acid sequence of SEQ ID NO:2 or a functional fragment or variant thereof. In some embodiments, the polypeptide includes one or more additional copies of the amino acid sequence of SEQ ID NO:3, or a functional fragment or variant thereof. In some embodiments, the polypeptide includes the amino acid sequence of

25   SEQ ID NO:4 or a functional fragment or variant thereof. In some embodiments, the polypeptide includes the amino acid sequence of SEQ ID NO:5 or a functional fragment or variant thereof. In some embodiments, the polypeptide includes the amino acid sequence of SEQ ID NO:6 or a functional fragment or variant thereof.

30   Exemplary heterologous amino acid sequences further include one or more molecules such as a chimeric antigen receptor (CAR), protein transduction domain, fusogenic polypeptide, targeting signal, expression and/or purification tag. In some embodiments, the heterologous sequence

includes a chimeric antigen receptor (CAR), and the amino acid sequence of
the IDR is present within the extracellular or intracellular region of the CAR.
CAR fusion proteins are modular in nature with several discrete domains
fused together. The IDR can be inserted or addended to any of the existing

5   domains of the CAR construct, preferably provided its addition does not
eliminate the activity of the construct (e.g., to bind to antigen, transduce
signal, active T cells, etc.)

      In some embodiments, the heterologous sequence includes a
chimeric antigen receptor (CAR), and the amino acid sequence of the IDR is

10   contiguous with the carboxyl terminus of the CAR. In some embodiments,
the heterologous sequence includes a chimeric antigen receptor (CAR)
having an intracellular component of CD3 zeta, and the amino acid sequence
of the IDR is contiguous with the intracellular component of CD3 zeta. In
one embodiment, the heterologous sequence includes a chimeric antigen

15   receptor (CAR) having a transmembrane domain of CD8α, cytosolic
signaling domains or a fragment there of derived from CD28, 41BB, and
CD3 zeta, and the amino acid sequence of the IDR is contiguous with the
intracellular component of CD3 zeta. In another embodiment, the
heterologous sequence includes a chimeric antigen receptor (CAR) having a

20   transmembrane domain of CD28, cytosolic signaling domains or a fragment
there of derived from CD28, 41BB, and CD3 zeta, and the amino acid
sequence of the IDR is contiguous with the intracellular component of CD3
zeta. In a further embodiment, the heterologous sequence includes a chimeric
antigen receptor (CAR) having a transmembrane domain of CD8α, cytosolic

25   signaling domains or a fragment there of derived from CD28 and CD3 zeta,
and the amino acid sequence of the IDR is contiguous with the intracellular
component of CD3 zeta. In exemplary embodiments, the polypeptide has or
includes the sequence of any one of SEQ ID NOs:7-27.

      In some embodiments, the CAR is specific for an antigen selected

30   from a cancer antigen, an inflammatory disease antigen, a neuronal disorder
antigen, HIV/AIDS, a diabetes antigen, a cardiovascular disease antigen, an
infectious disease antigen (including a viral antigen, a protozoan antigen, a
bacterial antigen, and an allergen), an autoimmune disease antigen and an

autoimmune disease antigen, or combinations thereof. Exemplary CAR

targets are selected from AFP, AKAP 4, ALK, Androgen receptor, B7H3,

BCMA, Bcr Abl, BORIS, Carbonic, CD123, CD138, CD174, CD19, CD20,

CD22, CD30, CD33, CD38, CD80, CD86, CEA, CEACAM5, CEACAM6,

5   Cyclin, CYP1B1, EBV, EGFR, EGFR806, EGFRvIII, EpCAM, EphA2,

ERG, ETV6 AML, FAP, Fos related antigen1, Fucosyl, fusion, GD2, GD3,

GloboH, GM3, gp100, GPC3, HER 2/neu, HER2, HMWMAA, HPV E6/E7,

hTERT, Idiotype, IL12, IL13RA2, IM19, IX, LCK, Legumain, lgK, LMP2,

MAD CT 1, MAD CT 2, MAGE, MelanA/MART1, Mesothelin, MET, ML

10   IAP, MUC1, Mutant p53, MYCN, NA17, NKG2D L, NY BR 1, NY ESO 1,

NY ESO 1, OY TES1, p53, Page4, PAP, PAX3, PAX5, PD L1, PDGFR β,

PLAC1, Polysialic acid, Proteinase3 (PR1), PSA, PSCA, PSMA, Ras

mutant, RGS5, RhoC, ROR1, SART3, sLe(a), Sperm protein 17, SSX2, STn,

Survivin, Tie2, Tn, TRP 2, Tyrosinase, VEGFR2, WT1, and XAGE. In some

15   embodiments, the antigen is a cancer antigen selected from 4 1BB, 5T4,

adenocarcinoma antigen, alpha fetoprotein, BAFF, B lymphoma cell, C242

antigen, CA 125, carbonic anhydrase 9 (CA IX), C MET, CCR4, CD 152,

CD 19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30

(TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74,

20   CD80, CEA, CNT0888, CTLA 4, DR5, EGFR, EpCAM, CD3, FAP,

fibronectin extra domain B, folate receptor 1, GD2, GD3 ganglioside,

glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor

kinase, IGF 1 receptor, IGF I, IgG1, L1 CAM, IL 13, IL 6, insulin-like growth

factor I receptor, integrin α5β1, integrin αvβ3, MORAb 009, MS4A1,

25   MUC1, mucin CanAg, N glycolylneuraminic acid, NPC 1C, PDGF R a,

PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON,

ROR1, SCH 900105, SDC1, SLAMF7, TAG 72, tenascin C, TGF beta 2,

TGF β, TRAIL R1, TRAIL R2, tumor antigen CTAA16.88, VEGF A,

VEGFR 1, VEGFR2, and vimentin. In exemplary embodiments, the CAR is

30   anti-CD19, anti-CD22, and/or anti-HER2.

        Nucleic acids including a nucleic acid encoding the polypeptides

are also described. Exemplary nucleic acids include a nucleic acid encoding

a polypeptide including a chimeric antigen receptor (CAR) and one or more

of SEQ ID NOs:1-27. In some embodiments, the nucleic acid is RNA or DNA. In some embodiments, the nucleic acid is mRNA, and/or includes an expression control sequence(s), and/or is, or is encoded by a vector or a transposon. An exemplary vector is a viral vector, such as a lentiviral vector,

5   an Adeno-associated virus (AAV) vector, or an adenovirus vector, or a Herpes Simplex virus (HSV) vector, or a vesicular stomatitis (VSV) vector, or a human Bocavirus vector (hBoV), or a chimeric vector including a combination of any two or more of a Adeno-associated virus (AAV) vector, Herpes Simplex virus (HSV) vector, vesicular stomatitis (VSV) vector, or a

10   human Bocavirus vector (hBoV). In some embodiments, the vector is a nucleic acid expression vector selected from a plasmid, a cosmid, and a replicon. In some embodiments, the nucleic acid includes one or more of a promotor, a protein transduction domain, fusogenic polypeptide, or targeting signal conjugated thereto.

15   Isolated cells including the polypeptides and/or nucleic acids are also provided. Exemplary cells include a T cell, hematopoietic stem cell (HSC), macrophage, natural killer cell (NK), or dendritic cell (DC). For example, in some embodiments, the T cell is a CD8+ T cell selected from effector T cells, memory T cells, central memory T cells, and effector

20   memory T cells. In other embodiments, the T cell is a CD4+ T cell selected from Th1 cells, Th2 cells, Th17 cells, and Treg cells. Isolated cells including a polypeptide encoding (i) an amino acid sequence encoding a CAR; and (ii) an IDR optionally have an amino acid sequence of one or more of SEQ ID NOs:1-6, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, or

25   99% identity to one or more of SEQ ID NOs:1-6, are also described. In some embodiments, the isolated cell includes a polypeptide including (i) an amino acid sequence encoding a CAR; and (ii) an amino acid sequence of one or more of SEQ ID NOs:1-6. In some embodiments, the amino acid sequence of any one of SEQ ID NOs: 1-6 is contiguous with the residue at the carboxyl

30   terminus of the CAR.

         Populations of cells derived by expanding an isolated cell including a polypeptide including: (i) an amino acid sequence encoding a CAR; and (ii) an amino acid sequence of one or more of SEQ ID NOs:1-6, or a variant

having at least 75 % identity to one or more of SEQ ID NOs:1-6 are also provided. In some embodiments, the amino acid sequence of one or more of SEQ ID NOs:1-6 is contiguous with the residue at the carboxyl terminus of the CAR.

5   Pharmaceutical compositions including (a) a population of cells expanded from an isolated cell including or expressing a polypeptide including: (i) an amino acid sequence encoding a CAR; and (ii) an amino acid sequence of one or more of SEQ ID NOs:1-6, or a variant having at least 75 % identity to one or more of SEQ ID NOs:1-6; and (b) a

10   pharmaceutically acceptable buffer, carrier, diluent or excipient are also provided.

          Methods of treating a subject having a disease, disorder, or condition including administering to the subject an effective amount of a pharmaceutical composition of cells are also provided. Typically, the

15   methods treat a subject having a disease, disorder, or condition associated with an elevated expression or specific expression of an antigen, by administering to the subject an effective amount of a T cell modified to include or express a polypeptide including: (i) an amino acid sequence encoding a CAR that targets the antigen; and (ii) an amino acid sequence of

20   one or more of SEQ ID NOs:1-6, or a variant having at least 75 % identity to one or more of SEQ ID NOs:1-6. In some embodiments, the cell was isolated from the subject having the disease, disorder, or condition. In other embodiments, the cell was isolated from a healthy donor.

          Typically, the subject is a human, such as a human with a disease

25   selected from cancer, an inflammatory disease, a neuronal disorder, HIV/AIDS, a diabetes, a cardiovascular disease, an infectious disease (including a viral, a protozoan, a bacterial disease, and an allergy), an autoimmune disease and an autoimmune disease, and a genetic disorder.

          In some embodiments, the subject has cancer or has been identified

30   as being at increased risk of developing cancer.

          Methods of introducing a fusion peptide including an IDR domain into a cell, are also provided. Typically, the method includes the steps of introducing to the cell: (i) a vector or transposon or mRNA encoding a

polypeptide encoding a fusion peptide including an IDR domain; and (ii) causing the polypeptide to be expressed in the cell.

Chimeric antigen receptors (CARs), including one or more IDR polypeptides (CAR-IDR) are described. In certain embodiments, the CAR-IDR targets CD19 and includes the amino acid sequence of any one of SEQ ID NOs:7-18. In a preferred embodiment, the CAR-IDR which targets CD19 and includes the amino acid sequence of any one of SEQ ID NOs:7-18 has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control. A suitable control has the same CAR targeting CD19 but without fusion to an IDR, for example, an exemplary control has the amino acid sequence of SEQ ID NO:19. In other embodiments, the CAR-IDR targets CD22 and includes the amino acid sequence of any one of SEQ ID NOs:20-22. In a preferred embodiment, the CAR-IDR which targets CD22 and includes the amino acid sequence of any one of SEQ ID NOs:20-22 has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control. An exemplary control has the amino acid sequence of SEQ ID NO:23. In further embodiments, the CAR-IDR targets HER2 and includes the amino acid sequence of any one of SEQ ID NOs:24-26. In a preferred embodiment, the CAR-IDR which targets HER2 and includes the amino acid sequence of any one of SEQ ID NOs:24-26 has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control. An exemplary control has the amino acid sequence of SEQ ID NO:27.

Nucleic acids, including a nucleic acid sequence encoding the chimeric antigen receptor including an IDR polypeptide (CAR-IDR), that targets CD19, CD22, and/or HER2 are also described. In some embodiments, the nucleic acid is a vector or a transposon. Isolated cells including a CAR including an IDR polypeptide (CAR-IDR), that targets CD19, CD22, and/or HER2, or a nucleic acid encoding the polypeptide are also described. Populations of cells derived from expanding the isolated cells including the CAR-IDRs that target CD19, CD22, and/or HER2 are also provided, and pharmaceutical compositions including the populations of cells and a

pharmaceutically acceptable buffer, carrier, diluent, or excipient are also described. Methods of treating a subject having a disease, disorder, or condition include administering to the subject an effective amount of the pharmaceutical composition. In some embodiments, the subject has cancer.

5   **BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1A-1K depict construction of IDR CARs. **Figure 1A** is a schematic of the CD19 CAR used in this study which contains an scFv targeting CD19 (FMC63), a CD8 hinge and transmembrane domain, an intracellular signaling domain composed of CD28, 41BB, and CD3ζ, an

10   intrinsically disordered region (IDR), and superfolder GFP. **Figures 1B-1I** are dot plot graphs showing FACS analyses of total and cell surface expression of IDR CARs, as determined by GFP and surface staining of FMC63, showing T cells with No CAR (**Figure 1B**); Control CAR (**Figure 1C**); CAR with FUS IDR (FUS) (**Figure 1D**); CAR with TAF15 IDR

15   (TAF15) (**Figure 1E**); CAR with EWS IDR (EWS) (**Figure 1F**); CAR with Nup98 IDR (Nup98) (**Figure 1G**); CAR with TDP43 IDR (TDP43) (**Figure 1H**); and CAR with Synthetic IDR (SynIDR) (**Figure 1I**), respectively. **Figure 1J** is a graph showing the results of TIRF microscopy to determine condensation of IDR CARs, with clustering of CARs quantified as

20   normalized variance (N = 48-50 cells) of the fluorescence (0-1500) for each of Control, FUS, TAF15, EWS, Nup98, TDP43 and SynIDR, respectively. Shown are mean + std. All comparisons in this and following figures were made between individual IDR CARs and the control CAR (n.s. p$>$=0.05, * $p<0.05$, ** $p<0.01$, *** $p<0.001$). **Figure 1K** is a graph showing the results

25   of CAR-T cell proliferation with or without IL-2. Quantification of the absolute cell number in the culture with or without IL-2. N=3 independent experiments. Shown are mean + std.

Figures 2A-2Q depict IDR-enhanced cytotoxicity of CD19 CAR-T in vitro and in vivo. **Figures 2A-2B** are graphs of the expression of CD19

30   CARs on the T cell surface as determined by an antibody recognizing FMC63 and the expression of CD19 on Nalm6 cells, showing numbers of CARs for each of EWS IDR CAR (EWS) , TAF15 IDR CAR (TAF15), FUS IDR CAR (FUS), Control CAR and No CAR, respectively (**Figure 2A**); and

numbers of CD19 on each of NalmCD19 high cells (34,517 molecules per
cell), NalmCD19 low cells (1,470 molecules per cell), and Null, respectively;
the average number of CD19 on each cell was determining by flow
cytometry using a BD Quantibrite Kit (**Figure 2B**). **Figures 2C-2D** are

5   graphs of cytotoxicity of CD19 CAR-Ts to CD19 high Nalm6 cells (**Figure
2C**), or CD19 low Nalm6 cells (**Figure 2D**), showing specific lysis (%) for
each Control (■), FUS (▲), TAF15 (▼) and EWS (◆) CART cells, at ratios
of 0.3:1 to 10:1, respectively, as reported by the luciferase assay. **Figures
2E-2I** are graphs showing production of cytotoxic factors by CAR-T. The

10   control or IDR CAR-Ts were co-cultured with CD19-low Nalm6 cells for 1
day at an E:T of 3:1. The cytokines released into the culture media were
quantified by flow cytometry using a LegendPlex assay kit. N=3 independent
experiments. Shown are mean + std (*p<0.05, **p<0.01, ***p<0.001).
Figure 2J is a schematic showing examination of the anti-tumor effect of

15   CD19 IDR CAR-Ts in a mouse xenograft model. 1×10^6 FLuc+ CD19 low
Raji B cells were engrafted into the immune-deficient NSG mice via tail
vein. Three days later, 8×10^6 CAR-T cells or PBS were injected into the
mice via tail vein. Cancer progression was monitored by bioluminescent
imaging. Blood was drawn at Day 16 to characterize T cell phenotypes.

20   **Figure 2K-2O** are graphs showing quantification of tumor progression in
vivo revealed by bioluminescent imaging. Both the individual and averaged
traces were shown. N=5 mice. Shown are mean + std (n.s. p>=0.05, ***
p<0.001). **Figure 2P** is a plot showing expression of T cell exhaustion
markers during cancer progression. Blood was collected at day 16 post CAR-

25   T infusion, stained with antibodies recognizing the exhaustion marker TIM3,
LAG3, and PD1, and analyzed by flow cytometry. N=5 mice. Shown are
mean + std (n.s. p>=0.05, *p<0.05, **p<0.01). **Figure 2Q** is a plot
showing quantification of T cells number in the blood. Blood was collected
at day 16 post CAR-T infusion, stained with antibodies recognizing human

30   CD3 and human CD45, and analyzed by flow cytometry. N=5 mice. Shown
are mean + std (n.s. p>=0.05).

          Figures 3A-3W depict IDRs enhancing cytotoxicity of HER2 CAR-
T in vitro and in vivo. **Figure 3A** is a schematic of the IDR CAR construct

targeting HER2 (H3B1), a CD28 hinge and transmembrane domain, an
intracellular signaling domain composed of CD28, 41BB and CD3ζ, and an
intrinsically disordered region (IDR). The control CAR contains a GFP tag at
the C-terminus so that the size and surface expression level of the control

5   and IDR CARs can be maintained in a similar level over time. **Figure 3B is** a
graph of the expression of the control or IDR CAR targeting HER2 in human
primary T cells. CAR expression was detected by recombinant HER2
proteins and measured by flow cytometry showing numbers of CARs for
each of EWS IDR CAR (EWS), TAF15 IDR CAR (TAF15), FUS IDR CAR

10   (FUS), Control CAR and Null, respectively (**Figure 3B**). **Figures 3C-3D** are
a pair of plots showing quantification of the HER2 level in K562 cells
expressing high (102,797 molecules per cell) or low (5,703 molecules per
cell) HER2, and in SKOV3 (77,679 molecules per cell) and HT29 (3,351
molecules per cell) cells. The average number of HER2 on each cell was

15   determining by flow cytometry using a BD Quantibrite Kit. **Figures 3E-3H**
are graphs of cytotoxicity of HER2 CAR-Ts to HER2 high or low K562,
HT29 or NCK292 cells as reported by the luciferase assay, showing specific
lysis (%) for each of Null, Control, FUS, TAF15 and EWS CART cells, at
ratios of 0.3:1 to 10:1, respectively, for each of CAR-T:HER2-K562-high

20   (**Figure 3E**); CAR-T:HER2-K562-low (**Figure 3F**); CAR-T:SKOV3
(**Figure 3G**); and CAR-T:HT29 (**Figure 3H**), CAR-T:NCI292 (**Figure 3W**)
respectively. **Figures 3I-3M** are graphs showing production of cytotoxic
factors by CAR-T. The control or IDR CAR-Ts were co-cultured with HT29
cells for 1 day at an E:T of 3:1. The cytokines released into the culture media

25   were quantified by flow cytometry using a LegendPlex assay kit. N=3
independent experiments. Shown are mean + std (n.s. $p >= 0.05$, *$p < 0.05$, **
$p < 0.01$, ***$p < 0.001$). **Figure 3N** is a schematic showing examination of the
anti-tumor effect of HER2 IDR CAR-Ts in a mouse xenograft model.
2.5×10^6 HT29 cells were engrafted subcutaneously into the immune-

30   deficient NSG mice. Eight days later, 8×10^6 CAR-T cells were injected into
the mice via tail vein, followed by a second injection of 4×10^6 CAR-T cells
five days after. Cancer progression was monitored by measuring the tumor
size using an electronic digital caliper. **Figures 3O-3T** are graphs showing

quantification of cancer progression in vivo after CAR-T treatment. Both the averaged and individual traces were shown. N=5 mice. Shown are mean + std ($*p<0.05$). **Figure 3U** is a plot showing quantification of T cell number in blood. Blood was drawn at Day 25, stained with antibodies recognizing

5   human CD3 and CD45. The percentile of human T cells was measured by flow cytometry. N=5 mice. Shown are mean + std. **Figure 3V** is a plot showing quantification of tumor-infiltrating T cells. At the end timepoint (the tumor size over 2000 mm3), tumors were dissected, digested, stained with antibodies recognizing human CD3 and CD45. The percentile of human

10 T cells was measured by flow cytometry. N=5 mice. Shown are mean + std.

         Figures 4A-4N depict IDRs enhanced the anti-tumor effect of CD22 CAR-T in vitro and in vivo. **Figure 4A** is a schematic of the CD22 CAR used in this study. It contains an scFv targeting CD22 (RFB4, low signaling efficiency), a CD28 hinge and transmembrane domain, an intracellular

15 signaling domain composed of CD28 and CD3ζ, and an intrinsically disordered region (IDR). The control CAR contained a GFP tag at the C-terminus so that the size and surface expression level of the control and IDR CARs were similar. **Figure 4B** is a graph showing numbers of CARs for each of TAF15 CAR, EWS IDR CAR (EWS), FUS IDR CAR (FUS),

20 Control CAR and Null, respectively. **Figure 4C** is a plot showing quantification of the CD22 level in the wild-type Raji B (21,297 molecules per cell) and Nalm6 (8,376 molecules per cell). The average number of CD22 on each cell was determining by flow cytometry using a BD Quantibrite Kit. **Figure 4D** is a graph of inhibition of cancer progression by

25 CAR-Ts, showing Radiance (p/sec/cm2/sr) over time (Days post CAR-T treatment) for each of PBS (●), Control (■), FUS (▲) and EWS (▼), respectively. **Figure 4E** is a bar graph of quantified flow cytometry analysis showing % Exhaustion markers of each of PD1, TIM3, and LAG3 on each of Control, FUS and EWS CAR-T cells, respectively, at day 19. N=5 mice.

30 Shown are mean + std (n.s. p>=0.05, $*p<0.05$). **Figures 4F-4G** are graph showing cytotoxicity of CD22 CAR-T in vitro. The control or IDR CAR-Ts were co-cultured with Raji B (**Figure 4F**) or Nalm6 (**Figure 4G**) cells expressing luciferase (Fluc+) for 3 or 1 day, respectively, with an effector to

target (E:T) ratio from 0.3:1 to 10:1. The percentile of lysed Naml6 cells were quantified by the luciferase assay and normalized to the group of Raji B or Nalm6 alone. N=3 independent experiments. Shown are mean + std (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). **Figures 4H-4L** are graphs showing

5   production of cytotoxic factors by CAR-T. The control or IDR CAR-Ts were co-cultured with the wild-type Nalm6 cells for 1 day at an E:T of 1:1. The cytokines released into the culture media were quantified by flow cytometry using a LegendPlex assay kit. N=3 independent experiments. Shown are mean + std (n.s. p>=0.05, * $p<0.05$). **Figure 4M** is a schematic showing

10 examination of the anti-tumor effect of CD22 IDR CAR-Ts in a mouse xenograft model. 1×10^6 FLuc+ Nalm6-WT cells were engrafted into the immune-deficient NSG mice via tail vein. Three days later, 8×10^6 CAR-T cells or PBS were injected into the mice via tail vein. Cancer progression was monitored by bioluminescent imaging. Blood was drawn at Day 10 and

15 19 to characterize T cell phenotypes. **Figure 4N** shows quantification of T cells in the blood. Blood was collected at day 10 post CAR-T infusion, stained with antibodies recognizing human CD3 and human CD45, and analyzed by flow cytometry. N=5 mice. Shown are mean + std (* $p<0.05$, ** $p<0.01$).

20   **Figures 5A-5L** depict IDR promoted membrane-proximal signaling in CAR-T. **Figure 5A** is a graph showing cell-cell conjugation between CAR-T and Nalm6 cells. CD19 CAR-Ts were co-cultured with CD19-low RajiB cells for 30 min at 37°C with an E:T ratio of 1:1. Cell conjugation was examined by confocal microscopy. N=3 independent experiments. Shown

25 are mean + std (n.s. p>=0.05). **Figures 5B-5J** are plots demonstrating phosphorylation kinetics of CD3ζ (pY142), LAT (pY171), and ERK (pT202/Y204). CAR-Ts were mixed with CD19-low Raji B cells at an E:T=1:1 at 37°C. Cells were fixed at 0, 5 or 15 min, stained and examined with flow cytometry. Displayed are traces for three independent experiments.

30 Relative pCD3z, pLAT, and pERK was calculated by dividing the geometric mean of fluorescence at indicated time point to that at 0 min. **Figure 5K** is a plot showing quantification of pCD3ζ at the CAR-T synapse. Normalized pCD3ζ was calculated by dividing the fluorescence of pCD3ζ to the

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fluorescence of CAR in the synapse. N=77-107 cells. Shown are mean + std
($**p<0.01$, $***p<0.001$). **Figure 5L** is a plot showing quantification of
pLAT at the CAR-T synapse. Normalized pLAT was calculated by dividing
the fluorescence of pLAT to the fluorescence of CAR in the synapse. N= 79-
105 cells. Shown are mean + std ($***p<0.001$).

Figures 6A-6I depict oligomerization by coiled-coil domain reduced
CAR surface localization and CAR-T activation. **Figure 6A** is a schematic of
the coiled-coil (cc) CD19 CAR which contains an scFv targeting CD19
(FMC63), a CD8 hinge, a LAT transmembrane domain, an intracellular
signaling domain composed of CD28, 41BB, and CD3ζ, and a coiled-coil
domain. **Figures 6B-6F** are graphs showing expression of coiled-coil CARs.
The localization of CAR on the T cell surface was detected via flow
cytometry using an anti-FMC63 antibody. A GFP tag was fused on the C-
terminus of each CAR to monitor the total expression of CAR. **Figure 6G** is
a plot showing cell-cell conjugation between CAR-T and Nalm6 cells. CAR-
Ts were mixed with Naml6-H8 clone (expressing 137,795 CD19 molecules
per cell) for 30 min at 37°C with an E:T =1:1. Cell conjugation was
monitored by confocal microscopy. N=3 independent experiments. Shown
are mean + std (n.s. p>=0.05, $*p<0.05$, $**p<0.01$). **Figures 6H-6I** are plots
showing activation of coiled-coil CAR-Ts assessed by CD69 expression and
IFNɣ release. CAR-Ts were co-cultured with Nalm6-H8 for 1 day at an E:T
= 1:1. CD69 expression was revealed by flow cytometry. IFNɣ release was
quantified by ELISA. N=3 independent experiments. Shown are mean + std
(n.s. p>=0.05, $*p<0.05$, $**p<0.01$).

Figures 7A-7D depict that IDR do not trigger tonic signaling.
Figures 7A-7B show graphs demonstrating activation of CD19 CAR-T in
the presence and absence of antigen. CAR-Ts were cultured alone or co-
cultured with the wild-type Raji B cells at E:T = 10:1 for 1 day. Expression
of CD69 was examined by flow cytometry. TNFα release was measured by
ELISA. N=3 independent experiments. Shown are mean + std (n.s. p>=0.05,
$*p<0.05$, $**p<0.01$). **Figures 7C-7D** are graphs showing activation of
HER2 CAR-T in the presence and absence of antigen. CAR-Ts were
stimulated by plate-coated HER2 protein for 1 day. Expression of CD69 was

examined by flow cytometry. TNFα release was measured by ELISA. N=3
independent experiments. Shown are mean + std (n.s. p>=0.05, $*p<0.05$,
$**p<0.01$, $***p<0.001$).

 Figures 8A-8P depict characterization of IDR CAR-Ts targeting

5 CD19. **Figure 8A** is a plot showing quantification of the CD19 level in Raji
B cells expressing high (37,172 molecules per cell) or low (2,638 molecules
per cell) level of CD19. The average number of CD19 on each cell was
determined by flow cytometry using a BD Quantibrite Kit. **Figures 8B-8C**
are graphs showing cytotoxicity of CD19 CAR-T against Raji B cells. The

10 control or IDR CAR-Ts were co-cultured with CD19-high or low Raji B
cells expressing luciferase (Fluc+) for 3 days with an effector to target (E:T)
ratio from 1:1 to 10:1. The percentile of lysed Raji B cells were quantified by
the luciferase assay and normalized to the group of Raji B alone. N=3
independent experiments. Shown are mean + std ($*p<0.05$, $**p<0.01$).

15 **Figure 8D** demonstrates representative heat map illustrating cytotoxic
factors released by CD19 CAR-Ts. The control or IDR CAR-Ts were co-
cultured with CD19-low Nalm6 cells for 1 day at an E:T of 3:1. The
cytokines released into the culture media were quantified by flow cytometry
using a LegendPlex assay kit. **Figures 8E-8G** are representative flow

20 cytometry plots showing T cell exhaustion markers. Blood was collected at
day 16 post CAR-T infusion, stained with antibodies recognizing the
exhaustion marker TIM3, LAG3, and PD1, and analyzed by flow cytometry.
Figures 8H-8K are representative flow cytometry plots showing T cell
population in the blood. Blood was collected at day 16 post CAR-T infusion,

25 stained with antibodies recognizing human CD3 and human CD45. **Figures
8L-8O** are representative flow cytometry plots showing T cell differentiation
in vivo. Blood was collected at day 16 post CAR-T infusion, stained with
antibodies recognizing CD45RA and CD62L, and analyzed by flow
cytometry. **Figure 8P** is a graph showing quantification of T cell differentiation

30 in vivo. N=5 mice. Shown are mean + std.

 Figures 9A-9K depict characterization of IDR CAR-Ts targeting
HER2. **Figure 9A** is a plot of representative heat map illustrating cytotoxic
factors released by HER2 CAR-Ts. The control or IDR CAR-Ts were co-
cultured with HT29 cells for 1 day at an E:T of 3:1. The cytokines released

into the culture media were quantified by flow cytometry using a
LegendPlex assay kit. **Figures 9B-9K** are representative flow cytometry
plots showing T cell population in the blood and tumor. Blood was draw at
day 25 and tumors were dissected at day 48, digested, stained with antibodies

5   recognizing human CD3 and CD45. The percentile of human T cells was
measured by flow cytometry.

      Figure 10A-10M depict characterization of IDR CAR-Ts targeting
CD22. **Figure 10A** is plot of representative heat map illustrating cytotoxic
factors released by CD22 CAR-Ts. The control or IDR CAR-Ts were co-

10   cultured with the wild-type Nalm6 cells for 1 day at an E:T of 1:1. The
cytokines released into the culture media were quantified by flow cytometry
using a LegendPlex assay kit. **Figures 10B-10E** are representative flow
cytometry plots showing T cell population in the blood. Blood was collected
at day 10 post CAR-T infusion, stained with antibodies recognizing human

15   CD3 and human CD45. **Figures 10F-10H** are representative flow cytometry
plots showing T cell exhaustion markers. Blood was collected at day 19 post
CAR-T infusion, stained with antibodies recognizing the exhaustion marker
TIM3, LAG3, and PD1, and analyzed by flow cytometry. **Figures 10I-10L**
are representative flow cytometry plots showing T cell differentiation in

20   vivo. Blood was collected at day 10 post CAR-T infusion, stained with
antibodies recognizing CD45RA and CD62L, and analyzed by flow
cytometry. **Figure 10M** shows quantification of T cell differentiation in
vivo. N=5 mice. Shown are mean + std.

      Figures 11A-11I depict phosphorylation kinetics of CD3ζ, LAT, and

25   ERK. CAR-Ts were mixed with CD19-low Raji B cells at an E:T=1:1 at
37°C. Cells were fixed at 0, 5 or 15 min, stained with phospho-antibodies
and examined with flow cytometry. Shown are representative flow plots.

      Figures 12A-12F depict oligomerization by coiled-coil domain
reduce CAR-T activation. **Figures 12A-12B** show representative flow

30   cytometry plots showing CD69 expression in coiled-coil CAR-Ts. CAR-Ts
were co-cultured with Nalm6-H8 for 1 day at an E:T = 1:1. **Figure 12C** is a
graph showing cell-cell conjugation between CAR-T and Raji B cells. CAR-
Ts were mixed with CD19-high Raji B cells for 30 min at 37°C with an E:T

=10:1. Cell conjugation was monitored by confocal microscopy. N=3 independent experiments. Shown are mean + std (*$p<0.05$, ***$p<0.001$). **Figures 12D-12F** are plots showing CD69 expression on coiled-coil CAR-Ts when engaged with Raji B cells. CAR-Ts were co-cultured with CD19-high Raji B cells for 1 day at an E:T = 10:1. CD69 expression was revealed by flow cytometry. N=3 independent experiments. Shown on are mean + std (n.s. $p>=0.05$, ***$p<0.001$). Representative flow cytometry plots were displayed.

DETAILED DESCRIPTION OF THE INVENTION

I.   Definitions

"Introduce" in the context of genome modification refers to bringing in to contact. For example, to introduce a gene editing composition to a cell is to provide contact between the cell and the composition. The term encompasses penetration of the contacted composition to the interior of the cell by any suitable means, *e.g.*, *via* transfection, electroporation, transduction, gene gun, nanoparticle delivery, *etc*.

As used herein, "homologous" means derived from a common ancestor. For example, a homologous trait is any characteristic of organisms that is inherited by two or more species from a common ancestor species. Homologous sequences can be orthologous or paralogous. Homologous sequences are orthologous if they were separated by a speciation event: when a species diverges into two separate species, the divergent copies of a single gene in the resulting species are said to be orthologous. Orthologs, or orthologous genes, are genes in different species that are similar to each other because they originated from a common ancestor. Homologous sequences are paralogous if they were separated by a gene duplication event: if a gene in an organism is duplicated to occupy two different positions in the same genome, then the two copies are paralogous.

"Heterologous" means having a different relation, relative position, or structure. Thus, unless otherwise specified, heterologous includes joining or linking of two or more amino acid or nucleic acid sequences from that organism (e.g., species) that are not normally found joined or linked (e.g.,

together) as well as joining or linking of two or more amino acid or nucleic acid sequences from different species.

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

5   "Exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "transmembrane domain" refers to any protein structure that is thermodynamically stable in a cell membrane, preferably a eukaryotic cell membrane. As used herein, the terms "extracellular domain" and

10   "ectodomain" refer to any protein structure that is thermodynamically stable in outside of the cell membrane (i.e., in the extracellular space). As used herein, an "intracellular domain" refers to any protein structure that is thermodynamically stable in inside of the cell membrane (i.e., in the intracellular cytosol).

15   The term "Chimeric Antigen Receptor", or alternatively "CAR", refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a cancer cell, or other specific cell, and with intracellular signal generation. In exemplary embodiments, a CAR includes at least an antigen binding

20   domain such as an extracellular binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to as "an intracellular signaling domain") including a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule. The term "antigen binding domain" is used in the context of a CAR to refer to the portion of a

25   CAR that specifically recognizes and binds to an antigen of interest. The "antigen binding domain" of a CAR may be derived from a binding protein such as an antibody or fragment thereof. In some embodiments, the "binding domain" of a CAR is a single-chain variable fragment (scFv). In certain embodiments, the "binding domain" of a CAR includes the complementarity

30   determining regions of a binding protein disclosed herein. In some embodiments, the stimulatory molecule is, or is derived from, the CD3ζ (zeta), also known as "zeta stimulatory domain", associated with a T cell receptor complex. In some embodiments, the cytoplasmic signaling domain

of the CAR further includes one or more functional signaling domains derived from at least one costimulatory molecule (*e.g.*, 4-1BB (*i.e.*, CD137), CD27 and/or CD28). In some embodiments, the CAR includes a chimeric fusion protein including an extracellular antigen binding domain, a

5   transmembrane domain and an intracellular signaling domain including a functional signaling domain derived from a stimulatory molecule. In various embodiments, CARs are fusion proteins of single-chain variable fragments (scFv) fused to a CD3-zeta transmembrane domain. However, other intracellular signaling domains such as CD28, 41-BB and Ox40 may be used

10   in various combinations to give the desired intracellular signal. Exemplary CAR-T cells include *Axicabtagene ciloleucel* (KTE-C19, Axi-cel), Tisagenlecleucel, Lisocabtagene Maraleucel (liso-cel; JCAR017).

The term "antigen" as used herein is defined as a molecule capable of being bound by an antibody or T-cell receptor. An antigen can additionally

15   be capable of provoking an immune response. This immune response can involve either antibody production, or the activation of specific immunologically competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from

20   recombinant or genomic DNA. A skilled artisan will understand that any DNA, which includes a nucleotide sequence or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full-length

25   nucleotide sequence of a gene. It is readily apparent that the disclosed compositions and methods includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not

30   be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid. In the context of cancer, "antigen" refers

to an antigenic substance that is produced in a tumor cell, which can therefore trigger an immune response in the host. These cancer antigens can be useful as markers for identifying a tumor cell, which could be a potential candidate/target during treatment or therapy. There are several types of

5   cancer or tumor antigens. There are tumor specific antigens (TSA) which are present only on tumor cells and not on healthy cells, as well as tumor associated antigens (TAA) which are present in tumor cells and on some normal cells. In some forms, the chimeric antigen receptors are specific for tumor specific antigens. In some forms, the chimeric antigen receptors are

10   specific for tumor associated antigens. In some forms, the chimeric antigen receptors are specific both for one or more tumor specific antigens and one or more tumor associated antigens.

The terms "CD3ζ", "CD3 zeta" or "CD3 eta" are used interchangeably to define the protein provided as GenBan Acc. No.

15   BAG36664.1, or the equivalent residues from a non-human species. A "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" is defined as the amino acid residues from the cytoplasmic domain of the zeta chain, or functional derivatives thereof, that are sufficient to functionally transmit an initial signal necessary for T cell activation.

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

25   some embodiments, the immune effector cell(s) is allogenic. In some embodiments, the immune effector cell(s) is autologous. Immune effector cells such as T cells may be activated and expanded generally using methods previously described, such as for example, as described in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466;

30   6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041, all of which are incorporated herein by reference in their entirety.

"Bi-specific chimeric antigen receptor" refers to a CAR that includes two domains, wherein the first domain is specific for a first ligand/antigen/target, and wherein the second domain is specific for a second ligand/antigen/target. In some forms, the ligand is a B-cell specific protein, a

5   tumor-specific ligand/antigen/target, a tumor associated ligand/antigen/target, or combinations thereof. A bispecific CAR is specific to two different antigens. A multi-specific or multivalent CAR is specific to more than one different antigen, *e.g.*, 2, 3, 4, 5, or more. In some forms, a multi-specific or multivalent CAR targets and/or binds three or more

10   different antigens.

"Encoding" or "encode" 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 polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*,

15   rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is

20   usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

As used herein, the term "locus" is the specific physical location of a DNA sequence (*e.g.*, of a gene) on a chromosome. It is understood that a

25   locus of interest can not only qualify a nucleic acid sequence that exists in the main body of genetic material (*i.e.*, in a chromosome) of a cell but also a portion of genetic material that can exist independently to said main body of genetic material such as plasmids, episomes, virus, transposons or in organelles such as mitochondria as non-limiting examples.

30   "Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An

isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell. An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring

5   state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *i.e.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, *e.g.*, RNA or DNA

10   or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*i.e.*, as a cDNA or a genomic or cDNA fragment produced by PCR

15   or restriction enzyme digestion) independent of other sequences. It also includes: a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence, complementary DNA (cDNA), linear or circular oligomers or polymers of natural and/or modified monomers or linkages, including deoxyribonucleosides, ribonucleosides, substituted and

20   alpha-anomeric forms thereof, peptide nucleic acids (PNA), locked nucleic acids (LNA), phosphorothioate, methylphosphonate, and the like.

In the context of cells, the term "isolated" also refers to a cell altered or removed from its natural state. That is, the cell is in an environment different from that in which the cell naturally occurs, *e.g.*, separated from its

25   natural milieu such as by concentrating to a concentration at which it is not found in nature. "Isolated cell" is meant to include cells that are within samples that are substantially enriched for the cell of interest and/or in which the cell of interest is partially or substantially purified.

As used herein, "transformed," "transduced," and "transfected"

30   encompass the introduction of a nucleic acid or other material into a cell by one of a number of techniques known in the art.

A "vector" is a composition of matter which includes an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the

interior of a cell. Examples of vectors include but are not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" encompasses an autonomously replicating plasmid or a virus. The term is also construed to

5   include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus (AAV) vectors, retroviral vectors, and the like.

10   "Tumor burden" or "tumor load" as used herein, refers to the number of cancer cells, the size or mass of a tumor, or the total amount of tumor/cancer in a particular region of a subject. Methods of determining tumor burden for different contexts are known in the art, and the appropriate method can be selected by the skilled person. For example, in some forms,

15   tumor burden can be assessed using guidelines provided in the Response Evaluation Criteria in Solid Tumors (RECIST).

          As used herein, "subject" includes, but is not limited to, animals, plants, parasites and any other organism or entity. The subject can be a vertebrate, more specifically a mammal (e.g., a human, horse, pig, rabbit,

20   dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent), a fish, a bird or a reptile or an amphibian. The subject can be an invertebrate, more specifically an arthropod (e.g., insects and crustaceans). The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. A patient refers

25   to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects. In some forms, the subject can be any organism in which the disclosed method can be used to genetically modify the organism or cells of the organism.

          The term "inhibit" or other forms of the word such as "inhibiting" or

30   "inhibition" means to decrease, hinder or restrain a particular characteristic such as an activity, response, condition, disease, or other biological parameter. It is understood that this is typically in relation to some standard or expected value, i.e., it is relative, but that it is not always necessary for the

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standard or relative value to be referred to. "Inhibits" can also mean to hinder or restrain the synthesis, expression or function of a protein relative to a standard or control. Inhibition can include, but is not limited to, the complete ablation of the activity, response, condition, or disease. "Inhibits" can also

5   include, for example, a 10% reduction in the activity, response, condition, disease, or other biological parameter as compared to the native or control level. Thus, the reduction can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53,

10   54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64,65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any amount of reduction in between as compared to native or control levels. For example, "inhibits expression" means hindering, interfering with or restraining the expression and/or

15   activity of the gene/gene product pathway relative to a standard or a control.

"Treatment" or "treating" means to administer a composition to a subject or a system with an undesired condition (e.g., cancer). The condition can include one or more symptoms of a disease, pathological state, or disorder. Treatment includes medical management of a subject with the

20   intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological state, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological state, or

25   disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological state, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological state, or disorder; and supportive

30   treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological state, or disorder. It is understood that treatment, while intended to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or

disorder, need not actually result in the cure, amelioration, stabilization or prevention. The effects of treatment can be measured or assessed as described herein and as known in the art as is suitable for the disease, pathological condition, or disorder involved. Such measurements and

5   assessments can be made in qualitative and/or quantitative terms. Thus, for example, characteristics or features of a disease, pathological condition, or disorder and/or symptoms of a disease, pathological condition, or disorder can be reduced to any effect or to any amount. "Prevention" or "preventing" means to administer a composition to a subject or a system at risk for an

10   undesired condition (e.g., cancer). The condition can include one or more symptoms of a disease, pathological state, or disorder. The condition can also be a predisposition to the disease, pathological state, or disorder. The effect of the administration of the composition to the subject can be the cessation of a particular symptom of a condition, a reduction or prevention of the

15   symptoms of a condition, a reduction in the severity of the condition, the complete ablation of the condition, a stabilization or delay of the development or progression of a particular event or characteristic, or reduction of the chances that a particular event or characteristic will occur.

As used herein, the terms "effective amount" or "therapeutically

20   effective amount" means a quantity sufficient to alleviate or ameliorate one or more symptoms of a disorder, disease, or condition being treated, or to otherwise provide a desired pharmacologic and/or physiological effect. Such amelioration only requires a reduction or alteration, not necessarily elimination. The precise quantity will vary according to a variety of factors

25   such as subject-dependent variables (e.g., age, immune system health, weight, etc.), the disease or disorder being treated, as well as the route of administration, and the pharmacokinetics and pharmacodynamics of the agent being administered.

By "pharmaceutically acceptable" is meant a material that is not

30   biologically or otherwise undesirable, i.e., the material can be administered to a subject along with the selected compound without causing any undesirable biological effects or interacting in a deleterious manner with any

of the other components of the pharmaceutical composition in which it is contained.

As used herein, the term "polypeptides" includes proteins and functional fragments thereof. Polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a three letter or a single letter code as indicated as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic Acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic Acid (Glu, E), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

As used herein, the term "functional fragment" or "functional variant" means a fragment or variant of a polypeptide, such as a full-length or native polypeptide, that retains one or more functional properties of the full-length or native polypeptide. For example, in some embodiments, a functional fragment or functional variant of an IDR polypeptide is a fragment or variant that retains the function of enhancing condensation of CAR as a CAR-IDR fusion polypeptide compared to a control CAR polypeptide without an IDR.

As used herein, the terms "variant" or "active variant" refers to a polypeptide or polynucleotide that differs from a reference polypeptide or polynucleotide, but retains one or more functional properties (*e.g.*, functional or biological activity). A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more modifications (*e.g.*, substitutions, additions, and/or deletions). A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be naturally occurring such as an allelic

variant, or it may be a variant that is not known to occur naturally. Modifications and changes can be made in the structure of the polypeptides of the disclosure and still obtain a molecule having similar characteristics as the polypeptide (*e.g.*, a conservative amino acid substitution). For example,

5   certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological or functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like

10   properties (*e.g.*, functional or biological activity).

       Modifications and changes can be made in the structure of the polypeptides of in disclosure and still obtain a molecule having similar characteristics as the polypeptide (*e.g.*, a conservative amino acid substitution). For example, certain amino acids can be substituted for other

15   amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like properties.

20   In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still

25   result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8);

30   tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known

5   in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly

10   preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly, where the biological functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. The following hydrophilicity values have been assigned to

15   amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It

20   is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5

25   are even more particularly preferred.

As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into

30   consideration are well known to those of skill in the art and include (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr),

(Thr: Ser), (Tip: Tyr), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents of a polypeptide as set forth above. In particular, embodiments of the polypeptides can include variants having about 50%, 60%, 70%, 80%, 90%,

5   and 95% sequence identity to the polypeptide of interest.

As used herein, "conservative" amino acid substitutions are substitutions wherein the substituted amino acid has similar structural or chemical properties.

As used herein, "non-conservative" amino acid substitutions are those

10   in which the charge, hydrophobicity, or bulk of the substituted amino acid is significantly altered.

As used herein, the term "identity," as known in the art, is a relationship between two or more polypeptide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of

15   sequence relatedness between polypeptide as determined by the match between strings of such sequences. "Identity" can also mean the degree of sequence relatedness of a polypeptide compared to the full-length of a reference polypeptide. "Identity" and "similarity" can be readily calculated by known methods, including, but not limited to, those described in

20   (Computational Molecular Biology, *Lesk, A. M.*, Ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, *Smith, D. W.*, Ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, *Griffin, A. M., and Griffin, H. G.*, Eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, *von Heinje, G.*,

25   Academic Press, 1987; and Sequence Analysis Primer, *Gribskov, M. and Devereux, J.*, Eds., M Stockton Press, New York, 1991; and *Carillo, H., and Lipman, D.*, SIAM J Applied Math., 48: 1073 (1988).

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity

30   and similarity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (*i.e.*, Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the *Needelman and*

Wunsch, (J. Mol. Biol., 48: 443-453, 1970) algorithm (*e.g.*, NBLAST, and XBLAST). The default parameters are used to determine the identity for the polypeptides of the present disclosure.

By way of example, a polypeptide sequence may be identical to the reference sequence, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from: at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in the reference polypeptide by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the reference polypeptide.

Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed method and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a ligand is disclosed and discussed and a number of modifications that can be made to a number of molecules including the ligand are discussed, each and every combination and permutation of ligand and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is

individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D.

5   Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Further, each of the materials, compositions, components, etc.

10   contemplated and disclosed as above can also be specifically and independently included or excluded from any group, subgroup, list, set, *etc.* of such materials.

These concepts apply to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed

15   compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

20   All methods described herein can be performed in any suitable order unless otherwise indicated or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the embodiments and does not pose a limitation on the scope of the embodiments unless otherwise

25   claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value

30   is incorporated into the specification as if it were individually recited herein.

Use of the term "about" is intended to describe values either above or below the stated value in a range of approx. +/- 10%; in other forms the values can range in value either above or below the stated value in a range of

approx. +/- 5%; in other forms the values can range in value either above or

below the stated value in a range of approx. +/- 2%; in other forms the values

can range in value either above or below the stated value in a range of

approx. +/- 1%. The preceding ranges are intended to be made clear by

5   context, and no further limitation is implied.

II.   Compositions

It has been established that polypeptides derived from intrinsically

disordered regions (IDRs) of proteins impart the function of enhanced

receptor signaling at the cell surface. Compositions including isolated

10   polypeptides of disordered regions of proteins ("IDR" polypeptides), and

fusion proteins incorporating the IDR polypeptides together with one or

more heterologous sequences are provided. Chimeric Antigen Receptors

(CAR) including one, two, three or more IDR polypeptides are provided (i.e.,

CAR-IDR). In preferred embodiments, the CAR-IDR fusion peptides include

15   at least one IDR polypeptide, for example, any of SEQ ID NOs:1-6. In

further preferred embodiments, the CAR-IDR fusion peptides have amino

acid sequence of any of SEQ ID NOs:7-27. Recombinant constructs

including nucleic acids expressing or encoding the polypeptides and fusion

proteins are also provided. Viral genomes including the recombinant

20   constructs, recombinant viruses including the constructs, and vaccine

formulations formed thereof are also provided.

The disclosed compositions and methods are especially applicable to

development of enhanced chimeric antigen receptor engineered T cell

therapy (CAR-T). Compositions of genetically modified T lymphocytes that

25   have enhanced anti-tumor activity are described.

A.   Intrinsically Disordered Regions (IDRs)

Intrinsically disordered regions (IDRs) are commonly present in

about 30% of the human proteome. They do not typically fold into a well-

defined 3-dimensional structure. However, some of them self-assemble via

30   weak inter and intra molecular interactions to form biomolecular

condensates, which drive the formation of membranelles organelles in the

intracellular environment. These condensates displayed unique biochemical

activities through enriching and organizing effector molecules to promote

signaling (Xiao, Q., McAtee, C. K. & Su, X. Nat Rev Immunol 22, 188-199;
Case, L. B., Ditlev, J. A. & Rosen, M. K. Annu Rev Biophys 48, 465-494,
doi:10.1146/annurev-biophys-052118-115534 (2019)).

Compositions including and encoding a polypeptide including a
5   fragment of the intrinsically disordered region gene product or variant
thereof, referred to herein as "IDR polypeptides", are provided. Typically,
the IDR polypeptide is between about 50 amino acids and about 500 amino
acids, or any specific integer number of amino acids therebetween,
including, but not limited to 60, 70, 80, 90, 100, 125, 150, 175, 200, 225,
10   250, 275, 300, 350, 400, or 450 amino acids; preferably, at least a length that
is sufficient to promote the condensation of CAR and enhance T cell
activation when forming a fusion protein with CAR. The IDR polypeptides,
nucleic acids encoding the same, and delivery vehicles thereof, and cells
including them can optionally include one or more additional heterologous
15   proteins, polypeptides, or other amino acid sequences. Typically, the
presence of one or more IDR polypeptides within the cytoplasmic domain of
a recombinant fusion peptide will impart an increased cell activation and/or
signaling to/from the fusion peptide. Thus, in some embodiments, IDR
domains are described as part of a fusion protein.
20   Recognition of the natural abundance and functional importance of
intrinsically disordered proteins (IDPs), and protein hybrids that contain both
intrinsically disordered regions (IDRs) and ordered regions, is changing
protein science. IDPs and IDRs, *i.e.,* functional proteins and protein regions
without unique structures, can often be found in all organisms, and typically
25   play vital roles in various biological processes. Disorder-based functionality
complements the functions of ordered proteins and domains. However, by
virtue of their existence, IDPs/IDRs, which are characterized by remarkable
conformational flexibility and structural plasticity, break multiple rules
established over the years to explain structure, folding, and functionality of
30   well-folded proteins with unique structures. Despite the general belief that
unique biological functions of proteins require unique 3D-structures (which
dominated protein science for more than a century), structure-less IDPs/IDRs

are functional, being able to engage in biological activities and perform tricks that are highly unlikely for ordered proteins.

The development of tools for reliable prediction of intrinsic disorder predisposition based on sequence data has presented the opportunity to

5   evaluate the commonness of IDPs/IDRs at the proteome level. Already, the first application of one of such tools to the Swiss Protein Database produced a very "big catch": long IDPRs of at least 40 consecutive residues were predicted to be present in over 15,000 proteins, and particularly high disorder scores were found for more than 1,000 proteins (Romero P, *et al.*, Pac Symp

10   Biocomput. (1998) 1998:437–48). Analysis of proteins encoded by 31 genomes from 3 kingdoms of life showed that eukaryotes typically have a higher disorder score than either archaea or prokaryotes, since 52–67% of eukaryotic proteins have long IDPRs as compared to 26–51 and 16–45% proteins with such long IDPRs in archaea and bacteria, respectively (Dunker

15   AK, *et al.*, Genome Inform Ser Workshop Genome Inform. (2000) 11:161–71; Oldfield CJ, *et al.*, Biochemistry (2005) 44:1989–2000). The natural abundance of intrinsic disorder was later supported by multiple comprehensive computational studies (Walsh I, *et al.*, Bioinformatics (2015) 31:201–8; Burra PV, *et al.*, PLoS ONE (2010) 5:e12069).

20   On multiple levels, ranging from amino acid composition to amino acid sequence, to structural content and spatial organization, ordered proteins, and domains are obviously much more complex than IDPs and IDRs. It seems that this global, multi-level simplicity of IDPs/IDRs is rooted in the peculiarities of their amino acid sequences, which are known to be

25   depleted in order-promoting residues (Trp, Cys, Tyr, Ile, Phe, Val, Asn, and Leu) and enriched in disorder-promoting residues (Arg, Pro, Gln, Gly, Glu, Ser, Ala, and Lys) and commonly contain repeats. Therefore, IDPs/IDRs are characterized by the reduced informational content of their amino acid sequences, and their amino acid alphabet is decreased in comparison with the

30   alphabet utilized in the amino acid sequences of ordered domains and proteins (Uversky VN. Intrins Disord Proteins (2016) 4:e1135015). Furthermore, since they do not need to gain any sort of ordered structure, IDPs/IDRs have a noticeably greater sequence space than that of ordered

proteins (Uversky VN. Biochim Biophys Acta (2013) 1834:932–51).
Therefore, the sequence simplicity associated with intrinsic disorder is
translated into an immensely expanded sequence space, which, in its turn,
gives rise to the enormous structural complexity of these proteins and

5   regions.

       Similarly, despite their inability to fold into unique structures in
isolation and despite their simplified spatial organization and globally
reduced structural content, IDPs are characterized by exceptional
spatiotemporal heterogeneity, where parts of an IDP are dis/ordered to

10   varying degrees at one moment in time, but can change state at a future point
in time (Uversky VN. Biochim Biophys Acta (2013) 1834:932–51; Uversky
VN. Intrins Disord Proteins (2016) 4:e1135015). Therefore, IDPs are not
homogeneous, but represent a very complex mixture of a broad variety of
partially foldable, potentially foldable, differently foldable, or completely

15   unfoldable segments. This behavior of an IDP as a highly frustrated system
that does not possess a singular folded state is reflected in its free energy
landscape, which is relatively flat and simple, lacks a deep energy minimum
that can be found within ordered proteins, and appears as a "hilly plateau,"
where the "hills" correspond to forbidden conformations (Fisher CK, Stultz

20   CM. Curr Opin Struct Biol. (2011) 21:426–31; Turoverov KK, Kuznetsova
IM, Uversky VN. Prog Biophys Mol Biol. (2010) 102:73–84; Uversky VN,
Oldfield CJ, Dunker AK. Annu Rev Biophys. (2008) 37:215–46). Such a
simplified and flattened energy landscape is extremely sensitive to different
environmental changes that can modify the landscape in several different

25   ways, lowering some energy minima while raising some energy barriers.
This explains the conformational plasticity of an IDP/IDR, its extreme
sensitivity to changes in the environment, its ability to interact with multiple
different partners, and consequently to fold in different ways. Therefore, this
exceptional spatio-temporal heterogeneity of IDPs/IDRs, where functional

30   elements have varying degrees of disorder and have unique responses to
environmental changes, is directly related the astonishing multifunctionality
of disordered proteins that are able to control, regulate, interact with, as well
as be controlled and regulated by, a plethora of structurally unrelated

partners. In summary, the multilevel structural, spatiotemporal, and sequential simplicity of IDPs/IDRs and their simplified energy landscapes define the structural heterogeneity and exceptional functional complexity of disordered proteins and regions.

5   Online tools can be used to identify regions of proteins that are intrinsically disordered. Exemplary online tools include IUPred2A. IUPred2A is a combined prediction tool designed to discover intrinsically disordered or conditionally disordered proteins and protein regions. Methods of analyzing protein disorder using IUPred2A has been previously described

10   (Mészáros B et al., Nucleic Acids Research 2018;46(W1):W329-W337; Erdős G and Dosztányi Z, Curr Protoc Bioinformatics. 2020 Jun;70(1):e99). In some embodiments, suitable IDRs are first determined using IUPred2A prior to using functional assays to assess their ability to promote condensation or CAR, T cell activation, and/or tumor cell cytotoxicity as

15   described in the Examples.

1.   Exemplary IDRs and Variants

In some embodiments, the IDR is one derived from RNA-binding protein FUS. An exemplary amino acid sequence of IDR derived from FUS is:

20   MASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYG
QSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQ
QPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQ
GYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGG
SGGYGQQDRG (SEQ ID NO:1)

25   In some embodiments, the IDR is one derived from RNA-binding protein EWS. RNA-binding protein EWS is a protein that in humans is encoded by the EWSR1 gene on human chromosome 22, specifically 22q12.2. An exemplary amino acid sequence of IDR derived from EWS is:
GYAQTTQAYGQQSYGTYGQPTDVSYTQAQTTATYGQTAYATSYGQPPTGYT

30   TPTAPQAYSQPVQGYGTGAYDTTTATVTTTQASYAAQSAYGTQPAYPAYGQ
QPAATAPTRPQDGNKPTETSQPQSSTGGYNQPSLGYGQSNYSYPQVPGSYP
MQPVTAPPSYPPTSYSSTQPTSYDQSSYSQQNTYGQPSSYGQQSSYGQQSS
YGQQPPTSYPPQTGSYSQAPSQYSQQSSSYG (SEQ ID NO:2)

In some embodiments, the IDR is one derived from TATA box
binding protein (TBP)-associated factor 15 (TAF15). An exemplary amino
acid sequence of IDR derived from TAF15 is:

MSDSGSYGQSGGEQQSYSTYGNPGSQGYGQASQSYSGYGQTTDSSYGQNYS

5   GYSSYGQSYSQSYGGYENQKQSSYSQQPYNNQGQQQNMESSGSQGGRAPSY

DQPDYGQQDSYDQQSGYDQHQGSYDEQSNYDQQHDSYSQNQQSYHSQRENY

SHHTQDDRRDVSRYGEDNRGYGGSQGG (SEQ ID NO:3)

In some embodiments, the IDR is one derived from nuclear pore
complex protein Nup98. An exemplary amino acid sequence of IDR derived

10   from Nup98 is:

FNKSFGTPFGGGTGGFGTTSTFGQNTGFGTTSGGAFGTSAFGSSNNTGGL

FGNSQTKPGGLFGTSSFSQPATSTSTGFGFGTSTGTANTLFGTASTGTSLF

SSQNNAFAQNKPTGFGNFGTSTSSGGLFGTTNTTSNPFGSTSGSLFGPSSF

TAAPTGTTIKFNPPTGTDTMVKAGVSTNISTKHQCITAMKEYESKSLEELR

15   LEDYQANRKGPQNQVGAGTTTGLFGSSPATSSATGLFSSSTTNSGFAYGQN

KTAFGTSTTGFGTNPGGLFGQQNQQTTSLFSKPFGQATTTQNTGFSFGNTS

TIGQPSTNTMGLFGVTQASQPGGLFGTATNTSTGT (SEQ ID NO:4)

In some embodiments, the IDR is one derived from TAR DNA
binding protein. An exemplary amino acid sequence of IDR derived from

20   TDP43 is:

SNRQLERSGRFGGNPGGFGNQGGFGNSRGGGAGLGNNQGSNMGGGMNFGAF

SINPAMMAAAQAALQSSWGMMGMLASQQNQSGPSGNNQNQGNMQREPNQAF

GSGNNSYSGSNSGAAIGWGSASNAGSGSGFNGGFGSSMDSKSSGWGM

(SEQ ID NO:5)

25   In some embodiments, the IDR is a synthetic polypeptide having the
desired functional properties. An exemplary amino acid sequence of a
synthetic IDR is:

SKGPGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPY

SGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGY (SEQ ID NO:6)

30   In some embodiments, the IDR is one, two, three, or more amino acid
sequences of SEQ ID NOs:1-6, or one, two, three or more copies of one,
two, three or more of SEQ ID NOs:1-6.

It has been discovered that the IDR polypeptide is sufficient to
promote the condensation of CAR and enhance T cell activation of a

recombinant fusion protein including the IDR polypeptide and one or more additional domains. Thus, compositions and methods of use of IDR peptide and fusion peptides thereof are provided. The compositions typically are, or include, an IDR polypeptide (e.g., SEQ ID NOs:1-6) or a functional

5   fragment or variant thereof, or a nucleic acid encoding the same. Functional fragments and variants can be, for example, any number of amino acids sufficient to drive enhanced condensation of CAR and T cell activation. The data below supports the conclusions that IDR enhances tumor cell killing by CAR-T cells having a CAR-IDR fusion, by increased condensation of CAR

10   and T cell activation.

        In some embodiments, the IDR polypeptide is between about 50 amino acids and about 500 amino acids, or any specific integer number of amino acids therebetween, including, but not limited to 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350, 400, or 450 amino acids;

15   preferably, at least a length that is sufficient to promote the condensation of CAR and enhance T cell activation. Variants can have, for example, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity to any one of SEQ ID NOs:1-6, or a functional fragment thereof; or the corresponding sequence of a homologue such as an orthologue or paralogue

20   of any of the foregoing sequences; or any combination thereof. In a particular embodiment, a variant IDR polypeptide has at least 75%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity to SEQ ID NO:1. In a particular embodiment, a variant IDR polypeptide has at least 75%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity to SEQ ID NO:2. In a

25   particular embodiment, a variant IDR polypeptide has at least 75%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity to SEQ ID NO:3. In a particular embodiment, a variant IDR polypeptide has at least 75%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity to SEQ ID NO:4. In a particular embodiment, a variant IDR polypeptide has at least 75%, 80%,

30   85%, 90%, 95%, 99% or 100% sequence identity to SEQ ID NO:5. In a particular embodiment, a variant IDR polypeptide has at least 75%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity to SEQ ID NO:6. Preferably variants maintain the ability to promote the condensation of CAR

and enhance T cell activation. In some embodiments, an IDR polypeptide variant is considered to be "functional" if it maintains the ability to promote the condensation of CAR and enhance T cell activation. In some embodiments, IDR polypeptide variants are identified as functional if they

5   enhance cytotoxicity *in vitro* or *in vivo* towards target cells.

Any of the IDR polypeptide sequences including the amino acid sequences of SEQ ID NOs:1-6 can include one or more amino acid substitutions. Typically, the amino acid substitutions do not impact the ability of the IDR domain of a fusion peptide to promote cell activation

10   and/or signaling, for example in the case of CAR-IDR fusion proteins. Amino acid substitutions within CT peptides are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions of amino acids within any of SEQ ID NOs:1-6 can include

15   (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Tip: Tyr), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological

20   equivalents of an IDR polypeptide, as set forth in any one of SEQ ID NOs:1-6. In particular embodiments, the IDR polypeptides can include variants having about 50%, 60%, 70%, 80%, 90%, and 95% sequence identity to the polypeptide of SEQ ID NOs:1-6. Such variants and fragments can be used alone or in combination with each other.

25   In preferred embodiments, suitable IDRs are those sufficient to induce biomolecular condensates inside a eukaryotic cell or on the cell surface.

B.   IDR Fusion Peptides

IDR fusion proteins, including one or more heterologous polypeptide

30   sequences fused to one or more IDR polypeptides are provided.

The term "IDR domain" is used exclusively in the context of fusion peptides that include one or more copies of one or more IDR polypeptides, to

39

refer to the component of the fusion peptide that includes the IDR polypeptide(s).

The term "IDR fusion protein" or "IDR fusion peptide" or "IDR fusion" refers to a polypeptide that includes an IDR domain and one or more heterologous amino acid sequences. A heterologous sequence for inclusion within an IDR fusion protein is not a component of an IDR peptide.

The term "Fusion peptide domain" refers to a heterologous sequence that is directly or indirectly fused to an IDR domain. Typically, a fusion peptide domain includes at least one amino acid that is on the extracellular side of the cell membrane, *i.e.*, constitutes an extracellular domain. In some embodiments, the fusion peptide domain also includes a transmembrane domain.

In some embodiments, IDR-fusion proteins are engineered to include one or more IDR domains as a cytosolic component of the IDR fusion protein. In preferred embodiments, IDR fusion peptides include one or more heterologous polypeptides fused to an intracellular component, including an IDR domain that includes one or more of SEQ ID NOs:1-6, or a functional homologue or variant thereof. In other embodiments, IDR-fusion proteins are engineered to include one or more IDR domains as an extracellular component of the IDR fusion protein.

An exemplary heterologous polypeptide for inclusion within IDR-fusion peptides includes the ectodomain and transmembrane domain of a cell-surface protein, such as an ectodomain of a cell-surface receptor that is not a component of the protein where the IDR is derived from.

In some embodiments, IDR-fusion proteins include one or more extracellular polypeptide domains, and one or more transmembrane domains, and optionally an intracellular domain, where the transmembrane domain or optionally the intracellular domain is fused to the IDR domain(s). In some embodiments, where a fusion protein includes one or more intracellular domains, the one or more IDR domains are fused to the amino (N) or carboxyl (C) terminus of the intracellular domains. In some embodiments, the fusion proteins include an entire endogenous protein fused to one or more IDR domains.

40

1.   Heterologous Sequences

Heterologous elements that can be associated with, linked, conjugated, or otherwise attached directly or indirectly to the IDR polypeptide sequence(s), or nucleic acids expressing the IDR polypeptides

5   are disclosed. Such molecules include, but are not limited to, protein domains, such as transduction domains, fusogenic peptides, targeting molecules, and sequences that enhance protein expression and/or isolation. Suitable protein domains include ectodomains, transmembrane domains, cytoplasmic domains of proteins and macromolecular structures including

10   combinations of ectodomains, transmembrane domains, and cytoplasmic domains. Typically, the other protein domains are not proteins from which the IDR domain is derived. In some embodiments, the other protein domains have or have potential for one or more molecular functions or activities. Such "functional" domains can be engineered to provide one or more

15   functions or activities, as desired. Exemplary functions include receptor or ligand binding, enzymic activity, and molecular transport, such as active transport of one or more molecules into or out of one or more cellular compartments. In some embodiments, the other protein domains within an IDR fusion protein bind to a specific substrate or molecule. An exemplary

20   molecule is an antigen or a cell-surface receptor.

Thus, in some embodiments, IDR fusion peptides include one or more heterologous peptide domains, such as receptors at the surface of a cell, optionally including a transmembrane domain that anchors or connects the ectodomain to the cell surface and connects with the intracellular IDR

25   domain.

Exemplary cell surface receptors coordinate the activity of cells upon interaction with other cells, such as immune cells, such as T cells. For example, in some embodiments, the heterologous domain is a recombinant or engineered chimeric antigen receptor (CAR). In other embodiments, the

30   heterologous domain includes a specific transmembrane domain (*e.g.*, transmembrane domain of CD8 or CD28) for further enhancing signaling and receptor sensitivity in the case of CAR. In further embodiments, the heterologous domain is a co-signaling domain. Exemplary co-signaling

domains have been previously described (Majzner, R. G. *et al.,* Cancer Discov, doi:10.1158/2159-8290.CD-19-0945 (2020); Heitzeneder, S. *et al.,* Cancer Cell 40, 53-69 e59, (2022); Priceman, S. J. *et al.,* Oncoimmunology 7, e1380764, (2018)) In some embodiments, the fusion peptides include multiple heterologous domains, such as a CAR domain (*e.g.,* a single chain variable fragment (scFv, FMC63) targeting CD19), a transmembrane domain of CD8α, cytosolic signaling domains or a fragment there of derived from CD28, 41BB, and CD3ζ.

a.   Chimeric Antigen Receptors (CAR)

In some forms, the fusion protein includes a Chimeric Antigen Receptor (CAR) fused with one or more IDR domains ("CAR-IDR"). Typically, CARs include a transmembrane domain and one or more intracellular/cytoplasmic domains.

In the first generation of CARs, there was only one intracellular signal component (e.g., CD3 zeta). The second generation of CAR added one costimulatory molecule on the basis of the first generation (e.g., CD28, 4-1BB). Based on the second generation of CARs, the third generation of CAR added another costimulatory molecule. Fourth-generation of CAR T cells can activate the downstream transcription factor to induce cytokine production after the CAR recognizes the target antigens. The fifth-generation of CARs, based on the second generation, uses gene editing to inactivate the TRAC gene, leading to the removal of the TCR alpha and beta chains. See, e.g., Zhao, Lijun & Cao, Yu. (2019). Engineered T Cell Therapy for Cancer in the Clinic. *Frontiers in Immunology.* 10. 10.3389/fimmu.2019.02250. Any of these CAR strategies and structures can serve as a starting point for the addition of one or more IDRs.

Thus, CAR fusion proteins are modular in structure, containing several discrete domains (e.g., additional examples discussed below). The IDR can be added or inserted into a CAR fusion protein at any location (e.g., between, before, or after existing domains) provided the CAR remains functional (e.g., the activity in antigen binding and in activating T cells). For example, the IDR can be inserted/added before or after the scFv or

equivalent domains that bind antigens, or any intracellular signaling domains (e.g., co-stimulatory domain(s)) of the CAR fusion protein.

Therefore, in some embodiments, a CAR-IDR fusion protein includes an entire CAR with one or more IDR domains fused at the C terminus. In
5   some embodiments, the CAR includes an intracellular domain that includes a component of endogenous CD28, 41BB, and/or CD3 zeta protein. Therefore, in some embodiments, a CAR-IDR fusion protein includes an entire CAR, with one or more IDR domains fused directly to the end of the CD3 zeta region, such that both the CD3 zeta and contiguous IDT domain(s) are
10   expressed in the cytoplasmic compartment. Additionally or alternatively, IDR(s) can be fused before or after (e.g., N-terminal or C-terminal to) the antigen binding domain, transmembrane domain, CD28, 41BB, CD3 zeta protein, or other intracellular or co-stimulatory domains.

i. CAR structure

15   CARs are engineered receptors that possess both antigen-binding and T-cell-activating functions. Immunotherapy using T cells genetically engineered to express a CAR is rapidly emerging as a superior new treatment for hematological and non- hematological malignancies. Based on the location of the CAR in the membrane of the cell, the CAR can be divided
20   into three main distinct domains, including an extracellular antigen-binding domain, followed by a space region, a transmembrane domain, and the intracellular signaling domain. The antigen-binding domain, most commonly derived from variable regions of immunoglobulins, typically contains VH and VL chains that are joined up by a linker to form the so-called "scFv."
25   The segment interposing between the antigen-binding domain (*e.g.*, scFv) and the transmembrane domain is a "spacer domain." The spacer domain can include the constant IgG1 hinge-CH2–CH3 Fc domain. In some cases, the spacer domain and the transmembrane domain are derived from CD8. The intracellular signaling domains mediating T cell activation can include a
30   CD3ζ co-receptor signaling domain derived from C-region of the TCR α and β chains and one or more costimulatory domains. In some forms, conjugation with one or more IDR domains includes addition of the one or more IDR domains immediately next to the costimulatory domains of the CAR.

In some forms, the antigen-binding domain is derived from an antibody. The term antibody herein refers to natural or synthetic polypeptides that bind a target antigen. The term includes polyclonal and monoclonal antibodies, including intact antibodies and functional (*e.g.*,

5   antigen-binding) antibody fragments, including Fab fragments, F(ab')$_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise

10   modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, *e.g.*, bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. The term also encompasses intact or full-length antibodies,

15   including antibodies of any class or subclass, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD. The antigen-binding domain of a CAR can contain complementary determining regions (CDR) of an antibody, variable regions of an antibody, and/or antigen binding fragments thereof. For example, the antigen-binding domain for a CD19 CAR can be derived from a

20   human monoclonal antibody to CD19, such as those described in U.S. Patent 7,109,304, which is specifically incorporated by reference herein in its entirety for use in accordance with the disclosed compositions and methods. In some forms, the antigen-binding domain can include an F(ab')2, Fab', Fab, Fv or scFv.

25   In some forms, the CAR includes one or more spacer domain(s) (also referred to as hinge domain) that is located between the extracellular antigen-binding domain and the transmembrane domain. A spacer domain is an amino acid segment that is generally found between two domains of a protein and may allow for flexibility of the protein and movement of one or

30   both of the domains relative to one another. Any amino acid sequence that provides such flexibility and movement of the extracellular antigen-binding domain relative to the transmembrane domain can be used. The spacer domain can be a spacer or hinge domain of a naturally occurring protein. In

some forms, the hinge domain is derived from CD8α, such as, a portion of the hinge domain of CD8α, *e.g.*, a fragment containing at least 5 (*e.g.*, 5, 10, 15, 20, 25, 30, 35, or 40) consecutive amino acids of the hinge domain of CD8α. Hinge domains of antibodies, such as an IgG, IgA, IgM, IgE, or IgD antibodies can also be used. In some forms, the hinge domain is the hinge domain that joins the constant CH1 and CH2 domains of an antibody. Non-naturally occurring peptides may also be used as spacer domains. For example, the spacer domain can be a peptide linker, such as a (GxS)n linker, wherein x and n, independently can be an integer of 3 or more, including 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more.

In some forms, the CAR includes a transmembrane domain that can be directly or indirectly fused to the antigen-binding domain. The transmembrane domain may be derived either from a natural or a synthetic source. In some forms, the transmembrane domain of the CAR includes a transmembrane domain of an alpha, beta, or zeta chain of a T-cell receptor, CD8, CD4, CD28, CD137, CD80, CD86, CD152 (CTLA-4) or PD1, or a portion thereof. Transmembrane domains can also contain at least a portion of a synthetic, non-naturally occurring protein segment. In some forms, the transmembrane domain is a synthetic, non-naturally occurring alpha helix or beta sheet. In some forms, the protein segment is at least about 15 amino acids, *e.g.*, at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acids. Examples of synthetic transmembrane domains are known in the art, for example in U.S. Patent No. 7,052,906 and PCT Publication No. WO 2000/032776.

The intracellular signaling domain is responsible for activation of at least one of the normal effector functions of the immune effector cell expressing the CAR. The term effector function refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. In some forms, an intracellular signaling domain includes the zeta chain of the T cell receptor or any of its homologs (*e.g.*, eta, delta, gamma, or epsilon), MB1 chain, B29, Fc RIII, Fc RI and combinations of signaling molecules such as CD3ζ and CD28, 4-1BB, OX40 and combination thereof, as well as other

similar molecules and fragments. Intracellular signaling portions of other members of the families of activating proteins can be used, such as FcγRIII and FcεRI.

5 Many immune effector cells require co-stimulation, in addition to stimulation of an antigen-specific signal, to promote cell proliferation, differentiation and survival, as well as to activate effector functions of the cell. Therefore, in some forms, the CAR includes at least one co-stimulatory signaling domain. The term co-stimulatory signaling domain, refers to at least a portion of a protein that mediates signal transduction within a cell to

10 induce an immune response such as an effector function. The co-stimulatory signaling domain can be a cytoplasmic signaling domain from a co-stimulatory protein, which transduces a signal and modulates responses mediated by immune cells, such as T cells, NK cells, macrophages, neutrophils, or eosinophils. In some forms, the co-stimulatory signaling

15 domain is derived from a co-stimulatory molecule selected from CD27, CD28, CD137, 0X40, CD30, CD40, CD3, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, ligands of CD83 and combinations thereof. In one form, the co-stimulatory signaling domain is derived from CD28.

CARs can be used in order to generate immuno-responsive cells, such

20 as T cells, specific for selected targets, such as malignant cells, with a wide variety of receptor chimera constructs having been described (see U.S. Patent Nos. 5,843,728; 5,851,828; 5,912,170; 6,004,811; 6,284,240; 6,392,013; 6,410,014; 6,753,162; 8,211,422; and PCT Publication WO 9215322, each of which is specifically incorporated by reference herein in its entirety).

25 Alternative CAR constructs can be characterized as belonging to successive generations. First-generation CARs typically include a single-chain variable fragment of an antibody specific for an antigen, for example including a VL linked to a VH of a specific antibody, linked by a flexible linker, for example by a CD8α hinge domain and a CD8α transmembrane domain, to the

30 transmembrane and intracellular signaling domains of either CD3ζ or FcRγ (scFv-CD3ζ or scFv- FcRγ; see U.S. Patent No. 7,741,465; U.S. Patent No. 5,912,172; U.S. Patent No. 5,906,936, each of which is specifically incorporated by reference herein in its entirety). Second-generation CARs

incorporate the intracellular domains of one or more costimulatory molecules, such as CD28, OX40 (CD134), or 4-1BB (CD137) within the endodomain (for example scFv-CD28/OX40/4-1BB-CD3ζ; see U.S. Patent Nos.8,911,993; 8,916,381; 8,975,071; 9,101,584; 9,102,760; 9,102,761, each

5   of which is specifically incorporated by reference herein in its entirety). Third-generation CARs include a combination of costimulatory endodomains, such a CD3ζ-chain, CD97, GDI la-CD18, CD2, ICOS, CD27, CD154, CDS, OX40, 4-1BB, or CD28 signaling domains (for example scFv-CD28-4-1BB-CD3ζ or scFv-CD28-OX40-CD3ζ; see U.S. Patent

10   No.8,906,682; U.S. Patent No.8,399,645; U.S. Pat. No. 5,686,281; PCT Publication No. WO2014134165; PCT Publication No. WO2012079000, each of which is specifically incorporated by reference herein in its entirety). Alternatively, co-stimulation can be orchestrated by expressing CARs in antigen-specific T cells, chosen so as to be activated and expanded following

15   engagement of their native αβTCR, for example by antigen on professional antigen-presenting cells, with attendant co-stimulation. Any of the first, second, or third generation CARs described above can be used in accordance with the disclosed compositions and methods.

ii. Exemplary Antigens and CAR Embodiments

20   The target specificity of the cell expressing a CAR is determined by the antigen recognized by the antibody/ectodomain . The disclosed compositions and methods can be used to create constructs, and cells expressing the constructs, that target any antigen. In the context of immunotherapy, particularly cancer immunotherapy, numerous antigens, and

25   suitable ectodomains for targeting them, are well known. Unlike the native TCR, the majority of scFv-based CARs recognize target antigens expressed on the cell surface rather than internal antigens that are processed and presented by the cells' MHC, however, CARs have the advantage over the classical TCR that they can recognize structures other than protein epitopes,

30   including carbohydrates and glycolipids Dotti, et al., *Immunol Rev.* 2014 January; 257(1): . doi:10.1111/imr.12131 (35 pages) thus increasing the pool of potential target antigens. Preferred targets include antigens that are only expressed on cancer cells or their surrounding stroma (Cheever, et al., *Clin*

Cancer Res.,15:5323–5337 (2009)), such as the splice variant of EGFR (EGFRvIII), which is specific to glioma cells (Sampson, et al., *Semin Immunol.*, 20(5):267-75 (2008)). However, human antigens meet this requirement, and the majority of target antigens are expressed either at low

5   levels on normal cells (e.g. GD2, CAIX, HER2) and/or in a lineage restricted fashion (e.g. CD19, CD20).

Preferred targets, and CARs that target them are known in the art (see, e.g., Dotti, et al., *Immunol Rev.* 2014 January; 257(1): . doi:10.1111/imr.12131 (35 pages). For example, CAR targets for

10   hematological malignancies include, but are not limited to, CD 19 (e.g., B-cell) (Savoldo, et al., *J Clin Invest.*, 121:1822-1826 (2011), Cooper, et al., *Blood*, 105:1622-1631 (2005); Jensen, et al., *Biol Blood Marrow Transplant* (2010), Kochenderfer, et al., *Blood*, 119:2709-2720 (2012), Brentjens, et al., *Molecular Therapy*, 17:S157 (2009), Brentjens, et al., *Nat Med.*, 9:279-286

15   (2003), Brentjens, et al., *Blood*, 118:4817-4828 (2011), Porter, et al., *N Engl J Med.*, 365:725-733 (2011), Kalos, et al., *Sci Transl Med.*, 3:95ra73 (2011), Brentjens, et al., *Sci Transl Med.*, 5:177ra38 (2013), Grupp, et al., *N Engl J Med* (2013)); CD20 (e.g., B-cell) (Jensen, et al., *Biol Blood Marrow Transplant* (2010), Till, et al., *Blood*, 112:2261-2271 (2008), Wang, et al.,

20   *Hum Gene Ther.*, 18:712-725 (2007), Wang, et al., *Mol Ther.*, 9:577-586 (2004), Jensen, et al., *Biol Blood Marrow Transplant*, 4:75-83 (1998)); CD22 (e.g., B-cell) (Haso, et al., *Blood*, 121:1165-1174 (2013)); CD30 (e.g., B-cell) (Di Stasi, et al., *Blood*, 113:6392-6402 (2009), Savoldo, et al., *Blood*, 110:2620-2630 (2007), Hombach, et al., *Cancer Res.*, 58:1116-1119 (1998));

25   CD33 (e.g., Myeloid) (Finney, et al., *J Immunol.*, 161:2791-2797 (1998)); CD70 (e.g., B-cell/T-cell) (Shaffer, et al., *Blood*, 117:4304-4314 (2011)); CD123 (e.g., Myeloid) (Tettamanti, et al., *Br J Haematol.*, 161:389-401 (2013)); Kappa (e.g., B-cell) (Vera, et al., *Blood*, 108:3890-3897 (2006)); Lewis Y (e.g., Myeloid) (Peinert, et al., *Gene Ther.*, 17:678-686 (2010),

30   Ritchie, et al., *Mol Ther.* (2013)); NKG2D ligands (e.g., Myeloid) (Barber, et al., *Exp Hematol.*, 36:1318-1328 (2008), Lehner, et al., *PLoS One.*, 7:e31210 (2012), Song, et al., *Hum Gene Ther.*, 24:295-305 (2013), Spear, et al., *J*

Immunol. 188:6389-6398 (2012)); ROR1 (e.g., B-cell) (Hudecek, et al., *Clin Cancer Res.* (2013)).

CAR targets for solid tumors include, but are not limited to, B7H3 (e.g., sarcoma, glioma) (Cheung, et al., *Hybrid Hybridomics*, 22:209–218

5   (2003)); CAIX (e.g., kidney) (Lamers, et al., *J Clin Oncol.*, 24:e20–e22. (2006)), Weijtens, et al., *Int J Cancer*, 77:181–187 (1998)); CD44 v6/v7 (e.g., cervical) (Hekele, et al., *Int J Cancer*, 68:232-238 (1996)), Dall, et al., *Cancer Immunol Immunother,* 54:51-60 (2005); CD171 (e.g., neuroblastoma) (Park, et al., *Mol Ther.*, 15:825-833 (2007)); CEA (e.g.,

10   colon) (Nolan, et al., *Clin Cancer Res.*, 5:3928-3941 (1999)); EGFRvIII (e.g., glioma) (Bullain, et al., *J Neurooncol.* (2009), Morgan, et al., *Hum Gene Ther.*, 23:1043-1053 (2012)); EGP2 (e.g., carcinomas) (Meier, et al., *Magn Reson Med.*, 65:756-763 (2011), Ren-Heidenreich, et al., *Cancer Immunol Immunother.*, 51:417-423 (2002)); EGP40 (e.g., colon) (Daly, et

15   al., *Cancer Gene Ther.*, 7:284-291 (2000); EphA2 (e.g., glioma, lung) (Chow, et al., *Mol Ther.*, 21:629-637 (2013)); ErbB2(HER2) (e.g., breast, lung, prostate, glioma) (Zhao, et al., *J Immunol.*, 183:5563-5574 (2009), Morgan, et al., *Mol Ther.*, 18:843-851 (2010), Pinthus, et al., 114:1774-1781 (2004), Teng, et al., *Hum Gene Ther.*, 15:699-708 (2004), Stancovski, et al.,

20   *J Immunol.*, 151:6577-6582 (1993), Ahmed, et al., *Mol Ther.*, 17:1779-1787 (2009), Ahmed, et al., *Clin Cancer Res.*, 16:474-485 (2010), Moritz, et al., *Proc Natl Acad Sci U.S.A.,* 91:4318-4322 (1994)); ErbB receptor family (e.g., breast, lung, prostate, glioma) (Davies, et al., *Mol Med.*, 18:565-576 (2012)); ErbB3/4 (e.g., breast, ovarian) (Muniappan, et al., *Cancer Gene*

25   *Ther.*, 7:128-134 (2000), Altenschmidt, et al., *Clin Cancer Res.*, 2:1001-1008 (1996)); HLA-A1/MAGE1 (e.g., melanoma) (Willemsen, et al., *Gene Ther.*, 8:1601-1608 (2001), Willemsen, et al., *J Immunol.*, 174:7853-7858 (2005)); HLA-A2/NY-ESO-1 (e.g., sarcoma, melanoma) (Schuberth, et al., *Gene Ther.*, 20:386-395 (2013)); FR-α (e.g., ovarian) (Hwu, et al., *J Exp Med.*,

30   178:361-366 (1993), Kershaw, et al., *Nat Biotechnol.*, 20:1221-1227 (2002), Kershaw, et al., *Clin Cancer Res.,* 12:6106-6115 (2006), Hwu, et al., *Cancer Res.*, 55:3369-3373 (1995)); FAP (e.g., cancer associated fibroblasts) (Kakarla, et al., *Mol Ther.* (2013)); FAR (e.g., rhabdomyosarcoma)

(Gattenlohner, et al., *Cancer Res.*, 66:24-28 (2006)); GD2 (e.g.,

neuroblastoma, sarcoma, melanoma) (Pule, et al., *Nat Med.*, 14:1264-1270

(2008), Louis, et al., *Blood*, 118:6050-6056 (2011), Rossig, et al., *Int J*

Cancer., 94:228-236 (2001)); GD3 (e.g., melanoma, lung cancer) (Yun, et

5   al., *Neoplasia.*, 2:449-459 (2000)); HMW-MAA (e.g., melanoma) (Burns, et

al., *Cancer Res.*, 70:3027-3033 (2010)); IL11Rα (e.g., osteosarcoma)

(Huang, et al., *Cancer Res.*, 72:271-281 (2012)); IL13Rα2 (e.g., glioma)

(Kahlon, et al., *Cancer Res.*, 64:9160-9166 (2004), Brown, et al., *Clin*

Cancer Res. (2012), Kong, et al., *Clin Cancer Res.*, 18:5949-5960 (2012),

10   Yaghoubi, et al., *Nat Clin Pract Oncol.*, 6:53-58 (2009)); Lewis Y (e.g.,

breast/ovarian/pancreatic) (Peinert, et al., *Gene Ther.*, 17:678-686 (2010),

Westwood, et al., *Proc Natl Acad Sci U.S.A.*, 102:19051-19056 (2005),

Mezzanzanica, et al., *Cancer Gene Ther.*, 5:401-407 (1998)); Mesothelin

(e.g., mesothelioma, breast, pancreas) (Lanitis, et al., *Mol Ther.*, 20:633-643

15   (2012), Moon, et al., *Clin Cancer Res.*, 17:4719-4730 (2011)); Muc1 (e.g.,

ovarian, breast, prostate) (Wilkie, et al., *J Immunol.*, 180:4901-4909 (2008));

NCAM (e.g., neuroblastoma, colorectal) (Gilham, et al., *J Immunother.*,

25:139-151 (2002)); NKG2D ligands (e.g., ovarian, sacoma) (Barber, et al.,

Exp Hematol., 36:1318-1328 (2008), Lehner, et al., *PLoS One*, 7:e31210

20   (2012), Song, et al., *Gene Ther.*, 24:295-305 (2013), Spear, et al., *J*

Immunol., 188:6389-6398 (2012)); PSCA (e.g., prostate, pancreatic)

(Morgenroth, et al., *Prostate*, 67:1121-1131 (2007), Katari, et al., *HPB*,

13:643-650 (2011)); PSMA (e.g., prostate) (Maher, et al., *Nat Biotechnol.*,

20:70-75 (2002), Gong, et al., *Neoplasia.*, 1:123-127 (1999)); TAG72 (e.g.,

25   colon) (Hombach, et al., *Gastroenterology*, 113:1163-1170 (1997),

McGuinness, et al., *Hum Gene Ther.*, 10:165-173 (1999)); VEGFR-2 (e.g.,

tumor vasculature) (*J Clin Invest.*, 120:3953-3968 (2010), Niederman, et al.,

Proc Natl Acad Sci U.S.A., 99:7009-7014 (2002)).

        In some forms, the CAR targets one or more antigens specific for

30   cancer, an inflammatory disease, a neuronal disorder, HIV/AIDS, diabetes, a

cardiovascular disease, an infectious disease, an autoimmune disease, or

combinations thereof. One of skill in the art, based on general knowledge in

the field and/or routine experimentation would be able to determine the

appropriate antigen to be targeted by a CAR for a specific disease, disorder, or condition.

Exemplary antigens specific for cancer that could be targeted by the CAR include, but are not limited to, 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD 152, CD 19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgGl, Ll-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, vimentin, and combinations thereof.

In preferred forms, the CAR targets CD19, CD22, HER2 or any combinations thereof.

The results presented below show that coiled-coiled domains can reduce membrane localization of and signaling from CAR fusion proteins. Thus, in some embodiments, the IDR fusion proteins, particularly the IDR-CAR fusion proteins, lack coiled-coiled domain(s).

b.   Exemplary Chimeric Antigen Receptors including IDR domains (CAR-IDR)

In some forms, the fusion peptide is a CAR including an IDR polypeptide conjugated to the carboxyl terminus of the CAR. An exemplary schematic for conjugation of a functional CAR with an IDR domain is provided in Figures 1A, 3A, and 4A.

In exemplary embodiments, the structure of a CAR-IDR is a fusion protein having the following features:

N-[CAR]-[IDR]_Z-C, where "N" and "C" refer to the amino (NH₂) and

Carboxyl (COOH) termini, respectively, "CAR" refers to a single contiguous

CAR fusion protein, "IDR" refers to an IDR and "Z" is an integer between

one and four, inclusive, and. Preferably, Z is 1 or 2.

5   In some embodiments, the structure of a CAR-IDR is a fusion protein

having the following features: N-[CAR part 1]-[IDR]z-[CAR part 2], where "N"

and "C" refer to the amino (NH₂) and Carboxyl (COOH) termini, respectively,

"IDR" refers to an IDR and "Z" is an integer between one and four, inclusive,

"CAR-part 1" includes one or more traditional CAR domains and "CAR-part 2"

10   includes one or more traditional CAR domains such that without the intervening

[IDR]z domain, CAR-part 1 and CAR-part 2 form a single contiguous CAR fusion

protein. Preferably, Z is 1 or 2.

         In some embodiments, a CAR-IDR including one IDR domain fused

to the cytoplasmic domain of the functional CAR includes the amino acid

15   sequence of any of SEQ ID NOs:1-6 fused to the last residue of the CAR.

         In some embodiments, the fusion peptide is a CAR including one or more

IDR domains. An exemplary CAR-IDR is an anti-CD19 CAR-IDR. An exemplary

amino acid sequence anti-CD19 CAR-IDR including an IDR derived from FUS is:

         MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

20   KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

         DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG

         LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS

         ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

         GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC

25   RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

         HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

         VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

         RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

         GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSGSGSGSGSMASNDY

30   TQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSS

         YGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSS

         TSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQN

         QYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQ

         QDRG (SEQ ID NO:7)

In some embodiments, the CAR-IDR is further conjugated to one or more fluorescent tags such as GFP. An exemplary amino acid sequence of an anti-CD19 CAR-IDR including an IDR derived from FUS and conjugated thereto a GFP is:

5

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE
DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

10

GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL
HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

15

GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSGSGSGSGSMASNDY
TQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSS
YGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSS
TSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQN
QYNSSSGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQ

20

QDRGGSGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLT
LKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQ
ERTISFKDDGTYKTRAEVKFEGDTLVNRIELKG
IDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQL
ADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAGI

25

THGMDELYK (SEQ ID NO:8)

Another exemplary amino acid sequence anti-CD19 CAR-IDR including an IDR derived from TAF15 is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

30

DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGGSGGTSMSDSGSYGQ
SGGEQQSYSTYGNPGSQGYGQASQSYSGYGQTTDSSYGQNYSGYSSYGQSY
SQSYGGYENQKQSSYSQQPYNNQGQQQNMESSGSQGGRAPSYDQPDYGQQD
SYDQQSGYDQHQGSYDEQSNYDQQHDSYSQNQQSYHSQRENYSHHTQDDRR
DVSRYGEDNRGYGGSQGG (SEQ ID NO:9)

In some embodiments, the CAR-IDR is further conjugated to one or more fluorescent tags such as GFP. An exemplary amino acid sequence of an anti-CD19 CAR-IDR including an IDR derived from TAF15 and conjugated thereto a GFP is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE
DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL
HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGGSGGTSMSDSGSYGQ
SGGEQQSYSTYGNPGSQGYGQASQSYSGYGQTTDSSYGQNYSGYSSYGQSY
SQSYGGYENQKQSSYSQQPYNNQGQQQNMESSGSQGGRAPSYDQPDYGQQD
SYDQQSGYDQHQGSYDEQSNYDQQHDSYSQNQQSYHSQRENYSHHTQDDRR
DVSRYGEDNRGYGGSQGGGSGSMSKGEELFTGVVPILVELDGDVNGHKFSV
RGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKR
HDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFK
EDGNILGHKLEYNFNSHNVYITADKQKNGIKAN
FKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKR
DHMVLLEFVTAAGITHGMDELYK (SEQ ID NO:10)

An exemplary amino acid sequence anti-CD19 CAR-IDR including an IDR derived from EWS is:

```
MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE
DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL
HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRTGSGSMGYAQTTQAY
GQQSYGTYGQPTDVSYTQAQTTATYGQTAYATSYGQPPTGYTTPTAPQAYS
QPVQGYGTGAYDTTTATVTTTQASYAAQSAYGTQPAYPAYGQQPAATAPTR
PQDGNKPTETSQPQSSTGGYNQPSLGYGQSNYSYPQVPGSYPMQPVTAPPS
YPPTSYSSTQPTSYDQSSYSQQNTYGQPSSYGQQSSYGQQSSYGQQPPTSY
PPQTGSYSQAPSQYSQQSSSYG  (SEQ ID NO:11)
```

In some embodiments, the CAR-IDR is further conjugated to one or more fluorescent tags such as GFP. An exemplary amino acid sequence of an anti-CD19 CAR-IDR including an IDR derived from EWS and a GFP conjugated thereto is:

```
MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE
DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL
HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRTGSGSMGYAQTTQAY
GQQSYGTYGQPTDVSYTQAQTTATYGQTAYATSYGQPPTGYTTPTAPQAYS
QPVQGYGTGAYDTTTATVTTTQASYAAQSAYGTQPAYPAYGQQPAATAPTR
PQDGNKPTETSQPQSSTGGYNQPSLGYGQSNYSYPQVPGSYPMQPVTAPPS
```

YPPTSYSSTQPTSYDQSSYSQQNTYGQPSSYGQQSSYGQQSSYGQQPPTSY

PPQTGSYSQAPSQYSQQSSSYGTSGSGSMSKGEELFTGVVPILVELDGDVN

GHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRY

PDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIEL

5   KGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSV

QLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAA

GITHGMDELYK (SEQ ID NO:12)

An exemplary amino acid sequence anti-CD19 CAR-IDR including an IDR derived from Nup98 is:

10 MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG

LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS

ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

15 GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

20 GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGGSGGFNKSFGTPFGG

GTGGFGTTSTFGQNTGFGTTSGGAFGTSAFGSSNNTGGLFGNSQTKPGGLF

GTSSFSQPATSTSTGFGFGTSTGTANTLFGTASTGTSLFSSQNNAFAQNKP

TGFGNFGTSTSSGGLFGTTNTTSNPFGSTSGSLFGPSSFTAAPTGTTIKFN

PPTGTDTMVKAGVSTNISTKHQCITAMKEYESKSLEELRLEDYQANRKGPQ

25 NQVGAGTTTGLFGSSPATSSATGLFSSSTTNSGFAYGQNKTAFGTSTTGFG

TNPGGLFGQQNQQTTSLFSKPFGQATTTQNTGFSFGNTSTIGQPSTNTMGL

FGVTQASQPGGLFGTATNTSTGT (SEQ ID NO:13)

In some embodiments, the CAR-IDR is further conjugated to one or more fluorescent tags such as GFP. An exemplary amino acid sequence of an

30 anti-CD19 CAR-IDR including an IDR derived from Nup98 and a GFP conjugated thereto is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG

LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS

ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

5   HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

VQTTQEEDGCSCRFPEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGGSGGFNKSFGTPFGG

GTGGFGTTSTFGQNTGFGTTSGGAFGTSAFGSSNNTGGLFGNSQTKPGGLF

10   GTSSFSQPATSTSTGFGFGTSTGTANTLFGTASTGTSLFSSQNNAFAQNKP

TGFGNFGTSTSSGGLFGTTNTTSNPFGSTSGSLFGPSSFTAAPTGTTIKFN

PPTGTDTMVKAGVSTNISTKHQCITAMKEYESKSLEELRLEDYQANRKGPQ

NQVGAGTTTGLFGSSPATSSATGLFSSSTTNSGFAYGQNKTAFGTSTTGFG

TNPGGLFGQQNQQTTSLFSKPFGQATTTQNTGFSFGNTSTIGQPSTNTMGL

15   FGVTQASQPGGLFGTATNTSTGTSGSMSKGEELFTGVVPILVELDGDVNG

HKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP

DHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELK

GIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQ

LADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAG

20   ITHGMDELYK (SEQ ID NO:14)

An exemplary amino acid sequence anti-CD19 CAR-IDR including
an IDR derived from TDP43 is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

25   DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG

LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS

ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

30   HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

VQTTQEEDGCSCRFPEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGSGSNRQLERSGRF

GGNPGGFGNQGGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQ

57

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AALQSSWGMMGMLASQQNQSGPSGNNQNQGNMQREPNQAFGSGNNSYSGSN
SGAAIGWGSASNAGSGSGFNGGFGSSMDSKSSGWGM (SEQ ID NO:15)
```

In some embodiments, the CAR-IDR is further conjugated to one or more fluorescent tags such as GFP. An exemplary amino acid sequence of an anti-CD19 CAR-IDR including an IDR derived from TDP43 and a GFP conjugated thereto is:

```
MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE
DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL
HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGSGSNRQLERSGRF
GGNPGGFGNQGGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQ
AALQSSWGMMGMLASQQNQSGPSGNNQNQGNMQREPNQAFGSGNNSYSGSN
SGAAIGWGSASNAGSGSGFNGGFGSSMDSKSSGWGMTSGSGSMSKGEELFT
GVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTL
VTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAE
VKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIK
ANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNE
KRDHMVLLEFVTAAGITHGMDELYK (SEQ ID NO:16)
```

Another exemplary amino acid sequence anti-CD19 CAR-IDR including a synthetic IDR of SEQ ID NO:6.

```
MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS
KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE
DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG
LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS
ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL
```

HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGSSKGPGRGDSPYS

GRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRG

DSPYSGRGDSPYSGRGDSPYSGY (SEQ ID NO:17)

   An exemplary amino acid sequence of an anti-CD19 CAR-IDR including a synthetic IDR of SEQ ID NO:6 and a GFP conjugated thereto is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG

LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS

ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGSSKGPGRGDSPYS

GRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRGDSPYSGRG

DSPYSGRGDSPYSGRGDSPYSGYTSGSGSMSKGEELFTGVVPILVELDGDV

NGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSR

YPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIE

LKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGS

VQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTA

AGITHGMDELYK (SEQ ID NO:18)

   In some forms, the function of the fusion peptide including one or more IDR domains is compared to a control peptide, *e.g.*, one having the same CAR domain but without an IDR domain. In some cases, a fluorescent tag is added to track or assess the control peptide, for example, a GFP can be fused to the C-terminus of the control peptide. An exemplary control CAR peptide with a C-terminal GFP, is an anti-CD19 CAR-GFP having the following amino acid sequence.

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE

DIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPG

LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNS

5 ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ

GTSVTVSSGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRSRLL

HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRP

VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG

10 RREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMK

GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSGSGSMSKGEELFTG

VVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLV

TTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEV

KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKA

15 NFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEK

RDHMVLLEFVTAAGITHGMDELYK (SEQ ID NO:19)

In some forms, the fusion peptide is a CAR including one or more IDR domains. An exemplary CAR-IDR is an anti-CD22 CAR-IDR. An exemplary amino acid sequence anti-CD22 CAR-IDR including an IDR

20 derived from FUS is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

NYLNWYQQKPDGTVKLLIYYTSILHSGVPSRFSGSGSGTDYSLTISNLEQE

DFATYFCQQGNTLPWTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGG

LVKPGGSLKLSCAASGFAFSIYDMSWVRQTPEKRLEWVAYISSGGGTTYYP

25 DTVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHSGYGSSYGVLFAY

WGQGTLVTVSAGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRP

EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRS

RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQ

GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKD

30 KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSGS

GSGSGSMASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQST

DTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSS

YPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQ

SYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQD

QSGGGGSGGYGQQDRG (SEQ ID NO:20)

Another exemplary amino acid sequence anti-CD22 CAR-IDR

including an IDR derived from EWS is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

NYLNWYQQKPDGTVKLLIYYTSILHSGVPSRFSGSGSGTDYSLTISNLEQE

DFATYFCQQGNTLPWTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGG

LVKPGGSLKLSCAASGFAFSIYDMSWVRQTPEKRLEWVAYISSGGGTTYYP

DTVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHSGYGSSYGVLFAY

WGQGTLVTVSAGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRP

EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRS

RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQ

GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKD

KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRTGS

GSMGYAQTTQAYGQQSYGTYGQPTDVSYTQAQTTATYGQTAYATSYGQPPT

GYTTPTAPQAYSQPVQGYGTGAYDTTTATVTTTQASYAAQSAYGTQPAYPA

YGQQPAATAPTRPQDGNKPTETSQPQSSTGGYNQPSLGYGQSNYSYPQVPG

SYPMQPVTAPPSYPPTSYSSTQPTSYDQSSYSQQNTYGQPSSYGQQSSYGQ

QSSYGQQPPTSYPPQTGSYSQAPSQYSQQSSSYG (SEQ ID NO:21)

Another exemplary amino acid sequence anti-CD22 CAR-IDR

including an IDR derived from TAF15 is:

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

NYLNWYQQKPDGTVKLLIYYTSILHSGVPSRFSGSGSGTDYSLTISNLEQE

DFATYFCQQGNTLPWTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGG

LVKPGGSLKLSCAASGFAFSIYDMSWVRQTPEKRLEWVAYISSGGGTTYYP

DTVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHSGYGSSYGVLFAY

WGQGTLVTVSAGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRP

EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRS

RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQ

GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKD

KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSGS

GSMMSDSGSYGQSGGEQQSYSTYGNPGSQGYGQASQSYSGYGQTTDSSYGQ

NYSGYSSYGQSYSQSYGGYENQKQSSYSQQPYNNQGQQQNMESSGSQGGRA

PSYDQPDYGQQDSYDQQSGYDQHQGSYDEQSNYDQQHDSYSQNQQSYHSQR

ENYSHHTQDDRRDVSRYGEDNRGYGGSQGG (SEQ ID NO:22)

In some forms, the function of the fusion peptide including one or more IDR domains is compared to a control peptide, *e.g.*, one having the

5   same CAR domain but without an IDR domain. In some cases, a fluorescent tag is added to track or assess the control peptide, for example, a GFP can be fused to the C-terminus of the control peptide. An exemplary control CAR peptide with a C-terminal GFP, is an anti-CD22 CAR-GFP having the following amino acid sequence.

10   MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDIS

NYLNWYQQKPDGTVKLLIYYTSILHSGVPSRFSGSGSGTDYSLTISNLEQE

DFATYFCQQGNTLPWTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGG

LVKPGGSLKLSCAASGFAFSIYDMSWVRQTPEKRLEWVAYISSGGGTTYYP

DTVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHSGYGSSYGVLFAY

15   WGQGTLVTVSAGSGSEQKLISEEDLGSTTTPAPRPPTPAPTIASQPLSLRP

EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSRSKRS

RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQ

GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKD

KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRTSGS

20   GSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICT

TGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISF

KDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVY

ITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS

TQSVLSKDPNEKRDHMVLLEFVTAAGITHGMDELYK (SEQ ID NO:23)

25   In some forms, the fusion peptide is a CAR including one or more IDR domains. An exemplary CAR-IDR is an anti-HER2 CAR-IDR. An exemplary amino acid sequence anti-HER2 CAR-IDR including an IDR derived from FUS is:

METDTLLLWVLLLWVPGSTGDKYLLPTAAAGLLLLAAQPAMAQVQLVQSGA

30   EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDTKY

SPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAK

WPEYFQHWGQGTLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSVSAAPGQKV

TISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSG

TSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLGGSGSEQKLI

SEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC

DFWVLVVVGGVLACYSLLVTVAFIIFWVSRSKRSRLLHSDYMNMTPRRPGP

TRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF

PEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ

GLSTATKDTYDALHMQALPPRTSGSGSGSGSMASNDYTQQATQSYGAYPTQ

PGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSSYGQSQNTGYGTQST

PQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSY

GQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGG

GGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRG (SEQ ID

NO:24)

An exemplary amino acid sequence anti-HER2 CAR-IDR including an IDR derived from TAF15 is:

METDTLLLWVLLLWVPGSTGDKYLLPTAAAGLLLLAAQPAMAQVQLVQSGA

EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDTKY

SPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAK

WPEYFQHWGQGTLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSVSAAPGQKV

TISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSG

TSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLGGSGSEQKLI

SEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC

DFWVLVVVGGVLACYSLLVTVAFIIFWVSRSKRSRLLHSDYMNMTPRRPGP

TRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF

PEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ

GLSTATKDTYDALHMQALPPRTSGSGSMMSDSGSYGQSGGEQQSYSTYGNP

GSQGYGQASQSYSGYGQTTDSSYGQNYSGYSSYGQSYSQSYGGYENQKQSS

YSQQPYNNQGQQQNMESSGSQGGRAPSYDQPDYGQQDSYDQQSGYDQHQGS

YDEQSNYDQQHDSYSQNQQSYHSQRE

NYSHHTQDDRRDVSRYGEDNRGYGGSQGG (SEQ ID NO:25)

An exemplary amino acid sequence anti-HER2 CAR-IDR including an IDR derived from EWS is:

METDTLLLWVLLLWVPGSTGDKYLLPTAAAGLLLLAAQPAMAQVQLVQSGA

EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDTKY

SPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAK

WPEYFQHWGQGTLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSVSAAPGQKV

TISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSG

TSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLGGSGSEQKLI

SEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC

5   DFWVLVVVGGVLACYSLLVTVAFIIFWVSRSKRSRLLHSDYMNMTPRRPGP

TRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF

PEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ

GLSTATKDTYDALHMQALPPRRTGSGSMGYAQTTQAYGQQSYGTYGQPTDV

10   SYTQAQTTATYGQTAYATSYGQPPTGYTTPTAPQAYSQPVQGYGTGAYDTT

TATVTTTQASYAAQSAYGTQPAYPAYGQQPAATAPTRPQDGNKPTETSQPQ

SSTGGYNQPSLGYGQSNYSYPQVPGSYPMQPVTAPPSYPPTSYSSTQPTSY

DQSSYSQQNTYGQPSSYGQQSSYGQQSSYGQQPPTSYPPQTGSYSQAPSQY

SQQSSSYG (SEQ ID NO:26)

15   In some forms, the function of the fusion peptide including one or
more IDR domains is compared to a control peptide, *e.g.*, one having the
same CAR domain but without an IDR domain. In some cases, a fluorescent
tag is added to track or assess the control peptide, for example, a GFP can be
fused to the C-terminus of the control peptide. An exemplary control CAR

20   peptide with a C-terminal GFP, is an anti-HER2 CAR-GFP having the
following amino acid sequence.

METDTLLLWVLLLWVPGSTGDKYLLPTAAAGLLLLAAQPAMAQVQLVQSGA

EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDTKY

SPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAK

25   WPEYFQHWGQGTLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSVSAAPGQKV

TISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSG

TSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLGGSGSEQKLI

SEEDLGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC

DFWVLVVVGGVLACYSLLVTVAFIIFWVSRSKRSRLLHSDYMNMTPRRPGP

30   TRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF

PEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ

GLSTATKDTYDALHMQALPPRTGSGSMSKGEELFTGVVPILVELDGDVNG

HKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP

DHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELK

GIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQ

LADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAG

ITHGMDELYK (SEQ ID NO:27).

C.   **Nucleic Acids**

Nucleic acids and vectors encoding or expressing IDR proteins and IDR fusion proteins are also described.

1.   **Isolated Nucleic Acid Molecules of IDR or IDR-Fusion Peptides**

Isolated nucleic acid sequences encoding IDR polypeptides and IDR fusion peptides are disclosed. In some embodiments, the isolated nucleic acid sequences encode a CAR-IDR, including a CAR fused with an IDR domain. In preferred embodiments, an isolated nucleic acid sequence encodes a CAR fused with an IDR domain including any one of SEQ ID NOs: 1-27.

The term "isolated nucleic acid" refers to a nucleic acid that is separated from other nucleic acid molecules that are present in a mammalian genome, including nucleic acids that normally flank one or both sides of the nucleic acid in a mammalian genome. An isolated nucleic acid can be, for example, a DNA molecule, provided one of the nucleic acid sequences normally found immediately flanking that DNA molecule in a naturally occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a DNA molecule that exists as a separate molecule independent of other sequences (*e.g.*, a chemically synthesized nucleic acid, or a cDNA or genomic DNA fragment produced by PCR or restriction endonuclease treatment), as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (*e.g.*, a retrovirus, lentivirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include an engineered nucleic acid such as a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid. A nucleic acid existing among hundreds to millions of other nucleic acids within, for example, a cDNA library or a genomic library, or a gel slice containing a

genomic DNA restriction digest, is not to be considered an isolated nucleic acid.

Nucleic acids can be in sense or antisense orientation, or can be complementary to a reference sequence encoding an IDR polypeptide or IDR

5   fusion peptide. Thus, nucleic acids encoding SEQ ID NOs:1-27, and fragments and variants thereof, in sense and antisense, and in single stranded and double stranded forms, are provided. The nucleic acids can be DNA, RNA, or nucleic acid analogs. Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone. Such modification can

10   improve, for example, stability, hybridization, or solubility of the nucleic acid. Modifications at the base moiety can include deoxyuridine for deoxythymidine, and 5-methyl-2'-deoxycytidine or 5-bromo-2'-deoxycytidine for deoxycytidine. Modifications of the sugar moiety can include modification of the 2' hydroxyl of the ribose sugar to form 2'-O-

15   methyl or 2'-O-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. See, for example, Summerton and

20   Weller (1997) *Antisense Nucleic Acid Drug Dev.* 7:187-195; and Hyrup *et al.* (1996) *Bioorgan. Med. Chem.* 4:5-23. In addition, the deoxyphosphate backbone can be replaced with, for example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone.

25   **2.   Vectors Expressing or Encoding IDR or IDR-Fusion Peptides**

In some embodiments, nucleic acids encoding IDR or IDR-Fusion Peptides are present within vectors. In some embodiments, the vectors encode or express a CAR-IDR, including a CAR fused with an IDR domain.

30   In preferred embodiments, a vector encodes or expresses a CAR fused with an IDR domain including any one of SEQ ID NOs: 1-27.

Vectors including an isolated polynucleotide encoding an IDR polypeptide and/or fusion polypeptide including an IDR domain and one or

more heterologous domains for the expression of an IDR or IDR fusion peptide within a host cell are described.

The term "vector" is a nucleic acid molecule used to carry genetic material into another cell, where it can be replicated and/or expressed. Any

5   vector known to those skilled in the art in view of the present disclosure can be used. Examples of vectors include, but are not limited to, plasmids, viral vectors (bacteriophage, animal viruses, and plant viruses), cosmids, and artificial chromosomes (*e.g.*, YACs). A vector can be a DNA vector or an RNA vector. In some embodiments, a vector is a DNA plasmid. One of

10   ordinary skill in the art can construct a vector of the application through standard recombinant techniques in view of the present disclosure.

In some embodiments, the vector including nucleic acids encoding an IDR domain or IDR fusion protein is an expression vector. The term "expression vector" refers to any type of genetic construct including a

15   nucleic acid coding for an RNA capable of being transcribed. Expression vectors include, but are not limited to, vectors for recombinant protein expression, such as a DNA plasmid or a viral vector, and vectors for delivery of nucleic acid into a subject for expression in a tissue of the subject, such as a DNA plasmid or a viral vector. It will be appreciated by those skilled in the

20   art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.*

In some embodiments, vectors contain one or more regulatory sequences. The term "regulatory sequence" refers to any sequence that

25   allows, contributes or modulates the functional regulation of the nucleic acid molecule, including replication, duplication, transcription, splicing, translation, stability and/or transport of the nucleic acid or one of its derivative (*i.e.* mRNA) into the host cell or organism. In the context of the disclosure, this term encompasses promoters, enhancers and other expression

30   control elements (*e.g.*, polyadenylation signals and elements that affect mRNA stability).

In some embodiments, the vector is a non-viral vector. Examples of non-viral vectors include, but are not limited to, DNA plasmids, bacterial

artificial chromosomes, yeast artificial chromosomes, bacteriophages, *etc*.
Examples of non-viral vectors include, but are not limited to, RNA replicon,
mRNA replicon, modified mRNA replicon or self-amplifying mRNA, closed
linear deoxyribonucleic acid, *e.g.*, a linear covalently closed DNA, *e.g.*, a

5   linear covalently closed double stranded DNA molecule. Preferably, a non-
viral vector is a DNA plasmid. A "DNA plasmid", which is used
interchangeably with "DNA plasmid vector," "plasmid DNA" or "plasmid
DNA vector," refers to a double-stranded and generally circular DNA
sequence that is capable of autonomous replication in a suitable host cell.

10   DNA plasmids used for expression of an encoded polynucleotide typically
include an origin of replication, a multiple cloning site, and a selectable
marker, which for example, can be an antibiotic resistance gene. Examples of
suitable DNA plasmids that can be used include, but are not limited to,
commercially available expression vectors for use in well-known expression

15   systems (including both prokaryotic and eukaryotic systems), such as
pSE420 (Invitrogen, San Diego, Calif.), which can be used for production
and/or expression of protein in Escherichia coli; pYES2 (Invitrogen, Thermo
Fisher Scientific), which can be used for production and/or expression in
Saccharomyces cerevisiae strains of yeast; MAXBAC®. complete

20   baculovirus expression system (Thermo Fisher Scientific), which can be used
for production and/or expression in insect cells; pcDNA™. or pcDNA3™
(Life Technologies, Thermo Fisher Scientific), which can be used for high
level constitutive protein expression in mammalian cells; and pVAX or
pVAX-1 (Life Technologies, Thermo Fisher Scientific), which can be used

25   for high-level transient expression of a protein of interest in most
mammalian cells. The backbone of any commercially available DNA
plasmid can be modified to optimize protein expression in the host cell, such
as to reverse the orientation of certain elements (*e.g.*, origin of replication
and/or antibiotic resistance cassette), replace a promoter endogenous to the

30   plasmid (*e.g.*, the promoter in the antibiotic resistance cassette), and/or
replace the polynucleotide sequence encoding transcribed proteins (*e.g.*, the
coding sequence of the antibiotic resistance gene), by using routine
techniques and readily available starting materials. (See *e.g.*, Sambrook et

al., Molecular Cloning a Laboratory Manual, Second Ed. Cold Spring Harbor
Press (1989)).

Preferably, a DNA plasmid is an expression vector suitable for
protein expression in mammalian host cells. Expression vectors suitable for
5   protein expression in mammalian host cells include, but are not limited to,
pcDNA™, pcDNA3™, pVAX, pVAX-1, ADVAX, NTC8454, *etc*. In some
embodiments, an expression vector is based on pVAX-1, which can be
further modified to optimize protein expression in mammalian cells. pVAX-
1 is a commonly used plasmid in DNA vaccines, and contains a strong
10   human immediate early cytomegalovirus (CMV-IE) promoter followed by
the bovine growth hormone (bGH)-derived polyadenylation sequence (pA).
pVAX-1 further contains a pUC origin of replication and a kanamycin
resistance gene driven by a small prokaryotic promoter that allows for
bacterial plasmid propagation.

15   In some embodiments, the vector is a viral vector. In general, viral
vectors are genetically engineered viruses carrying modified viral DNA or
RNA that has been rendered non-infectious, but still contains viral promoters
and transgenes, thus allowing for translation of the transgene through a viral
promoter. Because viral vectors are frequently lacking infectious sequences,
20   they require helper viruses or packaging lines for large-scale transfection.
Examples of viral vectors that can be used include, but are not limited to,
adenoviral vectors, adeno-associated virus vectors, pox virus vectors, enteric
virus vectors, Venezuelan Equine Encephalitis virus vectors, Semliki Forest
Virus vectors, Tobacco Mosaic Virus vectors, lentiviral vectors, arenavirus
25   viral vectors, replication-deficient arenavirus viral vectors or replication-
competent arenavirus viral vectors, bi-segmented or tri-segmented
arenavirus, infectious arenavirus viral vectors, nucleic acids which include
an arenavirus genomic segment wherein one open reading frame of the
genomic segment is deleted or functionally inactivated (and replaced by a
30   nucleic acid encoding a PC1-CTT polypeptide or another therapeutic
polypeptide as described herein), arenavirus such as lymphocytic
choriomeningitidis virus (LCMV), *e.g.*, clone 13 strain or MP strain, and
arenavirus such as Junin virus *e.g.*, Candid #1 strain, *etc*.

In some embodiments, the viral vector is an adenovirus vector, *e.g.*, a recombinant adenovirus vector. A recombinant adenovirus vector can for instance be derived from a human adenovirus (HAdV, or AdHu), or a simian adenovirus such as chimpanzee or gorilla adenovirus (ChAd, AdCh, or SAdV) or rhesus adenovirus (rhAd). Preferably, an adenovirus vector is a recombinant human adenovirus vector, for instance a recombinant human adenovirus serotype 26, or any one of recombinant human adenovirus serotype 5, 4, 35, 7, 48, etc. In other embodiments, an adenovirus vector is a rhAd vector, *e.g.*, rhAd51, rhAd52 or rhAd53. In some embodiments, a recombinant viral vector is prepared using methods known in the art in view of the present disclosure. For example, in view of the degeneracy of the genetic code, several nucleic acid sequences can be designed that encode the same polypeptide. In some embodiments, a polynucleotide encoding an IDR polypeptide or IDR-fusion polypeptide is codon-optimized to ensure proper expression in the host cell (*e.g.*, bacterial or mammalian cells). Codon-optimization is a technology widely applied in the art, and methods for obtaining codon-optimized polynucleotides will be well known to those skilled in the art in view of the present disclosure.

In some embodiments, the vectors, *e.g.*, a DNA plasmid or a viral vector (particularly an adenoviral vector), include any regulatory elements to establish conventional function(s) of the vector, including but not limited to replication and expression of the IDR polypeptide or IDR-fusion polypeptide encoded by the polynucleotide sequence of the vector.

3.   Regulatory Elements

In some embodiments, the disclosed nucleic acids, including RNAs and DNAs such as DNA vectors expressing or encoding an IDR polypeptide or IDR-fusion polypeptide include one or more regulatory elements.

Regulatory elements include, but are not limited to, a promoter, an enhancer, a polyadenylation signal, translation stop codon, a ribosome binding element, a transcription terminator, selection markers, origin of replication, *etc*. An isolated nucleic acid can be, and a vector can include one or more expression cassettes. An "expression cassette" is part of a nucleic acid such as a vector that directs the cellular machinery to make RNA and

protein. An expression cassette typically includes three components: a
promoter sequence, an open reading frame, and a 3'-untranslated region
(UTR) optionally including a polyadenylation signal. An open reading frame
(ORF) is a reading frame that contains a coding sequence of a protein of

5   interest (*e.g.*, IDR polypeptide or IDR-fusion polypeptide, *etc.*) from a start
codon to a stop codon. Regulatory elements of the expression cassette can be
operably linked to a polynucleotide sequence encoding an IDR polypeptide
or IDR-fusion polypeptide.

        As used herein, the term "operably linked" is to be taken in its

10   broadest reasonable context, and refers to a linkage of polynucleotide (or
polypeptide, *etc.*) elements in a functional relationship. A polynucleotide is
"operably linked" when it is placed into a functional relationship with
another polynucleotide. For instance, a promoter is operably linked to a
coding sequence if it affects the transcription of the coding sequence. Any

15   components suitable for use in an expression cassette described herein can be
used in any combination and in any order to prepare vectors of the
application.

                        a.   Promotors

        The disclosed nucleic acids, including vectors, can include a

20   promoter sequence, preferably within an expression cassette, to control
expression of an IDR polypeptide or IDR-fusion polypeptide. The term
"promoter" is used in its conventional sense and refers to a nucleotide
sequence that initiates the transcription of an operably linked nucleotide
sequence. A promoter is located on the same strand near the nucleotide

25   sequence it transcribes. Promoters can be a constitutive, inducible, or
repressible. Promoters can be naturally occurring or synthetic. A promoter
can be derived from sources including viral, bacterial, fungal, plants, insects,
and animals. A promoter can be a homologous promoter (*i.e.*, derived from
the same genetic source as the vector) or a heterologous promoter (*i.e.*,

30   derived from a different vector or genetic source). For example, if the vector
to be employed is a DNA plasmid, the promoter can be endogenous to the
plasmid (homologous) or derived from other sources (heterologous).

Preferably, the promoter is located upstream of the polynucleotide encoding an IDR polypeptide or IDR-fusion polypeptide within an expression cassette.

Examples of promoters that can be used include, but are not limited to, a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter (CMV-IE), Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. A promoter can also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metallothionein.

A promoter can also be a tissue specific promoter, such as a kidney specific promoter, preferably a kidney epithelial cell promoter, which can be natural or synthetic. Examples include, but are not limited to, the CDH 16 promoter, which is mostly kidney specific (it is also expressed in the thyroid) (Igarashi, et al., *Am J Physiol.*, 277(4):F599-610 (1999)); the Pax-8 promoter, which is also expressed primarily in the kidney as well as in the thyroid (Dehbi, et al., EMBO J., 15(16):4297-306 (1996)); the aquaporin 2 promoter, which drives expression specifically in principal cells of the renal collecting duct (which are the target of Tolvaptan) (Stricklett, et al., *Exp Nephrol.*, 7(1):67-74 (1999)), and kidney tubule-specific promoters in association with gene delivery viral vectors (Watanabe, et al., *PloS one,* vol. 12,3 e0168638 (2017)).

In some embodiments, the promoter is a strong eukaryotic promoter, such as cytomegalovirus immediate early (CMV-IE) promoter.

b.   Other Expression Control Elements

In some embodiments, the nucleic acids, including vectors, include additional polynucleotide sequences that stabilize the expressed transcript, enhance nuclear export of the RNA transcript, and/or improve transcriptional-translational coupling. Examples of such sequences include polyadenylation signals and enhancer sequences. A polyadenylation signal is typically located downstream of the coding sequence for an IDR polypeptide

or IDR-fusion polypeptide within an expression cassette of the vector. Enhancer sequences are regulatory DNA sequences that, when bound by transcription factors, enhance the transcription of an associated gene. An enhancer sequence is preferably located upstream of the polynucleotide

5   sequence encoding an IDR polypeptide or IDR-fusion polypeptide, but downstream of a promoter sequence within an expression cassette of the vector.

Any polyadenylation signal known to those skilled in the art in view of the present disclosure can be used. For example, the polyadenylation

10   signal can be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human beta-globin polyadenylation signal. Preferably, a polyadenylation signal is a bovine growth hormone (bGH) polyadenylation signal or a SV40 polyadenylation

15   signal.

Any enhancer sequence known to those skilled in the art in view of the present disclosure can be used. For example, an enhancer sequence can be a human actin, human myosin, human hemoglobin, human muscle creatine, or a viral enhancer, such as one from CMV, HA, RSV, or EBV.

20   Examples of particular enhancers include, but are not limited to, Woodchuck HBV Post-transcriptional regulatory element (WPRE), intron/exon sequence derived from human apolipoprotein A1 precursor (ApoAI), untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR), a splicing enhancer, a synthetic rabbit beta-globin

25   intron, or any combination thereof. Preferably, an enhancer sequence is a composite sequence of three consecutive elements of the untranslated R-U5 domain of HTLV-1 LTR, rabbit beta-globin intron, and a splicing enhancer, which is referred to herein as "a triple enhancer sequence."

A vector can include a polynucleotide sequence encoding a signal

30   peptide sequence. Preferably, the polynucleotide sequence encoding the signal peptide sequence is located upstream of the polynucleotide sequence encoding an IDR polypeptide or IDR-fusion polypeptide. Signal peptides typically direct localization of a protein, facilitate secretion of the protein

from the cell in which it is produced, and/or improve expression the
therapeutic polypeptide when expressed from the vector, but is cleaved off
by signal peptidase, e.g., upon secretion from the cell. An expressed protein
in which a signal peptide has been cleaved is often referred to as the "mature

5   protein." Any signal peptide known in the art in view of the present
disclosure can be used. For example, a signal peptide can be a cystatin S
signal peptide; an immunoglobulin (Ig) secretion signal, such as the Ig heavy
chain gamma signal peptide SPIgG or the Ig heavy chain epsilon signal
peptide SPIgE.

10   A vector, such as a DNA plasmid, can also include a bacterial origin
of replication and an antibiotic resistance expression cassette for selection
and maintenance of the plasmid in bacterial cells, e.g., E. coli. Bacterial
origins of replication and antibiotic resistance cassettes can be located in a
vector in the same orientation as the expression cassette encoding an IDR

15   polypeptide or IDR-fusion polypeptide, or in the opposite (reverse)
orientation. An origin of replication (ORI) is a sequence at which replication
is initiated, enabling a plasmid to reproduce and survive within cells.
Examples of ORIs suitable for use in the application include, but are not
limited to ColE1, pMB1, pUC, pSC101, R6K, and 15A, preferably pUC.

20   Expression cassettes for selection and maintenance in bacterial cells
typically include a promoter sequence operably linked to an antibiotic
resistance gene. Preferably, the promoter sequence operably linked to an
antibiotic resistance gene differs from the promoter sequence operably linked
to a polynucleotide sequence encoding a protein of interest, e.g., an IDR

25   polypeptide or IDR-fusion polypeptide. The antibiotic resistance gene can be
codon optimized, and the sequence composition of the antibiotic resistance
gene is normally adjusted to bacterial, e.g., E. coli, codon usage. Any
antibiotic resistance gene known to those skilled in the art in view of the
present disclosure can be used, including, but not limited to, kanamycin

30   resistance gene (Kan^r), ampicillin resistance gene (Amp^r), and tetracycline
resistance gene (Tet^r), as well as genes conferring resistance to
chloramphenicol, bleomycin, spectinomycin, carbenicillin, etc.

An expression vector can include a tag sequence, such as those
discussed above.

D.   Host Cells

In some embodiments, polypeptides, nucleic acids, or vectors

5   encoding IDR polypeptide or IDR-fusion polypeptides are present within a
host cells. In some embodiments, the cells include nucleic acids or vectors or
genes that encode or express a CAR-IDR, including a CAR fused with an
IDR domain. In preferred embodiments, the cells include nucleic acids or
vectors or genes that encode or express a CAR fused with an IDR domain

10   having SEQ ID NOs. 7-27. The term "host cell" is intended to include
prokaryotic and eukaryotic cells into which a recombinant expression vector
can be introduced. As used herein, "transformed" and "transfected"
encompass the introduction of a nucleic acid molecule (*e.g.*, a vector) into a
cell by one of a number of techniques. Although not limited to a particular

15   technique, a number of these techniques are well established within the art.
Prokaryotic cells can be transformed with nucleic acids by, for example,
electroporation or calcium chloride mediated transformation. Nucleic acids
can be transfected into mammalian cells by techniques including, for
example, calcium phosphate co-precipitation, DEAE-dextran-mediated

20   transfection, lipofection, electroporation, or microinjection. Host cells (*e.g.*, a
prokaryotic cell or a eukaryotic cell) can be used to, for example, produce
the IDR polypeptide or IDR-fusion polypeptides described herein.

In some forms, the cell is from an established cell line, or a primary
cell. The term "primary cell," refers to cells and cell cultures derived from a

25   subject and allowed to grow *in vitro* for a limited number of passages, *i.e.*,
splitting, of the culture.

1.   Human cells

In some embodiments, cells are obtained from a human subject.
Therefore, human cells expressing and/or including IDR-fusion polypeptides

30   are described. In preferred embodiments, the human cells include or express
a CAR-IDR, including a CAR fused with an IDR domain. In preferred
embodiments, the human cells include or express a CAR fused with an IDR
domain including one or more of SEQ ID NOs.:1-27. For example, in some

forms, the cells are autologous cells, *i.e.*, cells obtained from a subject prior to introduction of the IDR polypeptides or IDR-fusion polypeptides, and/or nucleic acids, or vectors encoding IDR polypeptides or IDR-fusion polypeptides, and re-introduction to the same subject following modification.

5   In other forms, the cells are heterologous cells, *i.e.*, cells obtained from a different subject than the intended recipient. In some forms, the cells are frozen prior to or after introduction of the IDR polypeptides or IDR-fusion polypeptides, and/or nucleic acids, or vectors encoding IDR polypeptides or IDR-fusion polypeptides. Methods and compositions for freezing and

10   thawing viable eukaryotic cells are known in the art. In some forms, the cells are autologous immune cells, such as T cells or progenitor cells/stem cells.

In some forms, cells are obtained from a healthy subject. In other forms, cells are obtained from a subject identified as having or at risk of having a disease or disorder, such as cancer and/or an auto-immune disease.

15   In preferred embodiments, the introduction of the IDR polypeptides or IDR-fusion polypeptides to the cells occurs through genetic modification of the cells. In some embodiments, genetic modification of the cell includes introduction of nucleic acids, or vectors encoding IDR polypeptides or IDR-fusion polypeptides to the cell for expression of the IDR polypeptides or

20   IDR-fusion polypeptides within the cell. In some embodiments, genetic modification of the cell includes transduction with a transposon encoding an IDR polypeptide or IDR-fusion polypeptide. In an exemplary embodiment, a CAR-IDR fusion peptide is introduced into a cell *in vitro* by transduction of the cell with a nucleic acid encoding a transposon including the CAR-IDR.

25   Therefore, genetically modified (transgenic) cells including IDR proteins, or IDR-fusion proteins according to the described compositions are described.

a.   T cells

In some forms, the cells are human immune cells, such as T cells. Therefore, human T cells that include or express IDR-fusion polypeptides

30   are described. In some forms, prior to expansion and genetic modification, T cells are obtained from a diseased or healthy subject. T cells can be obtained from a number of samples, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a

site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some

forms, T cells are obtained from a unit of blood collected from a subject

using any number of techniques known to the skilled artisan, such as

FICOLL™ separation. In one preferred form, cells from the circulating

5   blood of an individual are obtained by apheresis. The apheresis product

typically contains lymphocytes, including T cells, monocytes, granulocytes,

B cells, other nucleated white blood cells, red blood cells, and platelets. The

cells collected by apheresis can be washed to remove the plasma fraction and

to place the cells in an appropriate buffer or media for subsequent processing

10   steps. In some forms, the cells are washed with phosphate buffered saline

(PBS). In some forms, the wash solution lacks calcium and can lack

magnesium or can lack many if not all divalent cations. After washing, the

cells can be resuspended in a variety of biocompatible buffers, such as, for

example, Ca^{2+}-free, Mg^{2+}-free PBS, PLASMALYTE A, or other saline

15   solution with or without buffer. Alternatively, the undesirable components of

the apheresis sample are removed, and the cells directly resuspended in

culture media.

         In some forms, T cells are isolated from peripheral blood

lymphocytes by lysing the red blood cells and depleting the monocytes, for

20   example, by centrifugation through a PERCOLL™ gradient or by

counterflow centrifugal elutriation. In specific forms, a specific

subpopulation of T cells, such as CD3+, CD28+, CD4+, CD8+, CD45RA+,

and CD45RO+ T cells, is further isolated by positive or negative selection

techniques. For example, in some forms, T cells are isolated by incubation

25   with anti-CD3/anti-CD28-conjugated beads, such as DYNABEADS® M-450

CD3/CD28 T, for a time period sufficient for positive selection of the desired

T cells.

         In some embodiments, IDR-CAR cells have one or more of increased

secretion of one or more cytotoxic factors optionally included IFNγ,

30   Perforin, FasL, Granzyme A, and/or Granzyme B; activation of LAT and/or

pathway(s) downstream thereof, optionally comprising SLP76 activation,

ERK activation, RAS activation, and/or actin polymerization, optionally

wherein LAT activation comprises LAT phosphorylation; and/or reduction

of one or more T cell exhaustion markers optionally TIM3 and/or LAG3, relative to, for example, a control cell. Such a control cell can be a cell of the same cell type and the same heterologous fusion protein, but lacking the IDR sequence(s).

5   **b.   Delivery Vehicles**

Any of the disclosed compositions including, but not limited to IDR polypeptides or IDR-fusion proteins, such as CAR-IDR and/or nucleic acids, can be delivered to target cells using a delivery vehicle. The delivery vehicles can be, for example, polymeric particles, inorganic particles, silica

10   particles, liposomes, micelles, multilamellar vesicles, *etc.*

Delivery vehicles may be microparticles or nanoparticles. Nanoparticles are often utilized for intertissue application, penetration of cells, and certain routes of administration. The nanoparticles may have any desired size for the intended use. The nanoparticles may have

15   any diameter from 10 nm up to about 1,000 nm. The nanoparticle can have a diameter from 10 nm to 900 nm, from 10 nm to 800 nm, from 10 nm to 700 nm, from 10 nm to 600 nm, from 10 nm to 500 nm, from 20 nm from 500 nm, from 30 nm to 500 nm, from 40 nm to 500 nm, from 50 nm to 500 nm, from 50 nm to 400 nm, from 50 nm to 350 nm, from 50 nm to 300 nm, or

20   from 50 nm to 200 nm. In some embodiments the nanoparticles can have a diameter less than 400 nm, less than 300 nm, or less than 200 nm. The range can be between 50 nm and 300 nm.

Thus, in some embodiments, the delivery vehicles are nanoscale compositions, for example, 10 nm up to, but not including, about 1 micron.

25   However, it will be appreciated that in some embodiments, and for some uses, the particles can be smaller, or larger (*e.g.*, microparticles, etc.). Although many of the compositions disclosed herein are referred to as nanoparticle or nanocarrier compositions, it will be appreciated that in some embodiments and for some uses the carrier can be somewhat larger than

30   nanoparticles. Such compositions can be referred to as microparticulate compositions. For example, a nanocarriers according to the present disclosure may be a microparticle. Microparticles can a diameter between, for example, 0.1 and 100 μm in size.

E.   **Pharmaceutical Compositions**

Pharmaceutical compositions containing nucleic acids encoding, or a genetically modified cell or a population of genetically modified cells expressing, IDR polypeptides or IDR-fusion proteins such as CAR-IDR are provided.

In some embodiments, the pharmaceutical compositions include one or more of a pharmaceutically acceptable buffer, carrier, diluent, or excipients. In some forms, the pharmaceutical compositions include a specific number or population of cells, for example, expanded by culturing and expanding an isolated genetically modified cell (*e.g.*, CAR-IDR T cell), *e.g.*, a homogenous population. Therefore, in some embodiments, pharmaceutical compositions include a homogenous population of modified cells including and/or expressing an IDR peptide or IDR-fusion peptide, such as a CAR-IDR. In other forms, the pharmaceutical compositions include populations of cells that contain variable or different genetically modified cells, *e.g.*, a heterogeneous population. In some forms, the pharmaceutical compositions include cells that are bispecific or multi-specific. In some embodiments, the cells have been isolated from a diseased or healthy subject prior to genetic modification to express an IDR peptide or IDR-fusion peptide, such as a CAR-IDR.

The term "Pharmaceutically acceptable carrier" describes a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, in some forms the carrier is a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or a combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It must also be suitable for use in contact with any tissues or organs with which it may come in contact, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

In some embodiments, pharmaceutical compositions include buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextran, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating

5   agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. The pharmaceutical compositions can be formulated for delivery *via* any route of administration. The term "Route of administration" can refer to any administration pathway known in the art, including but not limited to aerosol, nasal, oral, intravenous, intramuscular, intraperitoneal,

10   inhalation, transmucosal, transdermal, parenteral, implantable pump, continuous infusion, topical application, capsules and/or injections. The pharmaceutical compositions are preferably formulated for intravenous administration.

Typically, the disclosed pharmaceutical compositions are

15   administered in a manner appropriate to a disease to be treated (or prevented). The quantity and frequency of administration is typically determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages can be determined by clinical trials.

20   The disclosed pharmaceutical compositions can be delivered in a therapeutically effective amount. The precise therapeutically effective amount is that amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the

25   characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers

30   in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for instance, by monitoring a subject's response to administration of a compound and adjusting the dosage

accordingly. For additional guidance, see Remington: The Science and Practice of Pharmacy (Gennaro ed. 20th edition, Williams & Wilkins PA, USA) (2000).

III.   Methods

Methods of using the disclosed compositions including, but not limited to, IDR proteins and IDR-fusion proteins are provided.

In some embodiments, the methods enhance the efficacy of cell receptor-mediated functions are provided. In particular embodiments, the methods provide enhanced anti-tumor activity through administration of CAR-T cells including CAR-IDR fusion peptides. It has been established that the antigen sensitivity of CAR can be enhanced by the presence of one or more IDRs, this can be utilized to develop a universal approach to reprogram T cell function. The features of the IDR were developed into a simple yet versatile approach to enhance Chimeric Antigen Receptor (CAR)-T cell function. Synthetic IDRs fused to the C-terminus of the CARs were engineered to promotes condensation of the CAR receptor (CAR-IDRs), which resulted in higher CAR-T activation and killing of low antigen-expressing cells.

As set forth in the Examples below, CAR-IDR cells showed significantly enhanced cytotoxicity towards tumor antigen-expressing cells *in vitro*; and CAR-IDR cells have the most durable *in vivo* anti-tumor effect, substantially more potent than control CAR-T cells. Together, these data here provide a distinct approach to engineer CAR-T cells with functional reprogramming via addition of one or more IDRs. The enhanced CAR-T function is (i) independent of CAR types and (ii) can be applied to a broad range of cell therapy. Furthermore, based on the modular feature of CAR, IDR can be combined with other engineering strategies for improving antigen sensitivity, including selecting specific transmembrane or co-signaling domains (Majzner, R. G. *et al.*, Cancer Discov, doi:10.1158/2159-8290.CD-19-0945 (2020); Heitzeneder, S. *et al.*, Cancer Cell 40, 53-69 e59, (2022); Priceman, S. J. *et al.*, Oncoimmunology 7, e1380764, (2018)), adding new signaling binding motifs (Salter, A. I. et al., Sci Signal 14, (2021)), replacing the intracellular part with that from TCR (HIT) (Mansilla-

Soto, J. et al., Nat Med 28, 345-352, (2022)), dual targeting by CAR together with chimeric costimulatory receptors (Katsarou, A. et al., Sci Transl Med 13, eabh1962, (2021)), implementing other signaling pathways (Wilkens, A. B. et al. Blood 140, 2261-2275, (2022)), and/or host cell gene knockdown or knockout (Mirzaei, et al., Cancer Lett., 2018 Jun 1;423:95-104. doi: 10.1016/j.canlet.2018.03.010. Epub 2018 Mar 12; "BATF Knockout in CAR T Cells Prevents Exhaustion in Solid Tumors"Cancer Discov (2022) 12 (12): OF9, doi.org/10.1158/2159-8290.CD-RW2022-188, Kamali, et al., BMC Biotechnol 21, 9 (2021). doi.org/10.1186/s12896-020-00665-4, Dai, et al., Nat Methods, . 2019 Mar;16(3):247-254. doi: 10.1038/s41592-019-0329-7. Epub 2019 Feb 25.) to increase activity, prevent exhaustion, reduce toxicity, or achieve more than additive effects.

A. Methods of Treatment

Methods of treatment including cells and other therapeutic agents including IDR polypeptides and IDR-fusion polypeptides are described. In preferred embodiments, the methods include Adoptive Cell Therapy (ACT) employing T cells expressing recombinant CAR-IDR fusion proteins. The CAR T cells including CAR-IDR fusion proteins have enhanced anti-tumor activity. For example, the CAR T cells including CAR-IDR fusion proteins show enhanced CAR-T activation and killing of cancer cells, particularly in killing of low antigen-expressing cells, as compared to CAR T cells including CAR proteins in the absence of IDR.

An exemplary method involves treating a subject (*e.g.*, a human) having a disease, disorder, or condition by administering to the subject an effective amount of a pharmaceutical composition including genetically modified cells including IDR polypeptides and/or IDR-fusion polypeptides. In some embodiments, the methods administer genetically manipulated T cells engineered to express recombinant CAR-IDR fusion proteins to a subject (*e.g.*, a human) having a disease, disorder, or condition in an amount effective to treat the disease, disorder, or condition. For example, in some embodiments, the methods treat a disease or disorder associated with an elevated expression or specific expression of an antigen by administering to the subject an effective amount of a pharmaceutical composition including

cells modified to express recombinant CAR-IDR fusion proteins. In some

embodiments, the methods treat a subject having a disease, disorder, or

condition associated with an elevated expression or specific expression of an

antigen by administering to the subject an effective amount of a

5   pharmaceutical composition including T cells modified to contain a CAR-

IDR that targets the antigen.

        Methods of using IDR-fusion proteins, such as a CAR-IDR, to treat a

disease or disorder by are provided. Typically, the methods enhance ACT,

for example, by providing CAR-IDR-bearing T cells with enhanced

10   therapeutic efficacy *in vivo*. The CAR-IDR-T cells have prolonged

survival/serum residency time *in vivo* relative to CAR-T cells lacking the

IDR fusion domain. Methods of treating a subject having a disease, disorder,

or condition including administering to the subject an effective amount of a

pharmaceutical composition including live, viable cells engineered to

15   express a CAR-IDR and/or another IDR-fusion protein are provided. In some

embodiments, when the methods treat a subject having a disease, disorder, or

condition associated with an elevated expression or specific expression of an

antigen, the methods include administering to the subject an effective

amount of a T cell modified to express a CAR-IDR that targets the antigen.

20   For example, in some forms, the methods treat a subject having a disease,

disorder, or condition by administering to the subject an effective amount of

a pharmaceutical composition having a genetically modified cell, where the

cell is modified by introducing to the cell:

        (i)   a vector, optionally including a transposon encoding a CAR-

25   IDR and/or other IDR-fusion protein; and

        (ii)   causing the CAR-IDR and/or other IDR-fusion protein to be

expressed by the cell. The cell can have been isolated from the subject

having the disease, disorder, or condition, or from a healthy donor, prior to

genetic modification.

30   In additional or alternative to ACT, CAR-IDR-expressing cells can

be created in vivo in a subject in need thereof by introducing an effective

amount of nucleic acids (e.g., RNA, viral vectors, etc.) encoding the CAR-

IDR into the subject. In some embodiments, the constructs are targeted to

the tumor microenvironment using, e.g., targeted delivery vehicles. See, e.g., Xin, et al., Front. Oncol., 10 February 2022, Sec. Cancer Immunity and Immunotherapy, Volume 12 - 2022 | doi.org/10.3389/fonc.2022.809754.

1.   Diseases to be treated

Methods of treating diseases and/or disorders in a subject in need thereof are provided. The subject to be treated can have a disease, disorder, or condition such as but not limited to, cancer, an immune system disorder such autoimmune disease, an inflammatory disease, a neuronal disorder, HIV/AIDS, diabetes, a cardiovascular disease, an infectious disease, or combinations thereof. The disease, disorder, or condition can be associated with an elevated expression or specific expression of an antigen.

a.   Cancer

In some embodiments, the methods treat or prevent cancer. In some forms, the methods treat or prevent cancer or other proliferative disease or disorder in a subject identified as having, or at risk of having cancer or other proliferative disease or disorder. Cancer is a disease of genetic instability, allowing a cancer cell to acquire the hallmarks proposed by Hanahan and Weinberg, including (i) self-sufficiency in growth signals; (ii) insensitivity to anti-growth signals; (iii) evading apoptosis; (iv) sustained angiogenesis; (v) tissue invasion and metastasis; (vi) limitless replicative potential; (vii) reprogramming of energy metabolism; and (viii) evading immune destruction (*Cell.*,144:646–674, (2011)).

Tumors, which can be treated in accordance with the disclosed methods, are classified according to the embryonic origin of the tissue from which the tumor is derived. Carcinomas are tumors arising from endodermal or ectodermal tissues such as skin or the epithelial lining of internal organs and glands. Sarcomas, which arise less frequently, are derived from mesodermal connective tissues such as bone, fat, and cartilage. The leukemias and lymphomas are malignant tumors of hematopoietic cells of the bone marrow. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. Malignant tumors may show up at numerous organs or tissues of the body to establish a cancer.

The disclosed compositions and methods of treatment thereof are generally suited for treatment of carcinomas, sarcomas, lymphomas and leukemias. The described compositions and methods are useful for treating, or alleviating subjects having benign or malignant tumors by delaying or inhibiting the growth/proliferation or viability of tumor cells in a subject, reducing the number, growth, or size of tumors, inhibiting, or reducing metastasis of the tumor, and/or inhibiting or reducing symptoms associated with tumor development or growth.

The types of cancer that can be treated with the provided compositions and methods include, but are not limited to, cancers such as vascular cancer such as multiple myeloma, adenocarcinomas, and sarcomas, of bone, bladder, brain, breast, cervical, colorectal, esophageal, kidney, liver, lung, nasopharyngeal, pancreatic, prostate, skin, stomach, and uterine. In some forms, the compositions are used to treat multiple cancer types concurrently. The compositions can also be used to treat metastases or tumors at multiple locations.

Exemplary tumor cells include, but are not limited to, tumor cells of cancers, including leukemias including, but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome, chronic leukemias such as, but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as, but not limited to, Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as, but not limited to, smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as, but not limited to, bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma,

liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors including, but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma,

5   meningioma, pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer, including, but not limited to,

10   pheochromocytoma and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer, including, but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers including, but not

15   limited to, Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers including, but not limited to, ocular melanoma such as iris melanoma, choroidal melanoma, and ciliary body melanoma, and retinoblastoma; vaginal cancers, including, but not limited to, squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer, including, but

20   not limited to, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers including, but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers including, but not limited to, endometrial carcinoma and uterine sarcoma; ovarian cancers including, but not limited to, ovarian epithelial

25   carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers including, but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers including,

30   but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers including, but not limited to, hepatocellular carcinoma

and hepatoblastoma, gallbladder cancers including, but not limited to, adenocarcinoma; cholangiocarcinoma including, but not limited to, papillary, nodular, and diffuse; lung cancers including, but not limited to, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma),

5   adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers including, but not limited to, germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers including, but not limited to, adenocarcinoma, leiomyosarcoma, and

10   rhabdomyosarcoma; penal cancers; oral cancers including, but not limited to, squamous cell carcinoma; basal cancers; salivary gland cancers including, but not limited to, adenocarcinoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma; pharynx cancers including, but not limited to, squamous cell cancer, and verrucous; skin cancers including, but not limited

15   to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers including, but not limited to, renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers

20   including, but not limited to, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, and carcinosarcoma. For a review of such disorders, see Fishman *et al.*, 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy *et al.*, 1997, Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery, Viking Penguin,

25   Penguin Books U.S.A., Inc., United States of America).

b.   Immune system disorders

In some embodiments, the methods administer modified T cells including CAR-IDR and/or other IDR-fusion protein(s) to treat or prevent one or more immune system disorders, including autoimmune diseases.

30   Under certain circumstances, the ability of the immune system to distinguish self from foreign antigens can be misdirected against healthy tissues, resulting in the undesirable attack and destruction of normal host cells (*i.e.*, autoimmune diseases). Autoimmune diseases include over 100

types of diseases, with varied etiology and prognoses based on factors such as the affected region, the age of onset, response to the therapeutic agents and clinical manifestation may vary among different people (Muhammad, *et al.*, Chimeric Antigen Receptor Based Therapy as a Potential Approach in Autoimmune Diseases: How Close Are We to the Treatment, Frontiers in Immunology, 11 (2020)).

Auto-antibody-secreting B lymphocytes and self-reactive T-lymphocytes play a key role in the development of autoimmune diseases. Based on the extent of tissue damage, autoimmunity is classified into two general categories, including organ-specific and systemic autoimmune. The former involves a specific area of the body such as type I diabetes (T1D), multiple sclerosis (MS), rheumatoid arthritis (RA), inflammatory bowel diseases (IBDs), and myasthenia gravis (MG), while the latter affects multiple regions of the body, causing systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). Therefore, in some forms, the methods treat or prevent one or more organ-specific autoimmune diseases in a subject. In other forms, the methods treat or prevent one or more systemic autoimmune diseases in a subject.

In some forms, the methods reduce or prevent one or more physiological processes associated with the development or progression of autoimmune disease in a subject. For example, in some forms, the methods reduce or prevent one or more of epitope spreading, for example, where infections alter the primary epitope into the secondary epitope or form several neoepitopes on antigen-presenting cells; bystander activation or pre-primed autoreactive T cell activation in a T cell receptor (TCR)-independent manner; persistent virus infection, or the constant presence of viral antigens that prompt immune responses; or immunological cross-reactivity between a host and pathogen, for example, due to shared immunologic epitopes or sequence similarities. Non-limiting examples of immune system disorders that can be treated or prevented by the methods include 22q11.2 deletion syndrome, Achondroplasia and severe combined immunodeficiency, Adenosine Deaminase 2 deficiency, Adenosine deaminase deficiency, Adult-onset immunodeficiency with anti-interferon-gamma autoantibodies,

Agammaglobulinemia, non-Bruton type, Aicardi-Goutieres syndrome,
Aicardi-Goutieres syndrome type 5, Allergic bronchopulmonary
aspergillosis, Alopecia, Alopecia totalis, Alopecia universalis, Amyloidosis
AA, Amyloidosis familial visceral, Ataxia telangiectasia, Autoimmune
lymphoproliferative syndrome, Autoimmune lymphoproliferative syndrome
due to CTLA4 haploinsuffiency, Autoimmune polyglandular syndrome type
1, Autosomal dominant hyper IgE syndrome, Autosomal recessive early-
onset inflammatory bowel disease, Autosomal recessive hyper IgE
syndrome, Bare lymphocyte syndrome 2, Barth syndrome, Blau syndrome,
Bloom syndrome, Bronchiolitis obliterans, C1q deficiency, Candidiasis
familial chronic mucocutaneous, autosomal recessive, Cartilage-hair
hypoplasia, CHARGE syndrome, Chediak-Higashi syndrome, Cherubism,
Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated
temperature, Chronic graft versus host disease, Chronic granulomatous
disease, Chronic Infantile Neurological Cutaneous Articular syndrome,
Chronic mucocutaneous candidiasis (CMC), Cohen syndrome, Combined
immunodeficiency with skin granulomas, Common variable
immunodeficiency, Complement component 2 deficiency, Complement
component 8 deficiency type 1, Complement component 8 deficiency type 2,
Congenital pulmonary alveolar proteinosis, Cryoglobulinemia, Cutaneous
mastocytoma, Cyclic neutropenia, Deficiency of interleukin-1 receptor
antagonist, Dendritic cell, monocyte, B lymphocyte, and natural killer
lymphocyte deficiency, Dyskeratosis congenital, Dyskeratosis congenita
autosomal dominant, Dyskeratosis congenita autosomal recessive,
Dyskeratosis congenita X-linked, Epidermodysplasia verruciformis, Familial
amyloidosis, Finnish type, Familial cold autoinflammatory syndrome,
Familial Mediterranean fever, Familial mixed cryoglobulinemia, Felty's
syndrome, Glycogen storage disease type 1B, Griscelli syndrome type 2,
Hashimoto encephalopathy, Hashimoto's syndrome, Hemophagocytic
lymphohistiocytosis, Hennekam syndrome, Hepatic venoocclusive disease
with immunodeficiency, Hereditary folate malabsorption, Hermansky Pudlak
syndrome 2, Herpes simplex encephalitis, Hoyeraal Hreidarsson syndrome,
Hyper IgE syndrome, Hyper-IgD syndrome, ICF syndrome, Idiopathic acute

eosinophilic pneumonia, Idiopathic CD4 positive T-lymphocytopenia,
IL12RB1 deficiency, Immune defect due to absence of thymus, Immune
dysfunction with T-cell inactivation due to calcium entry defect 1, Immune
dysfunction with T-cell inactivation due to calcium entry defect 2,

5   Immunodeficiency with hyper IgM type 1, Immunodeficiency with hyper
IgM type 2, Immunodeficiency with hyper IgM type 3, Immunodeficiency
with hyper IgM type 4, Immunodeficiency with hyper IgM type 5,
Immunodeficiency with thymoma, Immunodeficiency without anhidrotic
ectodermal dysplasia, Immunodysregulation, polyendocrinopathy and

10   enteropathy X-linked, Immunoglobulin A deficiency 2, Intestinal atresia
multiple, IRAK-4 deficiency, Isolated growth hormone deficiency type 3,
Kawasaki disease, Large granular lymphocyte leukemia, Leukocyte adhesion
deficiency type 1, LRBA deficiency, Lupus, Lymphocytic hypophysitis,
Majeed syndrome, Melkersson-Rosenthal syndrome, MHC class 1

15   deficiency, Muckle-Wells syndrome, Multifocal fibrosclerosis, Multiple
sclerosis, MYD88 deficiency, Neonatal systemic lupus erythematosus,
Netherton syndrome, Neutrophil-specific granule deficiency, Nijmegen
breakage syndrome, Omenn syndrome, Osteopetrosis autosomal recessive 7,
Palindromic rheumatism, Papillon Lefevre syndrome, Partial androgen

20   insensitivity syndrome, PASLI disease, Pearson syndrome, Pediatric multiple
sclerosis, Periodic fever, aphthous stomatitis, pharyngitis and adenitis,
PGM3-CDG, Poikiloderma with neutropenia, Pruritic urticarial papules
plaques of pregnancy, Purine nucleoside phosphorylase deficiency, Pyogenic
arthritis, pyoderma gangrenosum and acne, Relapsing polychondritis,

25   Reticular dysgenesis, Sarcoidosis, Say Barber Miller syndrome, Schimke
immunoosseous dysplasia, Schnitzler syndrome, Selective IgA deficiency,
Selective IgM deficiency, Severe combined immunodeficiency, Severe
combined immunodeficiency due to complete RAG1/2 deficiency, Severe
combined immunodeficiency with sensitivity to ionizing radiation, Severe

30   combined immunodeficiency, Severe congenital neutropenia autosomal
recessive 3, Severe congenital neutropenia X-linked, Shwachman-Diamond
syndrome, Singleton-Merten syndrome, SLC35C1-CDG (CDG-IIc), Specific
antibody deficiency, Spondyloenchondrodysplasia, Stevens-Johnson

90

syndrome, T-cell immunodeficiency, congenital alopecia and nail dystrophy, TARP syndrome, Trichohepatoenteric syndrome, Tumor necrosis factor receptor-associated periodic syndrome, Twin to twin transfusion syndrome, Vici syndrome, WHIM syndrome, Wiskott Aldrich syndrome, Woods Black

5   Norbury syndrome, X-linked agammaglobulinemia, X-linked lymphoproliferative syndrome, X-linked lymphoproliferative syndrome 1, X-linked lymphoproliferative syndrome 2, X-linked magnesium deficiency with Epstein-Barr virus infection and neoplasia, X-linked severe combined immunodeficiency, and ZAP-70 deficiency.

10   The disclosed compositions and methods can also be used to treat autoimmune diseases or disorders. Exemplary autoimmune diseases or disorders, which are not mutually exclusive with the immune system disorders described above, include Achalasia, Addison's disease, Adult Still's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis,

15   Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome, Autoimmune angioedema, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune hepatitis, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune oophoritis, Autoimmune orchitis, Autoimmune pancreatitis, Autoimmune retinopathy,

20   Autoimmune urticarial, Axonal & neuronal neuropathy (AMAN), Baló disease, Behcet's disease, Benign mucosal pemphigoid, Bullous pemphigoid, Castleman disease (CD), Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss Syndrome (CSS) or

25   Eosinophilic Granulomatosis (EGPA), Cicatricial pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis (EoE),

30   Eosinophilic fasciitis, Erythema nodosum, Essential mixed cryoglobulinemia, Evans syndrome, Fibromyalgia, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with

Polyangiitis, Graves' disease, Guillain-Barre syndrome, Hashimoto's

thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura (HSP), Herpes

gestationis or pemphigoid gestationis (PG), Hidradenitis Suppurativa (HS)

(Acne Inversa), Hypogammalglobulinemia, IgA Nephropathy, IgG4-related

5   sclerosing disease, Immune thrombocytopenic purpura (ITP), Inclusion body

myositis (IBM), Interstitial cystitis (IC), Juvenile arthritis, Juvenile diabetes

(Type 1 diabetes), Juvenile myositis (JM), Kawasaki disease, Lambert-Eaton

syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus,

Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus, Lyme disease

10   chronic, Meniere's disease, Microscopic polyangiitis (MPA), Mixed

connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann

disease, Multifocal Motor Neuropathy (MMN) or MMNCB, Multiple

sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neonatal Lupus,

Neuromyelitis optica, Neutropenia, Ocular cicatricial pemphigoid, Optic

15   neuritis, Palindromic rheumatism (PR), PANDAS, Paraneoplastic cerebellar

degeneration (PCD), Paroxysmal nocturnal hemoglobinuria (PNH), Parry

Romberg syndrome, Pars planitis (peripheral uveitis), Parsonnage-Turner

syndrome, Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis,

Pernicious anemia (PA), POEMS syndrome, Polyarteritis nodosa,

20   Polyglandular syndromes type I, II, III, Polymyalgia rheumatic,

Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy

syndrome, Primary biliary cirrhosis, Primary sclerosing cholangitis,

Progesterone dermatitis, Psoriasis, Psoriatic arthritis, Pure red cell aplasia

(PRCA), Pyoderma gangrenosum, Raynaud's phenomenon, Reactive

25   Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless

legs syndrome (RLS), Retroperitoneal fibrosis, Rheumatic fever,

Rheumatoid arthritis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma,

Sjögren's syndrome, Sperm & testicular autoimmunity, Stiff person

syndrome (SPS), Subacute bacterial endocarditis (SBE), Susac's syndrome,

30   Sympathetic ophthalmia (SO), Takayasu's arteritis, Temporal arteritis/Giant

cell arteritis, Thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome

(THS), Transverse myelitis, Type 1 diabetes, Ulcerative colitis (UC),

Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis,

Vitiligo, Vogt-Koyanagi-Harada Disease, and Wegener's granulomatosis (or Granulomatosis with Polyangiitis (GPA)).

c.   Other Disease or Disorders

In some forms the methods administer modified T cells including CAR-IDR and/or other IDR-fusion protein(s) to treat one or more additional disease or disorder in a subject in need thereof. For example, in some forms the methods treat one or more genetic disease or disorders in a subject, such as a hereditary genetic disease or disorder, or a somatic genetic disease or disorder in a subject.

Any of the methods can include treating a subject having an underlying disease or disorder. For example, in some forms, the methods treat a disease or disorder, such as a cancer or auto-immune disease in a patient having another disease or disorder, such as diabetes, a bacterial infection (*e.g.*, Tuberculosis), viral infection (*e.g.*, Hepatitis, HIV, HPV infection, *etc.*), or a drug-associated disease or disorder. In some forms, the methods treat an immunocompromised subject. In some forms, the methods treat a subject having a disease of the kidney, liver, heart, lung, brain, bladder, reproductive system, bowel/intestines, stomach, bones, or skin.

B.   Effective Amounts

In some forms the methods administer modified T cells including CAR-IDR and/or other IDR-fusion protein(s) in an effective amount. The effective amount or therapeutically effective amount of a pharmaceutical compositions including modified cells, such as therapeutic T cells, can be a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of a disease or disorder, such as a cancer or autoimmune disease, or to otherwise provide a desired pharmacologic and/or physiologic effect, for example, reducing, inhibiting, or reversing one or more of the underlying pathophysiological mechanisms underlying a disease or disorder, such as cancer or autoimmune disease.

In some forms, when administration of the pharmaceutical compositions including modified cells, such as therapeutic T cells, including CAR-IDR and/or other IDR-fusion protein(s) elicits an anti-cancer response, the amount administered can be expressed as the amount effective to achieve

a desired anti-cancer effect in the recipient. For example, in some forms, the
amount of the pharmaceutical compositions including modified cells, such as
therapeutic T cells, is effective to inhibit the viability or proliferation of
cancer cells in the recipient. In some forms, the amount of the

5   pharmaceutical composition including modified cells, such as therapeutic T
cells, is effective to reduce the tumor burden in the recipient, or reduce the
total number of cancer cells, and combinations thereof. In other forms, the
amount of the pharmaceutical compositions including modified cells, such as
therapeutic T cells, is effective to reduce one or more symptoms or signs of

10   cancer in a cancer patient, or signs of an autoimmune disease in a patient
having an autoimmune disease or disorder. Signs of cancer can include
cancer markers, such as PSMA levels in the blood of a patient.

The effective amount of the pharmaceutical compositions including
modified cells, such as therapeutic T cells, that is required will vary from

15   subject to subject, depending on the species, age, weight and general
condition of the subject, the severity of the disorder being treated, and its
mode of administration. Thus, it is not possible to specify an exact amount
for every pharmaceutical composition. However, an appropriate amount can
be determined by one of ordinary skill in the art using only routine

20   experimentation given the teachings herein. For example, effective dosages
and schedules for administering the pharmaceutical compositions including
therapeutic T cells can be determined empirically, and making such
determinations is within the skill in the art. In some forms, the dosage ranges
for the administration of the compositions including therapeutic T cells are

25   those large enough to effect reduction in cancer cell proliferation or viability,
or to reduce tumor burden for example.

The dosage should not be so large as to cause adverse side effects,
such as unwanted cross-reactions, anaphylactic reactions, and the like.
Generally, the dosage will vary with the age, condition, and sex of the

30   patient, route of administration, whether other drugs are included in the
regimen, and the type, stage, and location of the disease to be treated. The
dosage can be adjusted by the individual physician in the event of any
counter-indications. It will also be appreciated that the effective dosage of

the composition including therapeutic T cells used for treatment can increase or decrease over the course of a particular treatment. Changes in dosage can result and become apparent from the results of diagnostic assays.

5   Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the subject or patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and

10   repetition rates. Optimum dosages can vary depending on the relative potency of individual pharmaceutical compositions and can generally be estimated based on EC_{50}s found to be effective in *in vitro* and *in vivo* animal models.

           It can generally be stated that a pharmaceutical composition

15   containing CAR-IDR T cells described herein can be administered at a dosage of 10^4 to 10^9 cells/kg body weight, preferably 10^5 to 10^7 cells/kg body weight, including all integer values within those ranges. In some forms, patients can be treated by infusing a disclosed pharmaceutical composition containing CAR-IDR expressing cells (*e.g.*, T cells) in the range of about

20   10^4 to 10^{12} or more cells per square meter of body surface (cells/m).

           The infusion can be repeated as often and as many times as the patient can tolerate until the desired response is achieved. CAR-IDR T cell compositions can also be administered once or multiple times at these dosages. The cells can be administered by using infusion techniques that are

25   commonly known in immunotherapy (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319:1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly. In some forms, the unit dosage is in a unit dosage

30   form for intravenous injection. In some forms, the unit dosage is in a unit dosage form for oral administration. In some forms, the unit dosage is in a unit dosage form for inhalation. In some forms, the unit dosage is in a unit dosage form for intra-tumoral injection.

Treatment can be continued for an amount of time sufficient to achieve one or more desired therapeutic goals, for example, a reduction of the amount of cancer cells relative to the start of treatment, or complete absence of cancer cells in the recipient. Treatment can be continued for a

5   desired period of time, and the progression of treatment can be monitored using any means known for monitoring the progression of anti-cancer treatment in a patient. In some forms, administration is carried out every day of treatment, or every week, or every fraction of a week. In some forms, treatment regimens are carried out over the course of up to two, three, four or

10   five days, weeks, or months, or for up to 6 months, or for more than 6 months, for example, up to one year, two years, three years, or up to five years.

The efficacy of administration of a particular dose of the pharmaceutical compositions including modified cells, such as therapeutic T

15   cells, according to the methods described herein can be determined by evaluating the aspects of the medical history, signs, symptoms, and objective laboratory tests that are known to be useful in evaluating the status of a subject in need for the treatment of cancer or other diseases and/or conditions. These signs, symptoms, and objective laboratory tests will vary,

20   depending upon the particular disease or condition being treated or prevented, as will be known to any clinician who treats such patients or a researcher conducting experimentation in this field. For example, if, based on a comparison with an appropriate control group and/or knowledge of the normal progression of the disease in the general population or the particular

25   individual: (1) a subject's physical condition is shown to be improved (*e.g.*, a tumor has partially or fully regressed), (2) the progression of the disease or condition is shown to be stabilized, or slowed, or reversed, or (3) the need for other medications for treating the disease or condition is lessened or obviated, then a particular treatment regimen will be considered efficacious.

30   In some forms, efficacy is assessed as a measure of the reduction in tumor volume and/or tumor mass at a specific time point (*e.g.*, 1-5 days, weeks, or months) following treatment.

C.   Modes of Administration

In some embodiments the methods administer modified T cells
including CAR-IDR and/or other IDR-fusion protein(s) in combination with
a pharmaceutically acceptable carrier. The compositions described herein
5   can be conveniently formulated into pharmaceutical compositions composed
of one or more of the compounds in association with a pharmaceutically
acceptable carrier. See, *e.g., Remington's Pharmaceutical Sciences*, latest
edition, by E.W. Martin Mack Pub. Co., Easton, PA, which discloses typical
carriers and conventional methods of preparing pharmaceutical compositions
10   that can be used in conjunction with the preparation of formulations of the
therapeutics described herein and which is incorporated by reference herein.
These most typically would be standard carriers for administration of
compositions to humans. In one aspect, for humans and non-humans, these
include solutions such as sterile water, saline, and buffered solutions at
15   physiological pH. Other therapeutics can be administered according to
standard procedures used by those skilled in the art.

The pharmaceutical compositions including modified cells, such as
therapeutic T cells, described herein can include, but are not limited to,
carriers, thickeners, diluents, buffers, preservatives, surface active agents and
20   the like in addition to the therapeutic(s) of choice.

Pharmaceutical compositions containing one or more modified cells,
such as therapeutic T cells including CAR-IDR and/or other IDR-fusion
protein(s), and optionally one or more additional therapeutic agents can be
administered to the subject in a number of ways depending on whether local
25   or systemic treatment is desired, and on the area to be treated. Thus, for
example, a pharmaceutical composition including modified cells, such as
therapeutic T cells, can be administered as an intravenous infusion, or
directly injected into a specific site, for example, into or surrounding a
tumor. Moreover, a pharmaceutical composition can be administered to a
30   subject as an ophthalmic solution and/or ointment to the surface of the eye,
vaginally, rectally, intranasally, orally, by inhalation, or parenterally, for
example, by intradermal, subcutaneous, intramuscular, intraperitoneal,
intrarectal, intraarterial, intralymphatic, intravenous, intrathecal and

intratracheal routes. In some forms, the compositions are administered directly into a tumor or tissue, *e.g.*, stereotactically.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid

5   solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, *e.g.*, U.S. Patent No. 3,610,795, which is incorporated by reference herein. Suitable

10   parenteral administration routes include intravascular administration (*e.g.*, intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature); peri- and intra-tissue injection (*e.g.*, intraocular injection, intra-retinal injection, or sub-retinal injection); subcutaneous injection or deposition

15   including subcutaneous infusion (such as by osmotic pumps); direct application by a catheter or other placement device (*e.g.*, an implant including a porous, non-porous, or gelatinous material).

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions which can also contain

20   buffers, diluents, and other suitable additives. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions, or suspensions, including saline and buffered media. Parenteral vehicles include sodium

25   chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert

30   gases and the like.

Administration of the pharmaceutical compositions containing one or more genetically modified cells (*e.g.*, CAR-IDR T cells) can be localized

(i.e., to a particular region, physiological system, tissue, organ, or cell type) or systemic.

It is to be understood that the disclosed method and compositions are not limited to specific synthetic methods, specific analytical techniques, or to particular reagents unless otherwise specified, and, as such, can vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

D. Combination therapy

In some embodiments the methods administer modified T cells including CAR-IDR and/or other IDR-fusion protein(s) in combination with other therapeutic agents or treatment modalities. Any of the disclosed pharmaceutical compositions including modified cells, such as therapeutic T cells (*e.g.*, containing a population of CAR-IDR T- cells), can be used alone, or in combination with other therapeutic agents or treatment modalities, for example, chemotherapy or stem-cell transplantation. As used herein, "combination" or "combined" refer to either concomitant, simultaneous, or sequential administration of the therapeutics.

In some forms, the pharmaceutical compositions and other therapeutic agents are administered separately through the same route of administration. In other forms, the pharmaceutical compositions and other therapeutic agents are administered separately through different routes of administration. The combinations can be administered either concomitantly (*e.g.*, as an admixture), separately but simultaneously (*e.g.*, via separate intravenous lines into the same subject; one agent is given orally while the other agent is given by infusion or injection, *etc.*,), or sequentially (*e.g.*, one agent is given first followed by the second).

Examples of preferred additional therapeutic agents include other conventional therapies known in the art for treating the desired disease, disorder, or condition. In some forms, the therapeutic agent is one or more other targeted therapies (*e.g.*, a targeted cancer therapy) and/or immune-checkpoint blockage agents (*e.g.*, anti-CTLA-4, anti-PD1, and/or anti-PDL1 agents such as antibodies).

The compositions and methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of therapies known in the art, such as chemotherapy, surgery, radiation, gene therapy, immunotherapy, bone marrow transplantation, stem

5   cell transplantation, targeted therapy, cryotherapy, ultrasound therapy, photodynamic therapy, radio-frequency ablation or the like, in an adjuvant setting or a neoadjuvant setting.

The disclosed pharmaceutical compositions and/or other therapeutic agents, procedures or modalities can be administered during periods of active

10   disease, or during a period of remission or less active disease. The pharmaceutical compositions can be administered before the additional treatment, concurrently with the treatment, post-treatment, or during remission of the disease or disorder. When administered in combination, the disclosed pharmaceutical compositions, and the additional therapeutic agents

15   (e.g., second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, e.g., as a monotherapy. In certain forms, the administered amount or dosage of the disclosed pharmaceutical composition, the additional therapeutic agent (e.g., second or third agent), or all, is lower (e.g.,

20   at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, e.g., as a monotherapy (e.g., required to achieve the same therapeutic effect).

1.   Additional anti-cancer agents

In some embodiments, the methods administer one or more additional

25   anti-cancer agents to a subject.

In the context of cancer, targeted therapies are therapeutic agents that block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Many different targeted therapies

30   have been approved for use in cancer treatment. These therapies include hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immunotherapies, and toxin delivery molecules. Numerous antineoplastic drugs can be used in

combination with the disclosed pharmaceutical compositions. In some forms, the additional therapeutic agent is a chemotherapeutic or antineoplastic drug. The majority of chemotherapeutic drugs can be divided into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase

5   inhibitors, monoclonal antibodies, and other anti-tumor agents.

2.   Additional therapeutic agents against Autoimmune diseases

In some embodiments, the methods also include administering one or more conventional therapies for autoimmune diseases to the subject.

10   Exemplary therapies for autoimmune diseases include immunosuppressive agents, such as steroids or cytostatic drugs, analgesics, non-steroidal anti-inflammatory drugs, glucocorticoids, immunosuppressive and immunomodulatory agents, such as methotrexate, leflunomide, hydroxychloroquine, and sulfasalazine. In some forms, the methods

15   administer one or more disease-modifying antirheumatic drugs (DMARDs). In some forms, the methods administer one or more biologic agents for localized treatment (*i.e.*, agents that do not affect the entire immune system), such as TNF-α inhibitors, belimumab and rituximab depleting B cells, T-cell co-stimulation blocker, anti-interleukin 6 (IL-6), anti-IL-1, and protein

20   kinase inhibitors. In other forms, the methods also administer one or more monoclonal antibodies (mAbs), such as anti-TNFα, anti-CD19, anti-CD20, anti-CD22, and anti-IL6R, or other mAbs that target multiple B cell subtypes, and other aberrant cells in autoimmune diseases.

IV.   Kits

25   The compositions, reagents, and other materials for cellular genomic engineering can be packaged together in any suitable combination as a kit useful for performing, or aiding in the performance of, the methods. It is useful if the components in a given kit are designed and adapted for use together in the method. For example, kits with one or more compositions for

30   administration to a subject, may include a pre-measured dosage of the composition in a sterile needle, ampule, tube, container, or other suitable vessel. The kits may include instructions for dosages and dosing regimens.

Provided are kits containing a IDR-fusion peptide (*e.g.*, CAR-IDR)
within a vector (*e.g.*, a viral vector) and/or mRNA encoding the IDR-fusion
peptide (*e.g.*, CAR-IDR), and instructional material for use thereof. In
preferred forms, the kit includes a plurality of vectors, where each vector

5   independently contains a IDR-fusion peptide (*e.g.*, CAR-IDR) for insertion
into a host cell genome, such as a CAR expression cassette. In some forms,
the kit contains a population of cells (*e.g.*, T cells) collectively containing the
IDR-fusion peptide (*e.g.*, CAR-IDR). The instructional material can include
a publication, a recording, a diagram, or any other medium of expression

10   which can be used to communicate the usefulness of the compositions and
methods of the kit. For example, the instructional material may provide
instructions for methods using the kit components, such as performing
transfections, transductions, infections, and conducting screens. In some
forms, kits include a transposon that includes a promoter and/or

15   polyadenylation signal operationally linked to a reporter gene and/or a CAR;
in some forms, the kit includes a transposon including a CAR that is specific
for an antigen selected from a cancer antigen, an inflammatory disease
antigen, a neuronal disorder antigen, HIV/AIDS, a diabetes antigen, a
cardiovascular disease antigen, an infectious disease antigen (including a

20   viral antigen, a protozoan antigen, a bacterial antigen, and an allergen), an
autoimmune disease antigen and an autoimmune disease antigen, or
combinations thereof; for example, in some embodiments the CAR targets
one or more antigens selected from the group including AFP, AKAP 4,
ALK, Androgen receptor, B7H3, BCMA, Bcr Abl, BORIS, Carbonic,

25   CD123, CD138, CD174, CD19, CD20, CD22, CD30, CD33, CD38, CD80,
CD86, CEA, CEACAM5, CEACAM6, Cyclin, CYP1B1, EBV, EGFR,
EGFR806, EGFRvIII, EpCAM, EphA2, ERG, ETV6 AML, FAP, Fos related
antigen1, Fucosyl, fusion, GD2, GD3, GloboH, GM3, gp100, GPC3, HER
2/neu, HER2, HMWMAA, HPV E6/E7, hTERT, Idiotype, IL12, IL13RA2,

30   IM19, IX, LCK, Legumain, IgK, LMP2, MAD CT 1, MAD CT 2, MAGE,
MelanA/MART1, Mesothelin, MET, ML IAP, MUC1, Mutant p53, MYCN,
NA17, NKG2D L, NY BR 1, NY ESO 1, NY ESO 1, OY TES1, p53, Page4,
PAP, PAX3, PAX5, PD L1, PDGFR β, PLAC1, Polysialic acid, Proteinase3

(PR1), PSA, PSCA, PSMA, Ras mutant, RGS5, RhoC, ROR1, SART3, sLe(a), Sperm protein 17, SSX2, STn, Survivin, Tie2, Tn, TRP 2, Tyrosinase, VEGFR2, WT1, and XAGE.

In some embodiments, the kit includes a cell or vector including a

5   CAR-IDR polypeptide, or other IDR-fusion peptide or nucleic acid encoding a CAR- IDR, or other IDR-fusion peptide. In some embodiments, the CAR- IDR is specific for an antigen that is selected from a cancer antigen selected from 4 1BB, 5T4, adenocarcinoma antigen, alpha fetoprotein, BAFF, B lymphoma cell, C242 antigen, CA 125, carbonic anhydrase 9 (CA

10   IX), C MET, CCR4, CD 152, CD 19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA 4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter

15   factor receptor kinase, IGF 1 receptor, IGF I, IgGl, Ll CAM, IL 13, IL 6, insulin-like growth factor I receptor, integrin $\alpha 5 \beta 1$, integrin $\alpha v \beta 3$, MORAb 009, MS4A1, MUC1, mucin CanAg, N glycolylneuraminic acid, NPC 1C, PDGF R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG 72, tenascin C, TGF beta

20   2, TGF β, TRAIL R1, TRAIL R2, tumor antigen CTAA16.88, VEGF A, VEGFR 1, VEGFR2, and vimentin; in some forms, the CAR is bispecific or multivalent; in some forms, the CAR is anti-CD19, anti-CD22, and/or anti-HER2.

In exemplary embodiments, the kits include a nucleic acid and/or a

25   vector expressing or encoding the CAR-IDR and/or cells. Exemplary cells include a T cell, hematopoietic stem cell (HSC), macrophage, natural killer cell (NK), or dendritic cell (DC). In some embodiments, the T cell is a CD8+ T cell selected from effector T cells, memory T cells, central memory T cells, and effector memory T cells. In some embodiments, the T cell is a

30   CD4+ T cell selected from Th1 cells, Th2 cells, Th17 cells, and Treg cells.

The present invention can be further understood by the following numbered paragraphs.

1. A polypeptide including

(a) an amino acid sequence of an intrinsically disordered region (IDR) of a protein, optionally including between 50 and 500 amino acids, or a functional fragment or variant thereof; and

(b) an amino acid sequence that is heterologous to the IDR.

2. The polypeptide of paragraph 1, wherein the amino acid sequence of the IDR includes any one of SEQ ID NOs:1-6, or a functional variant thereof having an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical to any one of SEQ ID NOs:1-6.

3. The polypeptide of paragraphs 1 or 2, wherein the amino acid sequence of the IDR includes at least 70% and less than 100% sequence identity to any one of SEQ ID NOs:1-6, or a homologue thereof, or functional fragment of the foregoing.

4. The polypeptide of any one of paragraphs 1-3, wherein the heterologous sequence includes one or more of a chimeric antigen receptor (CAR), protein transduction domain, fusogenic polypeptide, targeting signal, expression, and/or purification tag.

5. The polypeptide of paragraph 4, wherein the heterologous sequence includes a chimeric antigen receptor (CAR), and wherein the amino acid sequence of the IDR is present within the extracellular or intracellular region of the CAR.

6. The polypeptide of paragraph 4 or 5, wherein the heterologous sequence includes a chimeric antigen receptor (CAR), and wherein the amino acid sequence of the IDR is contiguous with the carboxyl terminus or amino terminus of the CAR, or inserted between two domains of the CAR, optionally wherein the polypeptide includes a structure according to:

(i) N-[CAR]-[IDR]$_Z$-C, where "N" and "C" refer to the amino (NH$_2$) and Carboxyl (COOH) termini, respectively, "CAR" refers to a single contiguous CAR fusion protein, "IDR" refers to an IDR and "Z" is an integer between one and four, inclusive; or

(ii) N-[CAR part 1]-[IDR]$_Z$-[CAR part 2], where "N" and "C" refer to the amino (NH$_2$) and Carboxyl (COOH) termini, respectively, "IDR"

refers to an IDR and "Z" is an integer between one and four, inclusive, "CAR-part 1" includes one or more traditional CAR domains and "CAR-part 2" includes one or more traditional CAR domains such that without the intervening [IDR]z domain, CAR-part 1 and CAR-part 2 form a single

5   contiguous CAR fusion protein.

7.   The polypeptide of any one of paragraphs 4-6, wherein the heterologous sequence includes a chimeric antigen receptor (CAR) including a transmembrane domain of CD8α, cytosolic signaling domains or a fragment there of derived from CD28, 41BB, and CD3 zeta, and optionally

10   wherein the amino acid sequence of the IDR is contiguous with the intracellular component of CD3 zeta.

8.   The polypeptide of any one of paragraphs 4-7, wherein the CAR is specific for an antigen selected from the group consisting of a cancer antigen, an inflammatory disease antigen, a neuronal disorder antigen,

15   HIV/AIDS, a diabetes antigen, a cardiovascular disease antigen, an infectious disease antigen (including a viral antigen, a protozoan antigen, a bacterial antigen, and an allergen), an autoimmune disease antigen and an autoimmune disease antigen, or combinations thereof.

9.   The polypeptide of paragraph 8, wherein the CAR targets

20   one or more antigens selected from the group consisting of AFP, AKAP 4, ALK, Androgen receptor, B7H3, BCMA, Bcr Abl, BORIS, Carbonic, CD123, CD138, CD174, CD19, CD20, CD22, CD30, CD33, CD38, CD80, CD86, CEA, CEACAM5, CEACAM6, Cyclin, CYP1B1, EBV, EGFR, EGFR806, EGFRvIII, EpCAM, EphA2, ERG, ETV6 AML, FAP, Fos related

25   antigen1, Fucosyl, fusion, GD2, GD3, GloboH, GM3, gp100, GPC3, HER 2/neu, HER2, HMWMAA, HPV E6/E7, hTERT, Idiotype, IL12, IL13RA2, IM19, IX, LCK, Legumain, IgK, LMP2, MAD CT 1, MAD CT 2, MAGE, MelanA/MART1, Mesothelin, MET, ML IAP, MUC1, Mutant p53, MYCN, NA17, NKG2D L, NY BR 1, NY ESO 1, NY ESO 1, OY TES1, p53, Page4,

30   PAP, PAX3, PAX5, PD L1, PDGFR β, PLAC1, Polysialic acid, Proteinase3 (PR1), PSA, PSCA, PSMA, Ras mutant, RGS5, RhoC, ROR1, SART3, sLe(a), Sperm protein 17, SSX2, STn, Survivin, Tie2, Tn, TRP 2, Tyrosinase, VEGFR2, WT1, XAGE, Claudin-6, Claudin-18.2 and CD70.

10.   The polypeptide of paragraph 8, wherein the antigen is a
cancer antigen selected from the group consisting of 41BB, 5T4,
adenocarcinoma antigen, alpha fetoprotein, BAFF, B lymphoma cell, C242
antigen, CA 125, carbonic anhydrase 9 (CA IX), C MET, CCR4, CD152,
CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30
(TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74,
CD80, CEA, CNT0888, CTLA 4, DR5, EGFR, EpCAM, CD3, FAP,
fibronectin extra domain B, folate receptor 1, GD2, GD3 ganglioside,
glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor
kinase, IGF 1 receptor, IGF I, IgG1, L1 CAM, IL 13, IL 6, insulin-like growth
factor I receptor, integrin α5β1, integrin αvβ3, MORAb 009, MS4A1,
MUC1, mucin CanAg, N glycolylneuraminic acid, NPC 1C, PDGF R a,
PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON,
ROR1, SCH 900105, SDC1, SLAMF7, TAG 72, tenascin C, TGF beta 2,
TGF β, TRAIL R1, TRAIL R2, tumor antigen CTAA16.88, VEGF A,
VEGFR 1, VEGFR2, and vimentin.

11.   The polypeptide of paragraph 10, wherein the CAR is
anti-CD19, anti-CD22, or anti-HER2.

12.   The polypeptide of any one of paragraphs 1-11, including
the polypeptide sequence of any one of SEQ ID NOs:7-27.

13.   The polypeptide of any one of paragraphs 1-12, lacked a
coiled-coiled domain.

14.   A nucleic acid including a nucleic acid encoding the
polypeptide of any one of paragraphs 1-13.

15.   The nucleic acid of paragraph 14, wherein the nucleic
acid is RNA or DNA.

16.   The nucleic acid of paragraph 14 or 15, wherein the
nucleic acid is mRNA.

17.   The nucleic acid of any one of paragraphs 14-16, wherein
the nucleic acid includes an expression control sequence(s).

18.   The nucleic acid of any one of paragraphs 14-17, wherein
the nucleic acid is, or is encoded by a vector or a transposon.

19.   The nucleic acid of paragraph 18, wherein the vector is a viral vector.

20.   The nucleic acid of paragraph 19, wherein the viral vector is selected from the group consisting of a lentiviral vector, an Adeno-associated virus (AAV) vector, or an adenovirus vector, or a Herpes Simplex virus (HSV) vector, or a vesicular stomatitis (VSV) vector, or a human Bocavirus vector (hBoV), or a chimeric vector including a combination of any two or more of an Adeno-associated virus (AAV) vector, Herpes Simplex virus (HSV) vector, vesicular stomatitis (VSV) vector, or a human Bocavirus vector (hBoV).

21.   The nucleic acid of paragraph 18, wherein the vector is a nucleic acid expression vector selected from the group consisting of a plasmid, a cosmid, and a replicon.

22.   The nucleic acid of any one of paragraphs 14-21, wherein the nucleic acid includes a promotor.

23.   The nucleic acid of any one of paragraphs 14-22, including one or more of a protein transduction domain, fusogenic polypeptide, or targeting signal conjugated thereto.

24.   An isolated cell including the polypeptide of any one of paragraphs 1-12, or the nucleic acid of any one of paragraphs 14-23.

25.   The isolated cell of paragraph 24, wherein the cell is a T cell, hematopoietic stem cell (HSC), macrophage, natural killer cell (NK), or dendritic cell (DC).

26.   The isolated cell of paragraph 25, wherein the T cell is a CD8+ T cell selected from the group consisting of effector T cells, memory T cells, central memory T cells, and effector memory T cells.

27.   The isolated cell of paragraph 25, wherein the T cell is a CD4+ T cell selected from the group consisting of Th1 cells, Th2 cells, Th17 cells, and Treg cells.

28.   The isolated cell of any one of paragraphs 24-27, wherein the polypeptide includes

(i)   an amino acid sequence encoding a CAR; and

(ii) an amino acid sequence of one or more of SEQ ID NOs:1-6, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to one or more of SEQ ID NOs:1-6.

29. The isolated cell of any one of paragraphs 24-28, wherein the polypeptide includes an amino acid sequence of one or more of SEQ ID NOs:7-27, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to one or more of SEQ ID NOs: 7-27.

30. The isolated cells of any one of paragraphs 24-29, wherein the cell has increased secretion of one or more cytotoxic factors optionally included IFNγ, Perforin, FasL, Granzyme A, and/or Granzyme B; activation of LAT and/or pathway(s) downstream thereof, optionally including SLP76 activation, ERK activation, RAS activation, and/or actin polymerization, optionally wherein LAT activation includes LAT phosphorylation; and/or reduction of one or more T cell exhaustion markers optionally TIM3 and/or LAG3 relative to a control cell, optionally wherein the control cells is the same cell type including the same heterologous sequence without the IDR sequence.

31. A population of cells including a plurality of the cells of any one of paragraphs 24-30, optionally derived by expanding the cell of any one of paragraphs 24-30.

32. A pharmaceutical composition including the population of cells of paragraph 31 and a pharmaceutically acceptable buffer, carrier, diluent, or excipient.

33. A method of treating a subject having a disease, disorder, or condition including administering to the subject an effective amount of the pharmaceutical composition of paragraph 32.

34. A method of treating a subject having a disease, disorder, or condition associated with an elevated expression or specific expression of an antigen, the method including administering to the subject an effective amount of a cell of any one of paragraphs 27-30, wherein the CAR targets the antigen.

35.   The method of any one of paragraphs 33-34, wherein the cell is isolated from the subject having the disease, disorder, or condition prior to the introduction to the cell.

36.   The method of any one of paragraphs 33-34, wherein the cell is isolated from a healthy donor.

37.   The method of any one of paragraphs 33-36, wherein the subject is a human.

38.   The method of any one of paragraphs 33-37, wherein the subject has cancer.

39.   A method of introducing an IDR fusion peptide into a cell, the method including introducing to the cell:

(i)   a vector or transposon or mRNA encoding a polypeptide of any one of paragraphs 1-13; and

(ii)   causing the polypeptide to be expressed in the cell.

40.   A chimeric antigen receptor (CAR) including a polypeptide of an intrinsically disordered region (IDR) (CAR-IDR), wherein the CAR-IDR targets CD19 and includes the amino acid sequence of any one of SEQ ID NOs:7-18.

41.   The chimeric antigen receptor (CAR) of paragraph 40, wherein the CAR-IDR has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control, wherein the control has the same CAR targeting CD19 but without fusion to an IDR including the amino acid sequence of SEQ ID NO:19.

42.   A chimeric antigen receptor (CAR) including a polypeptide of an intrinsically disordered region (IDR) (CAR-IDR), wherein the CAR-IDR targets CD22 and includes the amino acid sequence of any one of SEQ ID NOs: 20-22.

43.   The chimeric antigen receptor (CAR) of paragraph 42, wherein the CAR-IDR has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control, wherein the control has the same CAR targeting CD22 but without fusion to an IDR including the amino acid sequence of SEQ ID NO:23.

44.   A chimeric antigen receptor (CAR) including a polypeptide of an intrinsically disordered region (IDR) (CAR-IDR), wherein the CAR-IDR targets HER2 and includes the amino acid sequence of any one of SEQ ID NOs:24-26.

45.   The chimeric antigen receptor (CAR) of paragraph 44, wherein the CAR-IDR has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control, wherein the control has the same CAR targeting HER2 but without fusion to an IDR including the amino acid sequence of SEQ ID NO:27.

46.   A nucleic acid, including a nucleic acid sequence encoding the chimeric antigen receptor of any one of paragraphs 40-45.

47.   The nucleic acid of paragraph 46, wherein the nucleic acid is a vector or a transposon.

48.   An isolated cell including the CAR of any one of paragraphs 40-45, or the nucleic acid of any one of paragraphs 46-47.

49.   A population of cells derived by expanding the cell of paragraph 48.

50.   A pharmaceutical composition including the population of cells of paragraph 49 and a pharmaceutically acceptable buffer, carrier, diluent, or excipient.

51.   A method of treating a subject having a disease, disorder, or condition including administering to the subject an effective amount of the pharmaceutical composition of paragraph 50.

52.   The method of paragraph 51, wherein the disease is a cancer.

53.   A composition or method as disclosed herein including in the text and drawings.

The present invention will be further understood by reference to the following non-limiting examples.

EXAMPLES

Previous studies indicated that CARs insufficiently induce membrane-proximal signaling including ZAP70 activation and LAT phosphorylation, and LAT is weakly engaged in the CAR pathway (Dong et
5   al., 2020; Gudipati et al., 2020; Salter et al., 2021). LAT enhances TCR signal transduction by promoting the macromolecular complex assembly and condensation of TCR signaling molecules (Houtman et al., 2006; Huang et al., 2019; Su et al., 2016; Zeng et al., 2021). These indicated an opportunity to engage condensation to improve CAR-T signaling, especially on its
10   response to low antigen-expressing cancers.

Intrinsically disordered regions (IDRs) have the ability to form biomolecular condensates. IDRs do not typically fold into a well-defined 3D structure. Instead, they form condensates via weak inter and intra molecular interactions (Borcherds et al., 2021; Pappu et al., 2023). These condensates
15   display unique biochemical activities through enriching and organizing effector molecules to promote cell signaling (Case et al., 2019; Xiao et al., 2022a). In the experiments below, CAR condensation was induced through constructing CAR-IDR fusion proteins. IDRs were identified, including those from FUS, EWS, and TAF15, that promote the membrane-proximal
20   signaling, cytotoxic factor production, and killing of CAR-Ts against low antigen-expressing cancers (CD19, CD22, and HER2). Moreover, no elevated tonic signaling was observed in IDR CAR-Ts, indicating a difference in signaling outcomes between IDR-induced condensation and previously reported CAR aggregation (Chen et al., 2023; Long et al., 2015).
25   Together, these results demonstrated that IDRs, though not originally linked to T cell signaling, can serve as a new modular motif to improve the anti-tumor effect of CAR-Ts.

Materials and Methods

A list of reagents used in this study could be found in **Tables 1-4**.
30

SEQ ID NO | Name | IDR Sequences

SEQ ID NO	Name	IDR Sequences
1	FUS	MASND YTQQATQSYGAYPTQPGQGY SQQSSQPYGQQSYSGYSQST DTSGYGQSSYSSYGQSQNTG YGTQSTPQGYGSTGGYGSSQ SSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQ P QSGSYSQQPSYGGQQQSYGQ QQSYNPPQGYGQQNQYNSSS GGGGGGGGGGNYGQDQSSMS SGGGSGGGYGNQDQSGGGGS GGYGQQDRG
2	EWS	GYAQTTQA YGQQSYGTYGQPTDVSYTQA QTTATYGQTAYATSYGQPPT GYTTPTAPQAYSQPVQGYGT GAYDTTTATVTTTQASYAAQ SAYGTQPAYPAYGQQPAATA PTRPQDGNKPTETSQPQSST GGYNQPSLGYGQSNYSYPQV PGSYPMQPVTAPPSYPPTSY SSTQPTSYDQSSYSQQNTYG QPSSYGQQSSYGQQSSYGQQ PPTSYPPQTGSYSQAPSQYSQQSSSYG
3	TAF15	MSDSGSYG QSGGEQQSYSTYGNPGSQGY GQASQSYSGYGQTTDSSYGQNYSGYSSYGQSYSQSYGGY E NQKQSSYSQQPYNNQGQQQN MESSGSQGGRAPSYDQPDYG QQDSYDQQSGYDQHQGSYDE QSNYDQQHDSYSQNQQSYHS QRENYSHHTQDDRRDVSRYG EDNRGYGGSQGG

4	**NUP98**	FNKSFGTPFG GGTGGFGTTSTFGQNTGFGT
		TSGGAFGTSAFGSSNNTGGL
		FGNSQTKPGGLFGTSSFSQP
		ATSTSTGFGFGTSTGTANTL
		FGTASTGTSLFSSQNNAFAQ
		NKPTGFGNFGTSTSSGGLFG
		TTNTTSNPFGSTSGSLFGPS
		SFTAAPTGTTIKFNPPTGTD
		TMVKAGVSTNISTKHQCITA
		MKEYESKSLEELRLEDYQAN
		RKGPQNQVGAGTTTGLFGSS
		PATSSATGLFSSSTTNSGFA
		YGQNKTAFGTSTTGFGTNPG
		GLFGQQNQQTTSLFSKPFGQ
		ATTTQNTGFSFGNTSTIGQP
		STNTMGLFGVTQASQPGGLF GTATNTSTGT
5	**TDP43**	SNRQLERSGR FGGNPGGFGNQGGFGNSRGG
		GAGLGNNQGSNMGGGMNFGA
		FSINPAMMAAAQAALQSSWG
		MMGMLASQQNQSGPSGNNQN
		QGNMQREPNQAFGSGNNSYS
		GSNSGAAIGWGSASNAGSGS
		GFNGGFGSSMDSKSSGWGM
6	**SynIDR**	SKGPGRGDSPY
		SGRGDSPYSGRGDSPYSGRG
		DSPYSGRGDSPYSGRGDSPY
		SGRGDSPYSGRGDSPYSGRG
		DSPYSGRGDSPYSGY

Plasmids and lentivirus

DNA fragments encoding CAR or antigen molecules was inserted into a pHR lentiviral vector with an SFFV promoter and a WPRE terminator. HEK293T cells, maintained in DMEM medium supplemented with 10% FBS and a Glutamine–Penicillin–Streptomycin mix, were co-transfected

with the pHR plasmids and second-generation lentiviral packaging plasmids pMD2.G and psPAX2 (Addgene plasmid #12259 and #12260) using Genejuice transfection reagent (EMD Millipore, #70967-3). 48 hrs after transfection, cell culture media containing viral particles were harvested,

5   centrifuged, and filtered through 0.45 µm pore size filters.

Generation of CAR-T cells

Pan T cells were isolated from PBMCs (Zenbio #SER-PBMC-200) from healthy donors using EasySep™ Human T Cell Isolation Kit (Stem Cell Cat#17951). T cell proliferation was stimulated with Human T-Activator

10   CD3/CD28 Dynabeads (ThermoFisher, #11161D). Cells were cultured in RPMI1640 supplemented with 10% FBS, 50 nM 2-mercaptoethanol, 300 U/mL Interleukin-2 (PEPROTECH, # 200-02). Two days after stimulation, the cells were infected with fresh lentivirus via spinoculation at 800x g for 90 min at 32 °C. Half of the cell culture media were changed with fresh T

15   cell culture medium 24 hours after infection. Five days after infection, Dynabeads were removed, and CAR-T cells were resuspended in a fresh culture medium. The medium was exchanged every two days after.

Generation of cancer cell lines

The Nalm6, Raji B, and K562 cells were cultured in RPMI1640

20   supplemented with 10% FBS and a Glutamine–Penicillin–Streptomycin mix. HT29 cells were cultured in DMEM supplemented with 10% FBS and a Glutamine–Penicillin–Streptomycin mix. The Nalm6 CD19-high and -low cell lines expressing GFP and Luciferase were used. The Raji B CD19-high and -low cells were generated by sorting the wild-type Raji B cells for high

25   and low CD19 expression level. The K562 HER2-high and -low cells were generated by infecting K562 cellls with lentivirus encoding the wild-type HER2, followed by single-cell sorting. The number of antigen molecules per cell was quantified using the BD Quantibrite PE Phycoerythrin Fluorescence Quantitation Kit (BD Bioscience, Cat #340495) by flow cytometry.

30   Luciferase-expressing cells were generated by infecting cancer cells with a lentiviral plasmid expressing Luciferase-GFP or Luciferase-mCherry, followed by FACS to generate stable cell lines.

In vitro cytotoxicity assay

Cytotoxicity was measured by luciferase assay. Cancer cells expressing luciferase were resuspended in RPMI medium supplemented with 10% FBS and mixed with CAR-T cells at an effector to target ratio from

5   0.3:1 to 10:1. After 24 hours of incubation at 37°C, cells were collected and washed with PBS. Cell pellets were lysed in a lysis reagent (Promega, Cat# 1531). The luminescence of lysates was detected by Luciferase Assay System (Promega, Cat# E1500) and analyzed using a plate spectrophotometer. The spontaneous release control was set up using cancer

10   cells alone. Cell lysis% = [1-(experimental readout- spontaneous readout) /spontaneous readout]x100.

Cytokine production

CAR-T cells and cancer cells were cocultured at indicated E:T ratios for 24 hours at 37°C. The supernatant was collected for cytokine

15   measurement using ELISA kits (IL2 ELISA kit, BioLegend #431801; IFNγ ELISA kit, BioLegend #430101; TNFα ELISA kit, BioLegend # 430204) or using LEGENDplex™ Human CD8/NK Panel Kit (Cat# 741186), according to the manufacturer's instruction.

Flow Cytometry

20   To determine the cell-surface expression, cells were collected and blocked with an anti-human Fc receptor binding inhibitory antibody in the staining buffer (PBS with 2% FBS and 1 mM EDTA) for 15 minutes at 4°C, which were further incubated with individual antibodies in the staining buffer for 30 minutes on ice. The stained cells were washed twice with the

25   staining buffer before sending for flow cytometry analysis. To determine the intracellular expression of targets of interest, cells were collected and fixed with Fixation/Permeabilization Solution (Cat# 554714) for 15 min on ice, blocked in the staining buffer for 30 min on ice, followed by antibody staining. To characterize T cells in the mice blood, blood was drawn from a

30   tail cut and diluted into PBS supplemented with 3 mM EDTA. Red blood cells were lysed with a red blood cell lysis buffer. The rest of cells were stained as described above and followed by flow cytometry. To characterize tumor-infiltrating T cells, tumors were dissected and digested with RPMI

medium containing 0.5 mg/mL Collagenase P and 1 μg/mL DNase per 100 mg of tumor tissues for 30 min at 37°C on a shaker. The digested tumor tissues were further homogenized and passed through a 40 μm strainer, followed by centrifugation to collect cell samples. Cells were further stained
5   and analyzed by flow cytometry as described above.

Mouse tumor xenograft

Mice were housed in pathogen-free conditions and cared for in accordance with US National Institutes of Health guidelines, and all procedures were approved by the Yale University Animal Care and Use
10   Committee. To generate liquid cancer models, 1 million of Raji B or Naml6 cells in 100 uL of PBS were intravenously injected into the NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice (~6 weeks old) via tail vein. Three days later, 8 million of the control or IDR CAR T cells in 100 uL PBS were injected by tail vein. In vivo imaging of bioluminescence was performed to
15   monitor tumor growth. Blood was drawn at indicated time points post CAR-T treatment for flow cytometry analysis of CAR-T. To generate solid tumor models, 2.5 million HT29 cells in 100uL PBS were subcutaneously injected into the right flank of NSG mice. Eight days later, when tumor became palpable, 8 million of CAR T cells in 100uL PBS were injected by tail vein,
20   which was followed by a second dose treatment of 4 million of CAR T cells after five days. Tumor growth was measured with calipers every two days, and the size was calculated as one-half of the product of perpendicular length and square width in cubic millimeters (Volume=1/2*L*W*W). Mice were euthanized when the tumor size exceeded 2,000 mm^3. Blood was collected
25   and tumors were dissected to quantify tumor-infiltrating T cells.

Signaling characterization in CAR-T

CAR-T cells were resuspend in image medium (RPMI1640 no phenol red supplemented with 20 mM HEPES pH 7.4) and co-cultured with cancer cells at E:T=1:1 for indicated time at 37°C, followed by fixation and
30   permeabilization on ice for 15 min, and staining with individual phosphor-antibodies before sending for flow cytometry analysis or confocal microscopy.

Microscopy

TIRF and Confocal microscopy was performed on a Nikon Ti2-E inverted motorized microscope stand equipped with motorized stage with stagetop Piezo, Nikon H-TIRF, Yokogawa CSU-X1 spinning disk confocal,

5   Agilent laser combiner with four lines, 405, 488, 561, and 640 nm, and scientific CMOS camera Photometrics Prime 95B. Images were acquired using Nikon Elements. To monitor CAR localization on the cell surface, glass was coated with 10 nM ICAM-1 (Sinobiological #10346-H08H) in PBS for 2 hrs at room temperature. CAR-T cells resuspended in imaging

10   medium (RPMI1640 no phenol red supplemented with 20 mM HEPES pH7.4) were dropped onto the glass and examined by TIRF microscopy. To examine cell-cell conjugates and synapse, CAR-T cells and cancer cells were fixed in imaging media at E:T=1:1 for 30 min at 37°C. The cell mixture was dropped onto glass and imaged by confocal microscopy. To measure

15   signaling in the CAR-T synapse, CAR-T cells were co-cultured with cancer cells for 5 min at 37°C before fixation and permeabilization for 15min on ice, followed by staining with phosphor- antibodies for 30 min on ice. The stained cells were dropped onto glass and imaged by confocal microscopy.

Image analysis

20   Microscopy images were analyzed in Fiji (ImageJ). The same brightness and contrast were applied to images within the same panels. CAR condensation was quantified as normalized variance, which equals to the square of standard deviation divided by the mean. Cell conjugation percentage was calculated by dividing the number of tumor cell-associated

25   GFP+ CAR-T cells by the number of total GFP+ CAR T cells. CAR activation was calculated by dividing the intensities of pCD3ζ (Y142) or pLAT (Y171) in the synapse by the intensities of CAR-GFP.

Statistical analysis

Student's T test or Mann–Whitney U test analysis was used to assess

30   significance, with $P < 0.05$ considered significant. Data were analyzed on GraphPad Prism. The statistical details for each experiment were provided in the associated figure legends.

Example 1: Construction and validation of Chimeric Antigen Receptors fused with Intrinsically disordered regions (CAR-IDR)

IDRs contain diverse sequence and structure features. To determine which IDR promotes the condensation of CAR, candidates were selected

5   from 6 well-characterized IDRs that were previously shown to induce condensation in a cellular environment. These include IDRs from FUS, EWS, TAF15, Nup98, TDP43, and a synthetic IDR (synIDR) (Chong et al., 2018; Dzuricky et al., 2020; Kato et al., 2012; Protter et al., 2018; Wang et al., 2018; Xu et al., 2021; You et al., 2020). CD19 CAR was selected, which

10   is commonly used in research and clinical practice, as an initial model. This CAR is composed of a single chain variable fragment (scFv, FMC63) that targets CD19, a stalk and transmembrane domain from CD8α, and cytosolic signaling domains from 41BB, CD28, and CD3ζ. The IDR was fused to the C-terminus of CD3ζ. A superfolder GFP tag, which promotes the folding of

15   fused client proteins (Pedelacq et al., 2006), was further attached on the C-terminus of IDR for visualization of CAR condensation (**Figure 1A**). The superfolder GFP tag facilitates live cell imaging, which avoids potential fixation-induced artifacts in characterizing IDR condensation (Irgen-Gioro et al., 2022). The DNA fragment encoding the control or IDR CAR was

20   packaged into lentivirus and introduced into primary CD3+ T cells purified from the human peripheral mononuclear cells (PBMCs). Flow cytometry revealed the total cellular expression (by GFP) versus cell surface localization (by FMC63) of individual CARs (**Figure 1B-1I**), demonstrating that fusion with IDR did not affect the trafficking of CAR to the cell surface.

25   To visualize the condensation of CAR on the cell surface, live CAR-T cells were stained with a plasma membrane dye CellMask deep red and performed total internal reflection fluorescence (TIRF) microscopy which effectively reduces the cytosolic background of fluorescence. It was found that CAR fused with FUS, EWS, or TAF15 displayed an enhanced condensation as

30   compared to the control CAR (**Figure 1J**). Therefore, these three CARs in the following functional assays were focused.

The aggregation of CARs targeting GD2 or CSPG4 induces tonic signaling, which triggers CAR-T activation in the absence of antigens (Chen

et al., 2023; Long et al., 2015). Therefore, if IDR-induced CAR condensation causes tonic signaling was assessed. It was found that the IDR CAR-Ts did not proliferate in the absence IL-2 (**Figure 1K**). The expression of CD69, a T cell activation marker, was similar between the control and IDR CAR-Ts

5   in the absence of antigen (**Figure 7A**). Moreover, IDR CAR-Ts did not secret detectable TNFα in the absence of antigen (**Figure 7B**). Together, assessed from cell proliferation, activation marker, and cytokine production, IDR-induced CAR condensation did not trigger tonic signaling.

Example 2: IDR-fusion enhances the cytotoxicity of CD19 CAR

10   To determine how IDRs affect the cytotoxicity of CAR-Ts against cancer cells, the control or IDR CAR-Ts (**Figure 2A**) were co-cultured with variants of Nalm6, a B cell leukemia line, that expresses either high or low CD19 (**Figure 2B**). The Nalm6 cells express a luciferase reporter which facilitates the quantification of cytotoxicity by the luciferase assay. The FUS

15   and EWS, but not TAF15 CAR, displayed a substantial higher cytotoxicity towards both CD19-high and low Nalm6 cells (**Figures 2C-2D**).

             This result was recapitulated using another B cell line Raji as the target (**Figures 8A - 8C**). The higher cytotoxicity of FUS and EWS CAR could be explained by their higher secretion of cytotoxic factors including

20   Granzyme A, Granzyme B, Perforin, FASL, and IFNγ, when CAR-Ts were engaged with CD19-low Nalm6 cells (**Figures 2E-2I** and **Figure 8D**). Together, these data showed that the FUS and EWS IDRs enhanced the cytotoxicity of CAR-Ts against CD19-low cells, which was accompanied with a higher secretion of cytotoxic factors.

25   To assess the tumor killing effect of IDR CAR-Ts toward CD19-low cancer cells in vivo, CD19-low Raji B cells expressing a luciferase reporter were injected into the immune-deficient NSG mice intravenously. Three days later, the control, FUS, or EWS CAR-T cells were infused through the tail vein. The cancer progression was monitored by bioluminescence imaging

30   (**Figure 2J**). It was determined that FUS CAR-T inhibited cancer proliferation better than the control CAR-T (**Figures 2K-2O**). The expression of T cell exhaustion marker TIM3 and LAG3 were lower in FUS CAR-T (**Figure 2P** and **Figure 8E-8G**). No significant difference was

detected in the T cell number (**Figure 2Q** and **Figure 8H-8K**) or memory phenotype (**Figure 8L-8P**). Together, these data indicated that FUS CAR-T exhibited an enhanced anti-cancer activity both in vitro and in vivo.

Example 3: IDRs enhanced cytotoxicity of HER2 CAR

5   To determine if the effect of IDR in promoting cytotoxicity applied to CARs beyond CD19, IDR CARs targeting HER2 (**Figure 3A**), an antigen commonly overexpressed in multiple solid tumors including breast, ovarian, lung and colorectal cancers, were constructed. Human primary T cells were infected with lentivirus encoding the control, FUS, TAF15, or EWS CAR
10   (**Figure 3B**). Similar to the case of CD19 CAR, IDR did not trigger tonic signaling of HER2 CAR as assessed by CD69 expression and TNFα expression (**Figures 7C-7D**). Next, multiple target cell lines were selected for testing cytotoxicity: the lymphoblast K562 cells ectopically expressing high or low HER2 (**Figure 3C**), an ovarian cancer cell line SKOV3
15   expressing high HER2 and a colon cancer cell line HT29 expressing low HER2 (**Figure 3D**). These target cells were co-cultured with the control or IDR CAR-Ts. It was found that FUS and TAF15 CAR-Ts displayed a higher cytotoxicity towards all four cell lines tested, as compared to the control CAR-T (**Figures 3E-3H**).
20   Consistent with that, FUS and TAF15 CAR-Ts secreted a higher level of cytotoxic factors including IFNγ, Perforin, and FasL, than the control CAR-T (**Figures 3I – 3M** and **Figure 9A**). Together, these data indicated that FUS and TAF15 enhanced the cytotoxicity of HER2-high and low CAR-Ts in vitro.
25   To determine the anti-tumor effect of IDR CAR-Ts toward HER2-low cells in vivo, HT-29 cells were injected into the immune-deficient NSG mice subcutaneously. Eight days later, the control or IDR CAR-T cells were infused through the tail vein. The tumor progression was monitored by electronic caliper over 7 weeks (**Figure 3N**). It was determined that FUS and
30   TAF15 CAR-T inhibited tumor growth better than the control CAR-T (**Figures 3O – 3T**), which is consistent with the in vitro killing results. Although EWS CAR-T did not show a higher cytotoxicity in vitro, it did display a higher anti-tumor effect than the control CAR-T. This could be

potentially explained by the higher cytotoxic factors, including IFNγ, that were secreted by EWS CAR-T (**Figure 3I**). The numbers of circulating and tumor-infiltrating T cells were comparable between the control and IDR CAR-Ts (**Figures 3U-3V** and **Figures 9B-9K**), indicating that the enhanced killing was not related to an increased cell number but to an increased cytotoxicity (**Figure 3H**). Together, these data indicated that IDR CAR-Ts exhibited an enhanced anti-cancer efficacy towards HER-low cells in vivo.

Example 4: IDRs enhanced anti-tumor effect of CD22 CAR *in vivo*

In addition to the anti-CD19 and anti-HER2 CAR which target membrane-proximal epitopes, how IDRs affect the cytotoxicity of an anti-CD22 CAR (RFB4) were tested, which showed very low signaling efficiency due to the membrane-distal epitope position on CD22 (James et al., 2008; Xiao et al., 2022b). Using a similar design strategy to the CD19 CAR, the IDR CARs targeting CD22 were constructed by fusing the IDR on the C-terminus (**Figure 4A**). Human primary T cells were infected with lentivirus encoding the control, FUS, EWS, or TAF15 CAR (**Figure 4B**). The wild-type Raji B or Nalm6 cells which express a medium level of CD22 (**Figure 4C**) were co-cultured with CAR-Ts.

It was determined that FUS and EWS CAR-T displayed a higher cytotoxicity as compared to the control CAR-T when co-cultured with either Raji B or Nalm6 cells (**Figures 4F-4G**). The cytotoxic factors release was measured and found that FUS and EWS CAR-Ts released a slightly higher Granzyme A and B, and FasL than the control CAR-T, though it was not statistically significant (**Figures 4H-4L** and **Figure 10A**). Together, these data showed that FUS and EWS enhanced the cytotoxicity of a low-signaling CD22 CAR-T.

To determine if IDRs improve the anti-tumor efficacy of the above low-signaling CD22 CAR in vivo, the wild-type Nalm6 cells were injected into the NSG mice intravenously. Three days later, the control, FUS or EWS CAR-T cells were infused through the tail vein. The cancer progression was monitored by bioluminescence imaging (**Figure 4M**). Similar to the in vitro killing result, FUS and EWS CAR-T inhibited the tumor growth better than the control CAR-T (**Figure 4D**). The circulating T cell number was higher in

the FUS and EWS group than the control group (**Figure 4N** and **Figures 10B-10E**). The expression of exhaustion markers (**Figure 4E** and **Figures 10F-10H**) or memory phenotypes (**Figures 10I-10M**) was not significantly different between the control and IDR CAR-Ts. Together, these data

5   indicated that IDRs from FUS and EWS promote the anti-tumor effect of a low-signaling CD22 CAR.

Example 5: IDR enhanced CAR-T activation by promoting CD3ζ and LAT phosphorylation.

To determine the mechanism by which IDRs promote CAR-T

10   activation, membrane-proximal signaling in CAR-Ts were monitored upon engagement with CD19. Firstly, the cell-cell conjugation efficiency between the CAR-T and cancer cells was determined as a reflection of binding strength between CAR and antigen. It was determined that IDRs did not affect the cell-cell conjugation (**Figure 5A**), indicating that IDRs promote

15   CAR-T activation post CAR-antigen interactions. Following a CAR-antigen engagement, the CD3ζ domain on CAR is phosphorylated, which leads a cascade of biochemical reactions occurring at the T cell synapse and intracellular space. CAR-Ts were co-cultured with CD19-low Raji B cells and the antigen-dependent signaling kinetics by flow cytometry was

20   monitored. It was determined that the FUS, EWS, and TAF15 IDR enhanced the phosphorylation of CD3ζ, LAT, ERK, which lined up a pathway that mediates IDR CAR-T activation (**Figures 5B-5J** and **Figures 11A-11I**). Because CD3ζ and LAT are two transmembrane proteins enriched in the CAR-T synapse, their phosphorylation level in the synapse by confocal

25   microscopy was examined. Consistent with the flow cytometry measurement, all three IDRs enhanced the phosphorylation of CD3ζ and LAT in the synapse (**Figures 5K-5L**). Together, these data indicated that IDRs promote CAR-T activation against low-antigen cancer cells by promoting membrane-proximal signaling pathways.

30   **Example 6: Oligomerization of CAR by coiled-coil domains caused reduced surface localization**

In addition to IDR-mediated protein condensation, the coiled-coil domain is a commonly used tool to induce oligomerization of protein of

interest. To test if coiled-coil domains could promote CAR-T activation, coiled-coil (cc) domains was fused that mediate dimerization, tetramerization, or hexamerization (Deng et al., 2006; Spencer and Hochbaum, 2017) to the C-terminus of a CD19 CAR (**Figure 6A**) and

5   introduced these cc-CARs into human primary T cells. Whereas the cc-dimer maintained a similar cell surface expression level as compared to the control CAR, cc-tetramer and hexamer CAR showed a dramatic reduction in the cell surface localization (**Figure 6B-6F**). Consequently, the cell-cell conjugation percentage between CAR-T and Nalm6 cells was significantly reduced in the

10   cc-tetramer and hexamer as compared to the control CAR (**Figure 6G**). In consistency, CAR-T activation, as evaluated by CD69 expression (**Figure 6H** and **Figures 12A-12B**) and IFNγ secretion (**Figure 6I**), was significantly reduced in cc-tetramer or hexamer. The reduction in cell-cell conjugation and CAR-T activation were recapitulated when using Raji B as a target cell

15   (**Figures 12C-12F**). Similar to CAR, the oligomerization-induced receptor internalization was frequently observed in other transmembrane receptors including EGFR and FGFR (Hofman et al., 2010; Pozniak et al., 2020). The fact that IDRs do not affect cell surface expression of CAR indicated that IDRs present a unique advantage to promote CAR clustering without causing

20   enhanced receptor internalization.

Summary

Chimeric antigen receptor (CAR)-T cell-based therapies demonstrate remarkable efficacy for the treatment of otherwise intractable cancers, particularly B-cell malignancies. However, existing FDA-approved CAR-Ts

25   are limited by low antigen sensitivity, rendering their insufficient targeting to low antigen-expressing cancers. To improve the antigen sensitivity of CAR-Ts, CARs targeting CD19, CD22, and HER2 were engineered by including intrinsically disordered regions (IDRs) that promote signaling condensation. The "IDR CARs" triggered enhanced membrane-proximal signaling in the

30   CAR-T synapse, which led to an increased release of cytotoxic factors, a higher killing activity towards low antigen-expressing cancer cells in vitro, and an improved anti-tumor efficacy in vivo. No elevated tonic signaling was observed in IDR CAR-Ts. Together, IDRs are a new tool set to enhance

CAR-T cytotoxicity and to broaden CAR-T's application to low antigen-expressing cancers.

Biomolecular condensation has been demonstrated to regulate diverse cellular signaling processes (Case et al., 2019; Li et al., 2012; Li et al., 2022). Previous work showed that condensation of TCR signaling molecules promotes membrane-proximal signaling including LAT phosphorylation, RAS activation, actin polymerization, and ERK activation (Huang et al., 2019; Su et al., 2016; Zeng et al., 2021). The data and results presented herein identified IDR-induced condensation as a new strategy to improve the function of CAR-T. In this study, it was demonstrated the proof-of-concept application of implementing IDRs to enhance the cytotoxicity of CAR-Ts without inducing tonic signaling. IDRs are present in 30% of the human proteins, and it is believed that many more IDRs can modulate the condensation, antigen-binding, and conformation of CARs.

In this work, it was demonstrated that several well-characterized IDRs including those from FUS, EWS, and TAF15, promoted the condensation of CARs on the T cell membranes, which leads to an enhanced cytotoxicity towards low antigen-expressing cancer cells. Because the three above IDR-containing proteins are naturally functioning in the nucleus and none of these IDRs have been reported to have a function in T cell signaling or cytotoxicity, current strategy can serve as an orthogonal means to enhance the low signaling efficiency of CAR-T, in combination with other strategies, including selecting specific transmembrane or co-signaling domains (Heitzeneder et al., 2022; Majzner et al., 2020; Priceman et al., 2018), adding new signaling binding motifs (Salter et al., 2021), replacing the intracellular part with that from TCR (HIT) (Mansilla-Soto et al., 2022), dual targeting by CAR together with chimeric costimulatory receptors (Katsarou et al., 2021), or implementing other signaling pathways (Wilkens et al., 2022), to achieve extra combined effects.

Not all the IDRs tested in the current study promoted CAR condensation according to the tests utilized. FUS, EWS, or TAF15 promoted CAR condensation, but not NUP98, TDP43 or SynIDR according to the initial screening test. This could be attributed to the fact that the ability of

these IDRs to promote condensation was initially characterized in the 3D cytosolic environment, which is different from the 2D plasma membrane environment where CAR is localized. The local molecular crowdedness or the negative charge in the inner leaflet of plasma membrane could influence

5   the ability of individual IDRs to promote CAR condensation.

Current work showed that IDRs promoted LAT phosphorylation in the CAR synapse. LAT is one of the key adaptor proteins in the TCR pathway; it forms signaling condensates, recruits and activates a plethora downstream effector to amply TCR signaling (Bunnell et al., 2002; Zhang et

10   al., 1998). Previous studies indicated that LAT is poorly activated (phosphorylated) following CAR-antigen interactions (Dong et al., 2020; Salter et al., 2021), which could explain the low antigen sensitivity of CAR signaling. Here it was demonstrated that IDRs can significantly increase the activation of LAT and downstream pathways including SLP76 and ERK,

15   which finally lead to an enhanced cytotoxicity.

A difference was also revealed between the outcomes of different ways to induce CAR clusters. IDR-induced CAR condensation enhanced CAR phosphorylation and CAR-T activation whereas coiled-coil domains reduced cell surface expression of CAR and suppressed CAR-T activation.

20   One of the major differences between IDR and coiled-coil is that IDRs promote self-assembly of CAR through weak interactions and the condensates remain liquid-like or gel-like properties. In contrast, coiled-coil domains induced stable oligomerization which could either affect the trafficking of CAR to the cell surface or trigger instant receptor

25   internalization. Current work demonstrated that IDRs present a distinct advantage in promoting the assembly of CAR into a higher order structure without compromising their cell surface localization.

Table 1. Reagents

	Cell lines	Additional information
1	HEK293T	For lentivirus production
2	Raji B	
3	Raji B-CD19 high-ffLuc2-mCherry	For testing CD19 CAR
4	Raji B-CD19 low-ffLuc2-mCherry	For testing CD19 CAR
5	Nalm6-CD19 high-ffLuc2-GFP	For testing CD19 CAR

6	Nalm6-CD19 low-ffLuc2-GFP	For testing CD19 CAR
7	Nalm6-ffLuc2-GFP	For testing CD22 CAR
8	Nalm6-CD22 high-ffLuc2-mCherry	For testing CD22 CAR
9	Nalm6-CD22 low-ffLuc2-mCherry	For testing CD22 CAR
10	K562	
11	K562-HER2 high-ffLuc2-mCherry	For testing CD22 CAR
12	K562-HER2 low-ffLuc2-mCherry	For testing CD22 CAR
13	SKOV3	
14	SKOV3-ffLuc2-mCherry	For testing CD22 CAR
15	HT29	
16	HT29-ffLuc2-mCherry	For testing CD22 CAR

Table 2: Plasmids

1	pMD2.G	Viral packaging
2	poPAX	Viral packaging
3	pHR-mCherry-CAAX	Lentiviral vector
4	pFUGW-FerH-ffLuc2-mCherry	Lentiviral vector
5	pFUGW-FerH-ffLuc2-GFP	Lentiviral vector
6	pHR-CD19-mCherry	Lentiviral vector
7	pHAGE-ERBB2 (HER2)	Lentiviral vector
8	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-sGFP	Lentiviral vector
9	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-FUS 1-214-sGFP	Lentiviral vector
10	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-TAF15 1-180-sGFP	Lentiviral vector
11	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-EWS 28-262-sGFP	Lentiviral vector
12	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-Nup98 1-340-sGFP	Lentiviral vector

13	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-TDP43 266-414 speI-sGFP	Lentiviral vector
14	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-10xIDR-speI-sGFP	Lentiviral vector
15	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-FUS 1-214	Lentiviral vector
16	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-TAF15 1-180	Lentiviral vector
17	pHR NdeI-SP-CD19 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-41BB-CD3z cyto-speI-EWS 28-262	Lentiviral vector
18	pHR NdeI-SP-CD22 RFB4 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-CD3z cyto-speI-sGFP	Lentiviral vector
19	pHR NdeI-SP-CD22 RFB4 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-CD3z cyto-speI-FUS 1-214	Lentiviral vector
20	pHR NdeI-SP-CD22 RFB4 ScFv-BamHI-myc-CD8 Stalk-CD8 TM- NruI-CD28-CD3z cyto-speI-EWS 28-262	Lentiviral vector
21	pHR NdeI-SP-CD22 RFB4 ScFv-BamHI-myc-CD8 Stalk-CD8 TM-NruI-CD28-CD3z cyto-speI-TAF15 1-180	Lentiviral vector
22	pHR NdeI-SP- HER2 ScFv -BamHI-myc-CD8 Stalk-CD28 TM-NruI-CD28-41BB-CD3z cyto-sGFP	Lentiviral vector
23	pHR NdeI-SP-HER2 ScFv -BamHI-myc-CD8 Stalk-CD28 TM-NruI-CD28-41BB-CD3z cyto-speI-TAF15 1-180	Lentiviral vector
24	pHR NdeI-SP-HER2 ScFv-BamHI-myc-CD8 Stalk-CD28 TM-NruI-CD28-41BB-CD3z cyto-speI-FUS 1-214	Lentiviral vector
25	pHR NdeI-SP-HER2 ScFv-BamHI-myc-CD8 Stalk-CD28 TM-NruI-41BB-CD3z cyto-speI-EWS 28-262	Lentiviral vector

Table 3: Antibodies

1	PE anti-human CD19 Antibody	BioLegend	#392506
2	PE anti-human CD22 Antibody	BioLegend	#363503
3	PE anti-human CD340 (erbB2/HER- 2) Antibody	BioLegend	#324405
4	APC anti-human CD19 Antibody	BioLegend	#392504

5	APC anti-human CD22 Antibody	BioLegend	#363506
6	Alexa Fluor® 647 anti-human CD340 (crbB2/HER-2) Antibody	BioLegend	#324412
7	APC anti-human CD69 Antibody (FN50)	BioLegend	#310910
8	Brilliant Violet 421™ anti-human CD69 Antibody (FN50)	BioLegend	#310930
9	APC anti-human CD366 (TIM3) Monoclonal Antibody (F38-2E2)	Invitrogen	#17-3109-42
10	PE anti-human CD279 (PD-1) Monoclonal Antibody (MIH4)	Invitrogen	#12-9969-42
11	PE-Cyanine7 anti-human CD223 (LAG-3) Monoclonal Antibody (3DS223H)	Invitrogen	#25-2239-42
12	Pacific Blue™ Mouse Anti-Human CD3 Clone UCHT1	BioLegend	#300417
13	PerCP/Cyanine5.5 anti-human CD45RA Antibody	BioLegend	#304122
14	Pacific Blue™ anti-human CD4 Antibody	BioLegend	#317424
15	BD Horizon™ BUV496 Mouse Anti-Human CD4	BDbiosciences	#612936
16	Brilliant Violet 785™ anti-human CD8 Antibody	BioLegend	#344739
17	PE/Cy7 anti-human CD8a [RPA-T8] 100 tests	BioLegend	#301012
18	APC/Fire™ 750 anti-human CD62L Antibody	BioLegend	#304845
19	Brilliant Violet 750™ anti-human CD45RA Antibody, 100 tests	BioLegend	#304166
20	BD Phosflow™ Alexa Fluor® 647 Mouse anti-CD247 (pY142)	BDbiosciences	#558489
21	Alexa Fluor® 647 anti-LAT Phospho (Tyr171) Antibody	BioLegend	#946603

Table 4: Others

1	EasySep™ Human T Cell Isolation Kit	Stem Cell	#17951
2	Human T-Activator CD3/CD28 Dynabeads	ThermoFisher	#11161D
3	Recomninant Interleukin-2	PEPROTECH	# 200-02
4	Human Recombinant Biotinylated Her2/ERBB2 protein	Sino Biological	#10004-H08H-B
5	Human Recombinant Biotinylated CD22 protein	Sino Biological	#11958-H41H-B
6	Luciferase Assay System	Promega	# E1500
7	Cell lysis reagent	Promega	# 1531
8	Red Blood cell lysis buffer	BioLegend	#420301
9	D-Luciferin, Sodium Salt	Gold Biotechnology	#LUCNA-100
10	Fixation/Permeabilization Solution	BD Bioscience	# 554714

11	TNFα ELISA kit	BioLegend	# 430204
12	BD Quantibrite PE Phycoerythrin Fluorescence Quantitation Kit	BD Bioscience	#340495
13	LEGENDplex™ Human CD8/NK Panel (13-plex)	BioLegend	#741186

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Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by

10   reference.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

15

We claim:

1.   A polypeptide comprising

        (a)   an amino acid sequence of an intrinsically disordered region (IDR) of a protein, optionally comprising between 50 and 500 amino acids, or a functional fragment or variant thereof; and

        (b)   an amino acid sequence that is heterologous to the IDR.

2.   The polypeptide of claim 1, wherein the amino acid sequence of the IDR comprises any one of SEQ ID NOs:1-6, or a functional variant thereof having an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical to any one of SEQ ID NOs:1-6.

3.   The polypeptide of claims 1 or 2, wherein the amino acid sequence of the IDR comprises at least 70% and less than 100% sequence identity to any one of SEQ ID NOs:1-6, or a homologue thereof, or functional fragment of the foregoing.

4.   The polypeptide of any one of claims 1-3, wherein the heterologous sequence comprises one or more of a chimeric antigen receptor (CAR), protein transduction domain, fusogenic polypeptide, targeting signal, expression, and/or purification tag.

5.   The polypeptide of claim 4, wherein the heterologous sequence comprises a chimeric antigen receptor (CAR), and wherein the amino acid sequence of the IDR is present within the extracellular or intracellular region of the CAR.

6.   The polypeptide of claim 4 or 5, wherein the heterologous sequence comprises a chimeric antigen receptor (CAR), and wherein the amino acid sequence of the IDR is contiguous with the carboxyl terminus or amino terminus of the CAR, or inserted between two domains of the CAR, optionally wherein the polypeptide comprises a structure according to:

        (i) N-[CAR]-[IDR]$_Z$-C, where "N" and "C" refer to the amino (NH$_2$) and Carboxyl (COOH) termini, respectively, "CAR" refers to a single contiguous CAR fusion protein, "IDR" refers to an IDR and "Z" is an integer between one and four, inclusive; or

        (ii) N-[CAR part 1]-[IDR]$_Z$-[CAR part 2], where "N" and "C" refer to the amino (NH$_2$) and Carboxyl (COOH) termini, respectively, "IDR"

refs to an IDR and "Z" is an integer between one and four, inclusive, "CAR-part 1" includes one or more traditional CAR domains and "CAR-part 2" includes one or more traditional CAR domains such that without the intervening [IDR]z domain, CAR-part 1 and CAR-part 2 form a single contiguous CAR fusion protein.

7.   The polypeptide of any one of claims 4-6, wherein the heterologous sequence comprises a chimeric antigen receptor (CAR) comprising a transmembrane domain of CD8α, cytosolic signaling domains or a fragment there of derived from CD28, 41BB, and CD3 zeta, and optionally wherein the amino acid sequence of the IDR is contiguous with the intracellular component of CD3 zeta.

8.   The polypeptide of any one of claims 4-7, wherein the CAR is specific for an antigen selected from the group consisting of a cancer antigen, an inflammatory disease antigen, a neuronal disorder antigen, HIV/AIDS, a diabetes antigen, a cardiovascular disease antigen, an infectious disease antigen (including a viral antigen, a protozoan antigen, a bacterial antigen, and an allergen), an autoimmune disease antigen and an autoimmune disease antigen, or combinations thereof.

9.   The polypeptide of claim 8, wherein the CAR targets one or more antigens selected from the group consisting of AFP, AKAP 4, ALK, Androgen receptor, B7H3, BCMA, Bcr Abl, BORIS, Carbonic, CD123, CD138, CD174, CD19, CD20, CD22, CD30, CD33, CD38, CD80, CD86, CEA, CEACAM5, CEACAM6, Cyclin, CYP1B1, EBV, EGFR, EGFR806, EGFRvIII, EpCAM, EphA2, ERG, ETV6 AML, FAP, Fos related antigen1, Fucosyl, fusion, GD2, GD3, GloboH, GM3, gp100, GPC3, HER 2/neu, HER2, HMWMAA, HPV E6/E7, hTERT, Idiotype, IL12, IL13RA2, IM19, IX, LCK, Legumain, IgK, LMP2, MAD CT 1, MAD CT 2, MAGE, MelanA/MART1, Mesothelin, MET, ML IAP, MUC1, Mutant p53, MYCN, NA17, NKG2D L, NY BR 1, NY ESO 1, NY ESO 1, OY TES1, p53, Page4, PAP, PAX3, PAX5, PD L1, PDGFR β, PLAC1, Polysialic acid, Proteinase3 (PR1), PSA, PSCA, PSMA, Ras mutant, RGS5, RhoC, ROR1, SART3, sLe(a), Sperm protein 17, SSX2, STn, Survivin, Tie2, Tn, TRP 2, Tyrosinase, VEGFR2, WT1, XAGE, Claudin-6, Claudin-18.2 and CD70.

10.   The polypeptide of claim 8, wherein the antigen is a cancer antigen selected from the group consisting of 41BB, 5T4, adenocarcinoma antigen, alpha fetoprotein, BAFF, B lymphoma cell, C242 antigen, CA 125, carbonic anhydrase 9 (CA IX), C MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA 4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF 1 receptor, IGF I, IgGl, Ll CAM, IL 13, IL 6, insulin-like growth factor I receptor, integrin $\alpha5\beta1$, integrin $\alpha v\beta3$, MORAb 009, MS4A1, MUC1, mucin CanAg, N glycolylneuraminic acid, NPC 1C, PDGF R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG 72, tenascin C, TGF beta 2, TGF β, TRAIL R1, TRAIL R2, tumor antigen CTAA16.88, VEGF A, VEGFR 1, VEGFR2, and vimentin.

11.   The polypeptide of claim 10, wherein the CAR is anti-CD19, anti-CD22, or anti-HER2.

12.   The polypeptide of any one of claims 1-11, comprising the polypeptide sequence of any one of SEQ ID NOs:7-27.

13.   The polypeptide of any one of claims 1-12, lacked a coiled-coiled domain.

14.   A nucleic acid comprising a nucleic acid encoding the polypeptide of any one of claims 1-13.

15.   The nucleic acid of claim 14, wherein the nucleic acid is RNA or DNA.

16.   The nucleic acid of claim 14 or 15, wherein the nucleic acid is mRNA.

17.   The nucleic acid of any one of claims 14-16, wherein the nucleic acid comprises an expression control sequence(s).

18.   The nucleic acid of any one of claims 14-17, wherein the nucleic acid is, or is encoded by a vector or a transposon.

19.   The nucleic acid of claim 18, wherein the vector is a viral vector.

20. The nucleic acid of claim 19, wherein the viral vector is selected from the group consisting of a lentiviral vector, an Adeno-associated virus (AAV) vector, or an adenovirus vector, or a Herpes Simplex virus (HSV) vector, or a vesicular stomatitis (VSV) vector, or a human Bocavirus vector (hBoV), or a chimeric vector comprising a combination of any two or more of an Adeno-associated virus (AAV) vector, Herpes Simplex virus (HSV) vector, vesicular stomatitis (VSV) vector, or a human Bocavirus vector (hBoV).

21. The nucleic acid of claim 18, wherein the vector is a nucleic acid expression vector selected from the group consisting of a plasmid, a cosmid, and a replicon.

22. The nucleic acid of any one of claims 14-21, wherein the nucleic acid comprises a promotor.

23. The nucleic acid of any one of claims 14-22, comprising one or more of a protein transduction domain, fusogenic polypeptide, or targeting signal conjugated thereto.

24. An isolated cell comprising the polypeptide of any one of claims 1-12, or the nucleic acid of any one of claims 14-23.

25. The isolated cell of claim 24, wherein the cell is a T cell, hematopoietic stem cell (HSC), macrophage, natural killer cell (NK), or dendritic cell (DC).

26. The isolated cell of claim 25, wherein the T cell is a CD8+ T cell selected from the group consisting of effector T cells, memory T cells, central memory T cells, and effector memory T cells.

27. The isolated cell of claim 25, wherein the T cell is a CD4+ T cell selected from the group consisting of Th1 cells, Th2 cells, Th17 cells, and Treg cells.

28. The isolated cell of any one of claims 24-27, wherein the polypeptide comprises

(i) an amino acid sequence encoding a CAR; and

(ii) an amino acid sequence of one or more of SEQ ID NOs:1-6, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to one or more of SEQ ID NOs:1-6.

29. The isolated cell of any one of claims 24-28, wherein the polypeptide comprises an amino acid sequence of one or more of SEQ ID NOs:7-27, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to one or more of SEQ ID NOs: 7-27.

30. The isolated cells of any one of claims 24-29, wherein the cell has increased secretion of one or more cytotoxic factors optionally included IFNγ, Perforin, FasL, Granzyme A, and/or Granzyme B; activation of LAT and/or pathway(s) downstream thereof, optionally comprising SLP76 activation, ERK activation, RAS activation, and/or actin polymerization, optionally wherein LAT activation comprises LAT phosphorylation; and/or reduction of one or more T cell exhaustion markers optionally TIM3 and/or LAG3 relative to a control cell, optionally wherein the control cells is the same cell type comprising the same heterologous sequence without the IDR sequence.

31. A population of cells comprising a plurality of the cells of any one of claims 24-30, optionally derived by expanding the cell of any one of claims 24-30.

32. A pharmaceutical composition comprising the population of cells of claim 31 and a pharmaceutically acceptable buffer, carrier, diluent, or excipient.

33. A method of treating a subject having a disease, disorder, or condition comprising administering to the subject an effective amount of the pharmaceutical composition of claim 32.

34. A method of treating a subject having a disease, disorder, or condition associated with an elevated expression or specific expression of an antigen, the method comprising administering to the subject an effective amount of a cell of any one of claims 27-30, wherein the CAR targets the antigen.

35. The method of any one of claims 33-34, wherein the cell is isolated from the subject having the disease, disorder, or condition prior to the introduction to the cell.

36. The method of any one of claims 33-34, wherein the cell is isolated from a healthy donor.

37.   The method of any one of claims 33-36, wherein the subject is a human.

38.   The method of any one of claims 33-37, wherein the subject has cancer.

39.   A method of introducing an IDR fusion peptide into a cell, the method comprising introducing to the cell:

        (i)   a vector or transposon or mRNA encoding a polypeptide of any one of claims 1-13; and

        (ii)   causing the polypeptide to be expressed in the cell.

40.   A chimeric antigen receptor (CAR) including a polypeptide of an intrinsically disordered region (IDR) (CAR-IDR), wherein the CAR-IDR targets CD19 and comprises the amino acid sequence of any one of SEQ ID NOs:7-18.

41.   The chimeric antigen receptor (CAR) of claim 40, wherein the CAR-IDR has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control, wherein the control has the same CAR targeting CD19 but without fusion to an IDR comprising the amino acid sequence of SEQ ID NO:19.

42.   A chimeric antigen receptor (CAR) including a polypeptide of an intrinsically disordered region (IDR) (CAR-IDR), wherein the CAR-IDR targets CD22 and comprises the amino acid sequence of any one of SEQ ID NOs: 20-22.

43.   The chimeric antigen receptor (CAR) of claim 42, wherein the CAR-IDR has increased activity in condensation of CAR and/or activation of T cells expressing the CAR-IDR compared to a control, wherein the control has the same CAR targeting CD22 but without fusion to an IDR comprising the amino acid sequence of SEQ ID NO:23.

44.   A chimeric antigen receptor (CAR) including a polypeptide of an intrinsically disordered region (IDR) (CAR-IDR), wherein the CAR-IDR targets HER2 and comprises the amino acid sequence of any one of SEQ ID NOs:24-26.

45.   The chimeric antigen receptor (CAR) of claim 44, wherein the CAR-IDR has increased activity in condensation of CAR and/or activation of

T cells expressing the CAR-IDR compared to a control, wherein the control has the same CAR targeting HER2 but without fusion to an IDR comprising the amino acid sequence of SEQ ID NO:27.

46.   A nucleic acid, comprising a nucleic acid sequence encoding the chimeric antigen receptor of any one of claims 40-45.

47.   The nucleic acid of claim 46, wherein the nucleic acid is a vector or a transposon.

48.   An isolated cell comprising the CAR of any one of claims 40-45, or the nucleic acid of any one of claims 46-47.

49.   A population of cells derived by expanding the cell of claim 48.

50.   A pharmaceutical composition comprising the population of cells of claim 49 and a pharmaceutically acceptable buffer, carrier, diluent, or excipient.

51.   A method of treating a subject having a disease, disorder, or condition comprising administering to the subject an effective amount of the pharmaceutical composition of claim 50.

52.   The method of claim 51, wherein the disease is a cancer.
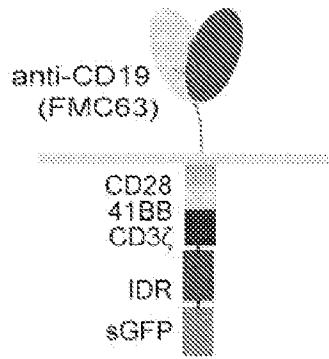
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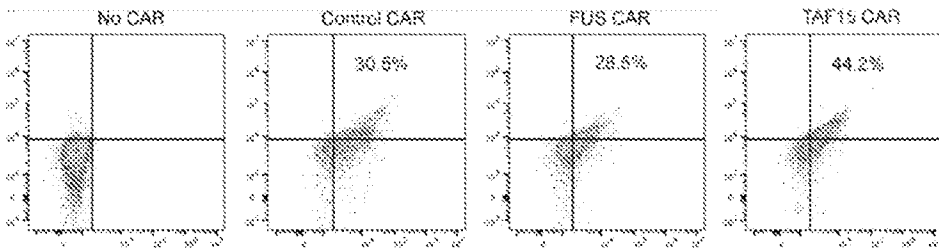
FIG. 1B   FIG. 1C   FIG. 1D   FIG. 1E
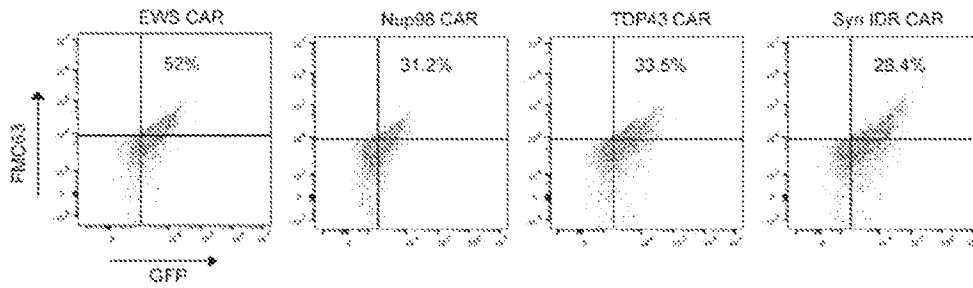
FIG. 1F   FIG. 1G   FIG. 1H   FIG. 1I
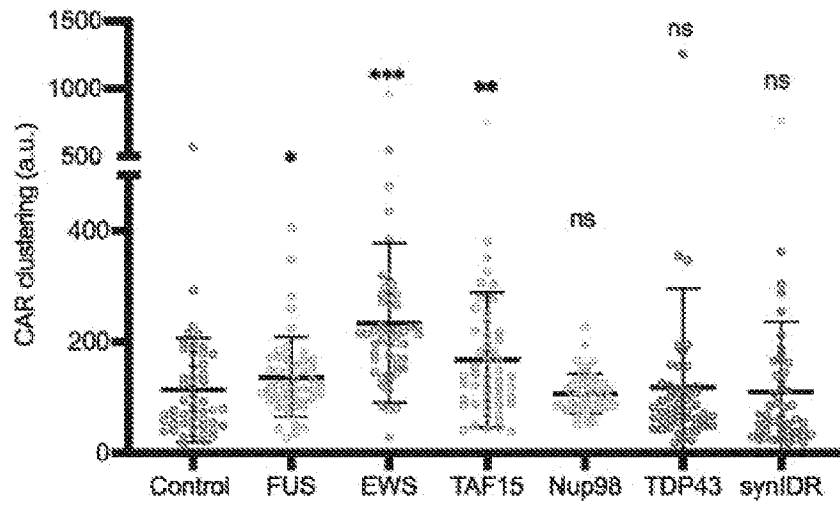
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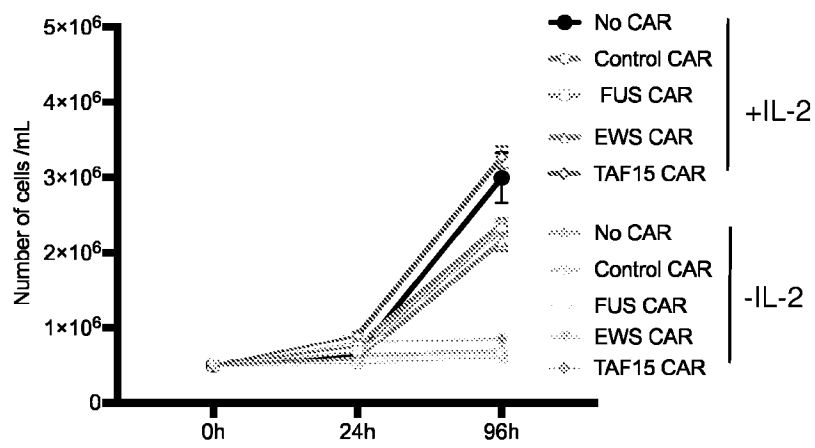
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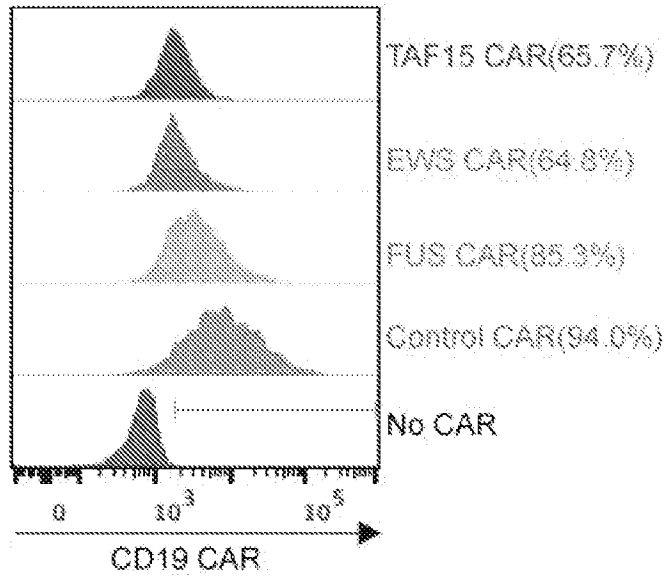
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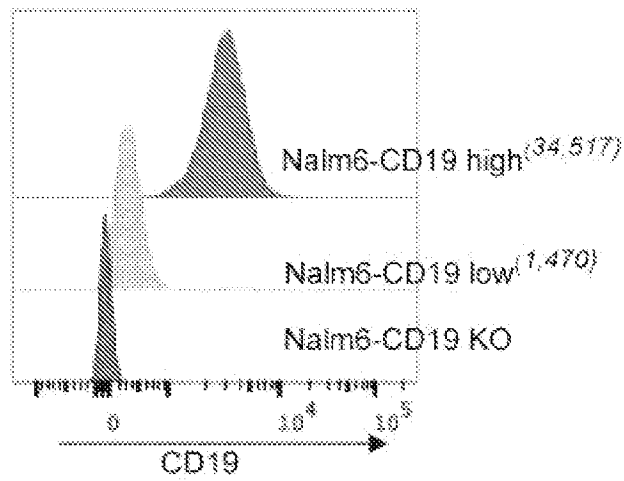
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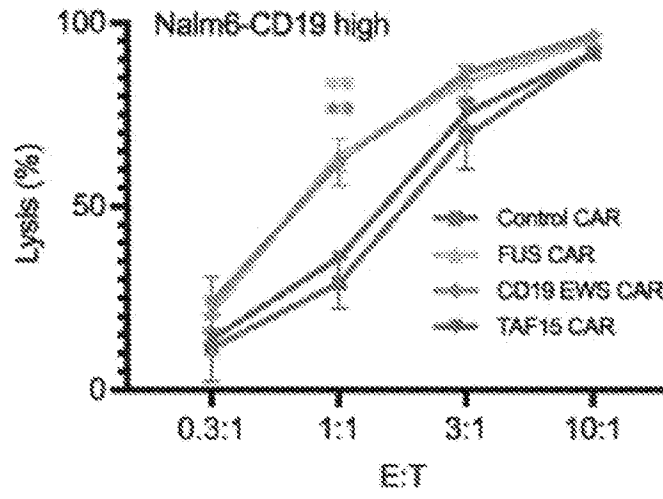
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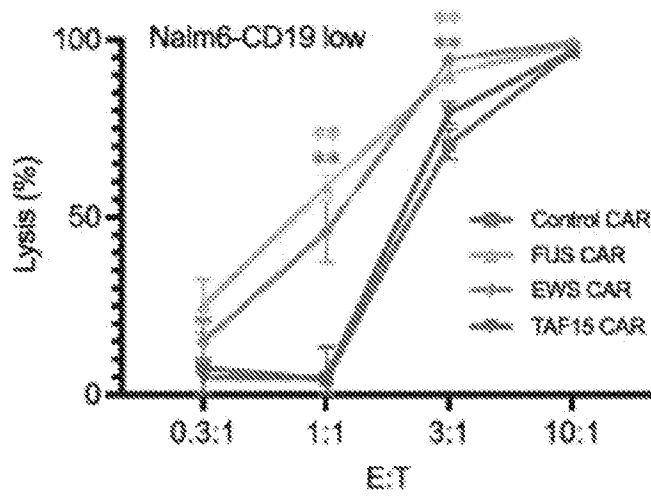
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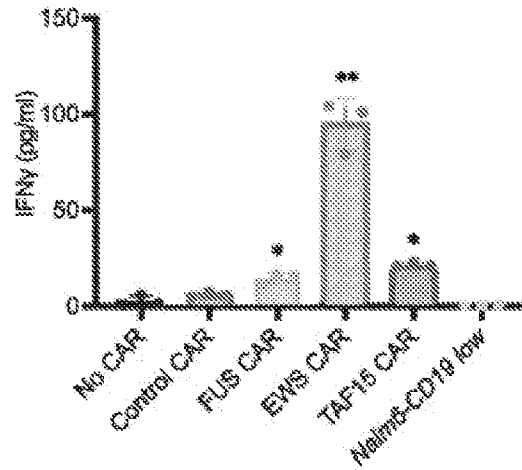
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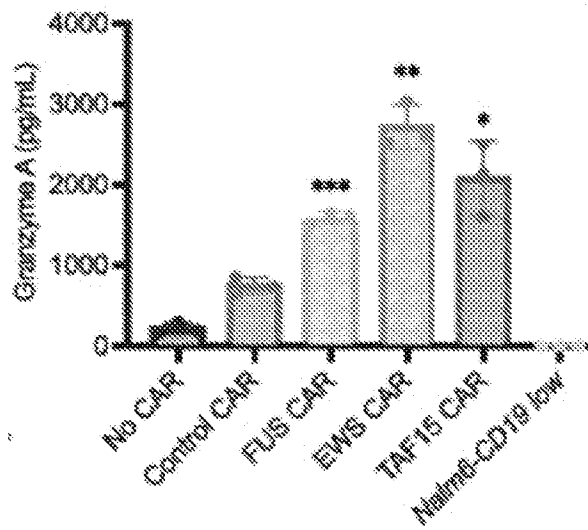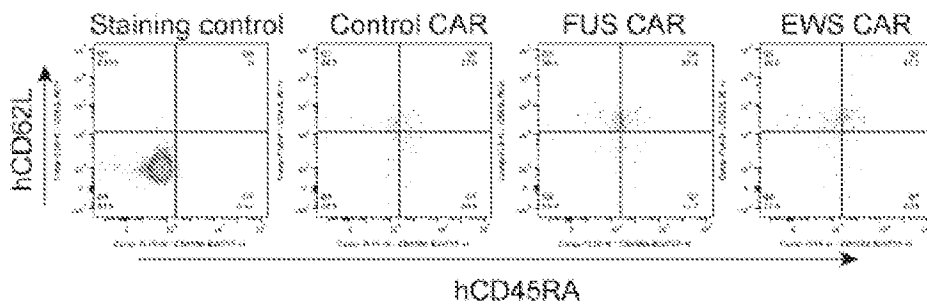

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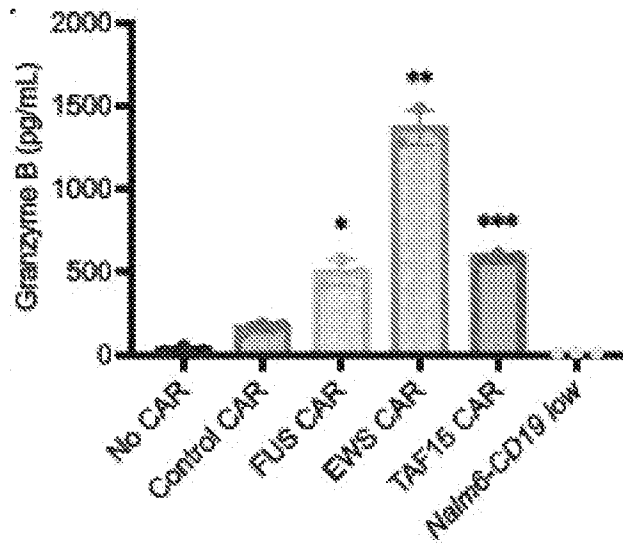
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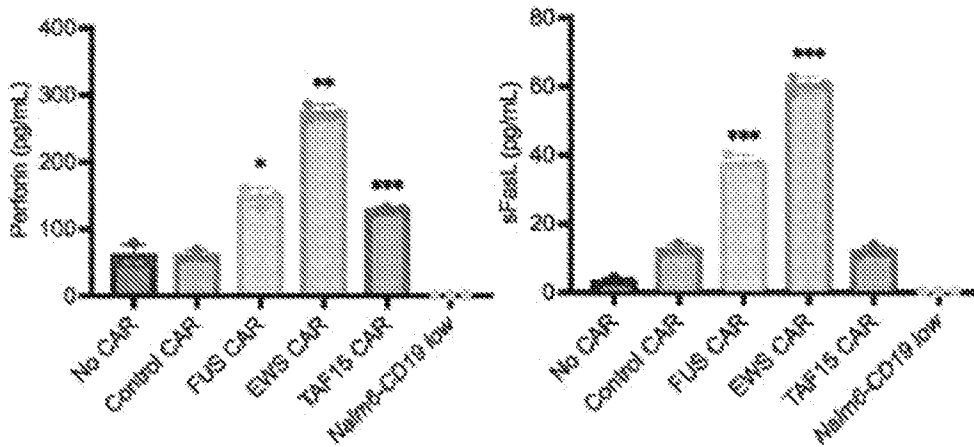
FIG. 2H

FIG. 2I
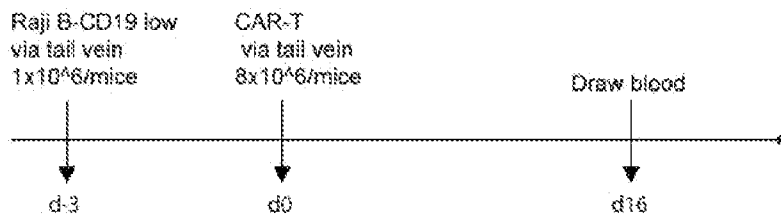
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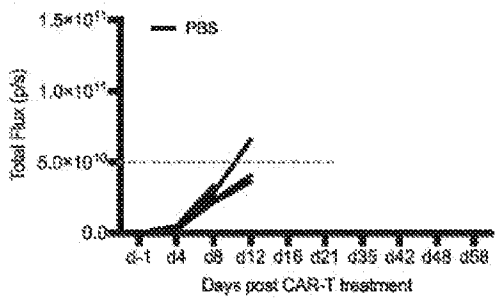
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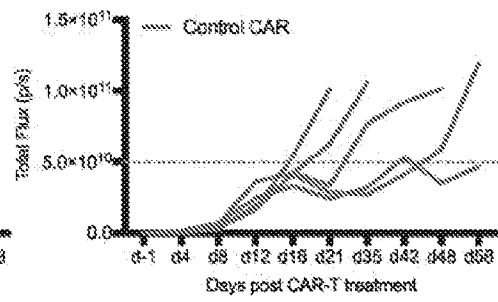
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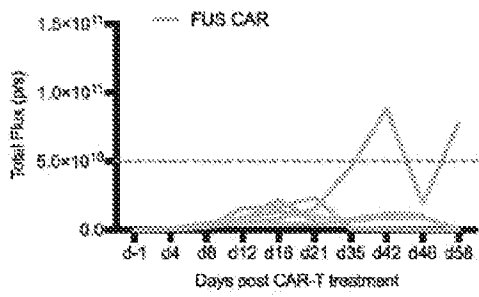
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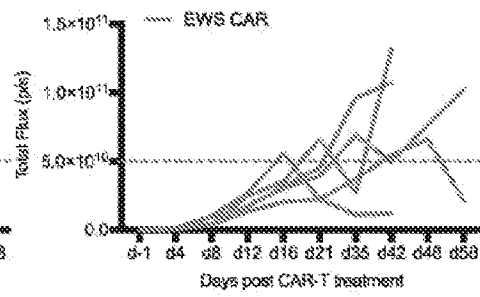
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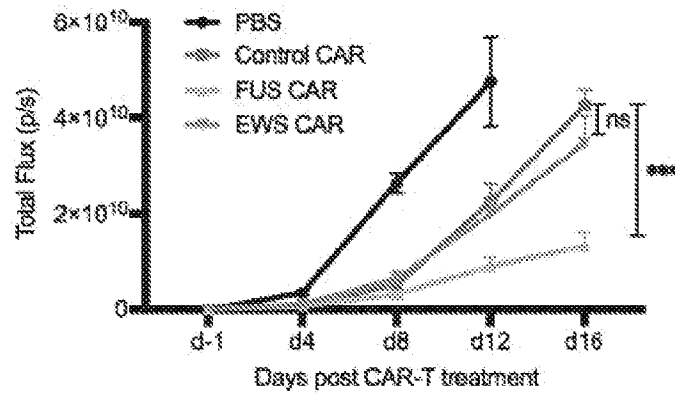
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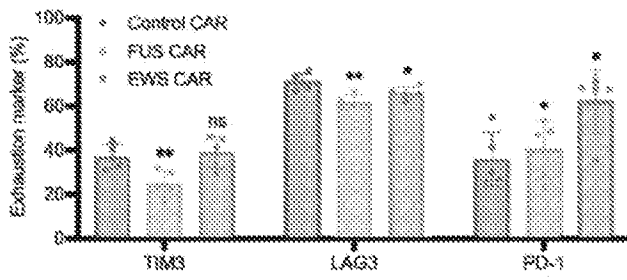
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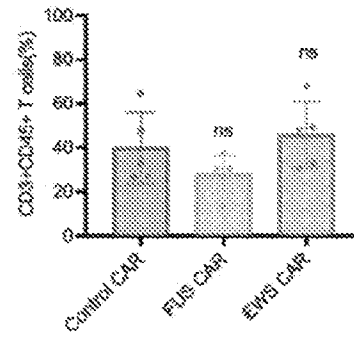
FIG. 2Q

FIG. 3A
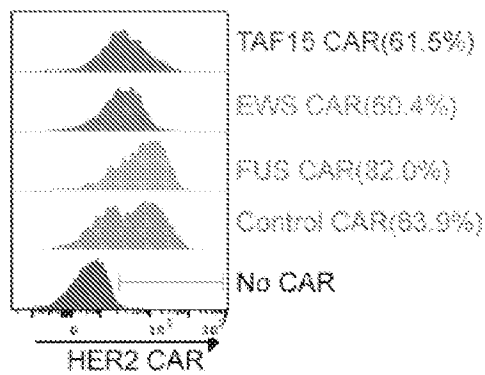
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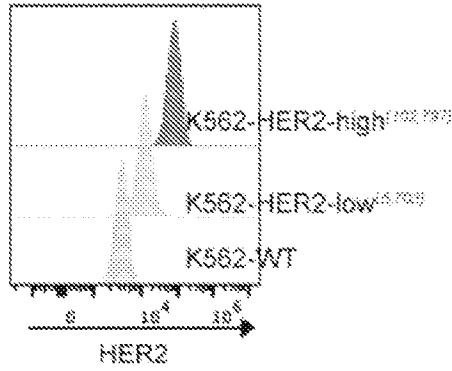
FIG. 3C
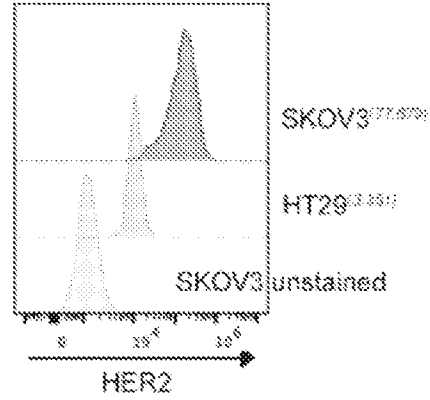
FIG. 3D
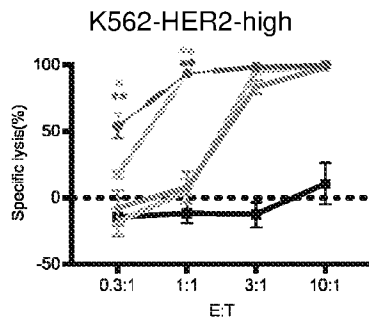
FIG. 3E
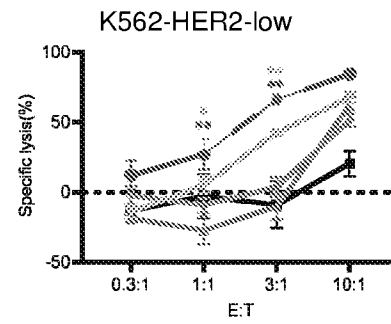
FIG. 3F
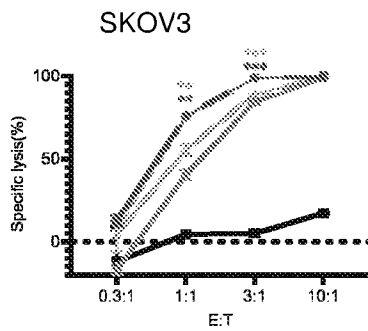
FIG. 3G
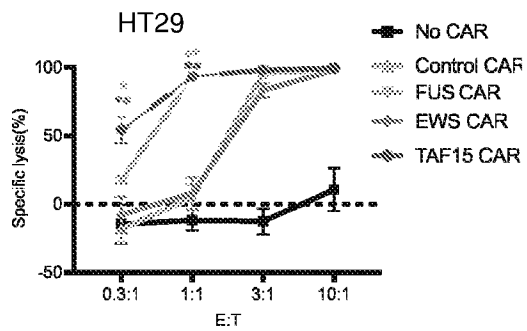
FIG. 3H
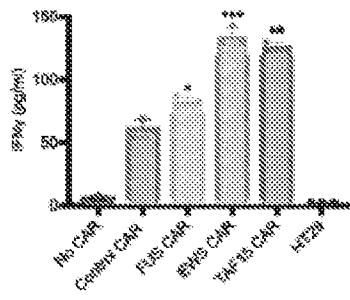
FIG. 3I
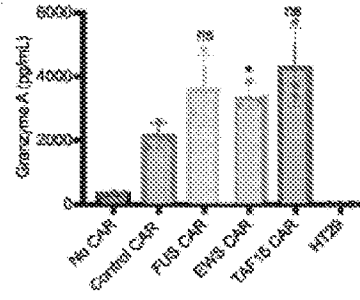
FIG. 3J
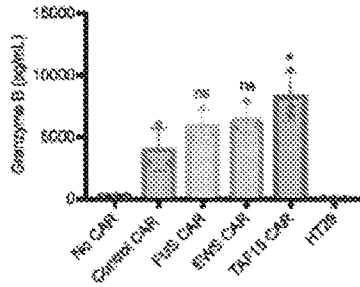
FIG. 3K
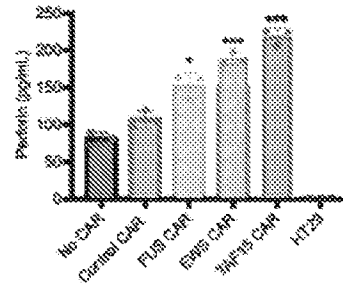
FIG. 3L

FIG. 3M

FIG. 3N

FIG. 3O
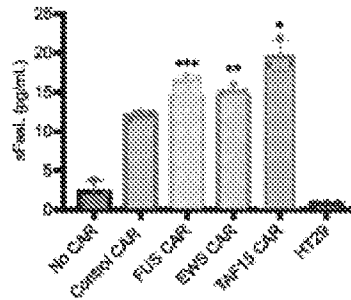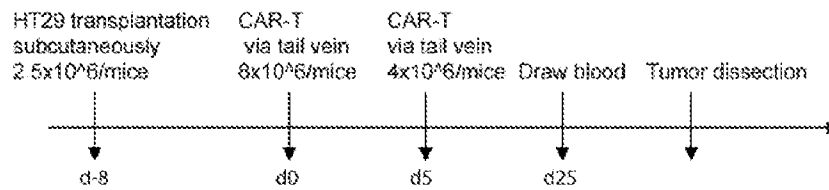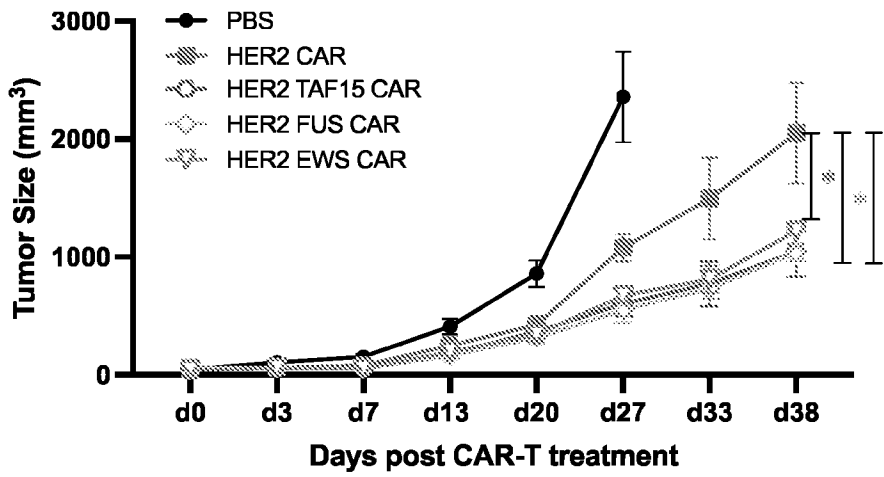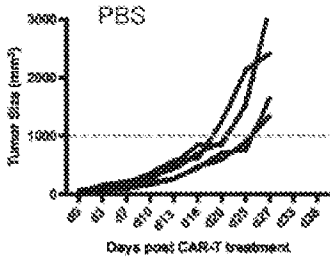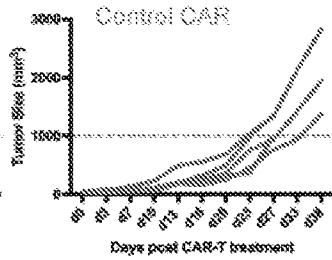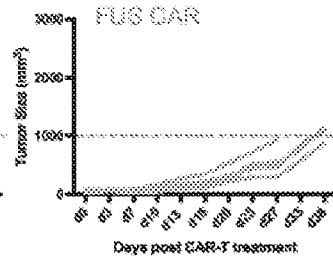
FIG. 3P   FIG. 3Q   FIG. 3R
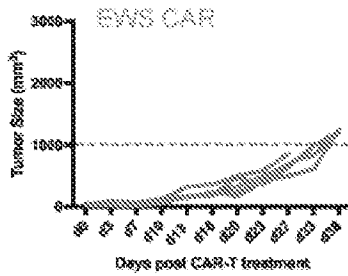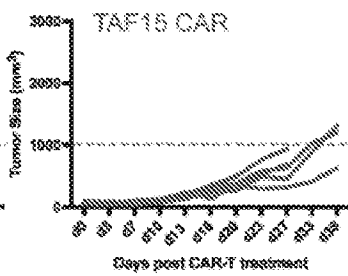
FIG. 3S   FIG. 3T
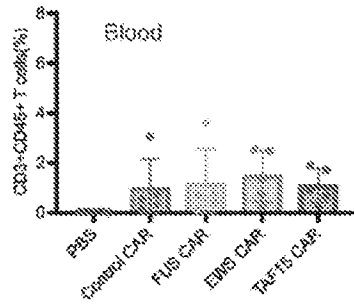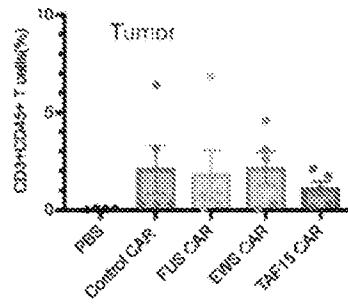
FIG. 3U   FIG. 3V
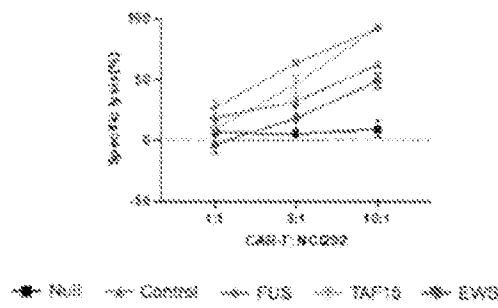
FIG. 3W
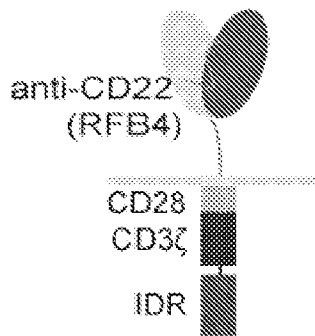
FIG. 4A
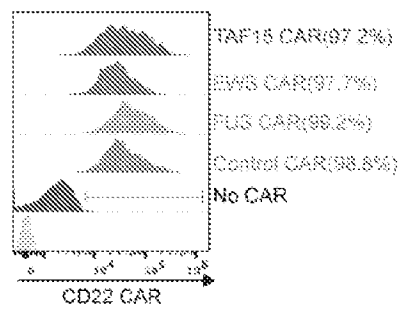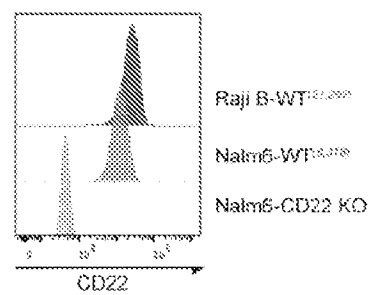
FIG. 4B   FIG. 4C
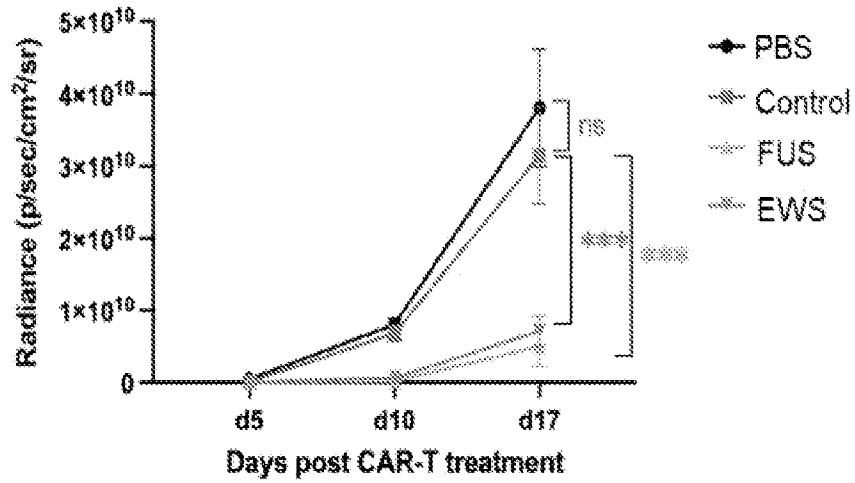
FIG. 4D
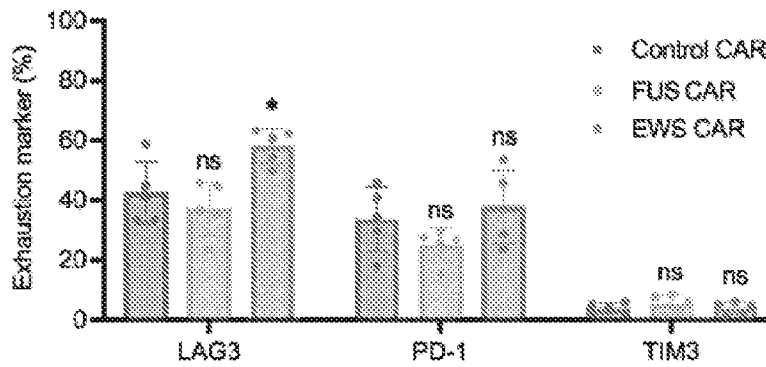
FIG. 4E
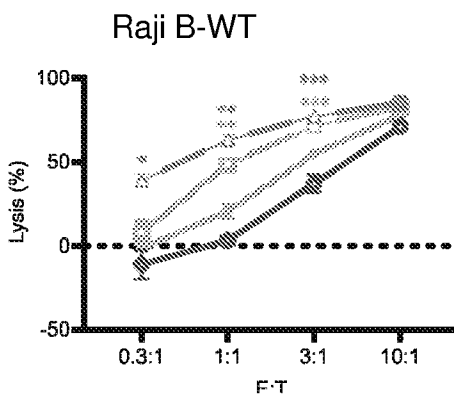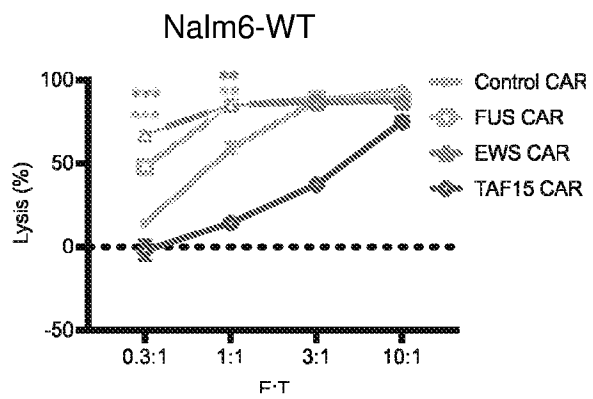
FIG. 4F   **FIG. 4G**
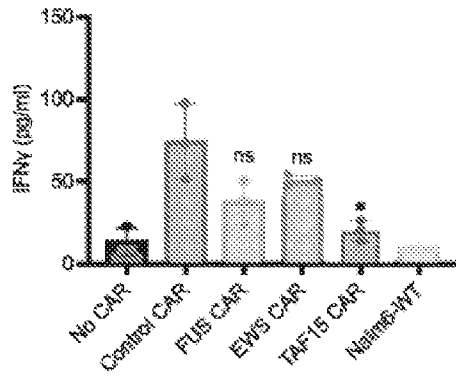
FIG. 4H
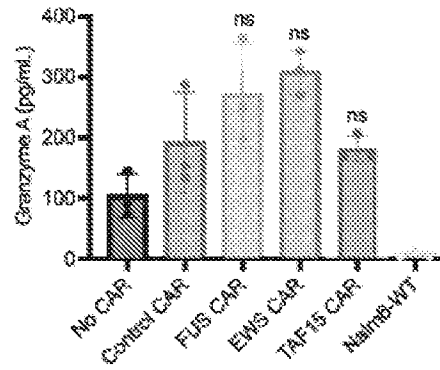
FIG. 4I
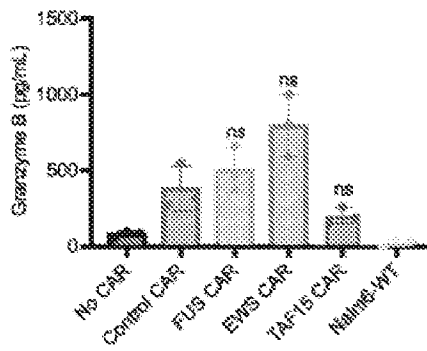
FIG. 4J
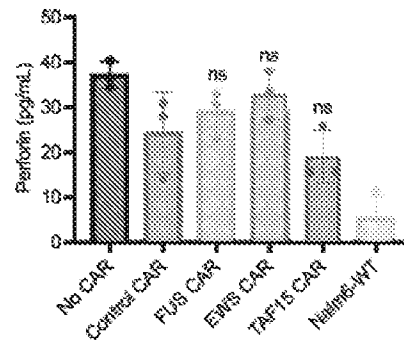
FIG. 4K
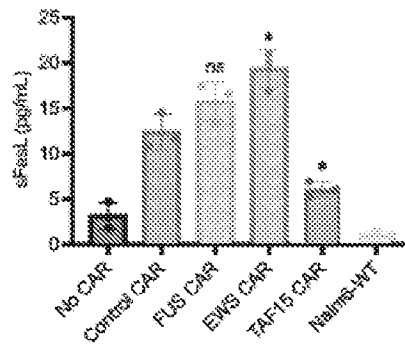
FIG. 4L
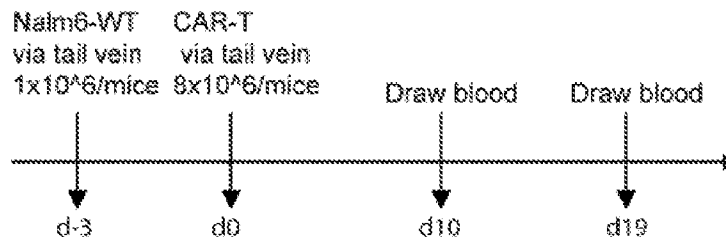
FIG. 4M
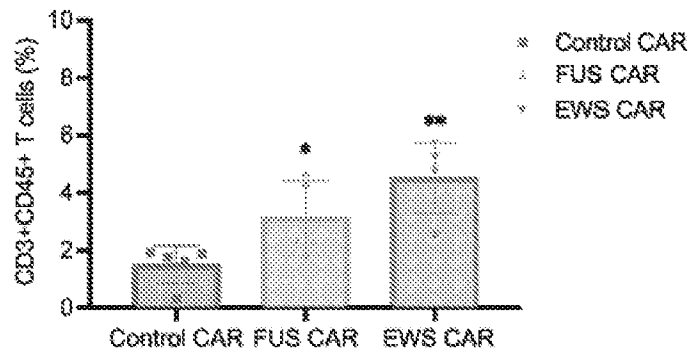
FIG. 4N
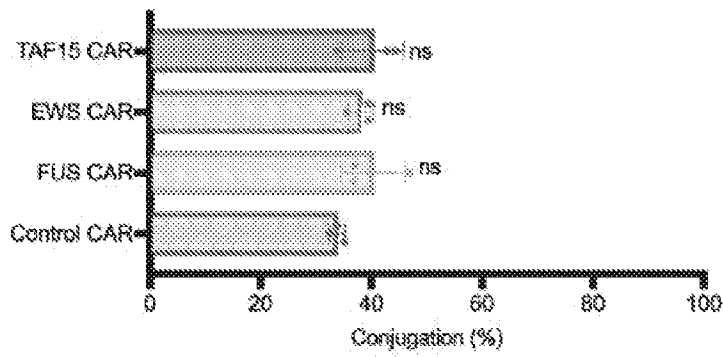
FIG. 5A
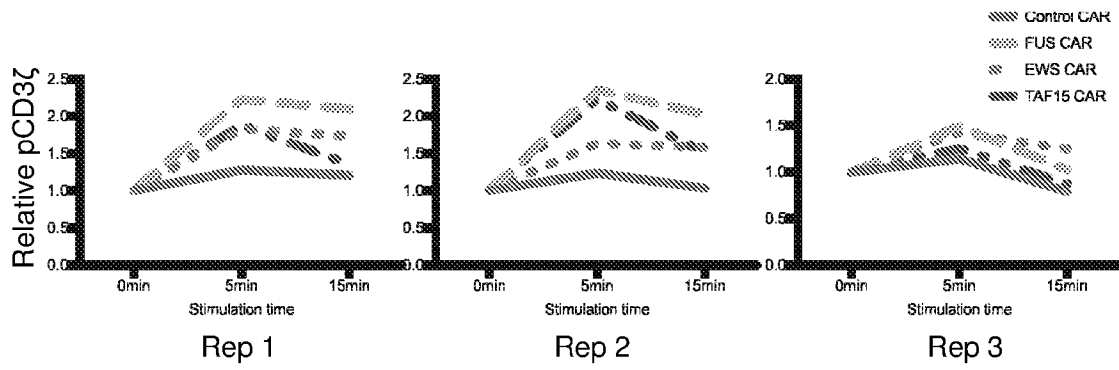
FIG. 5B

FIG. 5C

FIG. 5D
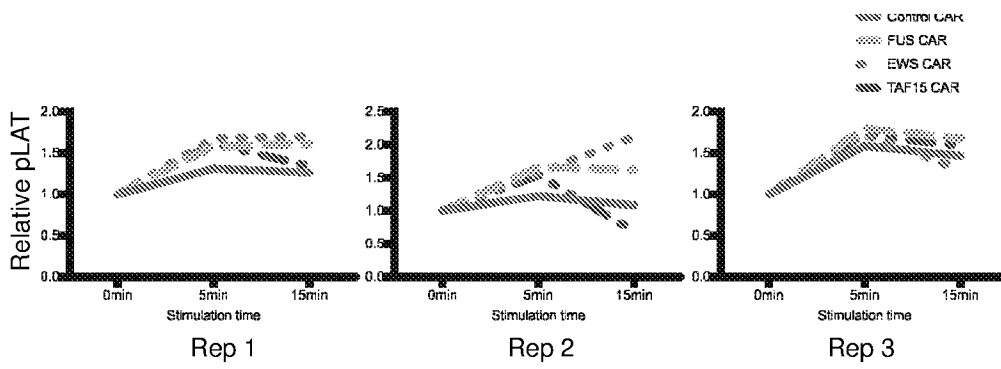
FIG. 5E

FIG. 5F

FIG. 5G
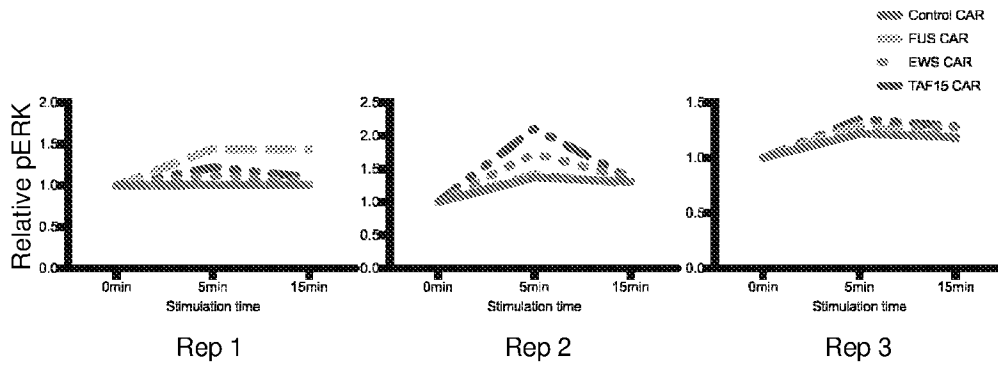
FIG. 5H

FIG. 5I

FIG. 5J
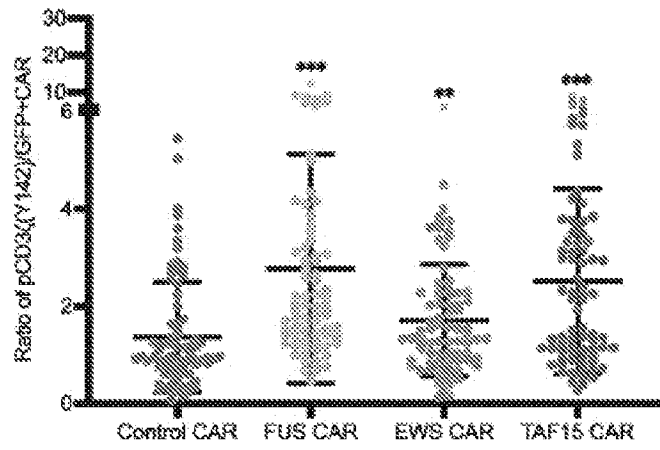
FIG. 5K
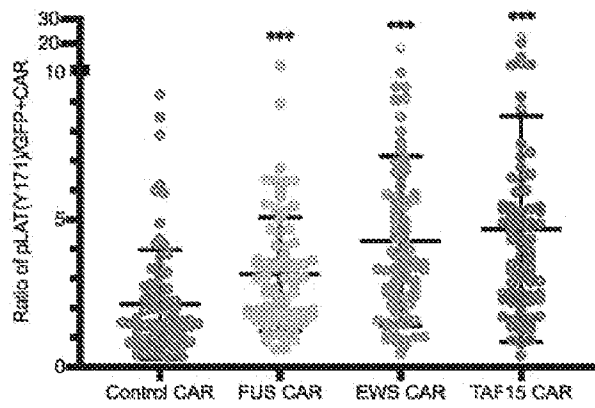
FIG. 5L
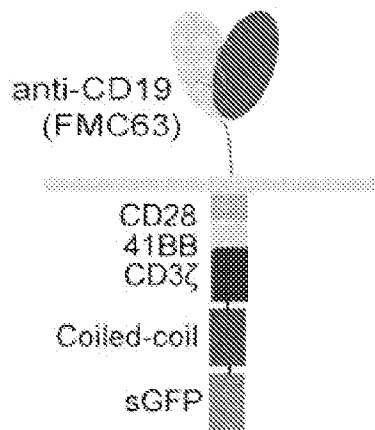
FIG. 6A
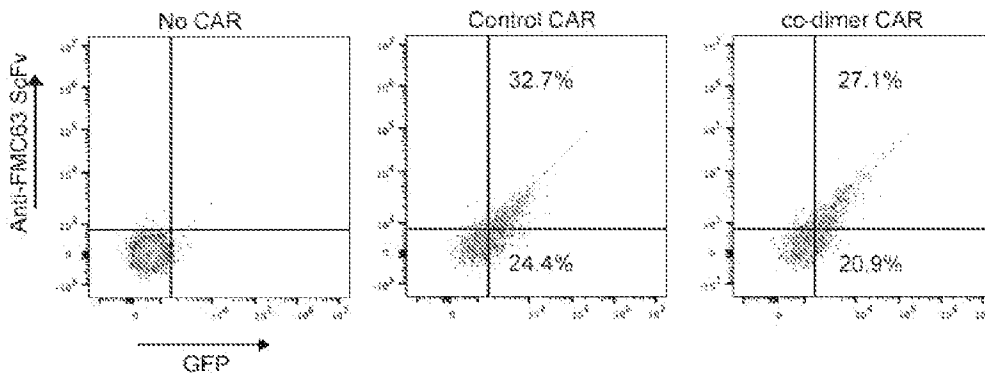
FIG. 6B   FIG. 6C   FIG. 6D
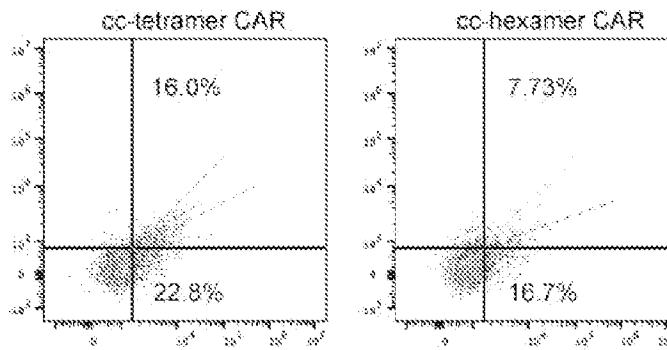
FIG. 6E   FIG. 6F
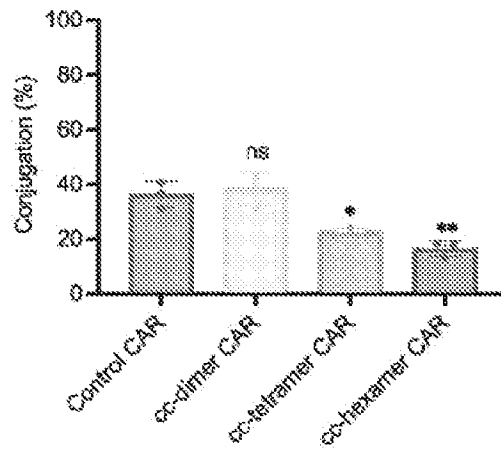
FIG. 6G
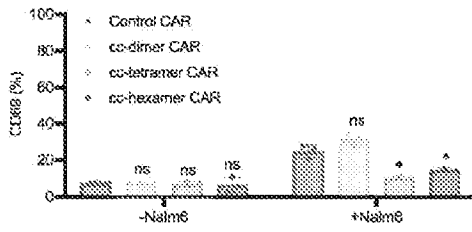
FIG. 6H
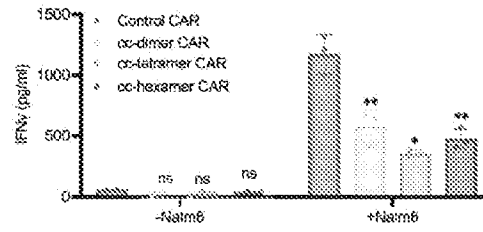
FIG. 6I
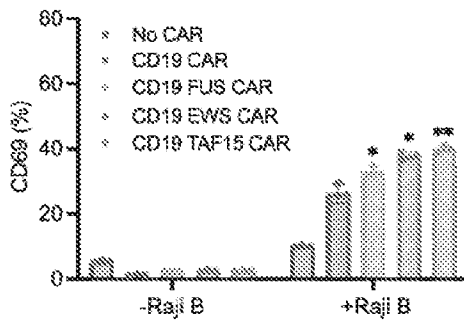
FIG. 7A
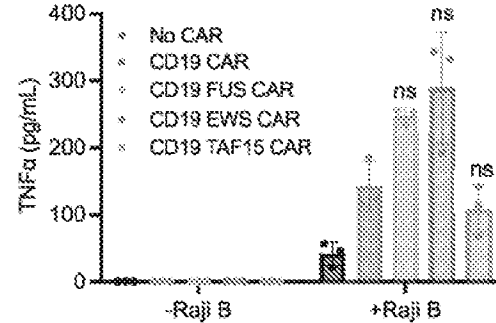
FIG. 7B
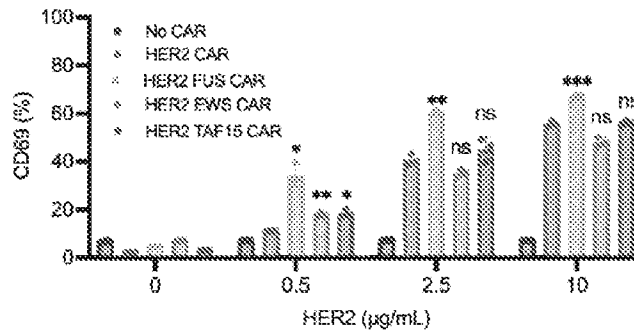
FIG. 7C
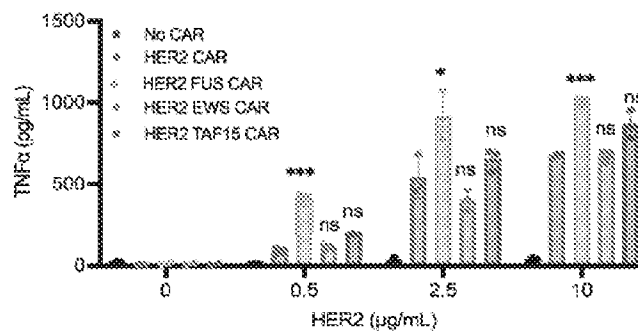
FIG. 7D
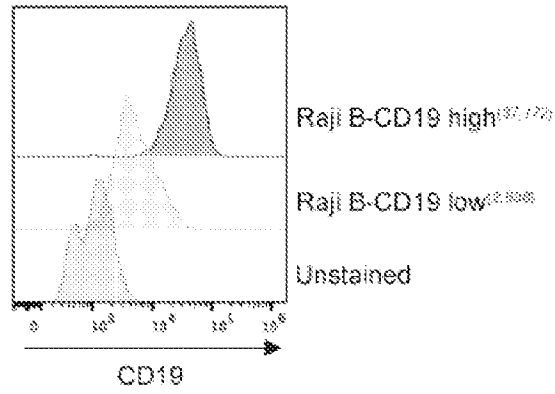
FIG. 8A
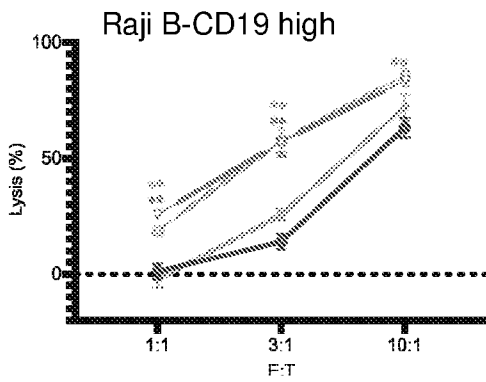
FIG. 8B
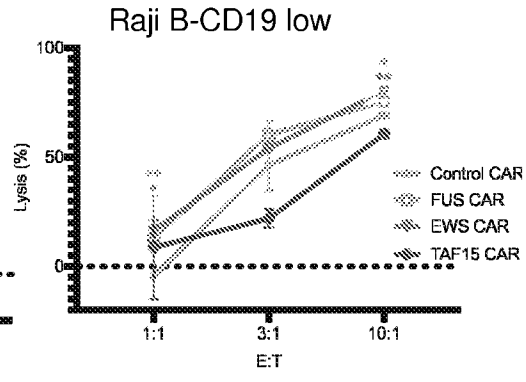
FIG. 8C
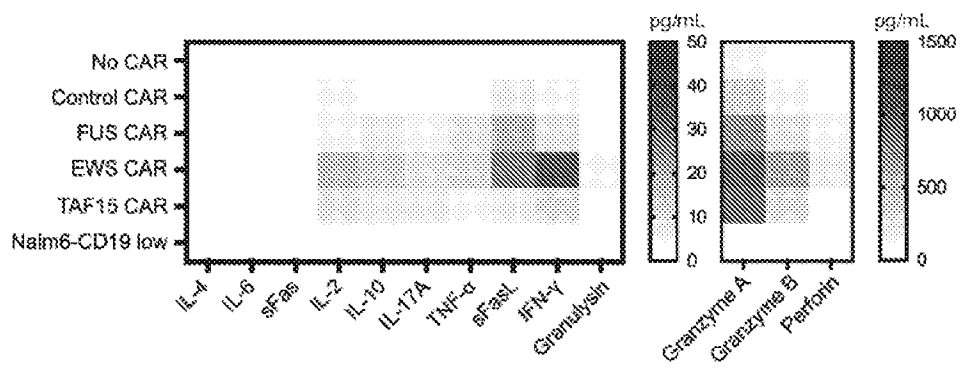
FIG. 8D
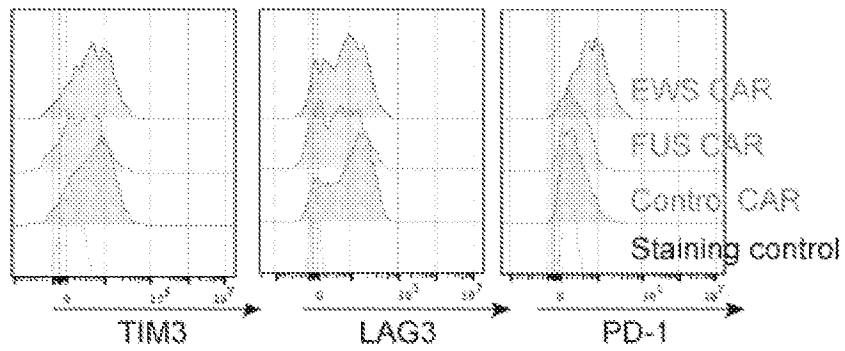
FIG. 8E   FIG. 8F   FIG. 8G
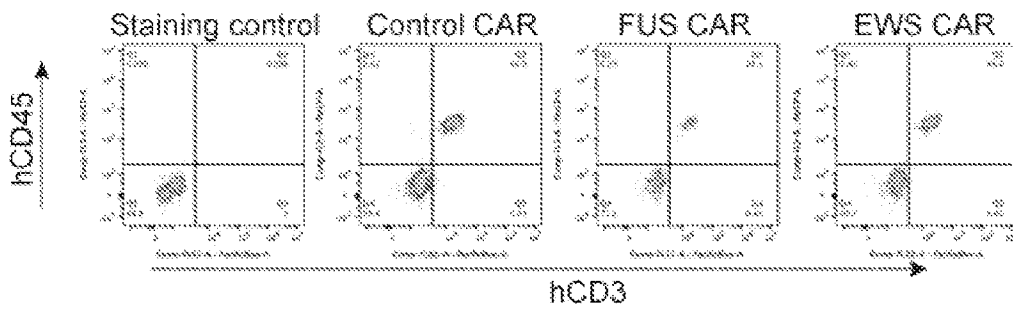
FIG. 8H   FIG. 8I   FIG. 8J   FIG. 8K
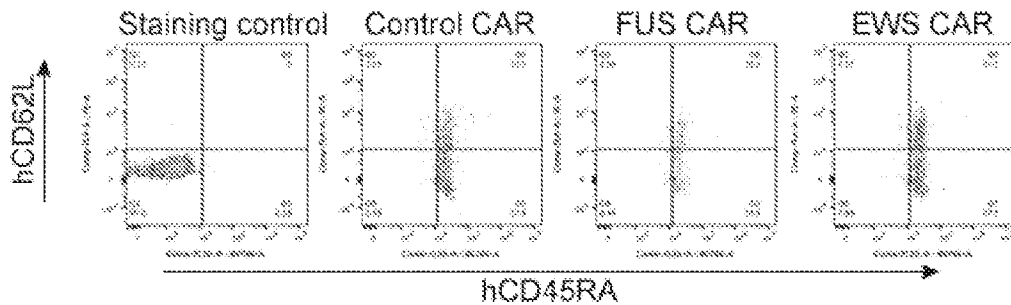
FIG. 8L   FIG. 8M   FIG. 8N   FIG. 8O
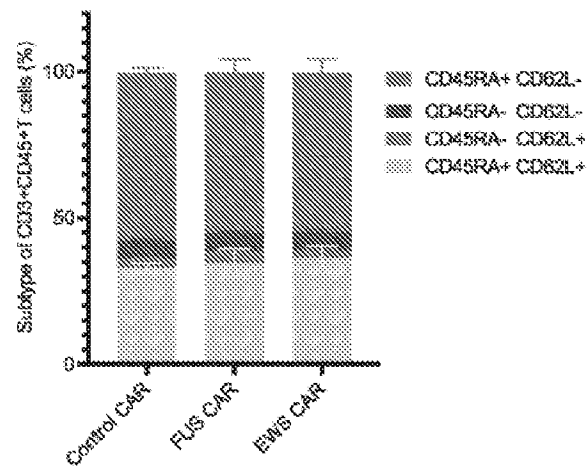
FIG. 8P
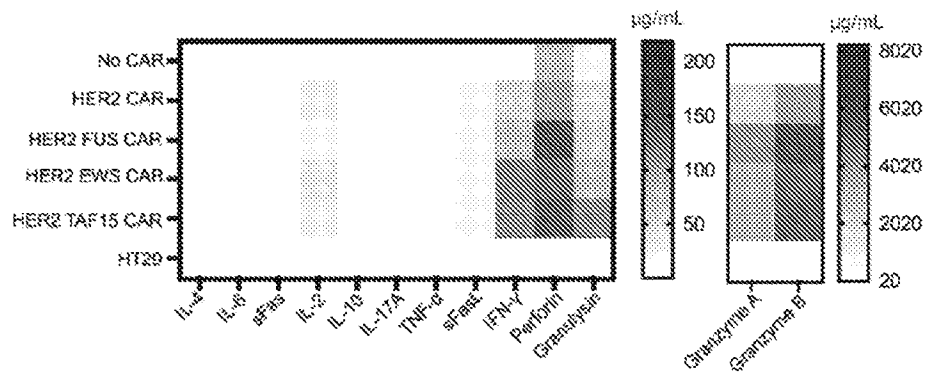
FIG. 9A
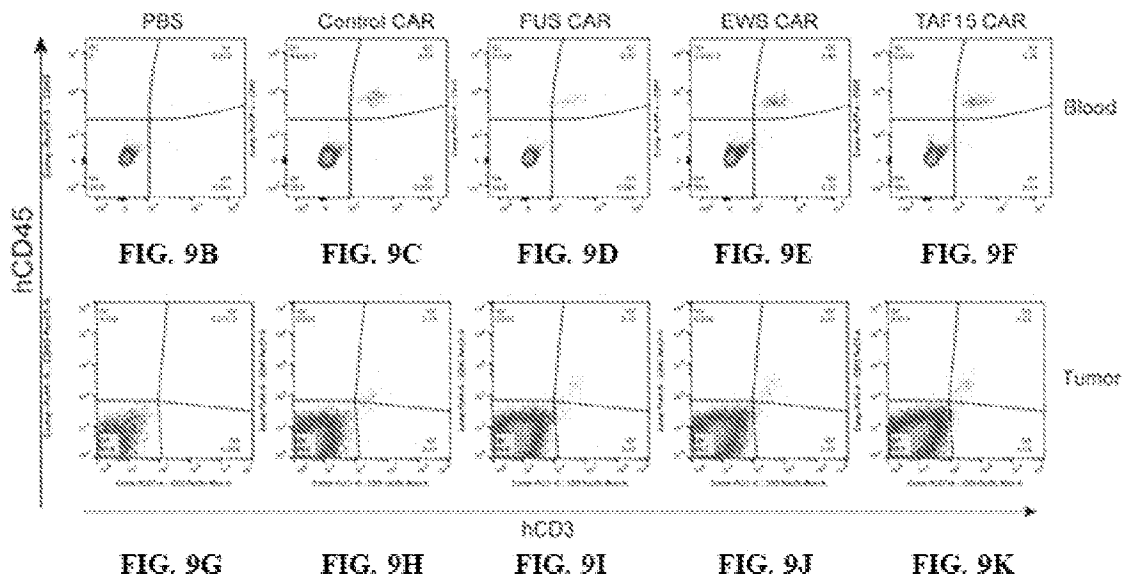
FIG. 9B   FIG. 9C   FIG. 9D   FIG. 9E   FIG. 9F

FIG. 9G   FIG. 9H   FIG. 9I   FIG. 9J   FIG. 9K
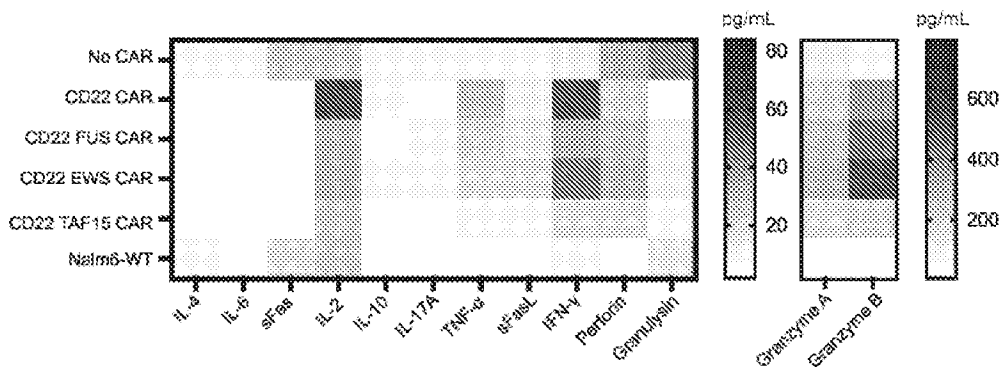
FIG. 10A
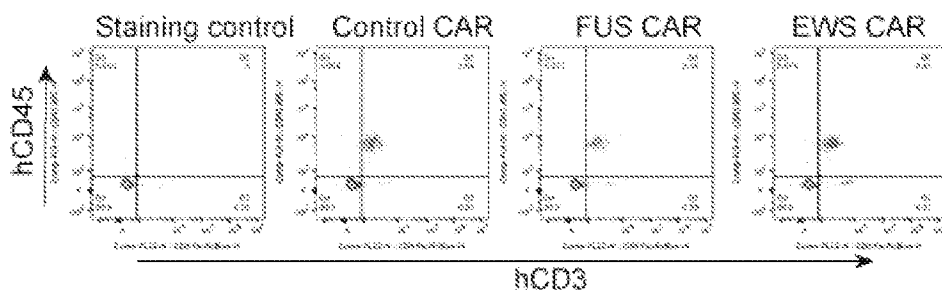
| **FIG. 10B** | **FIG. 10C** | **FIG. 10D** | **FIG. 10E** |

| **FIG. 10F** | **FIG. 10G** | **FIG. 10H** |

| **FIG. 10I** | **FIG. 10J** | **FIG. 10K** | **FIG. 10L** |
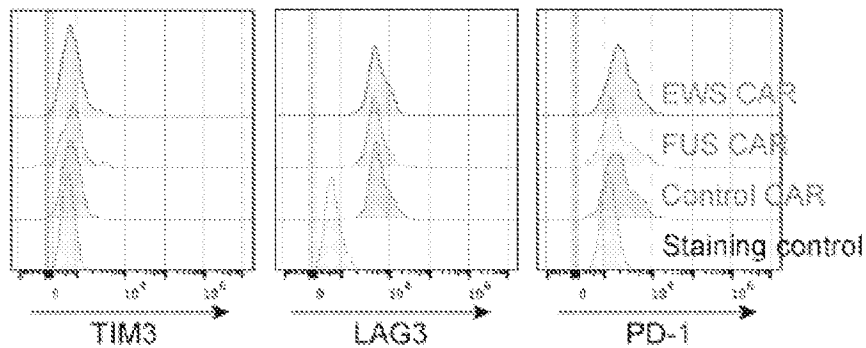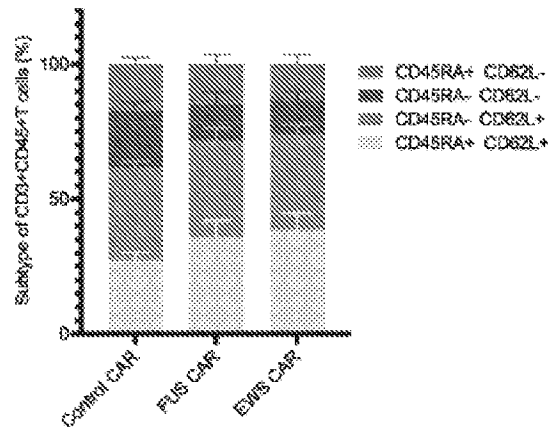
FIG. 10M
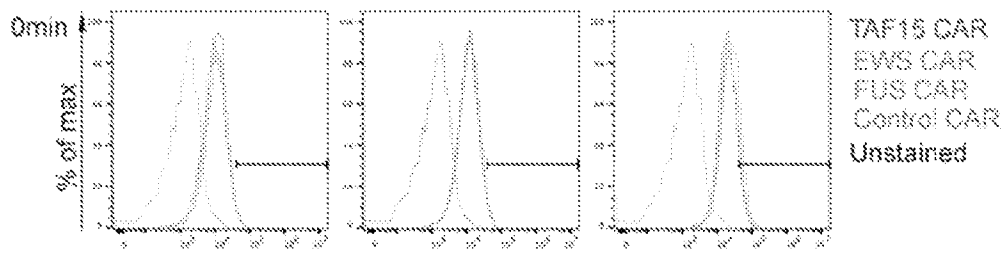
FIG. 11A	FIG. 11B	FIG. 11C
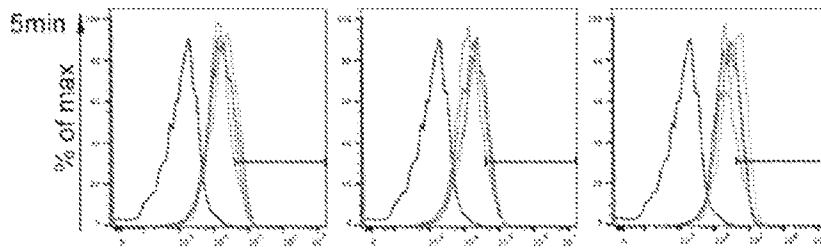
FIG. 11D	FIG. 11E	FIG. 11F
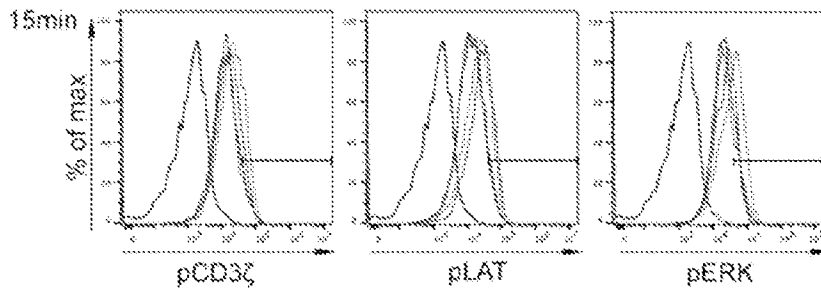
FIG. 11G	FIG. 11H	FIG. 11I
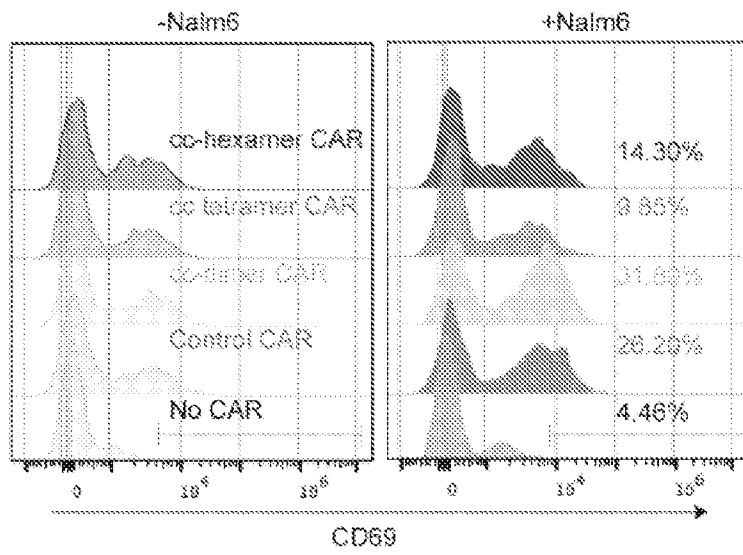
FIG. 12A   FIG. 12B
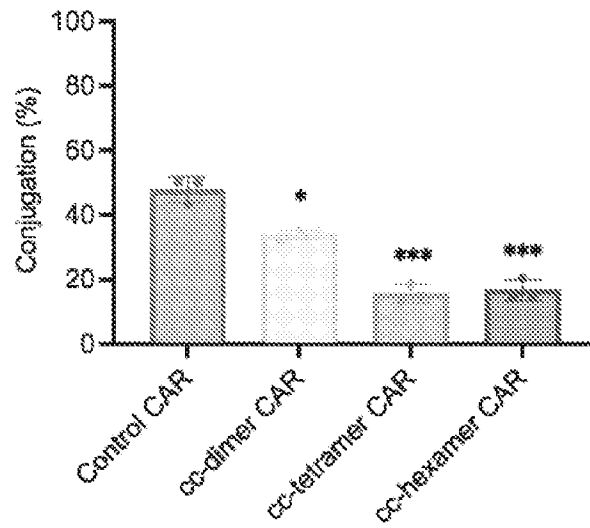
FIG. 12C
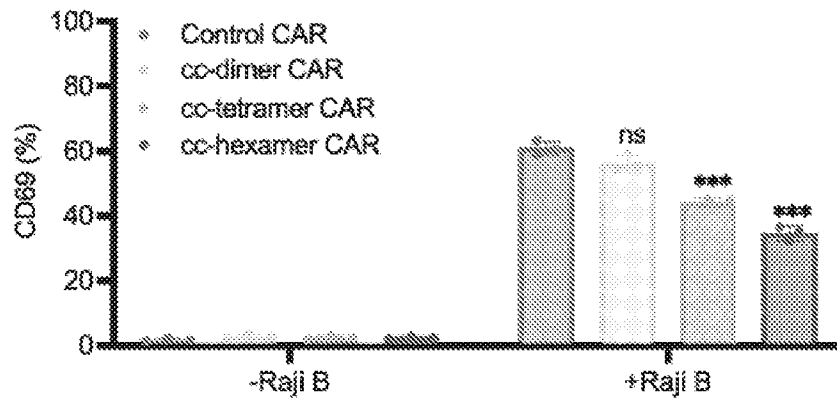
FIG. 12D
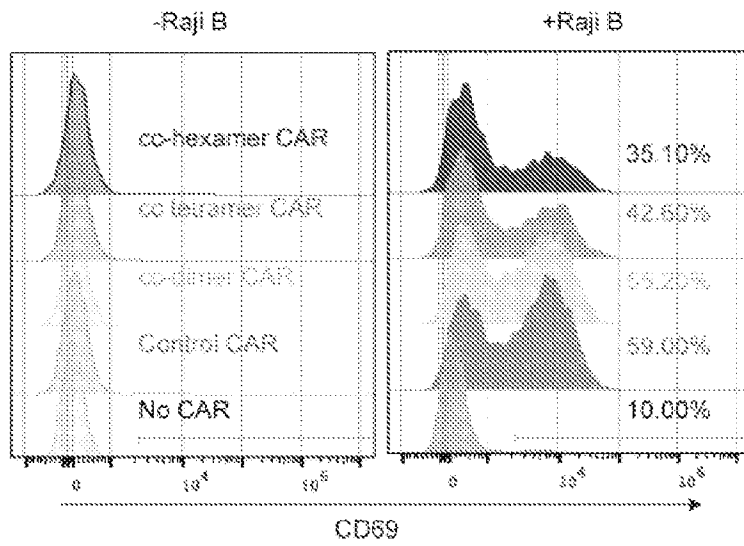
FIG. 12E   FIG. 12F

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00
ADD.

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)

A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIANG CHEN: "The CD8[alpha] hinge is intrinsically disordered with a dynamic exchange that includes proline cis-trans isomerization", JOURNAL OF MAGNETIC RESONANCE., 13 May 2022 (2022-05-13), page 107234, XP093156048, US ISSN: 1090-7807, DOI: 10.1016/j.jmr.2022.107234 Retrieved from the Internet: URL:https://pdf.sciencedirectassets.com/272577/1-s2.0-S1090780722X00062/1-s2.0-S1090780722000921/main.pdf?X-Amz-Security-Token=IQoJb3JpZ21uX2VjEFwaCXVzLWVhc3QtMSJGMEQCIG5LIGi90HDHOg5bdhQ5WPvK3nN8RMY3ZpLWtVnvL4VdAiBjQOkXsySavGerBIrJkeCSx9S%2B8JJ3W3nKeh5Nyfua2iq8BQil%2F%2F%2F%2F%2F%2F%2F%2F%2F%2F8BEAUaDDA -/--	1,4-6, 8-11, 13-15, 17-27, 30,31,39

[x] Further documents are listed in the continuation of Box C.

[x] 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	Date of mailing of the international search report
30 April 2024	23/05/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Barbosa, Rita

1

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	figure 1 figure 7 figure 4 figure 3 -----	
X	WO 2022/095916 A1 (CENTER FOR EXCELLENCE IN MOLECULAR CELL SCIENCE CHINESE ACAD OF SCIENC) 12 May 2022 (2022-05-12) figure 1; example 3 sequences 1, 3 -----	1-4,14, 15,17-24
X	SPIEGEL JAY Y ET AL: "CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial", NATURE MEDICINE, NATURE PUBLISHING GROUP US, NEW YORK, vol. 27, no. 8, 26 July 2021 (2021-07-26), pages 1419-1431, XP037538337, ISSN: 1078-8956, DOI: 10.1038/S41591-021-01436-0 [retrieved on 2021-07-26] "CAR construct and clinical trial design"; page 1420, right-hand column; figure 2 -----	1,4-29, 31-52
X,P	Xinyan Zhang: "IDR-induced CAR condensation improves the cytotoxicity of CAR-Ts against low-antigen cancers", bioRxiv, 28 October 2023 (2023-10-28), XP093156020, DOI: 10.1101/2023.10.02.560460 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2023.10.02.560460v2.full.pdf the whole document -----	1-52

1

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

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

1.   With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

　　　a.   [X]   forming part of the international application as filed.

　　　b.   [ ]   furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter.*1(a)).

　　　　　　[ ]   accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2.   [ ]   With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3.   Additional comments:

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 2022095916 A1	12-05-2022	CN	114437232 A	06-05-2022
		WO	2022095916 A1	12-05-2022