Functional Binders Synthesized and Secreted by Immune Cells

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 62/875,455, filed on July 17, 2019. The entire teachings of the above application are incorporated herein by reference.

INCORPORATION BY REFERENCE OF MATERIAL IN ASCII TEXT FILE

[0002] This application incorporates by reference the Sequence Listing contained in the following ASCII text file being submitted concurrently herewith:

a) File name: 44591154001_SEQUENCELISTING.txt; created July 15, 2020, 35 KB in size.

BACKGROUND

[0003] Cancer immunotherapy broadly relates to directing immune responses to selectively attack tumor cells. The immunotherapeutic toolbox to treat cancer has been significantly enriched by the advent of chimeric antigen receptor (CAR)-directed T lymphocytes. The clinical experience with CAR-T cells demonstrates that T-lymphocytes, when adequately activated, can overcome resistance to chemotherapy, leading to major reduction in tumor burden, disease stabilization and, in some patients with B-cell leukemia and lymphoma, tumor eradication.[18-26] In CAR-T cells, T cell stimulation occurs via the expression of chimeric molecules with antibody-like properties.

[0004] Current CAR T-cell methodologies do not harness the full potential of the immune system to target cancer cells.

SUMMARY

[0005] Described herein is a peptide that includes a single-chain variable fragment (scFv) domain; a fragment crystallizable (Fc) domain; and a hinge domain joining the scFv and Fc domains. Also described are nucleic acids encoding the peptides described herein; vectors that include the nucleic acids, which encode the peptide described herein; immune cells (e.g., natural killer cells and T cells) that express the peptides described herein; and methods of making immune cells that express the peptides described herein.

[0006] The scFv domain can include an immunoglobulin variable light (V_L) domain, an immunoglobulin variable heavy (V_H) domain, and a linker domain joining the V_L and V_H domains. The linker domain can be $(G_4S)_x$, wherein x is an integer from 1 to 100. The linker domain can be $(G_4S)_3$.

[0007] The scFv domain can bind CD19, CD20, CD22, CD38, CD7, CD2, CD3, epidermal growth factor receptor (EGFR), CD123, CD33, B-cell maturation antigen (BCMA), mesothelin, human epidermal growth factor receptor 2 (Her2), prostate-specific membrane antigen (PSMA), disialoganglioside (GD2), PD-L1 (CD274), CD80 or CD86.

[0008] The Fc domain can include an immunoglobulin constant heavy 2 (C_H2) domain and an immunoglobulin constant heavy 3 (C_H3) domain. The Fc domain can be human IgG1 Fc domain.

[0009] The peptide can further include a signal peptide that is N-terminal to the scFv domain.

[0010] The peptide can further include a self-cleaving peptide joining the Fc domain to a chimeric receptor, wherein the chimeric receptor includes: a receptor domain; a hinge and transmembrane domain; a co-stimulatory signaling domain; and a cytoplasmic signaling domain.

[0011] The self-cleaving peptide can be a 2A peptide. The receptor domain can be CD16. The hinge and transmembrane domain can be a CD8α hinge and transmembrane domain. The co-stimulatory domain can be 4-1BB co-stimulatory domain. The cytoplasmic signaling domain can be a CD3ζ cytoplasmic signaling. The chimeric receptor can be CD16V-4-1BB-CD3ζ.

[0012] In one particular embodiment, the scFv domain binds CD19 or CD20; the Fc domain is a human IgG1 Fc domain; and the hinge domain is an IgG1 hinge domain; the vector further includes a CD8α signal peptide that is N-terminal to the scFv domain; and the vector further includes a chimeric receptor that is CD16V-4-1BB-CD3ζ.

[0013] The vector can be a murine stem cell virus (MSCV).

[0014] The peptide can further include IL-15 joined to the Fc domain by a linker. The linker that joins IL-15 to the Fc domain is selected from the group consisting of SEQ ID NO: 51; $A(EAAK)_4ALEA(EAAAK)_4A$; $(EAAAK)_z$; $A(EAAAK)_zA$; and $(XP)_w$, wherein z is an integer from 1 to 100; X is any amino acid, and w is an integer from 1 to 100.

[0015] Described herein is a peptide that includes a T-cell receptor (TCR) β domain; a first fragment crystallizable (Fc) domain joined to the TCR β domain; a TCR α domain; a

self-cleaving peptide joining the Fc domain to the TCR α domain; and a second Fc domain joined to the TCR α domain. The peptide can further include a signal peptide joined to the T-cell receptor (TCR) β domain. The first Fc domain can be the same as the second Fc domain. The first Fc domain can be different from the second Fc domain. Also described are nucleic acids encoding the peptide and vectors that include the nucleic acid, which encodes the peptide.

[0016] Advantageously, the peptides described herein can be secreted by immune cells, such as T cells and NK cells. As a result, the immune cells can target and kill tumor cells without the need for exogenous administration of antibodies. NK cells can exert antibody-dependent cell cytotoxicity when the secreted peptides bind Fc receptors on the NK cell surface. T cells transduced with an Fc receptor can also exert antibody-dependent cell cytotoxicity. Moreover, the peptides can trigger phagocytosis of tumor cells by macrophages through interaction of Fc receptors on their cell surface. Finally, the peptides can kill tumor cells by inducing complement fixation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The foregoing will be apparent from the following more particular description of example embodiments, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments.

[0018] FIGs. 1A-C show design and expression of in vivo functional ligands (IFLs). FIG. 1A is a schematic representation of an IFL construct. A single-chain variable fragment (scFv) composed of variable domains of a light chain (VL) and a heavy chain (VH) is fused with a modified fragment crystallizable domain (Fc) composed of two of the three constant domains of heavy chain (CH2, CH3) of immunoglobulin G1 (IgG1) through a IgG1 hinge. FIG. 1B is flow cytometric dot plots that illustrate expression of GFP and IFL, detected by intracellular staining with an anti-human IgG Fc antibody, in NK cells transduced with GFP alone ("Control", left panel), anti-CD20 IFL gene ("aCD20 IFL", middle panel), or anti-CD19 IFL gene ("aCD19 IFL", right panel). Percentage of cells in each quadrant is shown. FIG. 1C is the same staining as in FIG. 1B in transduced T lymphocytes.

[0019] FIG. 2 shows that IFLs are specific for their cognate binder. FIG. 2 is flow cytometric histograms show labelling of Jurkat (CD20-, CD19-), Ramos (CD20+, CD19+), and RS4;11 (CD20-, CD19+) cells after incubation incubated with culture supernatant

obtained from either NK cells (top panel) or T cells (bottom panel), which had been transduced with GFP alone ("Control"), anti-CD20 IFL, or anti-CD19 IFL. IFLs bound to the surface of target cells were detected by a goat-anti human IgG antibody conjugated to phycoerythrin.

[0020] FIGs. 3A-C show synthesis and glycosylation of IFL. FIG. 3A is a plot showing levels of IFL secreted by NK cells or T cells form the same donor transduced with anti-CD20 IFL. Each symbol represents results from 1 of 3 donors tested. FIG. 3B is pie charts showing the percentage of fucosylated glycan (dark blue) and afucosylated glycan (light blue) of IFL secreted from transduced NK cells (left panel) or T cells (middle panel) according to N-glycan profiling by MALDI-TOF MS. Results with rituximab are shown for comparison. FIG. 3C is bar diagrams illustrating percentage of relative intensity each various types of glycan in IFL secreted by transduced NK cells or T cells, or in rituximab. Schematic structures show various types of glycan.

[0021] FIGs. 4A-B show CDC and ADCP mediated by immune cell-derived IFL. FIG. 4A is charts showing results when Ramos (left panel) and SUDHL-4 cells (right panel) were incubated with 0.05 μg/mL of rituximab or IFLs from NK cells or T cells in the presence or absence of 5% complement. Cell killing was measured by counting viable cells by flow cytometry. FIG. 4B is plots showing results when IFL from NK cells or T cells (0.1 μg/mL) were added to Ramos cells co-cultured with or without THP-1 cells for 48 hours. Cell killing was measured by counting viable target cells with Incucyte.

[0022] FIGs. 5A-C show ADCC mediated by immune cell-derived IFL. FIG. 5A is a graph of showing results when Raji cells were cultured with NK cells transduced with GFP alone ("NK-GFP") or anti-CD20 IFL ("NK-IFL") at a 1:1 E:T ratio. As a control, Ramos was cultured without NK cells ("no NK") or with NK-GFP in the presence of 1 μg/ml of rituximab. The number of viable Ramos cells was counted every 8 hours for 72 hours using Incucyte. FIG. 5B is a plot showing cytotoxicity of NK cells transduced with GFP alone or anti-CD19 IFL against RS4;11, OP-1 and Nalm-6. Shown are data for 4-hour assays at a E:T 2:1 ratio. Each symbol represents the results obtained with NK cells from 1 donor; bars correspond to the median value. FIG. 5C is a plot showing results when RS4;11 cells were incubated with medium alone or NK cells transduced with GFP alone or anti-CD20 or anti-CD19 IFLs at a E:T 2:1 ratio for 4 hours. Each symbol represents results obtained with NK cells from one donor.

[0023] FIGs. 6A-D show ADCC mediated by immune cell-derived IFL. FIG. 6A is a schematic representation of the gene construct containing IFL with CD16V-4-1BB-CD3ζ. FIG. 6B is flow cytometric dot plots showing surface expression of CD16 in T cells transduced with GFP alone ("Control") or IFL-P2A-CD16-41BB-CD3ζ ("IFL+CD16R"). Percentage of cells in each quadrant is shown. FIG. 6C is flow cytometric dot plots showing expression of IFL after intracellular staining with anti-human Ig Fc antibody in the same cells. FIG. 6D is a graph of results when Ramos cells were co-cultured with or without T cells transduced with various constructs as indicated. The number of viable Ramos cells was counted every 8 hours for 72 hours by Incucyte.

[0024] FIGs. 7A-B show plasma concentration and antitumor activity of IFL in vivo. FIG. 7A is a graph showing results when NOD-SCID-IL2RGnull mice were injected intravenously with 2 x 10^7 T cells transduced with anti-CD20 IFL-P2A-CD16-41BB-CD3ζ. Levels of IFL in plasma were measured by ELISA. FIG. 7B is a graph showing results when NOD-SCID-IL2RGnull mice (n = 18) received one intraperitoneal (i.p.) injection of 2 x 10^5 Daudi labelled with luciferase. In 12 mice, we administered two i.p. injections of 2 x 10^7 T cells transduced with either anti-CD20 IFL-P2A-CD16V-4-1BB-CD3ζ (n = 6) or GFP alone (n = 6), 3 and 6 days after Daudi injection; 6 additional mice were injected with medium alone. Kaplan-Meier curves show the percentage of disease-free survival in the different groups.

[0025] FIGs. 8A-E are examples of IFL variants. FIG. 8A is a schematic of IFL with polymorphisms to increase the affinity for Fc receptor or complement. FIG. 8B is a schematic of IFL with polymorphisms to promote formation of hexamers. FIG. 8C is a schematic of IFL fusing with cytokine through a linker. FIG. 8D is a schematic of extracellular domain of TCR α and β chains as binders for IFL. FIG. 8E is a schematic of IFL fusing with a ligand that binds a co-stimulatory molecule.

[0026] Figs. 9A-B show ADCC mediated by anti-CD20 IFL linked to interleukin-15 (IL-15) (see FIG. 8C) and secreted by immune cells. The graphs show results of experiments in which the CD20+ lymphoma cells Ramos were cultured with NK cells transduced with GFP alone ("NK-GFP"), anti-CD20 IFL ("NK-IFL") or anti-CD20 IFL linked to IL-15 ("NK-IFL-IL15") at a 1:1 E:T ratio. As a control, Ramos cells were cultured without NK cells ("no NK"). In the experiment of FIG. 9A, IL-2 was not added; in the experiment of FIG. 9B, cultures were performed with 40 IU/mL IL-2. The number of viable Ramos cells was counted

every 8 hours for 72 hours using life-cell imaging measured with an Incucyte System instrument.

DETAILED DESCRIPTION

[0027] A description of example embodiments follows.

[0028] Monoclonal antibodies are integral to the contemporary treatment of cancer. Antibodies exert anti-tumor activity via several mechanisms including direct induction of cell death, complement activation, and engagement of immune cells. Antibodies bound to tumor cells can trigger antibody-dependent cell cytotoxicity (ADCC).[1-6] ADCC, which results from the engagement of Fc receptors (FcγR) expressed on the surface of natural killer (NK) cells,[7] is central to the clinical efficacy of antibodies; polymorphisms of the gene coding FcγRIIIa (*FCRG3A* or *CD16*) leading to receptors with higher affinity for Fc have been associated with better tumor responses in patients.[2,8-16] Other important mechanisms underlying the anti-tumor activity of antibodies include clearance of tumor cells by macrophages through antibody-dependent cell phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC).[7,17]

[0029] The immunotherapeutic toolbox to treat cancer has been significantly enriched by the advent of chimeric antigen receptor (CAR)-directed T lymphocytes. The clinical experience with CAR-T cells demonstrates that T-lymphocytes, when adequately activated, can overcome resistance to chemotherapy, leading to major reduction in tumor burden, disease stabilization and, in some patients with B-cell leukemia and lymphoma, tumor eradication.[18-26] In CAR-T cells, T cell stimulation occurs via the expression of chimeric molecules with antibody-like properties.[27-31] Another approach leading to tumor-specific T cell activation is through the expression of high-affinity CD16 as a component of a chimeric receptor including both stimulatory and co-stimulatory signals.[32] Such receptor has the potential to significantly augment the anti-tumor effect of antibody therapy. Compared to CAR-T cells, it works in combination with other antibody-mediated mechanism, such as ADCP and CDC, resulting in a concerted anti-tumor effect. Moreover, by using multiple antibodies against weakly expressed antigens, vigorous T-cell responses can be elicited.

[0030] Described herein are methods that allow immune cells to produce binders with antibody-like function. These in vivo functional ligands (IFLs) are capable of triggering ADCC, ADCP and CDC, as well as cytokine stimulation. These can be expressed in NK cells and T cells, and in conjunction with CD16 chimeric receptors, to optimize effector functions.

[0031] While the particular examples described herein target CD20+ and CD19+ B-cells as a paradigm, the approach is applicable to targeting other antigens that are markers of cells in the pathogenesis of cancer and other diseases.

B-cell Non-Hodgkin lymphoma and CD20 and CD19

[0032] B-cell non-Hodgkin lymphoma (NHL) is a cancer of lymphoid blood cells. NHL inevitably progresses and is fatal if untreated. Standard treatment includes chemotherapy, antibody therapy, tyrosine kinase inhibitor therapy, and hematopoietic stem cell transplant. CD20 and CD19 are B-cell–specific antigens that are widely expressed in B-cell NHL (also referred to as B-NHL).

[0033] The vectors described herein can be used to generate modified T cells, which, in turn, can be used for targeted treatment of NHL. The processes described herein can be used to create transgenic T cells that can target CD20+ and CD19+ B-cells for destruction, thereby eradicating NHL and/or decreasing its severity.

Acute Lymphoblastic Leukemia and CD19

[0034] Acute lymphoblastic leukemia (ALL) is also a cancer of lymphoid blood cells. ALL progresses rapidly and is fatal if untreated. Standard treatment includes chemotherapy and hematopoietic stem cell transplant. CD19 is a B-cell–specific antigen that is expressed on all leukemic cells in the majority of cases of ALL.

[0035] The vectors described herein can be used to generate modified T cells, which, in turn, can be used for targeted treatment of ALL. The processes described herein can be used to create transgenic T cells that can target CD19+ B-cells for destruction, thereby eradicating ALL and/or decreasing its severity.

[0036]

Nucleic Acids

[0037] As used herein, the term "nucleic acid" refers to a polymer comprising multiple nucleotide monomers (*e.g.*, ribonucleotide monomers or deoxyribonucleotide monomers). "Nucleic acid" includes, for example, DNA (*e.g.*, genomic DNA and cDNA), RNA, and DNA-RNA hybrid molecules. Nucleic acid molecules can be naturally occurring, recombinant, or synthetic. In addition, nucleic acid molecules can be single-stranded, double-stranded or triple-stranded. In certain embodiments, nucleic acid molecules can be

modified. In the case of a double-stranded polymer, "nucleic acid" can refer to either or both strands of the molecule.

[0038] The terms "nucleotide" and "nucleotide monomer" refer to naturally occurring ribonucleotide or deoxyribonucleotide monomers, as well as non-naturally occurring derivatives and analogs thereof. Accordingly, nucleotides can include, for example, nucleotides comprising naturally occurring bases (*e.g.*, adenosine, thymidine, guanosine, cytidine, uridine, inosine, deoxyadenosine, deoxythymidine, deoxyguanosine, or deoxycytidine) and nucleotides comprising modified bases known in the art.

[0039] As used herein, the term "sequence identity," refers to the extent to which two nucleotide sequences, or two amino acid sequences, have the same residues at the same positions when the sequences are aligned to achieve a maximal level of identity, expressed as a percentage. For sequence alignment and comparison, typically one sequence is designated as a reference sequence, to which a test sequences are compared. The sequence identity between reference and test sequences is expressed as the percentage of positions across the entire length of the reference sequence where the reference and test sequences share the same nucleotide or amino acid upon alignment of the reference and test sequences to achieve a maximal level of identity. As an example, two sequences are considered to have 70% sequence identity when, upon alignment to achieve a maximal level of identity, the test sequence has the same nucleotide or amino acid residue at 70% of the same positions over the entire length of the reference sequence.

[0040] Alignment of sequences for comparison to achieve maximal levels of identity can be readily performed by a person of ordinary skill in the art using an appropriate alignment method or algorithm. In some instances, the alignment can include introduced gaps to provide for the maximal level of identity. Examples include the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444 (1988), computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), and visual inspection (see generally Ausubel *et al., Current Protocols in Molecular Biology*).

[0041] When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequent coordinates are designated, if necessary, and sequence

algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. A commonly used tool for determining percent sequence identity is Protein Basic Local Alignment Search Tool (BLASTP) available through National Center for Biotechnology Information, National Library of Medicine, of the United States National Institutes of Health. (Altschul *et al.*, *J Mol Biol.* 215(3):403-10 (1990)).

[0042] In various embodiments, two nucleotide sequences, or two amino acid sequences, can have at least, *e.g.*, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity. When ascertaining percent sequence identity to one or more sequences described herein, the sequences described herein are the reference sequences.

Vectors

[0043] The terms "vector", "vector construct" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence. Vectors typically comprise the DNA of a transmissible agent, into which foreign DNA encoding a protein is inserted by restriction enzyme technology. A common type of vector is a "plasmid", which generally is a self-contained molecule of double-stranded DNA that can readily accept additional (foreign) DNA and which can readily introduced into a suitable host cell. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts.

The terms "express" and "expression" mean allowing or causing the information in a gene or DNA sequence to become manifest, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an "expression product" such as a protein. The expression product itself, e.g. the resulting protein, may also be said to be "expressed" by the cell. A polynucleotide or polypeptide is expressed recombinantly, for example, when it is expressed or produced in a foreign host cell under the control of a foreign or native promoter, or in a native host cell under the control of a foreign promoter. Gene delivery vectors generally include a transgene (e.g., nucleic acid encoding an enzyme)

operably linked to a promoter and other nucleic acid elements required for expression of the transgene in the host cells into which the vector is introduced. Suitable promoters for gene expression and delivery constructs are known in the art. Recombinant plasmids can also comprise inducible, or regulatable, promoters for expression of an enzyme in cells.

[0044] Various gene delivery vehicles are known in the art and include both viral and non-viral (e.g., naked DNA, plasmid) vectors. Viral vectors suitable for gene delivery are known to those skilled in the art. Such viral vectors include, e.g., vector derived from the herpes virus, baculovirus vector, lentiviral vector, retroviral vector, adenoviral vector, adeno-associated viral vector (AAV), and murine stem cell virus (MSCV). The viral vector can be replicating or non-replicating. Such vectors may be introduced into many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art.

[0045] Non-viral vectors for gene delivery include naked DNA, plasmids, transposons, and mRNA, among others. Non-limiting examples include pKK plasmids (Clonetech), pUC plasmids, pET plasmids (Novagen, Inc., Madison, Wis.), pRSET or pREP plasmids (Invitrogen, San Diego, Calif.), pMAL plasmids (New England Biolabs, Beverly, Mass.). Such vectors may be introduced into many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art.

[0046] In certain embodiments, the vector comprises an internal ribosome entry site (IRES). In some embodiments, the vector includes a selection marker, such as an ampicillin resistance gene (Amp). In some embodiments, the nucleic acid encodes a fluorescent protein, such as green fluorescent protein (GFP) or mCherry. In some embodiments, the nucleic acid is suitable for subcloning into pMSCV-IRES-GFP between EcoRI and XhoI. In some embodiments, the vector contains a multiple cloning site (MCS) for the insertion of the desired gene.

[0047] Although the genetic code is degenerate in that most amino acids are represented by multiple codons (called "synonyms" or "synonymous" codons), it is understood in the art that codon usage by particular organisms is nonrandom and biased towards particular codon triplets. Accordingly, in some embodiments, the vector includes a nucleotide sequence that has been optimized for expression in a particular type of host cell (*e.g.*, through codon optimization). Codon optimization refers to a process in which a polynucleotide encoding a protein of interest is modified to replace particular codons in that polynucleotide with codons that encode the same amino acid(s), but are more commonly used/recognized in the host cell

in which the nucleic acid is being expressed. In some aspects, the polynucleotides described herein are codon optimized for expression in T cells.

In Vivo Functional Ligands

[0048] FIG. 1A is a schematic representation of an *in vivo* functional ligand (IFL) construct. The IFL includes a single chain variable fragment (scFv) domain, a modified fragment crystallizable (Fc) domain, and a hinge domain joining the scFv and the modified Fc domains. Preferably, an N-terminal signal peptide (leader peptide) is included that targets the IFL towards the secretory pathway and, ultimately, secretion by the cell. Signal peptides of surface proteins are generally suitable, and an example is a CD8α signal peptide.

[0049] The scFv domain typically includes an immunoglobulin variable light (V_L) domain, an immunoglobulin variable heavy (V_H) domain, and a linker domain joining the V_L and V_H domains. The relative positions of the V_L and V_H domains can be reversed, but they are both N' to the modified Fc domain, as illustrated in FIG. 1A.

[0050] The scFv domain targets an antigen of interest, such as an antigen of a tumor cell. One particular scFv described herein is an anti-CD19 single-chain variable fragment (anti-CD19 scFv). Another particular scFv described herein is an anti-CD20 single-chain variable fragment (anti-CD20 scFv).

[0051] While the embodiments described herein pertain to anti-CD19 construct and an anti-CD20 construct, a similar approach can be applied to generate constructs for other target antigens, such as CD22, CD123, CD33, B-cell maturation antigen (BCMA), mesothelin, human epidermal growth factor receptor 2 (Her2), prostate-specific membrane antigen (PSMA), disialoganglioside (GD)-2, PD-L1 (CD274), CD80 or CD86. For example, based on the schema in FIG. 5A, the anti-CD19 scFv portion can be replaced with a different scFv that specifically binds to a different target antigen. Other targets are suitable, including CD22, CD123, CD33, B-cell maturation antigen (BCMA), mesothelin, human epidermal growth factor receptor 2 (Her2), prostate-specific membrane antigen (PSMA), disialoganglioside (GD2), PD-L1 (CD274), CD80 or CD86.

[0052] A hinge domain joins the scFv and modified Fc domains, though in some instances the hinge domain may be considered part of the Fc domain. An example of a hinge domain is the IgG hinge domain. The construct can also include an N-terminal signal peptide, such as a CD8α signal peptide (see SEQ ID NOS: 21 and 22).

[0053] A variety of linker domains between V_L and V_H domains are suitable. In some embodiments, the linker domain can be $(G_4S)_x$, wherein x is an integer from 1 to 100; preferably, x is an integer from 1 to 10; even more preferably, x is an integer from 2 to 5. In some embodiments, the linker domain can be $(G_4S)_3$. In other embodiments, the linker domain can be one or more glycine residues (e.g., $(G)_y$, where y is an integer from 2 to 100. In other embodiments, the linker domain can be $(EAAAK)_3$. $(G_4S)_x$, $(G_4S)_3$, and $(G)_y$ are examples of flexible linkers, while $(EAAAK)_3$ is an example of a more rigid linker.

[0054] A variety of hinge domains are suitable. In some embodiments, the hinge domain can be a IgG hinge domain. In some embodiments, the hinge can be a plurality of amino acid residues. In some embodiments, the hinge domain can be a hinge domain from IgE, IgA, IgD, or CD8α.

[0055] In some embodiment, the construct is a bicistronic vector that also encodes a chimeric receptor, as illustrated in FIG. 6A. The chimeric receptor can include a receptor domain, a hinge and transmembrane domain, a co-stimulatory signaling domain, and a cytoplasmic signaling domain. In the construct of FIG. 6A, the chimeric receptor is joined with the modified Fc domain by a 2A peptide, which is a self-cleaving peptide. By joining the chimeric receptor with the scFv and Fc domains, coexpression of both proteins can be expressed from a single vector. Examples of 2A peptides are P2A (SEQ ID NOS: 43 and 44), T2A (SEQ ID NOS: 45 and 46), E2A (SEQ ID NOS: 47 and 48), and F2A (SEQ ID NOS: 49 and 50), though other 2A peptides are known in the art.

Modifications to IFL design

[0056] The design of the IFL construct tested in this study can be further modified to enhance some its functions and/or widen the range of its specificities. For example, the modified Fc can be further altered to increase its affinity for Fc receptors in NK cells and macrophages, thus enhancing ADCC and ADCP, and/or to increase its capacity to fix complement.[41-43]

[0057] In one modification (FIG. 8A), examples of two mutations introduced in the C_H2 domain of the Fc (serine in place of aspartic acid in position 239, S239D; and isoleucine in place of glutamic acid in position 332, I332E) are made to improve affinity for Fc receptors; two other mutations (S267E and H268F) are made to increase complement fixation.[41,43]

[0058] In another modification (FIG. 8B), examples of mutations introduced in the C_H2 domain of the Fc (E345K, E430G and S440Y) are made to promote hexamer formation. IFL hexamer could increase ADCC and CDC.[44,48]

[0059] In another modification (FIG. 8C), IL-15 is added to the IFL construct; this cytokine promotes activation and expansion of immune cells.[45,46] The IFL and IL-15 are joined by a linker. A variety of linker domains between the IFL construct and cytokine are suitable. In some embodiments, the linker domain is the amino acid of SEQ ID NO: 52, produced by its corresponding nucleotide sequence (SEQ ID NO: 51). In some embodiments, the linker domain can be $A(EAAK)_4ALEA(EAAAK)_4A$. In other embodiments, the linker domain can be $(EAAAK)_z$ and $A(EAAAK)_zA$, wherein z is an integer from 1 to 100; preferably, z is an integer from 2 to 5. In other embodiments, the linker domain can be $(XP)_w$, with X designating any amino acid; preferably, X is alanine, lysine, or glutamic acid, wherein w is an integer from 1 to 100.

[0060] In another modification (FIG. 8E), ligands that bind co-stimulatory molecules of immune cells such as 4-1BB (CD137), CD28, or OX40 (CD134), are added to the IFL construct. The IFL and the co-stimulatory ligand are joined by a linker. A variety of linker domains between the IFL construct and co-stimulatory ligand are suitable, and are generally the same linker domains that are suitable for between the IFL construct and cytokine of FIG. 8C.

[0061] FIG. 8D shows a construct in which the binding domain of the IFL is the extracellular domain of a T-cell receptor (TCR) directed against Epstein-Barr virus.[47] Such IFLs can recognize peptides produced by virally-infected or oncogenically transformed cells and can be expressed on the cell membrane in the context of MHC/HLA molecules. Therefore, such IFLs could be used to target viral peptides or peptides produced by cancer cells that cannot be recognized by antibodies or scFv derived from antibodies.

Methods of Making Transgenic Host Cells

[0062] Described herein are methods of making a transgenic host cell, such as transgenic natural killer (NK) cells or transgenic T cells. The transgenic host cells can be made, for example, by introducing one or more of the vector embodiments described herein into the host cell.

[0063] In one embodiment, the method comprises introducing into a host cell a vector that includes a nucleic acid that encodes an IFL. In some embodiments, a nucleic acid, such

as a bicistronic vector, expresses the IFL along with a chimeric receptor. In some embodiments, two separate vectors can be used to create a transgenic cell, such as a transgenic T cell, that expresses an IFL and a chimeric receptor.

[0064] In some embodiments, one or more of the nucleic acids are integrated into the genome of the host cell. In some embodiments, the nucleic acids to be integrated into a host genome can be introduced into the host cell using any of a variety of suitable methodologies known in the art, including, for example, homologous recombination, CRISPR-based systems (e.g., CRISPR/Cas9; CRISPR/Cpf1) and TALEN systems.

Host Cells

[0065] A variety of host cells are suitable for use in making transgenic host cells. Most commonly, the host cells are immune cells, such as natural killer (NK) cells or T lymphocyte cells.

[0066] As used herein, "natural killer cells" ("NK cells") refer to a type of cytotoxic lymphocyte of the immune system. NK cells provide rapid responses to virally infected cells and respond to transformed cells. Typically, immune cells detect peptides from pathogens presented by major histocompatibility complex (MHC) molecules on the surface of infected cells, triggering cytokine release, causing lysis or apoptosis. NK cells are unique, however, as they have the ability to recognize stressed cells regardless of whether peptides from pathogens are present on MHC molecules. They were named "natural killers" because of the initial notion that they do not require prior activation in order to kill target. NK cells are large granular lymphocytes (LGL) and are known to differentiate and mature in the bone marrow from where they then enter into the circulation. NK cell can also kill tumor cells if antigens on the surface of tumor cells are bound by antibodies; the Fc portion of the antibody bind Fc receptors (CD16) on the surface of NK cells and triggers cytotoxicity, a process known as antibody-dependent cell cytotoxicity (ADCC).

[0067] As used herein, “T lymphocytes” or “T cells” refers to lymphocytes that mature in the thymus. T cells can be further characterized into subpopulations, including T helper (T_H) cells, T cytotoxic (T_C) cells, and T regulatory (T_{reg}) cells. T_H and T_C cells can be characterized according to the presence or absence of membrane glycoproteins CD4 and CD8. Generally, T_H cells express CD4 on their surface, while T_C cells express CD8 on their surface. T helper cells can be further characterized as T_H1 cells and T_H2 cells. T cells can also exert ADCC if transduced with a receptor encoding CD16 and signaling molecules.[32]

[0068] In some aspects, the NK cell or the T cells are mammalian cells. Examples of "mammalian" or "mammals" include primates (e.g., human), canines, felines, rodents, porcine, ruminants, and the like. Specific examples include humans, dogs, cats, horses, cows, sheep, goats, rabbits, guinea pigs, rats and mice. In a particular aspect, the mammalian T or NK cell is a human T or NK cell.

[0069] Upon introducing into a host cell, a vector that includes a nucleic acid that encodes an IFL, the host cell becomes a transgenic host cell that expresses the IFL. Typically, the IFL is secreted by the transgenic host cell.

Values and Ranges

[0070] Unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in various embodiments, unless the context clearly dictates otherwise. "About" in reference to a numerical value generally refers to a range of values that fall within ±8%, in some embodiments ±6%, in some embodiments ±4%, in some embodiments ±2%, in some embodiments ±1%, in some embodiments ±0.5% of the value unless otherwise stated or otherwise evident from the context.

EXEMPLIFICATION

Materials and Methods

Cells

[0071] Human cell lines RS4;11 and Nalm-6 (B-cell leukemia), Ramos, Raji and Daudi (B-cell lymphoma) and Jurkat (T-cell leukemia) were obtained from the American Type Culture Collection (Rockville, MD). The B-cell leukemia cell line OP-1 was established at our laboratory.[33] We transduced Nalm-6 and Daudi with a murine stem cell virus (MSCV)-internal ribosome entry site (IRES)-green fluorescent protein (GFP) retroviral vector (from the Vector Development and Production Shared Resource of St. Jude Children's Research Hospital, Memphis, TN) containing firefly luciferase gene. We also transduced Ramos and Raji with the MSCV retroviral vector containing mCherry gene. Transduced cells were selected for their GFP or mCherry expression, respectively, using a MoFlo cell sorter (Beckman Coulter, Brea, CA). To have Nalm-6 express CD20 on the surface, we subcloned human CD20 gene in cytomegalovirus plasmid (pCMV6) vector (Origene, Rockville, MD)

into MSCV-IRES-GFP vector and transduced Nalm-6 with the CD20 gene. Nalm-6 cells expressing CD20 were selected using MoFlo sorter after staining with anti-CD20 antibody (BD Biosciences, San Jose, CA). Cell lines were cultured in RPMI-1640 (ThermoFisher Scientific, Waltham, MA) with 10% fetal bovine serum (FBS, Thermo Fisher Scientific) and 1% penicillin-streptomycin.

[0072] Peripheral blood was obtained from discarded products of platelet donations from healthy donors at the National University Hospital Blood Bank, Singapore. Mononucleated cells were isolated by a density gradient centrifugation with Lymphoprep (Axis-Shield, Oslo, Norway) and washed twice in RPMI-1640. For viral transduction, NK cells were expanded from the isolated mononucleated cells with the genetically modified K562-mb15-41BBL, previously established in our laboratory.[34,35] T cells were activated by T cell TransAct (Miltenyi Biotec, Bergisch Gladbach, Germany) and cultured in TexMACS medium (Miltenyi Biotec) with interleukin-2 (IL-2, Proleukin, Novartis, Basel, Switzerland, 100 IU/mL).

Plasmids and viral transduction

[0073] We designed IFLs composed of single-chain variable fragment (scFv) linking with a modified fragment crystallizable domain (Fc) of human immunoglobulin G1 (IgG1). The amino acid sequence of the signal peptide, scFv againstCD20 and modified Fc of IgG1 was obtained from the sequence of rituximab described in DrugBank (http://www.drugbank.ca; Accession No. DB00073). The scFv sequence against CD19 was from anti-CD19-41BB-CD3ζ CAR previously developed in our laboratory.[31] The variable domains of heavy and light chain were connected by a flexible linker sequence encoding $(Gly_4Ser)_3$. The linked scFv was joined to the signal peptide and the hinge followed by constant heavy domains 2 and 3 (C_H2, C_H3) of IgG1. The anti-CD20 IFL was fused with CD16V-4-1BB-CD3ζ, which could have T cells exert ADCC as previously described by our laboratory, through a self-cleaving 2A peptide (P2A).[36] The gene was subcloned into the MSCV vector with or without GFP.

[0074] Gene transduction by retroviral vector was performed as previously described.[37] Briefly, MSCV retroviral vector was added to RetroNectin-coated (Takara, Otsu, Japan) tubes and incubated at 4°C for 16 hours. Then, activated NK cells or T lymphocytes were added to the tubes after removal of the supernatant and incubated at 37°C in 5% CO_2 for 24

hours. The transduction procedure was repeated one more time on the following day. Transduced cells were maintained in RPMI-1640, 10% FBS with IL-2.

Determination of IFL expression and specificity

[0075] To detect the IFL expression, transduced cells were stained with phycoerythrin (PE)-conjugated anti-human IgG antibody (SouthernBiotech, West Grove, PA) after permeabilizing by 8E reagent (a permeabilization reagent developed in our laboratory). CD16 and CD3 expression on the cell surface were determined by anti-CD16-PE (clone B73.1, BD Biosciences) and anti-CD3-APC (clone SK7, BD Biosciences), respectively.

[0076] For the specificity of IFLs, culture supernatant from transduced cells was added to Jurkat (CD20 negative, CD19 negative), Ramos (CD20 positive, CD19 positive), or RS4;11 (CD20 negative, CD19 positive) at 1 μg/mL and incubated for 10 minutes. The IFLs bound on the cell surface were detected with PE-conjugated anti-human IgG antibody. Cell staining was analyzed using BD LSRFortessa (BD Biosciences).

Measurement of IFL concentration and glycosylation analysis

[0077] The IFL concentration in culture supernatant from transduced cells was measured by enzyme-linked immunosorbent assay (ELISA). Briefly, culture supernatant containing IFL or rituximab was incubated on plates coated with PE-conjugated anti-human IgG antibody for one hour and washed. Subsequently, horseradish peroxidase (HRP)-conjugated anti-Rituximab antibody (MB2A4, Bio-Rad, Hercules, CA) was added to the plates and incubated for one hour. Fluorescence was measured by Infinite 200 PRO (Tecan, Mannedorf, Switzerland) after adding QuantaBlu Fluorogenic Peroxidase Substrate (Thermo Fisher). The IFL concentration was determined by the standard curve prepared with rituximab.

[0078] Glycosylation analysis was performed by Proteodynamics (Riom, France). Briefly, IFLs in culture supernatant of transduced cells were concentrated by a dialysis membrane (Amicon Ultra-15 Centrifugal Filter Units, Merck Millipore, Burlington, MA) and purified using NAB Protein G Spin kit (Thermo Fisher). The purified IFLs were denatured in 0.5% sodium dodecyl sulfate (SDS) and 1% β–mercaptoethanol and deglycosylated by PNGase F (Promega, Fitchburg, WI). The PNGase released N-glycans were purified on Hypercarb Hypersep 200 mg (Thermo Fisher) and permethylated by sodium hydroxide, dimethyl sulfoxide (DMSO) and methyl iodide (ICH3), before MALDI-TOF MS analysis using an Autoflex speed mass spectrometer (Bruker, Billerica, MA).

Cytotoxicity assays in vitro

[0079] For CDC assay, Ramos or SUDHL-4 in RPMI / 10% FBS medium with or without 5% complement (Sigma-Aldrich, Saint Louis, MO) were plated, and Rituximab or anti-CD20 IFL was added at 0.05 μg/ml. Viable cells were counted by Accuri CD6 (BD Biosciences) after incubation at 37°C in 5% CO_2 for 2 hours.

[0080] To test ADCP, Ramos cells labelled with mCherry were cultured with or without THP-1 at a 1:1 ratio for 48 hours in the presence of anti-CD20 IFL or rituximab at 0.1 μg/ml. Ramos cells were counted by IncuCyte Zoom System (Essen BioScience, Ann Arbor, MI).

[0081] For ADCC assay, target cells stained with calcein AM (Thermo Fisher) were co-cultured with transduced NK cells or T lymphocytes at a 2:1 effector-to-target (E:T) ratio for 4 hours. Viable target cells were counted by flow cytometry. In other tests, target cells expressing mCherry were incubated with NK cells or T lymphocytes with IL-2 (200 IU/mL for NK cells, 100 IU/mL for T cells) at 37°C in 5% CO_2. As a control, rituximab was added to NK cells with GFP alone at 1.0 μg/ml. The target cells were counted using IncuCyte Zoom System every 8 hours for 3 days.

IFL kinetics and dynamics in mouse model

[0082] To measure plasma concentration of IFL secreted from T cells, NOD.Cg-Prkdc^{scid} IL2rg^{tm1Wjl}/SzJ (NOD/scid IL2RGnull) mice (The Jackson Laboratory, Bar Harbor, ME) we injected intravenously (i.v.) 2 x 10^7 T cells transduced with anti-CD20 IFL-P2A-CD16V-4-1BB-CD3ζ, followed by 2 x 10^5 Nalm-6 expressing CD20 two days later. Mice also received 20,000 IU of IL-2 intraperitoneally every 2 days for three weeks. IFL in plasma was measured by ELISA.

[0083] To examine antitumor activity in vivo, luciferase-labelled Daudi was injected in NOD/scid IL2RGnull mice at 2 x 10^5 cells per mouse intraperitoneally (i.p.). Three and 6 days later, mice received T cells transduced with anti-CD20 IFL-P2A-CD16V-4-1BB-CD3ζ at 2 x 10^7 cells per mouse i.p. Other mice received 2 x 10^7 T cells transduced with GFP or 0.2 ml of RPMI 1640 only, instead of T cells. All mice received 20,000 IU of IL-2 every 2 days for one or three weeks. Growth of Daudi cells was measured using the Xenogen IVIS-200 System (Caliper Life Sciences, Waltham, MA) after injection of D-luciferin potassium salt (Perkin Elmer, Waltham, MA). Luminescence was analyzed with the Living Image 3.0

software (Perkin Elmer). Mice were euthanized when luminescence reached 1 x 10^{11} photons per second or physical signs warranting euthanasia appeared.

Results

Design and expression of IFLs

[0084] We first generated a scFv fragment from the public sequence of the anti-CD20 antibody rituximab. This scFv, as well as an anti-CD19 scFv previously developed in our laboratory,[31] were linked to the hinge and heavy chain constant domain 2 (C_H2) and 3 (C_H3) of human IgG1 (FIG. 1A). We inserted the IFL genes into an MSCV retroviral vector also containing the GFP gene, and transduced them in expanded NK cells. To determine whether IFLs were synthesized by the transduced cells, we performed intracellular staining targeting the Fc component. As shown in FIG. 1B, most NK GFP+ cells also expressed either the anti-CD20 or the anti-CD19 IFL. Similar results were seen when peripheral blood T lymphocytes were transduced with the same construct (FIG. 1C).

[0085] We determined whether the IFLs could bind their cognate target. As shown in Fig. 2, the secreted anti-CD19 IFL labelled CD19+ cells Ramos and RS4;11, while the anti-CD20 IFL, labelled only the CD20+ Ramos cell line but not the CD20- RS4;11 cell line. Neither labelled the CD19- CD20- T cell line Jurkat (FIG. 2).

IFL characterization

[0086] To measure the capacity of immune cells to produce IFLs, we collected the culture media from anti-CD20 IFL transduced cells and measured the concentration of antibody by ELISA, using an anti-idiotypic rituximab antibody. As shown in Fig. 3A, both NK and T cells secreted the anti-CD20 IFL. Notably, the amount of IFL measured in the T cells' supernatant was significantly higher than that measured in the NK cells' supernatant ($P < 0.01$). The amount of IFL secreted from 1 x 10^6 transduced NK cells for 24 hours was equivalent to 23.5 ng of rituximab (range, 15.1-36.8 ng, n = 3). That secreted by T cells was equivalent to 74.3 ng (range, 62.8-93.2 ng, n = 3).

[0087] To define the type of post-translational modification profile of the constructs produced by immune cells, we performed an analysis of the N-linked glycans bound to the modified Fc domain using MALDI-TOF. Twelve N-glycan structures were detected for the NK cell IFL, and 8 for the T cell IFL. For both, the dominant structure was a di-sialylated bi-antennary N-glycan ($[M+Na]^+$ 2792) without core-fucose, containing 2 galactose and 2

terminal sialic acid named G2S2. Interestingly, 79% and 59% of Fc glycans were afucosylated when IFL were produced by NK and T cells, respectively. (Fig. 3B, C). As a control, we also tested the N-linked glycan pattern of Rituximab; the detected N-glycans were two fucosylated bi-antennary N-glycans ([M+Na] +1836 =G0F, 2040= G1F).

IFLs mediate CDC, ADCP and ADCC

[0088] To test whether IFLs could mediate CDC, we incubated the CD20+ B-lymphoma cell lines Ramos, SUDHL-4 and Raji with different concentrations of anti-CD20 IFL (collected from supernatants of NK cells or T cells transduced with IFL) and 5% complement for 2 hours. In parallel tests, the anti-CD20 IFL was replaced by rituximab. As shown in Fig. 4A, IFL triggered massive lysis of both Ramos and SUDHL-4 cell lines (known to be susceptible to complement lysis), while the complement-resistant Raji cells remained largely unaffected.[38,39]

[0089] ADCP was tested by co-culturing Ramos with the monocytic cell line, THP-1, which can exert phagocytosis of tagged target cells.[40] As shown in Fig. 4B, IFLs derived from either NK or T cells could promote Ramos cell elimination in the presence of THP-1 cells.

[0090] To determine whether IFLs produced by NK cells and T cells could mediate ADCC, we co-cultured CD20+ lymphoma cell line Raji with NK cells transduced with GFP alone or anti-CD20 IFL at a E:T 1:1 ratio, using rituximab at 1 μg/mL with NK-GFP cells as a control. As shown in Fig. 5A, IFL NK cells exerted powerful cytotoxicity. In other tests, we determined the cytotoxic capacity of NK cells transduced with anti-CD19 IFL against 3 CD19+ leukemic cells lines (RS4;11, OP-1 and Nalm-6). As shown in Fig. 5B, NK-IFL cells were significantly more powerful than NK cells transduced with GFP alone and cell killing against the CD19+ CD20- cell line RS4;11 was mediated only by the anti-CD19 IFL (Fig. 5C).

T cells expressing CD16 receptors exert ADCC through self-produced IFLs

[0091] We prepared a bicistronic construct containing anti-CD20 IFL and the CD16 (V158)-41BB-CD3ζ receptor, separated by P2A (Fig. 6A). CD16-41BB-CD3ζ had been previously generated in our laboratory and shown to confer ADCC capacity to T lymphocytes.[32] We transduced T lymphocytes with the construct achieving expression of both components (Fig. 6B, C). When challenged against Ramos cells in long-term cultures, T

lymphocytes expressing both IFL and CD16-41BB-CD3ζ eradicated lymphoma cells while T cell expressing only one of the genes, or GFP did not (Fig. 6D). Additional information regarding CD16-41BB-CD3ζ can be found US Patent No. 10,144,770 B2 and U.S. Patent Publication No. 2015/0139943, both of which are incorporated herein by reference in their entirety.

[0092] We next determined the levels of plasma IFL that can be measured in mouse plasma after intravenous injection of 2 x 10^7 T lymphocytes transduced with anti-CD20 IFL in NOD-SCID-IL2RGnull immunodeficient mice. As shown in Fig. 7A, IFL could be detected in plasma 50 days after cell injection, indicating the IFL secretion is durable. We assessed whether T lymphocytes expressing both IFL and CD16-41BB-CD3ζ could exert anti-tumor activity NOD-SCID-IL2RGnull immunodeficient mice engrafted with the CD20+ B-cell lymphoma cell line Daudi intraperitoneally. After intraperitoneal injection of T cells, there was a strong anti-tumor activity in mice receiving those with IFL and CD16-41BB-CD3ζ while tumor grew rapidly in those receiving T cells transduced with GFP only or no T cells (Fig. 7B).

Modified IFLs

[0093] The IFL constructs were modified to enhance some its functions and/or widen the range of its specificities. For example, the modified Fc can be further altered to increase its affinity for Fc receptors in NK cells and macrophages, thus enhancing ADCC and ADCP, and/or to increase its capacity to fix complement. In particular, the modified IFLs of FIGs. 8A-D were constructed.

[0094] The results of the experiments shown in FIGs. 9A-B demonstrate that the addition of a sequence encoding IL-15 to the anti-CD20 IFL secreted by NK cells markedly increases the killing activity against CD20+ lymphoma cells in 3-day co-cultures. In these experiments, Ramos cell numbers were maximally reduced when NK cells were transduced with IFL-IL15; these cells were more powerful that those transduced with ILF lacking IL-15, which in turn were more powerful than NK cells transduced with GFP alone. The superiority of IFL-IL15 was observed regardless of whether IL-2 was present in the cultures. These results demonstrate that the function of IFLs can be augmented by linking them to other functional molecules.

ADDITIONAL EMBODIMENTS

1. A peptide comprising:
   a) a single-chain variable fragment (scFv) domain;
   b) a fragment crystallizable (Fc) domain; and
   c) a hinge domain joining the scFv and Fc domains.

2. The peptide of Embodiment 1, wherein the scFv domain comprises an immunoglobulin variable light (V_L) domain, an immunoglobulin variable heavy (V_H) domain, and a linker domain joining the V_L and V_H domains.

3. The peptide of Embodiment 2, wherein the linker domain is $(G_4S)_x$, wherein x is an integer from 1 to 100.

4. The peptide of Embodiment 3, wherein the linker domain is $(G_4S)_3$.

5. The peptide of any one of Embodiments 1 through 4, wherein the scFv domain binds CD19.

6. The peptide of any one of Embodiments 1 through 4, wherein the scFv domain binds CD20.

7. The peptide of any one of Embodiments 1 through 4, wherein the scFv domain binds CD22, CD38, CD7, CD2, CD3, epidermal growth factor receptor (EGFR), CD123, CD33, B-cell maturation antigen (BCMA), mesothelin, human epidermal growth factor receptor 2 (Her2), prostate-specific membrane antigen (PSMA), disialoganglioside (GD2), PD-L1 (CD274), CD80 or CD86.

8. The peptide of any one of Embodiments 1 through 4, wherein the Fc domain comprises an immunoglobulin constant heavy 2 (C_H2) domain and an immunoglobulin constant heavy 3 (C_H3) domain.

9. The peptide of any one of Embodiments 1 through 4, wherein the Fc domain is human IgG1 Fc domain.

10. The peptide of any one of Embodiments 1 through 4, further comprising a signal peptide that is N-terminal to the scFv domain.

11. The peptide of any one of Embodiments 1 through 4, further comprising a self-cleaving peptide joining the Fc domain to a chimeric receptor, wherein the chimeric receptor comprises a receptor domain, a hinge and transmembrane domain, a co-stimulatory signaling domain, and a cytoplasmic signaling domain.

12. The peptide of Embodiment 11, wherein the self-cleaving peptide is a 2A peptide.

13. The peptide of Embodiment 11, wherein the receptor domain is CD16.

14. The peptide of Embodiment 11, wherein the hinge and transmembrane domain is a CD8α hinge and transmembrane domain.

15. The peptide of Embodiment 11, wherein the co-stimulatory domain is 4-1BB co-stimulatory domain.

16. The peptide of Embodiment 11, wherein the cytoplasmic signaling domain is a CD3ζ cytoplasmic signaling.

17. The peptide of Embodiment 11, wherein the chimeric receptor is CD16V-4-1BB-CD3ζ.

18. The peptide of any one of Embodiments 1 through 4, wherein the scFv domain binds CD19 or CD20, the Fc domain is a human IgG1 Fc domain, and the hinge domain is an IgG1 hinge domain; the peptide further comprising a CD8α signal peptide that is N-terminal to the scFv domain; the peptide further comprising a chimeric receptor that is CD16V-4-1BB-CD3ζ.

19. The peptide of any one of Embodiments 1 through 4, further comprising one or more of the following mutations: S239D; S267E; H268F; or I332E.

20. The peptide of any one of Embodiments 1 through 4, further comprising one or more of the following mutations: E345K; E430G; or S440Y.

21. The peptide of any one of Embodiments 1 through 4, wherein the peptide further comprise IL-15 joined to the Fc domain by a linker.

22. The peptide of Embodiment 21, wherein the linker that joins IL-15 to the Fc domain is selected from the group consisting of SEQ ID NO: 51; $A(EAAK)_4ALEA(EAAAK)_4A$; $(EAAAK)_z$; $A(EAAAK)_zA$; and $(XP)_w$, wherein z is an integer from 1 to 100; X is any amino acid, and w is an integer from 1 to 100.

23. The peptide of any one of Embodiments 1 through 4, wherein the peptide further comprises a ligand that binds 4-1BB (CD37), CD28, or OX40 (CD134) joined to the Fc domain by a linker.

24. The peptide of Embodiment 23, wherein the linker that joins IL-15 to the Fc domain is selected from the group consisting of SEQ ID NO: 51; $A(EAAK)_4ALEA(EAAAK)_4A$; $(EAAAK)_z$; $A(EAAAK)_zA$; and $(XP)_w$, wherein z is an integer from 1 to 100; X is any amino acid, and w is an integer from 1 to 100.

25. A nucleic acid encoding the peptide of any of Embodiments 1 through 24.

26. A vector comprising a nucleic acid, the nucleic acid encoding the peptide of any of Embodiments 1 through 24.

27. The vector of Embodiment 26, wherein the vector is a murine stem cell virus (MSCV).

28. An immune cell that expresses a peptide, wherein the peptide comprises:

a) a T-cell receptor (TCR) β domain;

b) a first fragment crystallizable (Fc) domain joined to the TCR β domain;

c) a TCR α domain;

d) a self-cleaving peptide joining the Fc domain to the TCR α domain;

e) a second Fc domain joined to the TCR α domain.

29. The immune cell of Embodiment 28, further comprising a signal peptide joined to the T-cell receptor (TCR) β domain.

30. The immune cell of Embodiment 28, wherein the first Fc domain is the same as the second Fc domain.

31. A peptide comprising:

a) a T-cell receptor (TCR) β domain;

b) a first fragment crystallizable (Fc) domain joined to the TCR β domain;

c) a TCR α domain;

d) a self-cleaving peptide joining the Fc domain to the TCR α domain;

e) a second Fc domain joined to the TCR α domain.

32. The peptide of Embodiment 31, further comprising a signal peptide joined to the T-cell receptor (TCR) β domain.

33. The peptide of Embodiment 31, wherein the first Fc domain is the same as the second Fc domain.

34. A nucleic acid encoding the peptide of any one of Embodiments 31 through 33.

35. A vector comprising a nucleic acid, the nucleic acid encoding the peptide of any one of Embodiments 31 through 33.

36. A method of making a transgenic host cell, the method comprising introducing a vector into a host cell, the vector comprising a nucleic acid encoding the peptide of any of Embodiments 1 through 24 or Embodiments 31 through 33.

37. A method of enhancing antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC) in a subject, the method comprising administering to the subject a therapeutically effective amount of any of the immune cells described herein.

SEQUENCES

[0095] SEQ ID NO: 1: Anti-CD20 IFL, Rituximab signal peptide; cDNA:
ATGGACTTCCAGGTGCAGATCATCAGCTTTCTGCTGATCTCCGCCTCT

[0096] SEQ ID NO: 2: Anti-CD20 IFL, Rituximab signal peptide; amino acid:
MDFQVQIISFLLISAS

[0097] SEQ ID NO: 3: Anti-CD20 IFL, Immunoglobulin variable domain of rituximab light chain; cDNA:
GTGATCATGTCCAGGGGCCAGATCGTGCTGAGCCAGTCCCCAGCAATCCTGTCTG
CCAGCCCTGGAGAGAAGGTGACCATGACATGCCGCGCCAGCTCCTCTGTGAGCT
ACATCCACTGGTTCCAGCAGAAGCCCGGCAGCTCCCCTAAGCCCTGGATCTATGC
CACAAGCAACCTGGCCTCCGGCGTGCCTGTGCGGTTTTCCGGCTCTGGCAGCGGC
ACCTCCTACTCTCTGACAATCAGCAGAGTGGAGGCCGAGGATGCCGCCACCTACT
ATTGCCAGCAGTGGACCTCCAATCCCCCTACATTCGGCGGCGGCACCAAGCTGGA
GATCAAG

[0098] SEQ ID NO: 4: Anti-CD20 IFL, Immunoglobulin variable domain of rituximab light chain; amino acid:
VIMSRGQIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNL
ASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK

[0099] SEQ ID NO: 5: Anti-CD20 IFL, Linker; cDNA:
GGCGGCGGCGGCTCTGGAGGAGGAGGCAGCGGCGGAGGAGGCTCC

[00100] SEQ ID NO: 6: Anti-CD20 IFL, Linker; amino acid: GGGGSGGGGSGGGGS

[00101] SEQ ID NO: 7: Anti-CD20 IFL, Immunoglobulin variable domain of rituximab heavy chain; cDNA:
CAGGTGCAGCTGCAGCAGCCAGGAGCAGAGCTGGTGAAGCCAGGAGCCTCTGTG
AAGATGAGCTGTAAGGCCTCCGGCTACACCTTCACAAGCTATAACATGCACTGG
GTGAAGCAGACACCAGGAAGGGGCCTGGAGTGGATCGGAGCAATCTACCCTGGC
AACGGCGACACCTCCTATAATCAGAAGTTTAAGGGCAAGGCCACCCTGACAGCC
GATAAGTCTAGCTCCACAGCCTACATGCAGCTGTCTAGCCTGACCTCTGAGGACA
GCGCCGTGTACTATTGCGCCAGAAGCACATACTATGGCGGCGATTGGTACTTCAA
CGTGTGGGGAGCAGGCACCACAGTGACCGTGTCTGCC

[00102] SEQ ID NO: 8: Anti-CD20 IFL, Immunoglobulin variable domain of rituximab heavy chain; amino acid:
QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGN

GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNV
WGAGTTVTVSA

[00103] SEQ ID NO: 9: Anti-CD20 IFL, Hinge and constant heavy domain 2 and 3 of immunoglobulin G1; cDNA:

GAGCCAAAGAGCTGTGACAAGACCCACACATGCCCACCATGTCCAGCACCTGAG
CTGCTGGGAGGACCTTCCGTGTTCCTGTTTCCTCCAAAGCCAAAGGATACCCTGA
TGATCTCTAGGACCCCTGAGGTGACATGCGTGGTGGTGGACGTGAGCCACGAGG
ACCCCGAGGTGAAGTTTAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCA
AGACCAAGCCTCGGGAGGAGCAGTACAACTCCACATATAGAGTGGTGTCTGTGC
TGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTATAAGTGCAAGGTGT
CCAATAAGGCCCTGCCAGCCCCCATCGAGAAGACAATCTCTAAGGCCAAGGGCC
AGCCTAGGGAGCCACAGGTGTACACCCTGCCACCTTCCCGCGACGAGCTGACAA
AGAACCAGGTGTCTCTGACCTGTCTGGTGAAGGGCTTCTATCCATCTGACATCGC
CGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAATTACAAGACCACACCACC
CGTGCTGGACTCCGATGGCTCTTTCTTTCTGTATAGCAAGCTGACAGTGGACAAG
TCCCGGTGGCAGCAGGGCAACGTGTTTAGCTGTTCCGTGATGCACGAGGCCCTGC
ACAATCACTACACCCAGAAGTCTCTGAGCCTGTCCCCCGGCAAGTGA

[00104] SEQ ID NO: 10: Anti-CD20 IFL, Hinge and constant heavy domain 2 and 3 of immunoglobulin G1; amino acid:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

[00105] SEQ ID NO: 11: Anti-CD19 IFL, CD8α signal peptide; cDNA:

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCG
CCAGGCCG

[00106] SEQ ID NO: 12: Anti-CD19 IFL, CD8α signal peptide; amino acid:

MALPVTALLLPLALLLHAARP

[00107] SEQ ID NO: 13: Anti-CD19 IFL, Immunoglobulin variable domain of light chain; cDNA:

GACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAG
TCACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCA

GCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACCATACATCAAGATTACAC
TCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCA
CCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAA
TACGCTTCCGTACACGTTCGGAGGGGGGACCAAGCTGGAGATCACA

[00108] SEQ ID NO: 14: Anti-CD19 IFL, Immunoglobulin variable domain of light chain; amino acid:

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV
PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT

[00109] SEQ ID NO: 15: Anti-CD19 IFL, Linker; cDNA:

GGTGGCGGTGGCTCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCT

[00110] SEQ ID NO: 16: Anti-CD19 IFL, Linker; amino acid: GGGGSGGGGSGGGGS

[00111] SEQ ID NO: 17: Anti-CD19 IFL, Immunoglobulin variable domain of heavy chain; cDNA:

GAGGTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTG
TCCGTCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGA
TTCGCCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGGAGTAATATGGGGTAGTG
AAACCACATACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAA
CTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCC
ATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTATGCTATGGACTACT
GGGGCCAAGGAACCTCAGTCACCGTCTCCTCA

[00112] SEQ ID NO: 18: Anti-CD19 IFL, Immunoglobulin variable domain of heavy chain; amino acid:

EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETT
YYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSS

[00113] SEQ ID NO: 19: Anti-CD19 IFL, Hinge and constant heavy domain 2 and 3 of immunoglobulin G1; cDNA:

GAGCCAAAGAGCTGTGACAAGACCCACACATGCCCACCATGTCCAGCACCTGAG
CTGCTGGGAGGACCTTCCGTGTTCCTGTTTCCTCCAAAGCCAAAGGATACCCTGA
TGATCTCTAGGACCCCTGAGGTGACATGCGTGGTGGTGGACGTGAGCCACGAGG
ACCCCGAGGTGAAGTTTAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCA
AGACCAAGCCTCGGGAGGAGCAGTACAACTCCACATATAGAGTGGTGTCTGTGC
TGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTATAAGTGCAAGGTGT

CCAATAAGGCCCTGCCAGCCCCCATCGAGAAGACAATCTCTAAGGCCAAGGGCC
AGCCTAGGGAGCCACAGGTGTACACCCTGCCACCTTCCCGCGACGAGCTGACAA
AGAACCAGGTGTCTCTGACCTGTCTGGTGAAGGGCTTCTATCCATCTGACATCGC
CGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAATTACAAGACCACACCACC
CGTGCTGGACTCCGATGGCTCTTTCTTTCTGTATAGCAAGCTGACAGTGGACAAG
TCCCGGTGGCAGCAGGGCAACGTGTTTAGCTGTTCCGTGATGCACGAGGCCCTGC
ACAATCACTACACCCAGAAGTCTCTGAGCCTGTCCCCCGGCAAGTGA

[00114] SEQ ID NO: 20: Anti-CD19 IFL, Hinge and constant heavy domain 2 and 3 of immunoglobulin G1; amino acid:
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

[00115] SEQ ID NO: 21: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Rituximab signal peptide; cDNA: ATGGATTTCCAGGTCCAGATTATTTCCTTCCTGCTGATTAGTGCCAGT

[00116] SEQ ID NO: 22: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Rituximab signal peptide; amino acid: MDFQVQIISFLLISAS

[00117] SEQ ID NO: 23: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Immunoglobulin variable domain of rituximab light chain; cDNA:
GTGATTATGAGTAGAGGCCAGATTGTGCTGAGCCAGTCCCCAGCAATCCTGAGC
GCCTCCCCAGGAGAGAAGGTGACAATGACCTGCAGAGCCAGCTCCTCTGTGAGC
TACATCCACTGGTTCCAGCAGAAGCCCGGCAGCTCCCCAAAGCCCTGGATCTATG
CCACCTCCAACCTGGCCTCTGGCGTGCCTGTGAGATTTTCTGGCAGCGGCTCCGG
CACATCTTACAGCCTGACCATCAGCAGGGTGGAGGCAGAGGACGCAGCAACCTA
CTATTGCCAGCAGTGGACATCCAATCCCCCTACCTTCGGCGGCGGCACAAAGCTG
GAGATCAAGGGC

[00118] SEQ ID NO: 24: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Immunoglobulin variable domain of rituximab light chain; amino acid:
VIMSRGQIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNL
ASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK

[00119] SEQ ID NO: 25: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Linker; cDNA:
GGCGGCGGCTCTGGAGGAGGAGGAAGCGGAGGAGGAGGCTCC

[00120] SEQ ID NO: 26: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Linker; amino acid: GGGGSGGGGSGGGGS

[00121] SEQ ID NO: 27: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Immunoglobulin variable domain of rituximab heavy chain; cDNA:
CAGGTGCAGCTGCAGCAGCCTGGAGCAGAGCTGGTGAAGCCAGGAGCCAGCGTG
AAGATGTCCTGTAAGGCCTCTGGCTACACATTCACCAGCTATAACATGCACTGGG
TGAAGCAGACCCCAGGAAGAGGCCTGGAGTGGATCGGAGCCATCTACCCTGGCA
ACGGCGACACATCCTATAATCAGAAGTTTAAGGGCAAGGCCACACTGACCGCCG
ATAAGTCTAGCTCCACCGCCTACATGCAGCTGTCTAGCCTGACATCCGAGGACTC
TGCCGTGTACTATTGCGCCAGGAGCACCTACTATGGCGGCGATTGGTACTTCAAC
GTGTGGGGCGCCGGCACCACAGTGACAGTGTCTGCC

[00122] SEQ ID NO: 28: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Immunoglobulin variable domain of rituximab heavy chain; amino acid:
QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGN
GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNV
WGAGTTVTVSA

[00123] SEQ ID NO: 29: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Hinge and constant heavy domain 2 and 3 of immunoglobulin G1; cDNA:
GAGCCCAAGAGCTGTGACAAGACACACACCTGCCCACCATGTCCTGCACCAGAG
CTGCTGGGAGGACCATCCGTGTTCCTGTTTCCTCCAAAGCCCAAGGATACCCTGA
TGATCTCTCGCACACCTGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGG
ATCCAGAGGTGAAGTTTAACTGGTACGTGGACGGCGTGGAGGTGCACAATGCCA
AGACCAAGCCTAGAGAGGAGCAGTACAACAGCACCTATAGGGTGGTGTCCGTGC
TGACAGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTATAAGTGCAAGGTGT
CCAATAAGGCCCTGCCCGCCCCTATCGAGAAGACCATCTCTAAGGCAAAGGGAC
AGCCAAGGGAGCCACAGGTGTACACACTGCCCCCTAGCCGGGACGAGCTGACCA
AGAACCAGGTGTCCCTGACATGTCTGGTGAAGGGCTTCTATCCATCCGATATCGC
CGTGGAGTGGGAGTCTAATGGCCAGCCCGAGAACAATTACAAGACCACACCACC
CGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCTAAGCTGACCGTGGACAAG
AGCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCTGTGATGCACGAGGCCCTGC
ACAATCACTACACCCAGAAGAGCCTGTCCCTGTCTCCCGGCAAG

[00124] SEQ ID NO: 30: Anti-CD20 IFL-P2A-CD16V-BB-ζ, Hinge and constant heavy domain 2 and 3 of immunoglobulin G1; amino acid:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

[00125] SEQ ID NO: 31: Anti-CD20 IFL-P2A-CD16V-BB-ζ, P2A; cDNA:
GCCACAAACTTTAGCCTGCTGAAGCAGGCAGGCGACGTGGAGGAGAATCCAGGA

[00126] SEQ ID NO: 32: Anti-CD20 IFL-P2A-CD16V-BB-ζ, P2A; amino acid:
ATNFSLLKQAGDVEENPG

[00127] SEQ ID NO: 33: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD8α signal peptide; cDNA:
CCCGCCCTGCCAGTGACCGCCCTGCTGCTGCCTCTGGCCCTGCTGCTGCACGCAG
CCCGCCCA

[00128] SEQ ID NO: 34: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD8α signal peptide; amino acid: PALPVTALLLPLALLLHAARP

[00129] SEQ ID NO: 35: Anti-CD20 IFL-P2A-CD16V-BB-ζ, FCGR3A extracellular domain; cDNA:
GGCATGCGGACAGAGGATCTGCCCAAGGCCGTGGTGTTTCTGGAGCCTCAGTGG
TACCGCGTGCTGGAGAAGGACTCCGTGACCCTGAAGTGTCAGGGCGCCTATTCCC
CTGAGGATAACTCTACACAGTGGTTCCACAATGAGTCTCTGATCTCCTCTCAGGC
CAGCTCCTACTTTATCGACGCAGCAACCGTGGACGATAGCGGAGAGTATCGGTG
CCAGACAAACCTGTCTACCCTGAGCGATCCAGTGCAGCTGGAGGTGCACATCGG
ATGGCTGCTGCTGCAGGCACCTAGATGGGTGTTCAAGGAGGAGGATCCAATCCA
CCTGAGGTGTCACAGCTGGAAGAATACCGCCCTGCACAAGGTGACATACCTGCA
GAACGGCAAGGGCCGCAAGTACTTCCACCACAATTCCGACTTTTATATCCCAAAG
GCCACCCTGAAGGATAGCGGCTCCTATTTTTGCCGGGGCCTGGTGGGCTCCAAGA
ACGTGTCTAGCGAGACAGTGAATATCACAATCACCCAGGGCCTGGCCGTGTCTAC
AATCTCCTCTTTCTTTCCTCCAGGCTACCAG

[00130] SEQ ID NO: 36: Anti-CD20 IFL-P2A-CD16V-BB-ζ, FCGR3A extracellular domain; amino acid:
GMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASS
YFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHS

WKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETV
NITITQGLAVSTISSFFPPGYQ

[00131] SEQ ID NO: 37: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD8α hinge and transmembrane; cDNA:
ACCACAACCCCTGCACCAAGACCCCCTACACCAGCACCTACCATCGCAAGCCAG
CCACTGTCCCTGCGGCCCGAGGCCTGTAGGCCAGCAGCAGGAGGAGCAGTGCAC
ACCAGGGGCCTGGACTTCGCCTGCGATATCTATATCTGGGCACCTCTGGCAGGAA
CCTGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCCTGTACTGC

[00132] SEQ ID NO: 38: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD8α hinge and transmembrane; amino acid:
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV
LLLSLVITLYC

[00133] SEQ ID NO: 39: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD137 cytoplasmic domain; cDNA:
AAGAGAGGCAGGAAGAAGCTGCTGTATATCTTCAAGCAGCCTTTTATGCGCCCA
GTGCAGACAACCCAGGAGGAGGACGGCTGCTCCTGTCGGTTCCCAGAAGAGGAG
GAGGGAGGATGTGAGCTG

[00134] SEQ ID NO: 40: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD137 cytoplasmic domain; amino acid: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

[00135] SEQ ID NO: 41: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD3ζ cytoplasmic domain; cDNA:
AGGGTGAAGTTTTCTCGGAGCGCCGATGCACCAGCATACCAGCAGGGACAGAAC
CAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGAT
AAGAGGCGCGGCAGGGACCCCGAGATGGGAGGCAAGCCCCGGAGAAAGAACCC
TCAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTATAG
CGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGT
ACCAGGGCCTGTCAACAGCAACAAAAGACACTTACGACGCACTGCACATGCAGG
CTCTGCCCCCAAGATAA

[00136] SEQ ID NO: 42: Anti-CD20 IFL-P2A-CD16V-BB-ζ, CD3ζ cytoplasmic domain; amino acid:
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

[00137] SEQ ID NO: 43: P2A cDNA:
GCCACAAACTTTAGCCTGCTGAAGCAGGCAGGCGACGTGGAGGAGAATCCAGGA

[00138] SEQ ID NO: 44: P2A amino acid: ATNFSLLKQAGDVEENPG

[00139] SEQ ID NO: 45: T2A cDNA:
GGAAGCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAA
TCCTGGACCT

[00140] SEQ ID NO: 46: T2A amino acid: GSGEGRGSLLTCGDVEENPGP

[00141] SEQ ID NO: 47: E2A cDNA:
GGAAGCGGACAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGA
GCAACCCTGGACCT

[00142] SEQ ID NO: 48: E2A amino acid: GSGQCTNYALLKLAGDVESNPGP

[00143] SEQ ID NO: 49: F2A cDNA:
GGAAGCGGAGTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAGAC
GTGGAGTCCAACCCTGGACCT

[00144] SEQ ID NO: 50: F2A amino acid: GSGVKQTLNFDLLKLAGDVESNPGP

[00145] SEQ ID NO: 51: Linker of FIG. 8C nucleotide:
AGCTGCTGCTAAGGCACTGGAAGCAGAAGCCGCGGCTAAGGAGGCGGCTGCAAA
AGAAGCTGCAGCCAAGGAAGCAGCCGCGAAGGCA

[00146] SEQ ID NO: 52: Linker of FIG. 8C amino acid:
AEAAAKEAAAKEAAAKEAAAKALEAEAAAKEAAAKEAAAKEAAAKA

[00147] SEQ ID NO: 53: IL-15 of FIG. 8C nucleotide:
AACTGGGTGAATGTGATCTCCGACCTGAAGAAGATCGAGGATCTGATCCAGTCT
ATGCACATCGACGCCACCCTGTACACAGAGTCCGATGTGCACCCCTCTTGCAAGG
TGACCGCCATGAAGTGTTTTCTGCTGGAGCTGCAGGTCATCTCCCTGGAGTCTGG
CGACGCCAGCATCCACGATACAGTGGAGAACCTGATCATCCTGGCCAACAATTCT
CTGTCCTCTAACGGCAATGTGACCGAGAGCGGCTGCAAGGAGTGTGAGGAGCTG
GAGGAGAAGAATATCAAAGAGTTCCTGCAGAGTTTCGTCCATATCGTCCAGATGT
TTATCAATACCTCC

[00148] SEQ ID NO: 54: IL-15 of FIG. 8C amino acid:
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDAS
IHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

INCORPORATION BY REFERENCE; EQUIVALENTS

[00149] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

[00150] While example embodiments have been particularly shown and described, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the embodiments encompassed by the appended claims.

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