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(54) Title: CD33 BINDING POLYPEPTIDES WITH STEFIN A PROTEIN

(57) Abstract: The present disclosure relates to engineered CD33-binding Stefin A polypeptide variants, polynucleotides encoding the engineered CD33-binding Stefin A polypeptide variants, cells expressing the polypeptide variants, pharmaceutical preparations of the polypeptide variants, and uses of the polypeptide variants in the treatment of various human conditions, including cancer.

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CD33 BINDING POLYPEPTIDES WITH STEFIN A PROTEIN

RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of US 63/185,463, filed May 7, 2021, which is incorporated by reference herein in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE

The instant application contains a Sequence Listing which has been submitted in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 2, 2022, is named A122470012WO00-SEQ-JXV and is 483,410 bytes in size.

BACKGROUND

Several surface molecules expressed by cancers cells have been identified and may be useful for directing tumor-specific immunotherapies. CD33 is a myeloid-specific sialic acid-binding receptor that is displayed on acute myeloid leukemia (AML) blasts in approximately 90% of patients and on AML stem cells. This antigen has been validated as an effective target for antibody-based therapies in clinical trials. Yet, CD33 is a challenging target in part because of its low expression and slow internalization; these characteristics limit antibody-dependent cell-mediated cytotoxicity and intracellular drug accumulation and, consequently, the activity of unlabeled and toxin-carrying antibodies. Improved molecules that can efficiently and effectively target CD33 are needed.

SUMMARY

Provided herein, in some aspects, are engineered polypeptides, referred to as CD33 AFFIMER® polypeptides or engineered CD33-binding Stefin A polypeptide variants, that are based on naturally occurring proteins (Stefin A cystatin) and engineered to stably display two loops that create a binding surface with high specificity and high affinity for CD33. The data provided herein show that these CD33 AFFIMER® polypeptides bind CD33 with a K_d of less than $1 \times 10^{-6}M$, or even less than $1 \times 10^{-7}M$. The CD33 AFFIMER® polypeptides of the present disclosure are useful for targeting cells that express CD33, such as such as those identified in acute myeloid leukemia (AML) and Alzheimer's Disease (AD) patients. These CD33 AFFIMER® polypeptides have several advantages over antibodies, for example. They are smaller (~14 kDa), simpler (no disulfide bridges and no posttranslational modifications), and more robust (thermally and chemically) than antibodies. These high affinity (single-digit nM)

CD33 AFFIMER® polypeptides can be generated in only a few weeks, exhibit exquisite specificity, are easily modified (chemically and as fusion proteins), and are easily manufactured in bacterial, yeast, or mammalian systems with high expression yields. Further, the core AFFIMER® polypeptides are non-immunogenic.

Some aspects of the present disclosure provide an engineered polypeptide that binds specifically to CD33 with a K_d of 1×10^{-6} M or less, wherein the engineered polypeptide is a variant of a Stefin A protein.

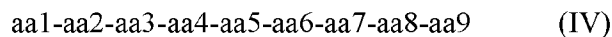
In some embodiments, engineered polypeptide of claim 1 comprising an amino acid sequence having at least 80%, at least 90%, at least 95, or at least 98% identity to an amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA-(Xaa)_n-STNYIKVRAGDNKYMHLKVFNGP-(Xaa)_m-ADRVLTGYQVDKNKDDDEL TGF (SEQ ID NO: 5), wherein Xaa, individually for each occurrence, is an amino acid residue; and n and m are each, independently, an integer from 3 to 20.

In some embodiments, the engineered polypeptide comprises the amino acid sequence of SEQ ID NO: 5.

In some embodiments, the engineered polypeptide binds to CD33 with a K_d of 1×10^{-7} M.

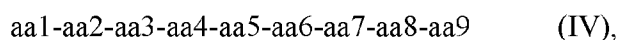
In some embodiments, the engineered polypeptide binds to CD33 with a K_d of 1×10^{-8} M.

In some embodiments, (Xaa)_n is represented by Formula (IV)



wherein aa1 is F; aa2 is Q, R, or T; aa3 is W; aa4 is G; aa5 is S; aa6 is S; aa7 is Y; aa8 is H; and aa9 is E or Q.

In some embodiments, (Xaa)_m is represented by Formula (IV)



wherein aa1 is I, K, V, or Y; aa2 is D, E, or Q; aa3 is D, N, S or Y; aa4 is D, E, N, or Q; aa5 is F, K, N, V or Y; aa6 is A or N; aa7 is L; aa8 is I or T; and aa9 is W.

In some embodiments, engineered polypeptide comprises the amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FRWGSSYHQST** NYIYIKVRAGDNKYMHLKVFNGP**VQDEFALI**WADRVLTGYQVDKNKDDDEL TGF (SEQ ID NO: 150)

In some embodiments, engineered polypeptide comprises the amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FRWGSSYHQST** NYIYIKVRAGDNKYMHLKVFNGP**YDNDVAL**TWADRVLTGYQVDKNKDDDEL TGF (SEQ ID NO: 300)

In some embodiments, engineered polypeptide comprises the amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FRWGSSYHQST** NYIYIKVRAGDNKYMHLKVFNG**PIESDFNLIW**ADRVLTGYQVDKNKDDELDTGF (SEQ ID NO: 297)

In some embodiments, engineered polypeptide comprises the amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FQWGSSYHEST** NYIYIKVRAGDNKYMHLKVFNG**PVEYDYNLIW**ADRVLTGYQVDKNKDDELDTGF (SEQ ID NO: 343)

In some embodiments, engineered polypeptide comprises the amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FTWGSSYHEST** NYIYIKVRAGDNKYMHLKVFNG**PVEYDYNLIW**ADRVLTGYQVDKNKDDELDTGF (SEQ ID NO: 346)

In some embodiments, (Xaa)_n is an amino acid sequence having at least 75% or at least 85% identity to the amino acid sequence of any one of SEQ ID NOs: 6-65.

In some embodiments, (Xaa)_n is the amino acid sequence of any one of SEQ ID NOs: 6-65.

In some embodiments, (Xaa)_m is an amino acid sequence having at least 75% or at least 85% identity to the amino acid sequence of any one of SEQ ID NOs: 66-129.

In some embodiments, (Xaa)_m is the amino acid sequence of any one of SEQ ID NOs: 66-129.

In some embodiments, engineered polypeptide further comprises a signal peptide.

Other aspects of the present disclosure provide a fusion protein comprising the engineered polypeptide as described herein linked to a therapeutic or diagnostic moiety.

In some embodiments, the therapeutic moiety is a therapeutic protein or peptide, optionally an antibody.

In some embodiments, the diagnostic moiety is a fluorescent protein.

In some embodiments, the engineered polypeptide is linked to the therapeutic or diagnostic moiety through a linker.

Further aspects of the present disclosure provide a multispecific fusion protein comprising: (a) the engineered polypeptide as described herein; and (b) an immune cell binding domain.

In some embodiments, the immune cell binding domain is a T cell binding domain.

In some embodiments, the T cell binding domain specifically binds to CD3, optionally a CD3ε chain.

In some embodiments, the immune cell binding domain is an antibody.

In some embodiments, the antibody is selected from whole antibodies, antibody fragments, and antibody mimetics.

In some embodiments, the multispecific fusion protein comprises a peptide linker.

In some embodiments, multispecific fusion protein comprises (a) the engineered polypeptide as described herein; (b) the peptide linker; and (b) an antibody, optionally single-chain variable fragment (scFv), that specifically binds to a CD3 ϵ chain.

In some embodiments, the immune cell binding domain is a Natural Killer (NK) cell binding domain.

In some embodiments, the NK cell binding domain specifically binds to CD16.

In some embodiments, the immune cell binding domain is an antibody.

In some embodiments, the antibody is selected from whole antibodies, antibody fragments, and antibody mimetics.

In some embodiments, the multispecific fusion protein further comprises a peptide linker.

In some embodiments, the multispecific fusion protein comprises: (a) the engineered polypeptide as described herein; (b) the peptide linker; and (c) an antibody, optionally single-chain variable fragment (scFv), that specifically binds to a CD16.

In some embodiments, the engineered polypeptide and the NK cell binding domain are crosslinked to an interleukin-15 (IL-15) protein.

In some embodiments, the multispecific fusion protein comprises (a) the engineered polypeptide of any one of the preceding claims; (b) IL-15 protein; and (b) an antibody, optionally single-chain variable fragment (scFv), that specifically binds to CD16, wherein the engineered polypeptide and the antibody are crosslinked to the IL-15 protein.

In some embodiments, the multispecific fusion protein further comprises an antibody, optionally scFv, that specifically binds to CD133.

In some embodiments, the multispecific fusion protein further comprises an immune cell binding domain that specifically binds to NKGD2.

In some embodiments, the multispecific fusion protein comprises: (a) the engineered polypeptide as described herein; (b) an antibody, optionally a Fab fragment, that specifically binds NKGD2; and (c) an antibody, optionally an Fc domain, that specifically binds to a CD16, optionally wherein the engineered polypeptide is linked to the antibody of (b) or (c) through a linker.

In some embodiments, the linker comprises Ala-Ser or Gly-Ala-Ser.

Other aspects of the present disclosure provide a T cell comprising the multispecific fusion protein as described herein.

Still other aspects of the present disclosure provide an NK cell comprising the multispecific fusion protein as described herein.

Yet other aspects of the present disclosure provide a chimeric antigen receptor, comprising: (a) the engineered polypeptide as described herein; (b) a linker sequence; (c) a transmembrane domain; and (d) a CD3z signaling domain.

In some embodiments, the chimeric antigen receptor further comprises a CD28 co-stimulatory domain, a 4-1BB co-stimulatory domain, or both a CD28 co-stimulatory domain and a 4-1BB co-stimulatory domain.

Other aspects of the present disclosure provide a T cell comprising the chimeric antigen receptor described herein.

Still other aspects of the present disclosure provide a fusion protein comprising: (a) a first engineered polypeptide as described herein; (b) an antibody Fc domain; and (c) a second engineered polypeptide as described herein.

In some embodiments, the engineered polypeptide or the fusion protein further comprises a half-life extension moiety.

In some embodiments, the half-life extension moiety is selected from antibody Fc domains, human serum albumin, serum binding proteins.

In some embodiments, the half-life extension moiety is an engineered polypeptide is a variant of a Stefin A protein that binds specifically to human serum albumin with a K_d of $1 \times 10^{-6} \text{M}$ or less.

Also provided herein, in some aspects, is a polynucleotide or set of polynucleotides encoding the engineered polypeptide as described herein, the fusion protein of the preceding paragraphs, or the chimeric antigen receptor as described herein.

In some embodiments, the polynucleotide is a DNA or an RNA.

In some embodiments, the RNA is an mRNA.

Some aspects of the present disclosure provide a delivery vehicle comprising the polynucleotide as described herein.

In some embodiments, the delivery vehicle is a viral delivery vehicle.

In some embodiments, the viral delivery vehicle is an adenoviral vector, a retroviral vector, a lentiviral vector, or an adeno-associated viral (AAV) vector.

In some embodiments, the delivery vehicle is a non-viral delivery vehicle.

In some embodiments, the non-viral delivery vehicle is a liposome or a lipid nanoparticle.

Other aspects of the present disclosure provide a plasmid or minicircle comprising the polynucleotide as described herein.

Yet other aspects of the present disclosure provide a messenger RNA (mRNA) comprising an open reading frame encoding the engineered polypeptide as described herein.

In some embodiments, the mRNA further comprises an open reading frame encoding an immune cell binding.

Other aspects of the present disclosure provide a lipid nanoparticle, optionally a cationic lipid nanoparticle, comprising the mRNA as described herein.

Other aspects of the present disclosure provide a conjugate comprising: (a) the engineered polypeptide as described herein; and (b) a chemotherapeutic agent.

Yet other aspects of the present disclosure provide a pharmaceutical composition comprising the engineered polypeptide, the fusion protein, the cell, or the delivery vehicle as described herein, and optionally a pharmaceutically acceptable excipient.

Still other aspects of the present disclosure provide a method comprising administering to a subject the pharmaceutical composition.

In some embodiments, the subject has a CD33⁺ cancer.

In some embodiments, the CD33⁺ cancer is acute myeloid leukemia.

In some embodiments, the subject has Alzheimer's disease.

In some embodiments, the pharmaceutical composition is administered subcutaneously, intravenously, or intramuscularly.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows dose-response curves of the percentage of CD33⁺ U937 cells bound by AFFIMER® Clones 21, 115, 162, 165, 168, 174, 182, and 183 at varying concentrations of each, as measured by flow cytometry analysis.

FIG. 2 shows dose-response curves of the percentage of CD33⁺ U937 cells bound by AFFIMER® Clones 21, 80, 115, and 208 at varying concentrations of each, as measured by flow cytometry analysis.

FIG. 3 shows dose-response curves of the percentage of CD33⁺ U937 cells bound by AFFIMER® Clones 21, 82, 115, and 211 at varying concentrations of each, as measured by flow cytometry analysis.

FIGs. 4A–4B show epitope binning analysis, based on Octet measurement of affinity, of AFFIMER® Clones 208 and 155. **FIG. 4A** shows nanometer (nm) shift when AFFIMER® Clone 208 or buffer is added to CD33-coated streptavidin sensors. The left vertical line in **FIG.**

4A indicates the point at which AFFIMER® Clone 155 or buffer is added to the sensors, and the right vertical line indicates the point at which a second AFFIMER® clone or buffer is added.

FIG. 4B shows the nm shift, relative to the optical densities measured in **FIG. 4A**, when a second AFFIMER® clone or buffer is added to the sensor measure in **FIG. 4A**. The left vertical line in **FIG. 4B** indicates the point at which the second AFFIMER® clone or buffer was added, and the right vertical line indicates the point at which any bound AFFIMERS® clone were allowed to dissociate from the sensors. Curves are labeled in the format “X -> Y” where X is the first reagent added to the sensors, and Y is the second reagent added to the sensors.

FIGs. 5A–5B show epitope binning analysis using AFFIMER® Clones 155, and 208.

FIG. 5A shows nm shift when AFFIMER® Clone 155 or buffer is added to CD33-coated streptavidin sensors. The left vertical line in **FIG. 5A** indicates the point at which AFFIMER® Clone 155, Clone 208, or buffer is added to the sensors, and the right vertical line indicates the point at which a second AFFIMER® clone or buffer is added. **FIG. 5B** shows the nm shift, relative to the optical densities measured in **FIG. 5A**, when a second AFFIMER® clone or buffer is added to the sensor measure in **FIG. 5A**. The left vertical line in **FIG. 5B** indicates the point at which the second AFFIMER® clone or buffer was added, and the right vertical line indicates the point at which any bound AFFIMERS® clone were allowed to dissociate from the sensors. Curves are labeled in the format “X -> Y” where X is the first reagent added to the sensors, and Y is the second reagent added to the sensors.

DETAILED DESCRIPTION

I. Overview

CD33 (sialic acid binding Ig-like lectin 3, SIGLEC3, SIGLEC-3, gp67, p67) is a member of the immunoglobulin superfamily and plays a role in mediating cell-cell interactions and in maintaining immune cells in a resting state. A transmembrane receptor, CD33 is expressed on myeloid stem cells (CFU-GEMM, CFU-GM, CFU-G, and E-BFU), myeloblasts and monoblasts, monocytes/macrophages, granulocyte precursors (with decreasing expression with maturation), and mast cells. Drugs are quickly internalized upon binding to CD33, and CD33 is expressed on the majority of myeloid leukemia cells, as well as normal myeloid and monocytic precursors; thus, CD33 is a therapeutic target for myeloid cell-based pathologies, such as acute myeloid leukemia (AML).

AML is a complex heterogeneous disease characterized by clonal expansion of functionally abnormal immature myeloid progenitors (precursors). The current treatments in use date back to the 1980s and include chemotherapy regimens primarily including an induction

regimen based on a daunorubicin and cytarabine backbone, followed by consolidation chemotherapy. While complete remission is achieved in most cases, patients frequently relapse within weeks or months. For example, complete remission is greater than 80% in younger patients; however, the five-year overall survival rate is approximately 40% due to the relapse rate (Thein et al., Cancer, 2013, 119: 2720-2727). There is an urgent need for molecules that can efficiently and effectively target CD33 in AML patients.

CD33 is also expressed on dendritic cells and microglial cells. It is a microglial receptor, the mutations and differential expression of which have been strongly associated with an increased risk of developing Alzheimer's Disease (AD). CD33 is one of the top-ranked AD risk genes identified by genome-wide association studies. AD affects nearly 50 million people worldwide, and currently no disease-modifying treatment is available. With continuous failure of anti-amyloid-beta- or tau-based therapies, identification of new targets has become an urgent necessity for AD prevention and therapy.

The present disclosure addresses the urgent need in the art for targeting molecules capable of binding to CD33 with high specificity and high affinity. Provided herein are CD33 AFFIMER® polypeptides, engineered polypeptide variants of the Stefin A protein, that bind CD33 with a K_d of less than 1×10^{-6} M. The CD33 AFFIMER® polypeptides of the present disclosure, in some embodiments, may be fused or otherwise linked to therapeutic molecules to be used for the treatment of diseases and/or disorders characterized at least in part by the presence of CD33-positive cells. In other embodiments, the CD33 AFFIMER® polypeptides can be used as therapeutic agents.

II. Certain Definitions of the Present Disclosure

Stefin polypeptides encompass a subgroup of proteins in the cystatin superfamily, a family which encompasses proteins that contain multiple cystatin-like sequences. The Stefin subgroup of the cystatin family includes relatively small (around 100 amino acids) single domain proteins. They receive no known post-translational modification, and lack disulfide bonds, suggesting that they will be able to fold identically in a wide range of extracellular and intracellular environments. Stefin A itself is a monomeric, single chain, single domain protein of 98 amino acids. The structure of Stefin A has been solved, facilitating the rational mutation of Stefin A into the AFFIMER® polypeptide. The only known biological activity of cystatins is the inhibition of cathepsin activity, which allowed for exhaustive testing for residual biological activity of the engineered proteins.

An “AFFIMER® polypeptide” (also referred to as an “AFFIMER® protein”) refers to a small, highly stable protein that is an engineered variant of a Stefin polypeptide. AFFIMER® proteins display two peptide loops and an N-terminal sequence that can all be randomized to bind to desired target proteins with high affinity and specificity, in a similar manner to monoclonal antibodies. Stabilization of the two peptides by the Stefin A protein scaffold constrains the possible conformations that the peptides can take, increasing the binding affinity and specificity compared to libraries of free peptides. These engineered non-antibody binding proteins are designed to mimic the molecular recognition characteristics of monoclonal antibodies in different applications. Variations to other parts of the Stefin A polypeptide sequence can be carried out, with such variations improving the properties of these affinity reagents, such as increase stability, make them robust across a range of temperatures and pH and the like. In some embodiments, an AFFIMER® polypeptide includes a sequence derived from Stefin A, sharing substantial identity with a Stefin A wild type sequence, such as human Stefin A. It will be apparent to a person skilled in the art that modifications may be made to the scaffold sequence without departing from the disclosure. In particular, an AFFIMER® polypeptide can have an amino acid sequences that is at least 25%, 35%, 45%, 55% or 60% identity to the corresponding sequences to human Stefin A, for example, at least 70%, at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95% identical, e.g., where the sequence variations do not adversely affect the ability of the scaffold to bind to the desired target (such as CD33), and e.g., which do not restore or generate biological functions such as those which are possessed by wild type Stefin A but which are abolished in mutational changes described herein.

An “AFFIMER® agent” refers to a polypeptide that includes an AFFIMER® polypeptide sequence and any other modification(s) (e.g., conjugation, post-translational modifications, etc.) so as to represent a therapeutically active protein intended for delivery to an individual.

An “AFFIMER®-linked conjugate” refers to an AFFIMER® agent having at least one moiety conjugated thereto through a chemical conjugation other than through the formation of a contiguous peptide bond through the C-terminus or N-terminus of the polypeptide portion of the AFFIMER® agent containing AFFIMER® polypeptide sequence. An AFFIMER®-linked conjugate may be an “AFFIMER® polypeptide-drug conjugate”, which refers to an AFFIMER® agent including at least one pharmacologically active moiety conjugated thereto. An AFFIMER®-linked conjugate may also be an “AFFIMER®-tag conjugate”, which refers to an AFFIMER® agent including at least one detectable moiety (e.g., detectable label) conjugated thereto.

An “encoded AFFIMER® construct” refers to a nucleic acid construct which, when expressed by cells in a patient’s body through a gene delivery process, produces an intended AFFIMER® agent *in vivo*.

Cluster of Differentiation 33 (CD33) is a protein that, in the case of humans, is encoded by the CD33 gene. CD33 is an antigenic determinant detectable on leukemia cells, as well on normal precursor cells of the myeloid lineage. The human amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot. For example, the amino acid sequence of human CD33 can be found as UniProt/Swiss-Prot. Accession No. P20138 and the nucleotide sequence encoding of the human CD33 can be found at Accession No. NM_001772.3. As used herein, “CD33” includes any native, mature CD33 which results from processing of a CD33 precursor protein in a cell. The term encompasses CD33 from any vertebrate source, including mammals such as primates (e.g., humans and cynomolgus monkeys) and rodents (e.g., mice and rats), unless otherwise indicated. The term also includes any CD33 proteins comprising mutations, e.g., point mutations, fragments, insertions, deletions, and splice variants of full length wild-type CD33.

CD33 is a member of the sialic acid binding, immunoglobulin-like lectin family. It is a 67-kDa glycosylated transmembrane protein expressed on most myeloid and monocytic leukemia cells in addition to committed myelomonocytic and erythroid progenitor cells. CD33 contains two tyrosine residues on its cytoplasmic tail, each of which is followed by hydrophobic residues similar to the immunoreceptor tyrosine-based inhibitory motif (ITIM) seen in many inhibitory receptors. The receptor plays a role in mediating cell-cell interactions and in maintaining immune cells in a resting state through its ITIMs. CD33 recognizes and binds alpha-2,3-linked sialic acid-bearing glycans as well as alpha-2, 6-linked sialic acid-bearing glycans. After binding ligands, such as Clq or sialylated glycoproteins, the two ITIMs are phosphorylated by Src-like kinases (e.g., LCK). Phosphorylation results in the emergence of docking sites for the recruitment and activation of protein-tyrosine phosphatases PTPN6/SHP-1 and PTPN11/SHP-2. Activating of SHP-1 and SHP-2 leads to downstream effects, including regulation of endocytosis, reduced cellular activation and proliferation, and increased receptor degradation.

A “CD33 AFFIMER® agent” refers to an AFFIMER® agent that comprises at least one AFFIMER® polypeptide that binds to CD33, particularly human CD33, with a dissociation constant (K_d) of at least 10⁻⁶M. In some embodiments, the CD33 AFFIMER® agent binds CD33 with a K_d of 1×10⁻⁷M or less, K_d of 1×10⁻⁸M or less, K_d of 1×10⁻⁹M or less, or a K_d of 1×10⁻¹⁰M or less. It should be understood that the terms “CD33 AFFIMER® polypeptide” and “engineered CD33-binding Stefin A polypeptide variant” are used interchangeably herein. Thus,

a “CD33 AFFIMER® polypeptide” is an engineered polypeptide that binds specifically to CD33 with a K_d of 1×10^{-6} M or less, wherein the engineered polypeptide is a variant of a Stefin A protein.

A. Polypeptides

Polypeptides (which includes peptides and proteins) are polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing at least one analog of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art.

Amino acids (also referred to herein as amino acid residues) participate in one more peptide bonds of a polypeptide. In general, the abbreviations used herein for designating the amino acids are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (see *Biochemistry* (1972) 11:1726-1732). For instance, Met, Ile, Leu, Ala and Gly represent “residues” of methionine, isoleucine, leucine, alanine and glycine, respectively. By the residue is meant a radical derived from the corresponding α -amino acid by eliminating the OH portion of the carboxyl group and the H portion of the α -amino group. The term “amino acid side chain” is that part of an amino acid exclusive of the $-\text{CH}(\text{NH}_2)\text{COOH}$ portion, as defined by K. D. Kopple, “Peptides and Amino Acids”, W. A. Benjamin Inc., New York and Amsterdam, 1966, pages 2 and 33.

For the most part, the amino acids used in the application of this disclosure are those naturally occurring amino acids found in proteins, or the naturally occurring anabolic or catabolic products of such amino acids which contain amino and carboxyl groups. Particularly suitable amino acid side chains include side chains selected from those of the following amino acids: glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan, and those amino acids and amino acid analogs which have been identified as constituents of peptidoglycan bacterial cell walls.

Amino acid residues having “basic sidechains” include Arg, Lys and His. Amino acid residues having “acidic sidechains” include Glu and Asp. Amino acid residues having “neutral polar sidechains” include Ser, Thr, Asn, Gln, Cys and Tyr. Amino acid residues having “neutral non-polar sidechains” include Gly, Ala, Val, Ile, Leu, Met, Pro, Trp and Phe. Amino acid

residues having “non-polar aliphatic sidechains” include Gly, Ala, Val, Ile and Leu. Amino acid residues having “hydrophobic sidechains” include Ala, Val, Ile, Leu, Met, Phe, Tyr and Trp. Amino acid residues having “small hydrophobic sidechains” include Ala and Val. Amino acid residues having “aromatic sidechains” include Tyr, Trp and Phe.

Amino acid residues further include analogs, derivatives and congeners of any specific amino acid referred to herein, as for instance, the subject AFFIMER® polypeptides (particularly if generated by chemical synthesis) can include an amino acid analog such as, for example, cyanoalanine, canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxy-phenylalanine, 5-hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, diaminiopimelic acid, ornithine, or diaminobutyric acid. Other naturally occurring amino acid metabolites or precursors having side chains which are suitable herein will be recognized by those skilled in the art and are included in the scope of the present disclosure.

Also included are the (D) and (L) stereoisomers of such amino acids when the structure of the amino acid admits of stereoisomeric forms. The configuration of the amino acids and amino acid residues herein are designated by the appropriate symbols (D), (L) or (DL), furthermore when the configuration is not designated the amino acid or residue can have the configuration (D), (L) or (DL). It will be noted that the structure of some of the compounds of this disclosure includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry are included within the scope of this disclosure. Such isomers can be obtained in substantially pure form by classical separation techniques and by sterically controlled synthesis. For the purposes of this application, unless expressly noted to the contrary, a named amino acid shall be construed to include both the (D) or (L) stereoisomers.

The terms “identical” or percent “identity” in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two nucleic acids or polypeptides of the disclosure are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and

aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the amino acid sequences that is at least about 10 residues, at least about 20 residues, at least about 40-60 residues, at least about 60-80 residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a target protein or an antibody. In some embodiments, identity exists over a region of the nucleotide sequences that is at least about 10 bases, at least about 20 bases, at least about 40-60 bases, at least about 60-80 bases in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 bases, such as at least about 80-1000 bases or more, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as a nucleotide sequence encoding a protein of interest.

A conservative amino acid substitution is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Generally, conservative substitutions in the sequences of the polypeptides, soluble proteins, and/or antibodies of the disclosure do not abrogate the binding of the polypeptide, soluble protein, or antibody containing the amino acid sequence, to the target binding site. Methods of identifying amino acid conservative substitutions which do not eliminate binding are well-known in the art.

A polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, soluble proteins, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

A material is considered substantially pure if the material is at least 50% pure (e.g., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

A fusion polypeptide (e.g., a fusion protein) is a hybrid polypeptide expressed by a nucleic acid molecule comprising at least two open reading frames (e.g., from two individual molecules, e.g., two individual genes).

A linker (also referred to as a linker region) may be inserted between a first polypeptide (e.g., copies of a CD33 AFFIMER® polypeptide) and a second polypeptide (e.g., another AFFIMER® polypeptide, an Fc domain, a ligand binding domain, etc.). In some embodiments, a linker is a peptide linker. Linkers should not adversely affect the expression, secretion, or bioactivity of the polypeptides. In some embodiments, linkers are not antigenic and do not elicit an immune response.

An “AFFIMER® polypeptide-antibody fusion” is a fusion protein that includes an AFFIMER® polypeptide portion and a variable region of an antibody. AFFIMER® polypeptide-antibody fusions may include full length antibodies having, for example, at least one AFFIMER® polypeptide sequence appended to the C-terminus or N-terminus of at least one of its VH and/or VL chains, e.g., at least one chain of the assembled antibody is a fusion protein with an AFFIMER® polypeptide. AFFIMER® polypeptide-antibody fusions may also include at least one AFFIMER® polypeptide sequence as part of a fusion protein with an antigen binding site or variable region of an antibody fragment.

An antibody is an immunoglobulin molecule that recognizes and specifically binds a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or a combination of any of the foregoing, through at least one antigen-binding site wherein the antigen-binding site is usually within the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses intact (whole) polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')₂, and Fv fragments), single chain Fv (scFv) antibodies provided those fragments have been formatted to include an Fc or other FcγRIII binding domain, multispecific antibodies, bispecific antibodies, monospecific antibodies, monovalent antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen-binding site of an antibody (formatted to include an Fc or other FcγRIII binding domain), antibody mimetics, and any other modified immunoglobulin molecule comprising an antigen-binding site as long as the antibodies exhibit the desired biological activity.

While the antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu.

A variable region of an antibody may be a variable region of an antibody light chain or a variable region of an antibody heavy chain, either alone or in combination. Generally, the variable region of heavy and light chains include four framework regions (FR) and three complementarity determining regions (CDRs), also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding sites of the antibody. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (e.g., Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Edition, National Institutes of Health, Bethesda Md.), and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al Lazikani et al., 1997, J. Mol. Biol., 273:927-948). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

A humanized antibody is a form of a non-human (e.g., murine) antibody that is specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulins in which residues of the CDRs are replaced by residues from the CDRs of a non-human species (e.g., mouse, rat, rabbit, or hamster) that have the desired specificity, affinity, and/or binding capability. In some instances, the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species. The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or binding capability. The humanized antibody may comprise variable domains containing all or substantially all of the CDRs that correspond to the non-human immunoglobulin whereas all or substantially all of the framework regions are those of a human immunoglobulin sequence. In some embodiments, the variable domains comprise the framework regions of a human immunoglobulin sequence. In some embodiments, the variable domains comprise the framework regions of a human immunoglobulin consensus sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. A humanized antibody is usually considered distinct from a chimeric antibody.

An epitope (also referred to herein as an antigenic determinant) is the portion of an antigen capable of being recognized and specifically bound by a particular antibody, a particular AFFIMER® polypeptide or other particular binding domain. When the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5, 6, 7, or 8-10 amino acids in a unique spatial conformation.

"Specifically binds to" or "specific for" refers to measurable and reproducible interactions such as binding between a target (e.g., CD33) and an AFFIMER® polypeptide, antibody or other binding partner, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an AFFIMER® polypeptide that specifically binds to CD33 is an AFFIMER® polypeptide that binds CD33 with greater affinity, avidity (if multimeric formatted), more readily, and/or with greater duration than it binds to other targets.

"Conjugate," "conjugation" and grammatical variations thereof refers the joining or linking together of two or more compounds resulting in the formation of another compound, by any joining or linking methods known in the art. It can also refer to a compound that is generated by the joining or linking together two or more compounds. For example, a CD33 AFFIMER® polypeptide linked directly or indirectly to at least one chemical moiety or polypeptide is an exemplary conjugate. Such conjugates include fusion proteins, those produced by chemical conjugates and those produced by any other methods.

B. Polynucleotides

A polynucleotide (also referred to herein as a nucleic acid or a nucleic acid molecule) is a polymer of nucleotides of any length and may comprise DNA, RNA (e.g., messenger RNA (mRNA)) or a combination of DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

A polynucleotide encoding a polypeptide refers to the order or sequence of nucleotides along a strand of deoxyribonucleic acid deoxyribonucleotides. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (e.g., protein) chain. Thus, a nucleic acid sequence encodes the amino acid sequence.

When used in reference to nucleotide sequences, a “sequence” may comprise DNA and/or RNA (e.g., messenger RNA) and may be single and/or double stranded.

Nucleic acid sequences may be modified, e.g., mutated, relative to naturally occurring nucleic acid sequences, for example.

Nucleic acid sequence may have any length, for example 2 to 1,000,000 or more nucleotides (or any integral value above or between) a nucleic acid, for example a length of from about 100 to about 10,000, or from about 200 nucleotides to about 500 nucleotides.

Transfection is the process of introducing an exogenous nucleic acid into a eukaryotic cell. Transfection can be achieved by various means known in the art, including calcium phosphate-DNA co-precipitation, DEAE- dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, and biolistics technology (biolistics).

A vector is a construct that is capable of delivering, and usually expressing, at least one gene or sequence of interest in a host cell. Examples of vectors include but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes. A vector may, in some embodiments, be an isolated nucleic acid that can be used to deliver a composition to the interior of the cell. It is known in the art a number of vectors including, but not limited to the linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, a vector may be an autonomously replicating plasmid or virus. The term should also be construed to include facilitate transfer of nucleic acid into cells of the non-plasmid and non-viral compounds, for example, polylysine compounds, liposomes, and the like. Non-limiting examples of viral vectors include but are not limited to adenoviral vectors, adeno-associated virus vectors, and retroviral vectors.

An expression vector is a vector comprising a recombinant polynucleotide comprising expression control sequence and a nucleotide sequence to be expressed operably linked. The expression vector comprises sufficient cis-acting elements (cis-acting elements) used for expression; other elements for expression can be supplied by the host cell or in vitro expression system. Expression vectors include, for example, cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentivirus, retroviruses, adenoviruses and adeno-associated viruses).

Operably linked refers to functional linkage between the regulatory sequence and a heterologous nucleic acid sequence resulting in the expression of the latter. For example, if the promoter affects the transcription or expression of the coding sequence, the promoter is operably

linked to a coding sequence. Typically, DNA sequencing operably linked are contiguous, and may join two protein coding regions in the same reading frame.

A promoter is a DNA sequence recognized by the synthetic machinery required for the synthesis machinery of the cell specific transcription of a polynucleotide sequence or introduced.

Inducible expression refers to expression under certain conditions, such as activation (or inactivation) of an intracellular signaling pathway or the contacting of the cells harboring the expression construct with a small molecule that regulates the expression (or degree of expression) of a gene operably linked to an inducible promoter sensitive to the concentration of the small molecule. This is contrasted with constitutive expression, which refers to expression under physiological conditions (not limited by certain conditions).

Electroporation refers to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids or other oligonucleotide to pass from one side of the cellular membrane to the other.

C. Checkpoint Inhibitors, Co-stimulatory Agonists and Chemotherapeutics

A checkpoint molecule is a protein that is expressed by tissues and/or immune cells and reduce the efficacy of an immune response in a manner dependent on the level of expression of the checkpoint molecule. When these proteins are blocked, the “brakes” on the immune system are released and, for example, T cells are able to kill cancer cells more effectively. Examples of checkpoint proteins found on T cells or cancer cells include PD-1/PD-L1 and CTLA-4/B7-1/B7-2, PD-L2, NKG2A, KIR, LAG-3, TIM-3, CD96, VISTA and TIGIT.

A checkpoint inhibitor is a drug entity that reverses the immunosuppressive signaling from a checkpoint molecule.

A costimulatory molecule is an immune cell such as a T cell cognate binding partner that specifically binds to costimulatory ligands thereby mediating co-stimulation, such as, but not limited to proliferation. Costimulatory molecules are cell surface molecules other than the antigen receptor or ligand which facilitate an effective immune response. Co-stimulatory molecules include but are not limited to MHCI molecules, BTLA receptor and Toll ligands, and OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a / CD18), ICOS (CD278) and 4-1BB (CD137). Examples of costimulatory molecules include but are not limited to: CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8 α , CD8 β , IL2R β , IL2R γ , IL7R α , ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE / RANKL, DNAM1 (CD226), SLAMF4 (CD244,2B4), CD84,

CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG / Cbp, CD19a, and CD83 ligand.

A costimulatory agonist is a drug entity that activates (agonizes) the costimulatory molecule, such as costimulatory ligand would do, and produces an immunostimulatory signal or otherwise increases the potency or efficacy of an immune response.

A chemotherapeutic agent is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN), CPT-11 (irinotecan, CAMPTOSAR), acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; pemetrexed; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; TLK-286; CDP323, an oral alpha-4 integrin inhibitor; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, e.g., Nicolaou et al., *Angew. Chem Intl. Ed. Engl.*, 33: 183-186 (1994))); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid,

nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR), tegafur (UFTORAL), capecitabine (XELODA), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, and imatinib (a 2-phenylaminopyrimidine derivative), as well as other c-Kit inhibitors; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE, FILDESIN); dacarbazine; mannometrine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE), and doxorubicin (TAXOTERE); chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN); oxaliplatin; leucovorin; vinorelbine (NAVELBINE); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN) combined with 5-FU and leucovorin.

Chemotherapeutic agents also include anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX tamoxifen), raloxifene (EVISTA), droloxifene, 4-

hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON); anti-progesterones; estrogen receptor down-regulators (ERDs); estrogen receptor antagonists such as fulvestrant (FASLODEX); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as leuprolide acetate (LUPRON and ELIGARD), goserelin acetate, buserelin acetate and triptorelin; anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (MEGASE), exemestane (AROMASIN), formestane, fadrozole, vorozole (RIVISOR), letrozole (FEMARA), and anastrozole (ARIMIDEX). In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS or OSTAC), etidronate (DIDROCAL), NE-58095, zoledronic acid/zoledronate (ZOMETA), alendronate (FOSAMAX), pamidronate (AREDIA), tiludronate (SKELID), or risedronate (ACTONEL); as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); anti-sense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC- α , Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE vaccine and gene therapy vaccines, for example, ALLOVECTIN vaccine, LEUVECTIN vaccine, and VAXID vaccine; topoisomerase 1 inhibitor (e.g., LURTOTECAN); an anti-estrogen such as fulvestrant; a Kit inhibitor such as imatinib or EXEL-0862 (a tyrosine kinase inhibitor); EGFR inhibitor such as erlotinib or cetuximab; an anti-VEGF inhibitor such as bevacizumab; irinotecan; rmRH (e.g., ABARELIX); lapatinib and lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); 17AAG (geldanamycin derivative that is a heat shock protein (Hsp) 90 poison), and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A cytokine is a protein released by one cell that act on another cell as intercellular mediators or have an autocrine effect on the cells producing the proteins. Examples of such cytokines include lymphokines, monokines; interleukins ("ILs") such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-17A-F, IL-18 to IL-29 (such as IL-23), IL-31, including PROLEUKIN rIL-2; a tumor-necrosis factor such as TNF- α or TNF- β , TGF- β 1-3; and other polypeptide factors including leukemia inhibitory factor ("LIF"), ciliary neurotrophic factor ("CNTF"), CNTF-like cytokine ("CLC"), cardiotrophin ("CT"), and kit ligand ("KL").

A chemokine is a soluble factor (e.g., cytokine) that has the ability to selectively induce chemotaxis and activation of leukocytes. Chemokines also trigger processes of angiogenesis, inflammation, wound healing, and tumorigenesis. Non-limiting examples of chemokines include IL-8, a human homolog of murine keratinocyte chemoattractant (KC).

A growth factor is a substance, such as a vitamin or hormone, that is required for the stimulation of growth in living cells. Low expression of CD33 is a limiting factor in approaches that target CD33. Priming with myeloid growth factors (SCF, IL-3, G-CSF, and/or GM-CSF) enhances CD33 expression, rendering cancer cells more sensitive to the action of CD33-targeted treatments, such as the antibody-drug conjugate gemtuzumab ozogamicin. CD33 AFFIMER® polypeptides may be conjugated to a myeloid growth factor that enhances CD33 expression on the cell surface, to increase the effectiveness of subsequent treatment with another CD33-targeting therapy, such as AFFIMER® polypeptide-drug conjugate.

(10.1182/blood.V112.11.2638.2638). In some embodiments, the AFFIMER® polypeptide can be combined with a growth factor selected from the group consisting of: adrenomedullin (AM), angiopoietin (Ang), BMPs, BDNF, EGF, erythropoietin (EPO), FGF, GDNF, G-CSF, GM-CSF, GDF9, HGF, HDGF, IGF, migration-stimulating factor, myostatin (GDF-8), NGF, neurotrophins, PDGF, thrombopoietin, TGF- α , TGF- β , TNF- α , VEGF, PlGF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, and IL-18.

An enzyme is a substance produced by a living organism which acts as a catalyst to bring about a specific biochemical reaction. CD33 AFFIMER® polypeptides may be conjugated to a sialidase, for example, so that the sialidase will cleave sialic acid motifs from the surface of CD33+ cells. Targeted cleavage of sialic acid motifs on the surface of HER2+ breast cancer cells has been shown to increase sensitivity to NK cell-mediated killing and may have a similar effect on CD33+ cancer cells. (10.1073/pnas.1608069113)

D. Treatments

The term “dysfunctional” includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into down-stream T-cell effector functions, such as proliferation, cytokine production (e.g., IL-2) and/or target cell killing.

"Anergy" refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (e.g., increase in intracellular Ca^{+2} in the absence of ras-activation). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of costimulation. The unresponsive state

can often be overridden by the presence of Interleukin-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

"Exhaustion" refers to T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors.

"Enhancing T-cell function" means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include: increased secretion of γ -interferon from CD8+ T-cells, increased proliferation, increased antigen responsiveness (e.g., viral, pathogen, or tumor clearance) relative to such levels before the intervention. In some embodiments, the level of enhancement is at least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

"Tumor immunity" refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

"Sustained response" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the beginning of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5x, 2.0x, 2.5x, or 3.0x length of the treatment duration.

A cancer is a physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, blastoma, sarcoma, and hematologic cancers such as lymphoma and leukemia.

A tumor (also referred to as a neoplasm) is any mass of tissue that results from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions. Tumor growth is generally uncontrolled and progressive, does not induce or inhibit the proliferation of normal cells. Tumor can affect a variety of cells, tissues or organs, including but not limited to selected from bladder, bone, brain, breast, cartilage, glial cells, esophagus, fallopian tube, gall bladder, heart, intestine, kidney, liver, lung, lymph node, neural tissue, ovary, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testis,

thymus, thyroid, trachea, urethra, ureter, urethra, uterus, vagina organ or tissue or the corresponding cells. Tumors include cancers, such as sarcoma, carcinoma, plasmacytoma or (malignant plasma cells). Tumors of the present disclosure, may include but are not limited to leukemias (e.g., acute leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, acute myeloid leukemia, acute promyelocytic leukemia, acute myeloid - monocytic leukemia, acute monocytic leukemia, acute leukemia, chronic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia, polycythemia vera), lymphomas (Hodgkin's disease, non-Hodgkin's disease), primary macroglobulinemia disease, heavy chain disease, and solid tumors such as sarcomas cancer (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, chordoma, endothelium sarcoma, lymphangiosarcoma, angiosarcoma, lymphangioendothelium sarcoma, synovial sarcoma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, (including triple negative breast cancer), ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, carcinoma, bronchogenic carcinoma, medullary carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, spermatogonia Tumor, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma (including small cell lung carcinoma and non-small cell lung carcinoma or NSCLC), bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, retinoblastoma), esophageal cancer, gallbladder, kidney cancer, multiple myeloma. Preferably, a "tumor" includes, but is not limited to: pancreatic cancer, liver cancer, lung cancer (including NSCLC), stomach cancer, esophageal cancer, head and neck squamous cell carcinoma, prostate cancer, colon cancer, breast cancer (including triple negative breast cancer), lymphoma, gallbladder cancer, renal cancer, leukemia, multiple myeloma, ovarian cancer, cervical cancer and glioma.

Metastasis refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at the new location. A "metastatic" or "metastasizing" cell is one that loses adhesive contacts with neighboring cells and migrates via the bloodstream or lymph from the primary site of disease to invade neighboring body structures.

"Cancer cell" and "tumor cell" refers to the total population of cells derived from a cancer or tumor or pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk

of the cancer cell population, and tumorigenic stem cells (cancer stem cells). As used herein, the terms "cancer cell" or "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

A "complete response" or "CR" refers to disappearance of all target lesions; "partial response" or "PR" refers to at least a 30% decrease in the sum of the longest diameters (SLD) of target lesions, taking as reference the baseline SLD; and "stable disease" or "SD" refers to neither sufficient shrinkage of target lesions to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the smallest SLD since the treatment started.

"Progression free survival" (PFS) refers to the length of time during and after treatment during which the disease being treated (e.g., cancer) does not get worse. Progression-free survival may include the amount of time patients have experienced a complete response or a partial response, as well as the amount of time patients have experienced stable disease.

"Overall response rate" (ORR) refers to the sum of complete response (CR) rate and partial response (PR) rate.

"Overall survival" refers to the percentage of individuals in a group who are likely to be alive after a particular duration of time.

"Treatment" refers to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In the case of cancer or a tumor, a subject is successfully "treated" according to the methods of the present disclosure if the patient shows at least one of the following: an increased immune response, an increased anti-tumor response, increased cytolytic activity of immune cells, increased killing of tumor cells by immune cells, a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer cells into soft tissue and bone; inhibition of or an absence of tumor or cancer cell metastasis; inhibition or an absence of cancer growth; relief of at least one symptom associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity; reduction in the number or frequency of cancer stem cells; or some combination of effects.

“Subject,” “individual,” and “patient,” used interchangeably herein, refer to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, and rodents.

"Agonist" and "agonistic" refer to agents that are capable of, directly or indirectly, substantially inducing, activating, promoting, increasing, or enhancing the biological activity of a target or target pathway. "Agonist" is used herein to include any agent that partially or fully induces, activates, promotes, increases, or enhances the activity of a protein or other target of interest.

"Antagonist" and "antagonistic" refer to or describe an agent that is capable of, directly or indirectly, partially or fully blocking, inhibiting, reducing, or neutralizing a biological activity of a target and/or pathway. The term "antagonist" is used herein to include any agent that partially or fully blocks, inhibits, reduces, or neutralizes the activity of a protein or other target of interest.

"Modulation" and "modulate" refer to a change or an alteration in a biological activity. Modulation includes, but is not limited to, stimulating an activity or inhibiting an activity. Modulation may be an increase in activity or a decrease in activity, a change in binding characteristics, or any other change in the biological, functional, or immunological properties associated with the activity of a protein, a pathway, a system, or other biological targets of interest.

An immune response includes responses from both the innate immune system and the adaptive immune system. It includes both cell-mediated and/or humoral immune responses. It includes both T-cell and B-cell responses, as well as responses from other cells of the immune system such as natural killer (NK) cells, monocytes, macrophages, etc.

"Pharmaceutically acceptable" refers to a substance approved or approvable by a regulatory agency of the Federal government or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

"Pharmaceutically acceptable excipient" is an excipient, carrier or adjuvant that can be administered to a subject, together with at least one agent of the present disclosure, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic effect. In general, those of skill in the art and the U.S. FDA consider a pharmaceutically acceptable excipient, carrier, or adjuvant to be an inactive ingredient of any formulation.

An “effective amount” (also referred to herein as a “therapeutically effective amount” is an amount of an agent, such as a CD33 AFFIMER® agent, effective to treat a disease or disorder in a subject such as, a mammal. In the case of cancer or a tumor, the therapeutically effective amount of an CD33 AFFIMER® agent has a therapeutic effect and as such can boost the immune response, boost the anti-tumor response, increase cytolytic activity of immune cells, increase killing of tumor cells by immune cells, reduce the number of tumor cells; decrease tumorigenicity, tumorigenic frequency or tumorigenic capacity; reduce the number or frequency of cancer stem cells; reduce the tumor size; reduce the cancer cell population; inhibit or stop cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibit and stop tumor or cancer cell metastasis; inhibit and stop tumor or cancer cell growth; relieve to some extent at least one of the symptoms associated with the cancer; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

E. Miscellaneous

It is understood that wherever embodiments are described herein with the language "comprising" otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided. It is also understood that wherever embodiments are described herein with the language "consisting essentially of" otherwise analogous embodiments described in terms of "consisting of" are also provided.

As used herein, reference to "about" or "approximately" a value or parameter includes (and describes) embodiments that are directed to that value or parameter. For example, description referring to "about X" includes description of "X".

The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

The phrase “at least one” may be used interchangeably with “one or more.” It should be understood that “a” is not limited to one but rather means “at least one.”

III. CD33 AFFIMER® polypeptides

An AFFIMER® polypeptide is a scaffold based on a Stefin A polypeptide, meaning that it has a sequence which is derived from a Stefin A polypeptide, for example, a mammalian Stefin A polypeptide, for example, a human Stefin A polypeptide. Some aspects of the application

provide AFFIMER® polypeptides that bind CD33 (also referred to as “CD33 AFFIMER® polypeptides”) in which at least one of the solvent accessible loops from the wild-type Stefin A protein having the ability to bind CD33, preferably selectively, and preferably with K_d of 10^{-6} M or less.

In some embodiments, a CD33 AFFIMER® polypeptide is derived from the wild-type human Stefin A polypeptide having a backbone sequence and in which one or both of loop 2 [designated (Xaa)_n] and loop 4 [designated (Xaa)_m] are replaced with alternative loop sequences (Xaa)_n and (Xaa)_m, to have the general Formula (I)

FR1-(Xaa)_n-FR2-(Xaa)_m-FR3 (I)

wherein

FR1 is a polypeptide sequence represented by MIPGGLSEAK PATPEIQEIV DKVKPQLEEK TNETYGKLEA VQYKTQVLA (SEQ ID NO: 1) or a polypeptide sequence having at least 70% homology thereto;

FR2 is a polypeptide sequence represented by GTNYYIKVRA GDNKYMHLKV FKSL (SEQ ID NO: 2) or a polypeptide sequence having at least 70% homology thereto;

FR3 is a polypeptide sequence represented by EDLVL TGYQV DKNKDEL TGF (SEQ ID NO: 3) or a polypeptide sequence having at least 70% homology thereto; and

Xaa, individually for each occurrence, is an amino acid residue, n and m are each, independently, an integer from 3 to 20.

In some embodiments, FR1 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% homology with SEQ ID NO: 1. In some embodiments, FR1 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% identity with SEQ ID NO: 1; In some embodiments, FR2 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% homology with SEQ ID NO: 2. In some embodiments, FR2 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% identity with SEQ ID NO: 2; In some embodiments, FR3 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% homology with SEQ ID NO: 3. In some embodiments, FR3 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% identity with SEQ ID NO: 3.

In some embodiments, the CD33 AFFIMER® polypeptide has an amino acid sequence represented in the general Formula (II):

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA-(Xaa)_n-Xaa2-TNYYIKVRAGDNKYMHLKVF-Xaa3-Xaa4-Xaa5-(Xaa)_m-Xaa6-D-Xaa7-VLTGYQVDKNKDEL TGF (SEQ ID NO: 4)

wherein

Xaa, individually for each occurrence, is an amino acid residue; n and m are each, independently, an integer from 3 to 20; Xaa1 is Gly, Ala, Val, Arg, Lys, Asp, or Glu, more preferably Gly, Ala, Arg or Lys, and more even more preferably Gly or Arg; Xaa2 is Gly, Ala, Val, Ser or Thr, more preferably Gly or Ser; Xaa3 is Arg, Lys, Asn, Gln, Ser, Thr, more preferably Arg, Lys, Asn or Gln, and even more preferably Lys or Asn; Xaa4 is Gly, Ala, Val, Ser or Thr, more preferably Gly or Ser; Xaa5 is Ala, Val, Ile, Leu, Gly or Pro, more preferably Ile, Leu or Pro, and even more preferably Leu or Pro; Xaa6 is Gly, Ala, Val, Asp or Glu, more preferably Ala, Val, Asp or Glu, and even more preferably Ala or Glu; and Xaa7 is Ala, Val, Ile, Leu, Arg or Lys, more preferably Ile, Leu or Arg, and even more preferably Leu or Arg.

For example, the CD33 AFFIMER® polypeptide can have an amino acid sequence represented in the general Formula (III):

MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA-(Xaa)_n-STNYYIKVRAGDNKYMHLKVFNGP-(Xaa)_m-ADRVLTGYQVDKNKDELDTGF (SEQ ID NO: 5), wherein Xaa, individually for each occurrence, is an amino acid residue; n and m are each, independently, an integer from 3 to 20.

In some embodiments, n is 3 to 15, 3 to 12, 3 to 9, 3 to 7, 5 to 7, 5 to 9, 5 to 12, 5 to 15, 7 to 12 or 7 to 9.

In some embodiments, m is 3 to 15, 3 to 12, 3 to 9, 3 to 7, 5 to 7, 5 to 9, 5 to 12, 5 to 15, 7 to 12 or 7 to 9.

In some embodiments, Xaa, individually for each occurrence, is an amino acid that can be added to a polypeptide by recombinant expression in a prokaryotic or eukaryotic cell, and even more preferably one of the 20 naturally occurring amino acids.

In some embodiments of the above sequences and formulas, (Xaa)_n is an amino acid sequence selected from SEQ ID NOs: 6 to 129, or an amino acid sequence having at least 80%, 85%, 90%, 95% or even 98% homology with a sequence selected from SEQ ID NOs: 6 to 129. In some embodiments, (Xaa)_n is an amino acid sequence having at least 80%, 85%, 90%, 95% or even 98% identity with a sequence selected from SEQ ID NOs: 6 to 129.

Table 1. Loop 2 Sequences

Loop 2	SEQ ID NO:
VTSFNITDLH	6
SEYVNWEGE	7
DFYLNWEGP	8
WAWGSSFHY	9

Loop 2	SEQ ID NO:
QHPDWPSDLK	10
NKWRGRQKH	11
FHKRAYHFH	12
WPWGSSVHV	13
NDRHGWEFY	14
GSEQYTDLE	15
KHKQHHKPW	16
WPIKSGYEF	17
GRSKHAHNR	18
NDRRHHIRP	19
SKFKHHHHH	20
FYWGVSYHE	21
DSEIETDLD	22
NKYNRNYGK	23
FQWGSSYHQ	24
FRWGSSFHT	25
FQWGSSFHQ	26
WQWGSSFHE	27
FAWGSSYHE	28
FRWGSSFHR	29
FRWGSSFHE	30
FQWGSSFHR	31
FQWGSSFHT	32
WRWGSSFHE	33
FRWGSSYHS	34
GLDYTGLE	35
GLEEYTGLE	36
GLEEYTSLA	37
GLEEYTGLT	38
GYEDYTALQ	39
LWEEYTALS	40
GLDYTGLS	41
GLEQYTGLQ	42
GLDDYTGLP	43
GLSYTGLS	44

Loop 2	SEQ ID NO:
KWDGYTALP	45
GLEAYTALE	46
GWEQYTALA	47
GLDEYTALP	48
GLSYTSLA	49
GLDEYTVLE	50
GLEEYTQLP	51
SAVTVTDHH	52
GLEAYTGLA	53
FQWGSSFHS	54
FQWGSSFHE	55
GLEEYTALR	56
KWDGYTALP	57
ILEPYTALP	58
GLEEYTGLA	59
GLEEYTALQ	60
GLEDYTALL	61
GLDDYTGLE	62
FRWGSSYHQ	63
FQWGSSYHE	64
FTWGSSYHE	65

In some embodiments of the above sequences and formulas, (Xaa)_m is an amino acid sequence selected from SEQ ID NOs: 66 to 129, or an amino acid sequence having at least 80%, 85%, 90%, 95% or even 98% homology with a sequence selected from SEQ ID NOs: 66 to 129. In some embodiments, (Xaa)_m is an amino acid sequence having at least 80%, 85%, 90%, 95% or even 98% identity with a sequence selected from SEQ ID NOs: 66 to 129.

Table 2. Loop 4 Sequences

Loop 4	SEQ ID NO:
VVDQDIIIW	66
NLSHKWGIV	67
GDAAAYGVE	68
VEDQDSLW	69
FFSKEAYRW	70
VLRWGHQYK	71

Loop 4	SEQ ID NO:
WDPVKKDLK	72
YEHNNLAW	73
DFNEERYSP	74
YLNDWGLVW	75
LSKPFHAHF	76
WSGWRPDVV	77
KIFWGAVGT	78
DGKRSKQWV	79
SWHNYRYQD	80
KRTEESLTW	81
WWHQAIWQW	82
IKVKWRGHK	83
YEDDYNLVW	84
VETDLALTW	85
EETQNNLIW	86
VEDKNDLTW	87
YEDNLNLA	88
KEDHKSLVW	89
DQDNDNLIW	90
NDNNNNLTW	91
KETKENLTW	92
YENEDNLIW	93
YESEDNLIW	94
KDNNDNLVW	95
EDTHNSLVW	96
NESNESLVW	97
KEDNDSLII	98
VETEESLTW	99
DDSHDALVW	100
IDDDEALTW	101
YGDDWGVKW	102
VEYDYNLIN	103
VQDEFALIN	104
SEEHFIPID	105
FDAQDSLTI	106

Loop 4	SEQ ID NO:
IEDDKTLTW	107
KDDNETLVW	108
DDANDDLIW	109
LDSQESLIW	110
YLEDWGLNW	111
VQDEFALIW	112
YGDDWGVKW	113
NEYNDNLTW	114
YDNQNNLLW	115
KETNNNLTW	116
IEDNNNLTW	117
VEYDYNLIW	118
VDNEYNLTW	119
KETEDNLIW	120
FEDKKDLTW	121
KETNNDLIW	122
KEYQKNLTW	123
IESDFNLIW	124
YDNDVALTW	125
YENHYALTW	126
YEYELDLIW	127
YDYQDNLTW	128
LESNKTLVW	129

In some embodiments, the CD33 AFFIMER® polypeptide has an amino acid sequence selected from SEQ ID NOs: 130-356. In some embodiments, the CD33 AFFIMER® polypeptide has an amino acid sequence having at least 70%, 75% 80%, 85%, 90%, 95% or even 98% identity with a sequence selected from SEQ ID NOs: 130-356.

Table 3. Exemplary Anti-CD33 AFFIMER® Polypeptide Sequences

Clone #	Amino Acid Sequences	SEQ ID NO:
1	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIPAQTFPDWSTNYYI KVRAGDNKYMHLKVFNGPNFTVEVSEHHADRVLTGYQVDKNKDDELTGF	130
2	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAITLLETEIEYSTNYYIKV RAGDNKYMHLKVFNGPTGLSDSRFHADRVLTGYQVDKNKDDELTGF	131

Clone #	Amino Acid Sequences	SEQ ID NO:
3	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIKFEDETHDSTNYYIK VRAGDNKYMHLKVFNGPDYGMVQQFEADRVLTGYQVDKNKDDDELTF	132
4	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAITMLEGDFYESTNYYI KVRAGDNKYMHLKVFNGPDWQPQHKRLVADRVLTGYQVDKNKDDDELTF	133
5	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIAEGEELHSTNYYIK VRAGDNKYMHLKVFNGPEAEGRDFVADRVLTGYQVDKNKDDDELTF	134
6	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAILEGEPSTNYYIK VRAGDNKYMHLKVFNGPDNMLHSHFVADRVLTGYQVDKNKDDDELTF	135
7	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLATHGMVHELMSTNYY IKVRAGDNKYMHLKVFNGPTDYIFMEVQADRVLTGYQVDKNKDDDELTF	136
8	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVSPIHSHKFDSTNYYIK VRAGDNKYMHLKVFNGPFVPIREFWADRVLTGYQVDKNKDDDELTF	137
9	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANHNQTFINESTNYYI KVRAGDNKYMHLKVFNGPHDYHLHFEADRVLTGYQVDKNKDDDELTF	138
10	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAHYGRKFGWFHSTNY YIKVRAGDNKYMHLKVFNGPEKVQKKIWLADRVLTGYQVDKNKDDDELTF	139
11	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWYRAYGDTKSTNYYI KVRAGDNKYMHLKVFNGPHINKFTVHKADRVLTGYQVDKNKDDDELTF	140
12	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAHRHGKHFHYSTNYYI KVRAGDNKYMHLKVFNGPQPKHNDRKHADRVLTGYQVDKNKDDDELTF	141
13	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKWDGYTALPSTNYYI KVRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDDDELTF	142
14	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFIRFFHPHGSTNYYIK VRAGDNKYMHLKVFNGPWWNIKHQYAYRVLTGYQVDKNKDDDELTF	143
15	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAPWHRHGIPRGSTNY YIKVRAGDNKYMHLKVFNGPEFPYTTTQRADRVLTGYQVDKNKDDDELTF	144
16	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIFERKKRISSTNYYIKV RAGDNKYMHLKVFNGPYQNEFEWQSADRVLTGYQVDKNKDDDELTF	145
17	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADHLQWTDIVSTNYYI KVRAGDNKYMHLKVFNGPVVNSLTWYADRVLTGYQVDKNKDDDELTF	146
18	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVTSFNTDLHSTNYYIK VRAGDNKYMHLKVFNGPVVDQDIHWADRVLTGYQVDKNKDDDELTF	147
19	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKDEEKGEQSTNYYI KVRAGDNKYMHLKVFNGPLLKWFPIEADRVLTGYQVDKNKDDDELTF	148
20	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAHTQVLEDTGSTNYYI KVRAGDNKYMHLKVFNGPVVWYPTVRDADRVLTGYQVDKNKDDDELTF	149
21	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDDDELTF	150
22	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIKDEDFELYSTNYYIK VRAGDNKYMHLKVFNGPYWVRSLDYADRVLTGYQVDKNKDDDELTF	151
23	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAYSKNLDGVSSTNYYIK VRAGDNKYMHLKVFNGPEWTWVWWFADRVLTGYQVDKNKDDDELTF	152
24	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAEFFDRAYVSTNYYIK VRAGDNKYMHLKVFNGPWQPLAVQSYADRVLTGYQVDKNKDDDELTF	153
25	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGHRWDDWDASTNY YIKVRAGDNKYMHLKVFNGPSQWRIQKVAADRVLTGYQVDKNKDDDELTF	154
26	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASEYVNWEGESTNYYI KVRAGDNKYMHLKVFNGPNLSHKWGIVADRVLTGYQVDKNKDDDELTF	155
27	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQAKSTKRQTSTNYYI KVRAGDNKYMHLKVFNGPNVDDTISVPADRVLTGYQVDKNKDDDELTF	156

Clone #	Amino Acid Sequences	SEQ ID NO:
28	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAPWYPDQGLPSTNYYI KVRAGDNKYMHLKVFNGPWDNHGNVNWADRVLTGYQVDKNKDELDTGF	157
29	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADFYLNWEGPSTNYYI KVRAGDNKYMHLKVFNGPGDAAAYGVEADRVLTGYQVDKNKDELDTGF	158
30	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLARLPLSRAWWDSTNYYI KVRAGDNKYMHLKVFNGPEWDHFHHPNADRVLTGYQVDKNKDELDTGF	159
31	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLATKHHYNLYTSTNYYIK VRAGDNKYMHLKVFNGPGDQDFQEQFADRVLTGYQVDKNKDELDTGF	160
32	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIEQHDAYKASTNYYIK VRAGDNKYMHLKVFNGPNFKITFKHEADRVLTGYQVDKNKDELDTGF	161
33	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWAWGSSFHSTNYYI IKVRAGDNKYMHLKVFNGPVEDQDSLLWADRVLTGYQVDKNKDELDTGF	162
34	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAEHWYNHGLDSTNYYI IKVRAGDNKYMHLKVFNGPDWHQTVGNADRVLTGYQVDKNKDELDTGF	163
35	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQVQVETESTNYYI KVRAGDNKYMHLKVFNGPHWHSWQSKADRVLTGYQVDKNKDELDTGF	164
36	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLATSPPRDWVSTNYYI KVRAGDNKYMHLKVFNGPDFNSIITEADRVLTGYQVDKNKDELDTGF	165
37	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASREGPDSTSTNYYIK VRAGDNKYMHLKVFNGPWIQWFPFIEIADRVLTGYQVDKNKDELDTGF	166
38	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADHYHWFEGWSTNY YIKVRAGDNKYMHLKVFNGPGAYKAVAFYADRVLTGYQVDKNKDELDTGF	167
39	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQHPDWPSDLKSTNY YIKVRAGDNKYMHLKVFNGPFFSKEAYRWADRVLTGYQVDKNKDELDTGF	168
40	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWEKTFSGQSTNYYI KVRAGDNKYMHLKVFNGPDDQFWLNWEADRVLTGYQVDKNKDELDTGF	169
41	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADLPWQGTIWQFSS TNYYIKVRAGDNKYMHLKVFNGPDGWVFLGGKADRVLTGYQVDKNKDELDTGF	170
42	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAEDRRFPKASTNYYIK VRAGDNKYMHLKVFNGPFSSGQQGKADRVLTGYQVDKNKDELDTGF	171
43	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAHFHIKRNAESTNYYIK VRAGDNKYMHLKVFNGPKIPRVAHKDADRVLTGYQVDKNKDELDTGF	172
44	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANKWRGRQKHSTNYYI IKVRAGDNKYMHLKVFNGPVLRWGHQYKADRVLTGYQVDKNKDELDTGF	173
45	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAINDWETDLFSTNYYI KVRAGDNKYMHLKVFNGPVTTLNQLINWADRVLTGYQVDKNKDELDTGF	174
46	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAYEKDGDLPQSTNYYI KVRAGDNKYMHLKVFNGPFVRWFPIEADRVLTGYQVDKNKDELDTGF	175
47	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWVGHNAWVESTNY YIKVRAGDNKYMHLKVFNGPNAVYNYDHADRVLTGYQVDKNKDELDTGF	176
48	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLWASIRTKSTNYYIK VRAGDNKYMHLKVFNGPWGYWRHLFGADRVLTGYQVDKNKDELDTGF	177
49	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASYDLRKRHLSTNYYIK VRAGDNKYMHLKVFNGPKAYNKSQPADRVLTGYQVDKNKDELDTGF	178
50	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWEEEDQVVLSTNYYI KVRAGDNKYMHLKVFNGPILIQYHWPWADRVLTGYQVDKNKDELDTGF	179
51	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASIDNNVYISTNYYIK VRAGDNKYMHLKVFNGPDIPFAVISRADRVLTGYQVDKNKDELDTGF	180
52	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFHKRAYHFHSTNYYI KVRAGDNKYMHLKVFNGPWDVKKDLKADRVLTGYQVDKNKDELDTGF	181

Clone #	Amino Acid Sequences	SEQ ID NO:
53	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASDPDWGHIESTNYYI KVRAGDNKYMHLKVFNGPEEDWWGWRWADRVLTGYQVDKNKDDDELTF	182
54	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWPWGSSVHVSTNYY IKVRAGDNKYMHLKVFNGPYEYHNNLAWADRVLTGYQVDKNKDDDELTF	183
55	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANDRHGWFEYSTNYYI KVRAGDNKYMHLKVFNGPDFNEERYSPADRVLTGYQVDKNKDDDELTF	184
56	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADYAEVHQWYSTNYY IKVRAGDNKYMHLKVFNGPQVYCSEFGGADRVLTGYQVDKNKDDDELTF	185
57	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAEGDPVIVEYSTNYYIK VRAGDNKYMHLKVFNGPFVAIPWNNFADRVLTGYQVDKNKDDDELTF	186
58	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASAVTVDHHSTNYYI KVRAGDNKYMHLKVFNGPSEEHFIPIDADRVLTGYQVDKNKDDDELTF	187
59	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGESEQYTDLESTNYYIK VRAGDNKYMHLKVFNGPYLNDWGLVWADRVLTGYQVDKNKDDDELTF	188
61	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAPFSRWHGYRSTNYYI KVRAGDNKYMHLKVFNGPNRRHQEWQKADRVLTGYQVDKNKDDDELTF	189
62	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWKNYTFQSTNYYIKV RAGDNKYMHLKVFNGPYNYFPYRWADRVLTGYQVDKNKDDDELTF	190
63	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANAIRKLSSTNYYIK VRAGDNKYMHLKVFNGPVIQAIWSPQADRVLTGYQVDKNKDDDELTF	191
64	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQTAHHRKATSTNYYI KVRAGDNKYMHLKVFNGPWSAQWDFWVADRVLTGYQVDKNKDDDELTF	192
65	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKHKQHHPWSTNYY IKVRAGDNKYMHLKVFNGPLSKPFHAHFADRVLTGYQVDKNKDDDELTF	193
66	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKHYPTDLKSTNYYIK VRAGDNKYMHLKVFNGPYSLNLWGIWWADRVLTGYQVDKNKDDDELTF	194
67	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAWPIKSGYEFSTNYYIK VRAGDNKYMHLKVFNGPWGWRPDPVADRVLTGYQVDKNKDDDELTF	195
70	IPRGLSEAKPATPEIQEIVDNVVKPQLEEKTNETYGKLEAVQYKTQVLAHDYYTDSWASTNYYI KVRAGDNKYMHLKVFNGPWPRFLIKQADRVLTGYQVDKNKDDDELTF	196
71	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANDRRHHIRPSTNYYI KVRAGDNKYMHLKVFNGPDGKRKQWVADRVLTGYQVDKNKDDDELTF	197
72	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQALEPHAVKSTNYYI KVRAGDNKYMHLKVFNGPHFLFWFEDAADRVLTGYQVDKNKDDDELTF	198
73	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASKFKHHHHSTNYYI KVRAGDNKYMHLKVFNGPSWHNYRYQDADRVLTGYQVDKNKDDDELTF	199
74	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIEGLITDLQSTNYYIKV RAGDNKYMHLKVFNGPIDGESAWYWADRVLTGYQVDKNKDDDELTF	200
75	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFYWGVSYHESTNYYI KVRAGDNKYMHLKVFNGPKRTEESLTWADRVLTGYQVDKNKDDDELTF	201
76	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADSEIETDLSTNYYIK VRAGDNKYMHLKVFNGPWWHQAIWQWADRVLTGYQVDKNKDDDELTF	202
77	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANKYNRNYGKSTNYYI KVRAGDNKYMHLKVFNGPIKVKWRGHKADRVLTGYQVDKNKDDDELTF	203
78	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKVRPLRIHTSTNYYIK VRAGDNKYMHLKVFNGPFQPTIPNNLADRVLTGYQVDKNKDDDELTF	204
69	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGRSKHAHNSTNYYI KVRAGDNKYMHLKVFNGPKIFWGAVGTAADRVLTGYQVDKNKDDDELTF	205
79	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVVWRRHLHQKSTNYYI KVRAGDNKYMHLKVFNGPIIAHDGPYVADRVLTGYQVDKNKDDDELTF	206

Clone #	Amino Acid Sequences	SEQ ID NO:
25	IPRGLSEAKPATPEIQEIVDKVKPQLEEKGTETYGKLEAVQYKTQVLAGHRWDDWDASTNY YIKVRAGDNKYMHLKVFNGPSQWRIQKVAADRVLTGYQVDKNKDELDTGF	207
31	IPRGLSEAKPATPEIQEIVDKVKPQLEEKGTETYGKLEAVQYKTQVLA TKHHYNLYTSTNYYIK VRAGDNKYMHLKVFNGPGDQDFQEQFADRVLTGYQVDKNKDELDTGF	208
38	IPRGLSEAKPATPEIQEIVDKVKPQLEEKGTETYGKLEAVQYKTQVLADHYHWFEGWSTNY YIKVRAGDNKYMHLKVFNGPGAYKAVAFYADRVLTGYQVDKNKDELDTGF	209
58	IPRGLSEAKPATPEIQEIVDKVKPQLEEKGTETYGKLEAVQYKTQVLASAVTVDHHSTNYYI KVRAGDNKYMHLKVFNGPSEEHFIPIADRVLTGYQVDKNKDELDTGF	210
25	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGHRWDDWDASTNY YIKVRAGDNKYMHLKVFNGPSQWRIQKVAADRVLTGYQVDKNKDELDTGF	211
31	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA TKHHYNLYTSTNYYIK VRAGDNKYMHLKVFNGPGDQDFQEQFADRVLTGYQVDKNKDELDTGF	212
38	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADHYHWFEGWSTNY YIKVRAGDNKYMHLKVFNGPGAYKAVAFYADRVLTGYQVDKNKDELDTGF	213
58	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLASAVTVDHHSTNYYI KVRAGDNKYMHLKVFNGPSEEHFIPIADRVLTGYQVDKNKDELDTGF	214
80	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFQWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	215
81	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	216
82	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	217
83	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFQWGSSYHTSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	218
84	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFQWGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	219
85	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFQWGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	220
86	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFQWGSSFHSSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	221
87	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFQWGSSYHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	222
88	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAILEPYTALPSTNYYIKV RAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	223
89	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTGASTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	224
90	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEY TALQSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	225
91	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEY TALVSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	226
92	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYTALLSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	227
93	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEY TALPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	228
94	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEAYTGASTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	229
95	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEAYTALASTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	230
96	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLDDYTGLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELDTGF	231

Clone #	Amino Acid Sequences	SEQ ID NO:
97	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTALESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	232
98	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYALTSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	233
99	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLALEPYTVLPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	234
100	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTGLDSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	235
101	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVEDFYTDLISTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	236
102	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYTVLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	237
103	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTAALLSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	238
104	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTSLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	239
105	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTAIRSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	240
106	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTAALDSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	241
107	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTVLPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	242
108	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIEEFYTDLVSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	243
109	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAIRHVEVTDLHSTNYYI KVRAGDNKYMHLKVFNGPSEEHFIPIADRVLTGYQVDKNKDELDTGF	244
110	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKEVLVTDLHSTNYYIK VRAGDNKYMHLKVFNGPSEEHFIPIADRVLTGYQVDKNKDELDTGF	245
111	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPNEYNDNLTWADRVLTGYQVDKNKDELDTGF	246
112	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYDNQNNLLWADRVLTGYQVDKNKDELDTGF	247
113	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKETNNLTLWADRVLTGYQVDKNKDELDTGF	248
114	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPIEDNNLTLWADRVLTGYQVDKNKDELDTGF	249
115	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVEYDYNLIWADRVLTGYQVDKNKDELDTGF	250
116	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYENEVTLTWADRVLTGYQVDKNKDELDTGF	251
117	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKDDNETLVWADRVLTGYQVDKNKDELDTGF	252
118	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYDAQYSLAWADRVLTGYQVDKNKDELDTGF	253
119	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPFDQAQSLTWADRVLTGYQVDKNKDELDTGF	254
120	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVDNEYNLTLWADRVLTGYQVDKNKDELDTGF	255
121	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPLDSQESLIWADRVLTGYQVDKNKDELDTGF	256

Clone #	Amino Acid Sequences	SEQ ID NO:
122	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPDDANDDLIWADRVLTGYQVDKNKDELDTGF	257
123	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKETEDNLIWADRVLTGYQVDKNKDELDTGF	258
124	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPFEDKKDLTWADRVLTGYQVDKNKDELDTGF	259
125	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKETNNDLIWADRVLTGYQVDKNKDELDTGF	260
126	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWDGYTALPSTNYYI KVRAGDNKYMHLKVFNGPYLEDWGLNWADRVLTGYQVDKNKDELDTGF	261
127	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWDGYTALPSTNYYI KVRAGDNKYMHLKVFNGPYFEDWGLNWADRVLTGYQVDKNKDELDTGF	262
128	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKKEYQKNLTWADRVLTGYQVDKNKDELDTGF	263
129	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPEETNDNLTWADRVLTGYQVDKNKDELDTGF	264
130	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVENHFALTWADRVLTGYQVDKNKDELDTGF	265
131	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPIEDDKTLTWADRVLTGYQVDKNKDELDTGF	266
132	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPLETDYNLIWADRVLTGYQVDKNKDELDTGF	267
133	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPNETNNSLTWADRVLTGYQVDKNKDELDTGF	268
134	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPEEYHDALAWADRVLTGYQVDKNKDELDTGF	269
135	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWGGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	270
136	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	271
137	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSFHTSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	272
138	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYTGLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	273
139	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWGGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	274
140	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWGGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	275
141	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWGGSSFHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	276
142	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWGGSSFHTSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	277
143	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHSSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	278
144	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWGGSSYHSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELDTGF	279
145	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLDDYTGLPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	280
146	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEQYTGLOSTNYYI KVRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	281

Clone #	Amino Acid Sequences	SEQ ID NO:
147	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLESYALPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	282
148	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVEDFYTDLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	283
149	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGWEQYALASTNYYI KVRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	284
150	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYTGLSSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	285
151	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLESYTGLSSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	286
152	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTGLTSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	287
153	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLDEYALPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	288
154	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLALWEEYALSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	289
155	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEAYTALESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	290
156	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTSLASTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	291
157	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGYEDYALQSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	292
158	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTLQSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	293
159	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLESYTVLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	294
160	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLDEYTVLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	295
161	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYTVLPSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGVKWADRVLTGYQVDKNKDELDTGF	296
162	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPIESDFNLIWADRVLTGYQVDKNKDELDTGF	297
163	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPIDDEALTWADRVLTGYQVDKNKDELDTGF	298
164	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKETKENLTWADRVLTGYQVDKNKDELDTGF	299
165	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYDNDVALTWADRVLTGYQVDKNKDELDTGF	300
166	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVETDLALTWADRVLTGYQVDKNKDELDTGF	301
167	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYENEDNLIWADRVLTGYQVDKNKDELDTGF	302
168	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYENHYALTWADRVLTGYQVDKNKDELDTGF	303
169	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKEDHKSILVWADRVLTGYQVDKNKDELDTGF	304
170	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPEETQNNLIWADRVLTGYQVDKNKDELDTGF	305
171	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPEDTHNSLVWADRVLTGYQVDKNKDELDTGF	306

Clone #	Amino Acid Sequences	SEQ ID NO:
172	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPNDNNNNLTWADRVLTGYQVDKNKDELTGF	307
173	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVEDKNDLTWADRVLTGYQVDKNKDELTGF	308
174	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYEYELDIWADRVLTGYQVDKNKDELTGF	309
175	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYEDDYNLVWADRVLTGYQVDKNKDELTGF	310
176	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPVETEESLTWADRVLTGYQVDKNKDELTGF	311
177	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYETNLTWADRVLTGYQVDKNKDELTGF	312
178	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPDDSHDALVWADRVLTGYQVDKNKDELTGF	313
179	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPNESNESLVWADRVLTGYQVDKNKDELTGF	314
180	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKDNDNLVWADRVLTGYQVDKNKDELTGF	315
181	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPDQDNDNLWADRVLTGYQVDKNKDELTGF	316
182	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYDYQDNLWADRVLTGYQVDKNKDELTGF	317
183	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPLESNKTLVWADRVLTGYQVDKNKDELTGF	318
184	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYESEDNLIWADRVLTGYQVDKNKDELTGF	319
185	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPYEDNLNLAWADRVLTGYQVDKNKDELTGF	320
186	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSYHQSTNYYI KVRAGDNKYMHLKVFNGPKEDNDSLIWADRVLTGYQVDKNKDELTGF	321
187	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKWDGYTALPSTNYYI KVRAGDNKYMHLKVFNGPYTEDWGLNWADRVLTGYQVDKNKDELTGF	322
188	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLESYTSLASTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELTGF	323
189	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEAYTALDSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELTGF	324
190	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYTGLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELTGF	325
191	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFRWGSSFHRSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELTGF	326
192	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAQWSSFHRSTNYYI KVRAGDNKYMHLKVFNGPVQDEFALIWADRVLTGYQVDKNKDELTGF	327
193	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEAYTVLQSTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELTGF	328
194	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEQYTALESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELTGF	329
195	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEAYTVLESTNYYIK VRAGDNKYMHLKVFNGPYGDDWGKWAADRVLTGYQVDKNKDELTGF	330
196	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAKWDGYTALPSTNYYI KVRAGDNKYMHLKVFNGPYAEDWGLNWADRVLTGYQVDKNKDELTGF	331

Clone #	Amino Acid Sequences	SEQ ID NO:
197	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPYENEVTLTWADRVLTGYQVDKNKDDDELTF	332
198	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPYDAQYSLAWADRVLTGYQVDKNKDDDELTF	333
199	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPKDDNETLVWADRVLTGYQVDKNKDDDELTF	334
200	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLDDYTGLESTNYYIK VRAGDNKYMHLKVFNGPYLEDWGLNWADRVLTGYQVDKNKDDDELTF	335
201	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALPSTNYYIK VRAGDNKYMHLKVFNGPYLEDWGLNWADRVLTGYQVDKNKDDDELTF	336
202	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALDSTNYYIK VRAGDNKYMHLKVFNGPYLEDWGLNWADRVLTGYQVDKNKDDDELTF	337
203	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALQSTNYYIK VRAGDNKYMHLKVFNGPYENEVTLTWADRVLTGYQVDKNKDDDELTF	338
204	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPYDAQYSLAWADRVLTGYQVDKNKDDDELTF	339
205	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPYENEVTLTWADRVLTGYQVDKNKDDDELTF	340
206	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALQSTNYYIK VRAGDNKYMHLKVFNGPYLEDWGLNWADRVLTGYQVDKNKDDDELTF	341
207	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPKEYQKNLTWADRVLTGYQVDKNKDDDELTF	342
208	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPVEYDYNLIWADRVLTGYQVDKNKDDDELTF	343
209	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPIEDNNNLTWADRVLTGYQVDKNKDDDELTF	344
210	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPKEYQKNLTWADRVLTGYQVDKNKDDDELTF	345
211	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPVEYDYNLIWADRVLTGYQVDKNKDDDELTF	346
212	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAFTWGSSYHESTNYYI KVRAGDNKYMHLKVFNGPIEDNNNLTWADRVLTGYQVDKNKDDDELTF	347
213	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALQSTNYYIK VRAGDNKYMHLKVFNGPKEYQKNLTWADRVLTGYQVDKNKDDDELTF	348
214	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALQSTNYYIK VRAGDNKYMHLKVFNGPVEYDYNLIWADRVLTGYQVDKNKDDDELTF	349
215	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEEYALQSTNYYIK VRAGDNKYMHLKVFNGPIEDNNNLTWADRVLTGYQVDKNKDDDELTF	350
216	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYALLSTNYYIK VRAGDNKYMHLKVFNGPKEYQKNLTWADRVLTGYQVDKNKDDDELTF	351
217	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYALLSTNYYIK VRAGDNKYMHLKVFNGPIEDNNNLTWADRVLTGYQVDKNKDDDELTF	352
218	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAILEPYTALPSTNYYIKV RAGDNKYMHLKVFNGPKEYQKNLTWADRVLTGYQVDKNKDDDELTF	353
219	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGLEDYALLSTNYYIK VRAGDNKYMHLKVFNGPVEYDYNLIWADRVLTGYQVDKNKDDDELTF	354
220	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAILEPYTALPSTNYYIKV RAGDNKYMHLKVFNGPVEYDYNLIWADRVLTGYQVDKNKDDDELTF	355
221	IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAILEPYTALPSTNYYIKV RAGDNKYMHLKVFNGPIEDNNNLTWADRVLTGYQVDKNKDDDELTF	356

In some embodiments, the CD33 AFFIMER® polypeptide has an amino acid sequence that is encoded by a nucleic acid having a coding sequence at least 70%, 75% 80%, 85%, 90%, 95% or even 98% identical with a sequence selected from SEQ ID NOs: 357-583. In some embodiments, the CD33 AFFIMER® polypeptide has an amino acid sequence that is encoded by a nucleic acid that having a coding sequence that hybridizes to a sequence selected from SEQ ID NOs: 357-583 under stringent conditions (such as in the presence of 6X sodium chloride/sodium citrate (SSC) at 45°C followed by a wash in 0.2X SSC at 65°C).

Table 4. Exemplary Encoded Anti-CD33 AFFIMER® Sequences

Clone #	Nucleic Acid Sequences	SEQ ID NO:
1	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCCCGGCTCAGACCTTCCCGGA TTGGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGAACTTCACCGTTGAAGTTTCTGAACATCATGCGGACCGT GTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	357
2	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACCTGCTGGAAACCGGAAATCG AATACTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGACCGGTCTGTCTGATTCTCGTTTCCATGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	358
3	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCAAATTCGAAGATGAAACCC ATGATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGGATTACGGTATGGTTCAGCAGTTCGAAGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	359
4	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACCATGCTGGAAGGTGATTTCT ACGAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGGATTGGCCGCAGCATAAACGTCTGGTTGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	360
5	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCCTGGCTGAAGGTGAAGAAC TGCAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGGAAGCTGAAGGTCGTGATTTCTCGTTGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	361
6	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATGATCCTGGAAGGTGAACCGT CTCTGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT	362

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GAAAGTGTTC AACGGCCAGATAACATGCTGCATTCTCATTTCGTTGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
7	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACCCATGGTATGGTTCATGAAC TATGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGACCGATTACATCTTCATGGAAGTTCAGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	363
8	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGTTTCTCCGATCCATTCTAAATTC GATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGA AAGTGTTC AACGGCCCGTTCGTTCCGATCCGTGAAGATTCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	364
9	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAACCATAACCAGACCTTCATCAA CGAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGCATGATTACTACCTGCATCTGTTCAAGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	365
10	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACATTACGGTTCGTAAATTTGGTTG GTTTTCATTCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCAC CTGAAAGTGTTC AACGGCCCGGAAAAAGTTCAGCAGAAAAATCTGGCTGGCGGACCG TGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	366
11	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGTACCGTGCATACGGTGATA CCAAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGCATATCAACAAATTTACCGTTCATAAAGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	367
12	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACATCGTCATGGTAAACATTTTCA TTAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGCAGCCAAAACATAACGATCGTAAACATGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	368
13	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAATGGGATGGTTACACAGCAC TGCCATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	369
14	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTATCCGTTTTTTTCATCCACAT GGTTCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGA AAGTGTTC AACGGCCCGTGTTGGAACATCAAAATCCATCAGTACGCGTACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	370

Clone #	Nucleic Acid Sequences	SEQ ID NO:
15	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACCATGGCATCGTCATGGTATCCC ACGTGGTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCAC CTGAAAGTGTTCAACGGCCCCGGAATTTCCATACACCACCACCCAGCGTGCGGACCGT GTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	371
16	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCTTTGAACGTAAAAACGTAT CTCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGTACCAGAACGAATTTGAATGGCAGTCTGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	372
17	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATCATCTGCAGTGGACCGATA TCGTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCCGTTGTTAACTCTCTGCTGACCTACTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	373
18	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGTTACCTCTTTTAACACCGATCT GCATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGTTGTTGATCAGGATATCATCTCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	374
19	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAAGATGAAGAAAAAGGTA GAACAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCAC CTGAAAGTGTTCAACGGCCCCGTGCTGAAATGGTTTCCAATCGAAATCGCGGACCGT GTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	375
20	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACATACCCAGGTTCTGGAAGATA CCGGTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCCGTTGTTGGTACCAACCGTTCTGATGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	376
21	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGTTCAAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	377
22	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCAAAGATGAAGATTTTGAAC TGTA CTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCCGTACTGGGTTCTGTTCTCTGGATTACGCGGACCGTGTTCT GACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	378
23	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT	379

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATACTCTAAAAACCTGGATGGTGT TTCTTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGAATGGACCTGGGTTTGGTGGTTTGCGGACCGTGTTCTG ACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
24	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGAATTTTTGATCGTGCATACTA CGTTTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTGGCAGCCACTGGCAGTTCAGTCTTACGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	380
25	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCATCGTTGGGATGATTGGG ATGCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTCTCAGTGGCGTATCCAGAAAGTTGCAGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	381
26	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTGAATACGTTAACTGGGAAG GTGAATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGAACCTGTCTATAAATGGGGTATCGTTGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	382
27	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACAGGCAAAATCTACAAACGTC AGACCTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGAACGTTGATGATACCATCTCTGTTCCAGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	383
28	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACCATGGTACCCAGATCAGGGTC TGCCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTGGGATAACCATGGTAACGTTAACTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	384
29	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATTTTTACCTGAAGTGGGAAG GTCCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGGTGATGCAGCAGCATACGGTGTTGAAGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	385
30	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACGTCTGCCACTGTCTCGTGCATG GGATTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGAATGGGATCATTTTCATCATCCAAACGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	386
31	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACCAAAACATCATTACAACCTGTA CACCTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG	387

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	AAAGTGTTCAACGGCCCGGGTGATCAGGATTTTCAGGAACAGTTTGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
32	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCGAACAGCATGATGCATACAAGCATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGAACTTTAAATCACCTTTAAACATGAAGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	388
33	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGGCATGGGGTTCTTCTTTTCACTACTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGGTTGAAGATCAGGATTCTCTGCTGTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	389
34	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGAACATTGGTACAACCATGGTCTGGATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGGATTGGCATCAGACCTGGGTTGGTAACGCGGACCGGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	390
35	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACAGGTTCAAGTTGAAACCGAATACCGTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGCATTGGCATTCTCATTGGCAGTCTAAAGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	391
36	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACCACCTCTCCACCACGTGATTGGTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGGATTTAACTCTATCATCATCACCGAAGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	392
37	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTCGTGAAGGTCCAGATTCTACTCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGTGGATCCAGTGTTTCCAATCGAAATCGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	393
38	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATCATTACCATTTGGTTTGAAGGTTGGTCCACCAATTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCGGGTGCATACAAAGCAGTTGCATTTTACGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	394
39	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATCGTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACAGCATCCAGATTGGCCATCTGATCTGAAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACTGAAAGTGTTCAACGGCCCGTTTTTTCTAAAGAAGCATACCGTTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	395

Clone #	Nucleic Acid Sequences	SEQ ID NO:
40	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGAATGGGAAAAACCTTTTCTG GTCAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGATGATCAGTTTTGGCTGAACTGGGAAGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	396
41	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATCTGGATCCATGGCAGGGTA CCATCTGGCAGTTTTCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAA GTATATGCACCTGAAAGTGTTCAACGGCCCGGATGGTTGGTGGTTTCTGGGTGGTAA AGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGA CGGGTTTC	397
42	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGAAGATCGTCGTTTTTACCCAAA AGCATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTTTTCTTCTGGTCAGCAGCAGGGTAAAGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	398
43	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACATTTTCATATCAAACGTAACGC AGAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGAAAATCCACGTGTTGCACATAAAGATGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	399
44	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACAAATGGCGTGGTCTGACAGA AACATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGTTCTGCGTTGGGGTCATCAGTACAAAGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	400
45	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCAACGATTGGGAAACCGATC TGTTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGTTACCACCAACCTGCAGATCAACTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	401
46	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATACGAAAAAGATGGTGATCTGC CACAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTTTGTTCGTTGGTTTCAATCGAAATCGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	402
47	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGGTTGGTCATAACGCATGGG TTGAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGAACGCAGTTTACAATACTACGATCATGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	403

Clone #	Nucleic Acid Sequences	SEQ ID NO:
48	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGTGGGCATCTATCCGTAC CAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTGGGGTACTGGCGTCATCTGTTTGGTGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	404
49	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTTACGATCTGCGTAAACGTCT GCATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAAGCATACAACAAATCTCTGCAGCCAGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	405
50	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGGAAGAAGAAGATCAGGTT GTTCTGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACC TGAAAGTGTTCAACGGCCCGATCCTGATCCAGTACCATTGGCCATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	406
51	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTATCGATAACAACGTTTACTA CATCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGATATCCATTTGCAGTTATCTCTCGTGCGGACCGTGTTT TGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	407
52	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCATAAACGTGCATACCATTT TCATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTGGGATCCAGTTAAAAAAGATCTGAAAGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	408
53	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTGATCCAGATTGGGGTCATAT CGAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGAAGAAGATTGGTGGGGTTGGCGTTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	409
54	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGCCATGGGGTTCTTCTGTTCA TGTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTACGAATACCATAACAACCTGGCATGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	410
55	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACGATCGTCATGGTTGGGAAT TTTACTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGATTTTAACGAAGAACGTTACTCTCCAGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	411
56	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT	412

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATTACGCAGAAGTTCATCAGT GGTACTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGCCCCGCAGGTTTACTGTTCTGAATTTGGTGGTGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
57	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGAAGGTGATCCAGTTATCGTTG AATACTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGCCCCGTTGTTGCAATCCCATGGAACAACCTTTCGCGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	413
58	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTGCAGTTACCGTTACCGATCA TCATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGCCCCGTCTGAAGAACATTTTATCCCAATCGATGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	414
59	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTTCTGAACAGTACACCGATCT GGAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGCCCCGTACCTGAACGATTGGGGTCTGGTTTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	415
61	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACCATTTTCTCGTTGGCATGGTTA CCGTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGCCCCGAACCGTCGTCATCAGGAATGGCAGAAAGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	416
62	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGAACCACTACACCTTTAGTC CACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTGAAAGT GTTCAACGCCCCGTACTACAACCTTTCCATACCGTTGGGCGGACCGTGTTCTGACC GGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	417
63	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACGCAATCCGTAACCTGGAAC GTTCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGCCCCGTAATCCAGGCAATCGTTTCTCCACAGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	418
64	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACAGACCGCACATCATCGTAAAG CAACCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGCCCCGTGGTCTGCACAGTGGGATTTTGGGTTGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	419
65	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACATAAACAGCATCATAAAC CATGGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT	420

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GAAAGTGTTC AACGGCCCGCTGTCTAAACCATTTCATGCACATTTTGC GGACCGTGTTCTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
66	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAACATTACCCAGATACCGATCT GAAATCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTACTCTAACCTGTGGGGTATCTGGTGGGCGGACCGTG TTCTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	421
67	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGCCAATCAAACTCTGGTTACG AATTTTCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTGGTCTGGTTGGCGTCCAGATGTTGTTGCGGACCGTGT TCTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	422
70	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACATGATTACTACACCGATTCTTG GGCATCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTGGCCACGTTTTCTGATCATCAAACAGGCGGACCGTGT TCTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	423
71	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACGATCGTCGTCATCATATCCG TCCATCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGGATGGTAAACGTTCTAAACAGTGGGTTGCGGACCGTGTT CTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	424
72	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACAGGCACTGGAACCACATGCAG TTAAATCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGCATTTTCTGTTTTGGGAATTTGATGCAGCGGACCGTGT TCTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	425
73	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATCTAAATTTAAACATCATCATCA TCATTCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGTCTTGGCATAACTACCGTTACCAGGATGCGGACCGTGTT CTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	426
74	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATCGAAGGTCTGATCACCGATCT GCAGTCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGATCGATGGTGAATCTGCATGGTACTGGGCGGACCGTG TTCTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	427
75	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTACTGGGGTGTTCCTTACCA TGAATCCACCAACTATTACATTAAGGTTCTGCGCGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGAAACGTACCGAAGAATCTCTGACCTGGGCGGACCGTGTT CTGACCGGTTAC CAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	428

Clone #	Nucleic Acid Sequences	SEQ ID NO:
76	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATTCTGAAATCGAAACCGATCT GGATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTGGTGGCATCAGGCAATCTGGCAGTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	429
77	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAACAAATACAACCGTAACCTACG GTAAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGATCAAAGTTAAATGGCGTGGTCATAAAGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	430
78	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAAGTTCTGCTCACTGCGTATCCA TACCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTTTCAGCCAACCATCCCAAACAACCTGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	431
69	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCGTTCTAAACATGCACATAA CCGTTCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAAAATCTTTTGGGGTGCAATTGGTACCGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	432
79	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGTTGTTTGGCGTCTGTCATCA GAAATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGATCATCGCATGATGGTCCATACGTTGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	433
25	ATGATCCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCGGCGAAACCTACGGAAAG CTGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCAGGTCATCGTTGGGATGATTG GGATGCATCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCGTCTCAGTGGCGTATCCAGAAAGTTGCAGCGGACCG TGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	434
31	ATGATCCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCGGCGAAACCTACGGAAAG CTGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCAACCAAACATCATTACAACCTG TACACCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACC TGAAAGTGTTCAACGGCCCGGGTGATCAGGATTTTCAGGAACAGTTTGCAGGACCGT GTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	435
38	ATGATCCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCGGCGAAACCTACGGAAAG CTGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCAGATCATTACCATTGGTTTGAA GGTTGGTCCACCAATTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACC TGAAAGTGTTCAACGGCCCGGGTGATACAAAGCAGTTGCATTTTACGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	436
58	ATGATCCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCGGCGAAACCTACGGAAAG	437

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	CTGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCATCTGCAGTTACCGTTACCGAT CATCATTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACC TGAAAGTGTTCAACGGCCCGTCTGAAGAACATTTATCCCAATCGATGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
25	ATGATTCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCAACGAAACCTACGGAAAGC TGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCAGGTCATCGTTGGGATGATTGG GATGCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACC TGAAAGTGTTCAACGGCCCGTCTCAGTGCGGTATCCAGAAAGTTGCAGCGGACCGT GTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	438
31	ATGATTCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCAACGAAACCTACGGAAAGC TGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCAACCAACATCATTACAACCTGT ACACCTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGTTGATCAGGATTTTCAGGAACAGTTTGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	439
38	ATGATTCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCAACGAAACCTACGGAAAGC TGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCAGATCATTACCATTGGTTTGAAG GTTGGTCCACCAATTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGTTGCATACAAAGCAGTTGCATTTTACGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	440
58	ATGATTCCGAGGGGTCTGTCCGAAGCCAAGCCCGCTACCCCGGAGATCCAGGAGAT CGTGGACAAGGTCAAGCCCCAGCTCGAGGAAAAGACCAACGAAACCTACGGAAAGC TGGAAGCCGTGCAGTACAAGACCCAAGTGCTAGCATCTGCAGTTACCGTTACCGATC ATCATTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTCTGAAGAACATTTTATCCCAATCGATGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	441
80	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTTGAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	442
81	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTTTCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTTGAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	443
82	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTTGAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	444
83	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTGCTGGGGCTCTAGCTATCA TACGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG	445

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	AAAGTGTTC AACGCCCCG GTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
84	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCAGTGGGGCTCTAGCTTTCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG GTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	446
85	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCAGTGGGGCTCTAGCTTTCA TCATTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG GTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	447
86	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCAGTGGGGCTCTAGCTTTCA TTCGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG GTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	448
87	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTGAGTGGGGCGCTAGCTATC ATGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTC AACGCCCCG GTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	449
88	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATTTTGAACCGTATACCGCGCTGCCGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG TACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	450
89	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGGGCTGGCGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTC AACGCCCCG TACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	451
90	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGCTGCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTC AACGCCCCG TACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	452
91	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGCGCTGGTTTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTC AACGCCCCG TACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	453

Clone #	Nucleic Acid Sequences	SEQ ID NO:
92	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGATTATACCGCCC TGCTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	454
93	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGCGC TGCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	455
94	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGCGTATACCGGGC TGGCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	456
95	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGAAGCGTATACCGCGC TGGCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	457
96	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGATGATTATACCGGGC TGGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	458
97	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAAGAGTATACCGCGC TGGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	459
98	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAAGATTATACCGCGC TGACGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	460
99	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACTCTGGAACCGTATACCGTGCT GCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	461
100	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT	462

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGGCC TGGATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
101	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGTTGAGGATTTTATACCGACCT GATTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	463
102	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGATTATACCGTCC TGGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	464
103	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGCTGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	465
104	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAAGAGTATACCTCCC TGGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	466
105	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGAGGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	467
106	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGCCC TGGATTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	468
107	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAAGAGTATACCGTGC TGCCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	469
108	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATTGAGGAATTTTATACCGACCT GGTGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT	470

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GAAAGTGTTC AACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
109	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAGGCACGTGGAGGTGACCGATC TCCATTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTCTGAAGAACATTTTATCCCAATCGATGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	471
110	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAGGAGGTGTTGGTGACCGATC TCCATTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTCTGAAGAACATTTTATCCCAATCGATGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	472
111	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGAATGAATATAACGATAACCTGACCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	473
112	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGTATGACAATCAAAATAACCTGCTCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	474
113	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGAAGGAACTAACAATAACCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	475
114	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGATTGAAGATAACAATAACCTGACCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	476
115	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGGTGGAATATGACTATAACCTGATCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	477
116	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGTATGAAAATGAAAGTGACCCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	478

Clone #	Nucleic Acid Sequences	SEQ ID NO:
117	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGACGATAACGAGACCCTGGTCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	479
118	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGACGCTCAATATTCCTGGCCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	480
119	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTTTGACGCTCAAGATTCCTGACCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	481
120	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTTGACAATGAATATAACCTGACCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	482
121	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTTGGACTCTCAAGAGTCCCTGATCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	483
122	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGATGACGCTAACGATGACCTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	484
123	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGAACTGAAGATAACCTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	485
124	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTTTGAAGATAAAAAGGACCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	486
125	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACG GTAAGCT	487

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGAAGGAACTAACAATGACCTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
126	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAATGGGATGGTTACACAGCAC TGCCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCCGATTGAGGATTGGGGCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	488
127	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAATGGGATGGTTACACAGCAC TGCCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCCGATTGAGGATTGGGGCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	489
128	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGAAGGAATATCAAAAGAACCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	490
129	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGAGGAACTAACGATAACCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	491
130	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGTGGAAATCACTTTCCTGACCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	492
131	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGATTGAAGATGACAAGACCCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	493
132	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCCGTTGGAACTGACTATAACCTGATCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	494
133	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG	495

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	AAAGTGTTC AACGCCCCGAATGAACTAACAATTCCTGACCTGGGCGGACCGTGTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
134	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCGAGGAATATCACGATGCCCTGGCCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	496
135	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGCGGTGGGGCTCTAGCTTTC ATGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCGGTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	497
136	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTAGGTGGGGCTCTAGCTTTCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCGGTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	498
137	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGCTCTAGCTTTCA TACGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCGGTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	499
138	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGATTATACCGGGC TGGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCGTACGGTGATGATTGGGGTGTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	500
139	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCAGTGGGGCTCTAGCTATCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCGGTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	501
140	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCAGTGGGGCTCTAGCTTTCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCGGTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	502
141	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGCAGTGGGGCTCTAGCTTTC ATGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCGGTTCAGGATGAATTTGCACTGATCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	503

Clone #	Nucleic Acid Sequences	SEQ ID NO:
142	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTAGTGGGGCTCTAGCTTTCA TACGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTT CAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	504
143	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTGGTGGGGCTCTAGCTATCA TTCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTT CAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	505
144	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTGCGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTT CAGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	506
145	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGATGATTATACCGGGC TGCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	507
146	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAACAGTATACCGGGC TGCACTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	508
147	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTTTGAATCGTATACCGCGC TGCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	509
148	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGTTGAGGATTTTATACCGACCT GGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	510
149	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTGGAACAGTATACCGCCC TGCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	511
150	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT	512

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAAGATTATACCGGCC TGTCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
151	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGAATCGTATACCGGGC TGTCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	513
152	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGGGC TGACTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	514
153	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGATGAGTATACCGCGC TGCCCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	515
154	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACTGTGGGAAGAGTATACCGCCC TGAGTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	516
155	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGCTTATACCGCGC TGGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	517
156	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGAAGAGTATACCTCGC TGGCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	518
157	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTACGAAGATTATACCGCCC TGCAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	519
158	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGAAGAGTATACCCAGC TGCCCTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT	520

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GAAAGTGTTC AACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
159	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAATCGTATACCGTCC TGGAGTCCACCAACTATTACATTAAGGTTCTGTCAGGTGACAATAAGTATATGCACC TGAAAGTGTTC AACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGT GTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	521
160	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGATGAGTATACCGTGC TGGAGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	522
161	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTTTGGAAGATTATACCGTCT GCCGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	523
162	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGATTGAATCTGACTTTAACCTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	524
163	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGATTGACGATGACGAGGCCCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	525
164	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGAAGGAAACTAAAGAGAACCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	526
165	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGTATGACAATGACGTTGCCCTGACCTGGGCGGACCGTGT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	527
166	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCTGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTCTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGGCCCGGTGGAAGTACTGACCTGGCCCTGACCTGGGCGGACCGTGT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	528

Clone #	Nucleic Acid Sequences	SEQ ID NO:
167	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAAAATGAAGATAACCTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	529
168	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAAAATCACTATGCCCTGACCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	530
169	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGAAGATCACAAGTCCCTGGTCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	531
170	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGAGGAACTCAAAATAACCTGATCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	532
171	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGAGGACACTCACAATTCCTGGTCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	533
172	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAATGACAATAACAATAACCTGACCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	534
173	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTGGAAGATAAAAATGACCTGACCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	535
174	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAATATGAATTGGACCTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	536
175	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT	537

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAAGATGACTATAACCTGGTCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
176	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTGGAAGTGAAGAGTCCCTGACCTGGGCGGACCGTGTT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	538
177	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAAACTAAGTTCGACCTGGTCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	539
178	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGATGACTCTACGATGCCCTGGTCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	540
179	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAATGAATCTAACGAGTCCCTGGTCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	541
180	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGACAATAACGATAACCTGGTCTGGGCGGACCGTGTT TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	542
181	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGATCAAGATAACGATAACCTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	543
182	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGACTATCAAGATAACCTGACCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	544
183	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG	545

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	AAAGTGTTC AACGCGCCGTTGGAATCTAACAAGACCCTGGTCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
184	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCGCCGTTATGAATCTGAAGATAACCTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	546
185	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCGCCGTTATGAAGATAACTTGAACCTGGCCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	547
186	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGTTCTTCTTACCA TCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCGCCGGAAGGAAGATAACGATTCCTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	548
187	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAATGGGATGGTTACACAGCAC TGCCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCGCCGTTACTGAGGATTGGGGCTGAATTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	549
188	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAAAGTTATACCTCGC TGGCTTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCGCCGTTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	550
189	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGCTTATACCGCCC TGGATTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCGCCGTTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	551
190	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGGG CTGGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCAC CTGAAAGTGTTC AACGCGCCGTTACGGTGATGATTGGGGTGTTAAATGGGCGGACCG TGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	552
191	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTCGTTGGGGCTCTAGCTTTCA TAGGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCGCCGTTACGGATGAATTTGCACTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	553

Clone #	Nucleic Acid Sequences	SEQ ID NO:
192	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTTTCA TCGTTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTTACGGATGAATTTGCACTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	554
193	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGAAGCGTATACCGTCC TGCAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	555
194	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTCGAACAGTATACCGCCC TGGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	556
195	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCTGGAAGCTTATACCGTGC TGGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTACGGTGATGATTGGGGTGTTAAATGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	557
196	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAAATGGGATGGTTACACAGCAC TGCCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTATGCGGAGGATTGGGGCCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	558
197	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAAAATGAAGTGACCCTGACCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	559
198	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGACGCTCAATATCCCTGGCCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	560
199	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTTTCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGACGATAACGAGACCCTGGTCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	561
200	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT	562

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGATGATTATACCGGGC TGGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTATTTGGAGGATTGGGGCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
201	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGCGC TGCCGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTATTTGGAGGATTGGGGCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	563
202	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGTTGGAAGAGTATACCGCCC TGGATTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTATTTGGAGGATTGGGGCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	564
203	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGCACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTATGAAAATGAAGTGACCCCTGACCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	565
204	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGACGCTCAATATCCCTGGCCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	566
205	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGTATGAAAATGAAGTGACCCCTGACCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	567
206	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGCACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGTATTTGGAGGATTGGGGCTGAATTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	568
207	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGAATATCAAAAGAACCTGACCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	569
208	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTCAGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTG	570

Clone #	Nucleic Acid Sequences	SEQ ID NO:
	AAAGTGTTC AACGCCCCG GTGGAATATGACTATAACCTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	
209	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG ATTGAAGATAACAATAACCTGACCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	571
210	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG AAGGAATATCAAAAAGAACCTGACCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	572
211	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG GTGGAATATGACTATAACCTGATCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	573
212	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTACGTGGGGCTCTAGCTATCA TGAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTC AACGCCCCG ATTGAAGATAACAATAACCTGACCTGGGCGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	574
213	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGCAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCG AAGGAATATCAAAAAGAACCTGACCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	575
214	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGCAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCG GTGGAATATGACTATAACCTGATCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	576
215	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGAGTATACCGCGC TGCAGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCG ATTGAAGATAACAATAACCTGACCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	577
216	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTC AAGAGATC GTCGATAAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGATTATACCGCCC TGCTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTC AACGCCCCG AAGGAATATCAAAAAGAACCTGACCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	578

Clone #	Nucleic Acid Sequences	SEQ ID NO:
217	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGATTATACGCCC TGCTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGATTGAAGATAACAATAACCTGACCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	579
218	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATTTTGAACCGTATACGCGCT GCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGAAGGAATATCAAAAAGAACCTGACCTGGGCGGACCGTG TCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	580
219	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGGCTGGAAGATTATACGCCC TGCTTTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCT GAAAGTGTTCAACGGCCCGGTGGAATATGACTATAACCTGATCTGGGCGGACCGTG TTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	581
220	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATTTTGAACCGTATACGCGCT GCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGGTGGAATATGACTATAACCTGATCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	582
221	ATGATCCCGCGTGGCCTGTCTGAAGCTAAACCAGCAACTCCGGAAATTCAAGAGATC GTCGATAAGGTGAAACCGCAGCTGGAAGAGAAAACGAACGAAACCTACGGTAAGCT GGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAATTTTGAACCGTATACGCGCT GCCGTCCACCAACTATTACATTAAGGTTCTGCGCGGTGACAATAAGTATATGCACCTG AAAGTGTTCAACGGCCCGATTGAAGATAACAATAACCTGACCTGGGCGGACCGTGTT CTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	583

Furthermore, minor modifications may also include small deletions or additions – beyond the loop 2 and loop 4 inserts described above – to the Stefin A or Stefin A derived sequences disclosed herein, such as addition or deletion of up to 10 amino acids relative to Stefin A or the Stefin A derived AFFIMER® polypeptide.

In some embodiments, the AFFIMER® agent is a CD33 binding AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human CD33 as a monomer with a dissociation constant (K_D) of about 1 μ M or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, or about 0.1 nM or less.

In some embodiments, the AFFIMER® agent is a CD33 binding AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human CD33 as a monomer with an off-rate constant (K_{off}), such as measured by Biacore, of about 10^{-3} s^{-1} (e.g., unit of 1/second) or slower; of about 10^{-4} s^{-1} or slower or even of about 10^{-5} s^{-1} or slower.

In some embodiments, the AFFIMER® agent is a CD33 AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human CD33 as a monomer with an association constant (K_{on}), such as measured by Biacore, of at least about $10^3 \text{ M}^{-1}\text{s}^{-1}$ or faster; at least about $10^4 \text{ M}^{-1}\text{s}^{-1}$ or faster; at least about $10^5 \text{ M}^{-1}\text{s}^{-1}$ or faster; or even at least about $10^6 \text{ M}^{-1}\text{s}^{-1}$ or faster.

In some embodiments, the AFFIMER® agent is a CD33 AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human CD33 as a monomer with an IC50 in a competitive binding assay with human CD33 of 1 μM or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, or about 0.1 nM or less.

In some embodiments, the AFFIMER® agent has a melting temperature (T_m , e.g., temperature at which both the folded and unfolded states are equally populated) of 65°C or higher, and preferably at least 70°C , 75°C , 80°C or even 85°C or higher. Melting temperature is a particularly useful indicator of protein stability. The relative proportions of folded and unfolded proteins can be determined by many techniques known to the skilled person, including differential scanning calorimetry, UV difference spectroscopy, fluorescence, circular dichroism (CD), and NMR (Pace et al. (1997) "Measuring the conformational stability of a protein" in Protein structure: A practical approach 2: 299-321).

A. Fusions Proteins - General

In some embodiments, the AFFIMER® polypeptides may further comprise an additional insertion, substitution and/or deletion that modulates biological activity of the AFFIMER® polypeptide. For example, the additions, substitutions and/or deletions may modulate at least one property or activity of modified AFFIMER® polypeptide. For example, the additions, substitutions or deletions may modulate affinity for the AFFIMER® polypeptide, e.g., for binding to and inhibiting CD33, modulate the circulating half-life, modulate the therapeutic half-life, modulate the stability of the AFFIMER® polypeptide, modulate cleavage by proteases, modulate dose, modulate release or bioavailability, facilitate purification, decrease deamidation, improve shelf-life, or improve or alter a particular route of administration. Similarly, AFFIMER® polypeptides may comprise protease cleavage sequences, reactive groups, antibody-binding domains (including but not limited to, FLAG or poly-His) or other affinity-based sequences (including but not limited to, FLAG, poly-His, GST, etc.) or linked molecules (including but not limited to, biotin) that improve detection, purification or other traits of the polypeptide.

In some instances, these additional sequences are added to one end and/or the other of the AFFIMER® polypeptide in the form of a fusion protein. Accordingly, in certain aspects of the

disclosure, the AFFIMER® agent is a fusion protein having at least one AFFIMER® polypeptide sequence and at least one heterologous polypeptide sequence (“fusion domain” herein). A fusion domain may be selected so as to confer a desired property, such as secretion from a cell or retention on the cell surface (e.g., for an encoded AFFIMER® construct), to serve as substrate or other recognition sequences for post-translational modifications, to create multimeric structures aggregating through protein-protein interactions, to alter (often to extend) serum half-life, or to alter tissue localization or tissue exclusion and other ADME properties – merely as examples.

For example, some fusion domains are particularly useful for isolation and/or purification of the fusion proteins, such as by affinity chromatography. Well known examples of such fusion domains that facilitate expression or purification include, merely to illustrate, affinity tags such as polyhistidine (e.g., a His₆ tag), Strep II tag, streptavidin-binding peptide (SBP) tag, calmodulin-binding peptide (CBP), glutathione S-transferase (GST), maltose-binding protein (MBP), S-tag, HA tag, c-Myc tag, thioredoxin, protein A and protein G.

In order for the AFFIMER® agent to be secreted, it will generally contain a signal sequence that directs the transport of the protein to the lumen of the endoplasmic reticulum and ultimately to be secreted (or retained on the cell surface if a transmembrane domain or other cell surface retention signal). Signal sequences (also referred to as signal peptides or leader sequences) are located at the N-terminus of nascent polypeptides. They target the polypeptide to the endoplasmic reticulum and the proteins are sorted to their destinations, for example, to the inner space of an organelle, to an interior membrane, to the cell outer membrane, or to the cell exterior via secretion. Most signal sequences are cleaved from the protein by a signal peptidase after the proteins are transported to the endoplasmic reticulum. The cleavage of the signal sequence from the polypeptide usually occurs at a specific site in the amino acid sequence and is dependent upon amino acid residues within the signal sequence.

In some embodiments, the signal peptide is about 5 to about 40 amino acids in length (such as about 5 to about 7, about 7 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, or about 25 to about 30, about 30 to about 35, or about 35 to about 40 amino acids in length).

In some embodiments, the signal peptide is a native signal peptide from a human protein. In other embodiments, the signal peptide is a non-native signal peptide. For example, in some embodiments, the non-native signal peptide is a mutant native signal peptide from the corresponding native secreted human protein, and can include at least one (such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more) substitution, insertions and/or deletions.

In some embodiments, the signal peptide is a signal peptide or mutant thereof from a non-IgSF protein family, such as a signal peptide from an immunoglobulin (such as IgG heavy chain or IgG-kappa light chain), a cytokine (such as interleukin-2 (IL-2), or CD33), a serum albumin protein (e.g. HSA or albumin), a human azurocidin preprotein signal sequence, a luciferase, a trypsinogen (e.g. chymotrypsinogen or trypsinogen) or other signal peptide able to efficiently secrete a protein from a cell. Exemplary signal peptides include but are not limited to:

Table 5. Exemplary Signal Sequences

Native Protein	Signal Sequence	SEQ ID NO:
Human Serum Albumin (HSA)	MKWVTFISLLFLESSAYS	584
Ig kappa light chain	MDMRAPAGIFGFLVLFPGYRS	585
Human azurocidin preprotein	MTRLTVLALLAGLLASSRA	586
IgG heavy chain	MELGLSWIFLLAILKGVQC	587
IgG heavy chain	MELGLRWVFLVAILEGVQC	588
IgG heavy chain	MKHLWFFLLLVAAPRWVLS	589
IgG heavy chain	MDWTWRILFLVAAATGAHS	590
IgG heavy chain	MDWTWRFLFVVAATGVQS	591
IgG heavy chain	MEFGLSWLFLVAILKGVQC	592
IgG heavy chain	MEFGLSWVFLVALFRGVQC	593
IgG heavy chain	MDLLHKNMKHLWFFLLLVAAPRWVLS	594
IgG Kappa light	MDMRVPAQLLGLLLWLSGARC	595
IgG Kappa light	MKYLLPTAAAGLLLLAAQPAMA	596
Gaussia luciferase	MGVKVLFALICIAVAEA	597
Human chymotrypsinogen	MAFLWLLSCWALLGTTFG	598
sHuman interleukin-2	MQLLSCIALILALV	599
Human trypsinogen-2	MNLLLILT FVAAAVA	600
Human CD33	MPLLLLLPLLWAGALA	601
Prolactin	MDSKGSSQKGSRLLLLLVVSNNLLCQGVVS	602
Human tPA	MDAMKRGLCCVLLLCGAVFVSPS	603
Synthetic/Consensus	MLLLLLLLLLLALALA	604
Synthetic/Consensus	MWWRLWLLLLLLLLLWPMVWA	605

In some embodiments of a secreted AFFIMER® agent, the recombinant polypeptide comprises a signal peptide when expressed, and the signal peptide (or a portion thereof) is cleaved from the AFFIMER® agent upon secretion.

The subject fusion proteins may also include at least one linker separating heterologous protein sequences or domains. As used herein, the term "linker" refers to a linker amino acid sequence inserted between a first polypeptide (e.g., an AFFIMER® polypeptide) and a second polypeptide (e.g., a second AFFIMER® polypeptide, an Fc region, a receptor trap, albumin, etc.). Empirical linkers designed by researchers are generally classified into 3 categories according to their structures: flexible linkers, rigid linkers, and *in vivo* cleavable linkers. Besides

the basic role in linking the functional domains together (as in flexible and rigid linkers) or releasing free functional domain *in vivo* (as in *in vivo* cleavable linkers), linkers may offer many other advantages for the production of fusion proteins, such as improving biological activity, increasing expression yield, and achieving desirable pharmacokinetic profiles. Linkers should not adversely affect the expression, secretion, or bioactivity of the fusion protein. Linkers should not be antigenic and should not elicit an immune response.

Suitable linkers may include mixtures of glycine and serine residues and often include amino acids that are sterically unhindered. Other amino acids that can be incorporated into useful linkers include threonine and alanine residues. Linkers can range in length, for example from 1-50 amino acids in length, 1-22 amino acids in length, 1-10 amino acids in length, 1-5 amino acids in length, or 1-3 amino acids in length. In some embodiments, the linker may comprise a cleavage site. In some embodiments, the linker may comprise an enzyme cleavage site, so that the second polypeptide may be separated from the first polypeptide.

In some embodiments, the linker can be characterized as flexible. Flexible linkers are usually applied when the joined domains require a certain degree of movement or interaction. They are generally composed of small, non-polar (e.g., Gly) or polar (e.g., Ser or Thr) amino acids. See, for example, Argos P. (1990) "An investigation of oligopeptides linking domains in protein tertiary structures and possible candidates for general gene fusion" J Mol Biol. 211:943–958. The small size of these amino acids provides flexibility and allows for mobility of the connecting functional domains. The incorporation of Ser or Thr can maintain the stability of the linker in aqueous solutions by forming hydrogen bonds with the water molecules, and therefore reduces the unfavorable interaction between the linker and the protein moieties. The most commonly used flexible linkers have sequences consisting primarily of stretches of Gly and Ser residues ("GS" linker). An example of the most widely used flexible linker has the sequence of (Gly-Gly-Gly-Gly-Ser)_n (SEQ ID NO: 606). By adjusting the copy number "n", the length of this GS linker can be optimized to achieve appropriate separation of the functional domains, or to maintain necessary inter-domain interactions. Besides the GS linkers, many other flexible linkers have been designed for recombinant fusion proteins. As These flexible linkers are also rich in small or polar amino acids such as Gly and Ser but can contain additional amino acids such as Thr and Ala to maintain flexibility, as well as polar amino acids such as Lys and Glu to improve solubility.

In some embodiments, the linker can be characterized as rigid. While flexible linkers have the advantage to connect the functional domains passively and permitting certain degree of movements, the lack of rigidity of these linkers can be a limitation in certain fusion protein

embodiments, such as in expression yield or biological activity. The ineffectiveness of flexible linkers in these instances was attributed to an inefficient separation of the protein domains or insufficient reduction of their interference with each other. Under these situations, rigid linkers have been successfully applied to keep a fixed distance between the domains and to maintain their independent functions.

Many natural linkers exhibited α -helical structures. The α -helical structure was rigid and stable, with intra-segment hydrogen bonds and a closely packed backbone. Therefore, the stiff α -helical linkers can act as rigid spacers between protein domains. George et al. (2002) "An analysis of protein domain linkers: their classification and role in protein folding" *Protein Eng.* 15(11):871-9. In general, rigid linkers exhibit relatively stiff structures by adopting α -helical structures or by containing multiple Pro residues. Under many circumstances, they separate the functional domains more efficiently than the flexible linkers. The length of the linkers can be easily adjusted by changing the copy number to achieve an optimal distance between domains. As a result, rigid linkers are chosen when the spatial separation of the domains is critical to preserve the stability or bioactivity of the fusion proteins. In this regard, alpha helix-forming linkers with the sequence of (EAAAK)_n (SEQ ID NO: 776) have been applied to the construction of many recombinant fusion proteins. Another type of rigid linkers has a Pro-rich sequence, (XP)_n, with X designating any amino acid, preferably Ala, Lys, or Glu.

Merely to illustrate, exemplary linkers include:

Table 6. Exemplary Linkers

Type	Sequence	SEQ ID NO:
Flexible	(GGGGS) _n (e.g., n = 1-6)	606
Flexible	(Gly) ₈	607
Flexible	(Gly) ₆	608
Flexible	KESGSVSSEQLAQFRSLD	609
Flexible	EGKSSGSGSESKST	610
Flexible	GSAGSAAGSGEF	611
Rigid	(EAAAK) _n (e.g., n = 1-6)	612
Rigid	A(EAAAK) ₄ ALEA(EAAAK) ₄ A	613
Rigid	PAPAP	614
Rigid	AEAAAKEEAAKA	615
Rigid	(Ala-Pro) _n (10 to 34 aa)	616

Other linkers that may be used in the subject fusion proteins include but are not limited to, SerGly, GGSG (SEQ ID NO: 617), GSGS (SEQ ID NO: 618), GGGs (SEQ ID NO: 619), S(GGS)_n (SEQ ID NO: 620) where n is 1-7, GRA, poly(Gly), poly(Ala), GGGSGGG (SEQ ID NO: 621), ESGGGGV (SEQ ID NO: 622), LESGGGGV (SEQ ID NO: 623), GRAQVT

(SEQ ID NO: 624), WRAQVT (SEQ ID NO: 625), and ARGRAQVT (SEQ ID NO: 626). The hinge regions of the Fc fusions described below may also be considered linkers.

Various elements can be employed to anchor proteins on the plasma membrane of cells. For example, the transmembrane domains (TM) of type-I (oriented with the N-terminus outside the cell) and type-II (oriented with the N-terminus in the cytosol) integral membrane proteins can be used to target chimeric proteins to the plasma membrane. Proteins can also be attached to the cell surface by fusion of a GPI (glycophosphatidylinositol lipid) signal to the 3' end of genes. Cleavage of the short carboxy-terminal peptide allows attachment of a glycolipid to the newly exposed C-terminus through an amide linkage. See Udenfriend et al. (1995) "How Glycosylphosphatidylinositol Anchored Membrane Proteins are Made" *Annu Rev Biochem* 64:563–591.

In some embodiments, the fusion protein includes a transmembrane polypeptide sequence (a transmembrane domain). The distinguishing features of appropriate transmembrane polypeptides comprise the ability to be expressed at the surface of the cell on which the AFFIMER® agent is to be displayed. In some embodiments, that may be an immune cell, in particular lymphocyte cells or Natural killer (NK) cells, and once there to interact with CD33 so as to directing cellular response of the immune cell against a predefined target tumor cell on which CD33 is upregulated. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non limiting examples, the transmembrane polypeptide can be a subunit of the T cell receptor such as α , β , γ or δ , polypeptide constituting CD3 complex, IL2 receptor p55 (a chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fc γ receptor III or CD proteins. Alternatively, the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine.

In some embodiments, a polypeptide, a sequence that signals for the posttranslational addition of a glycosylphosphatidylinositol (GPI) anchor. GPI anchors are glycolipid structures that are added post-translationally to the C-terminus of many eukaryotic proteins. This modification to the AFFIMER® agent will cause it to be anchored (attached) on the extracellular surface of the cell membrane of the cell in which the AFFIMER® agent is re-expressed as a recombinant protein (e.g., an encoded AFFIMER® construct as described below). In these embodiments, the GPI anchor domain is C-terminal to the AFFIMER® polypeptide sequence, and preferably occurs at the C-terminus of the fusion protein.

In some embodiments, the GPI anchor domain is a polypeptide that signals for the posttranslational addition of a GPI anchor when the fusion protein of which it is a part is

expressed in a eukaryotic system. The GPI anchor signal sequence consists of a set of small amino acids at the site of anchor addition (the ω site) followed by a hydrophilic spacer and ending in a hydrophobic stretch (Low, (1989) FASEB J. 3:1600-1608). Cleavage of this signal sequence occurs in the ER before the addition of an anchor with conserved central components but with variable peripheral moieties (Homans et al., Nature, 333:269-272 (1988)). The C-terminus of a GPI-anchored protein is linked through a phosphoethanolamine bridge to the highly conserved core glycan, mannose(α 1-2)mannose(α 1-6)mannose(α 1-4)glucosamine(α 1-6)myo-inositol. A phospholipid tail attaches the GPI anchor to the cell membrane.

Exemplary GPI anchor domains that can be used in the subject AFFIMER® polypeptide-containing fusion proteins include:

SGTTS GTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 628)

SGTSPGLSAGATVGIMIGVLVGVALI (SEQ ID NO: 629)

SAPVLSAVATVGITIGVLARVALI (SEQ ID NO: 630)

SSPDLSAGTAVSIMIGVLGMAI (SEQ ID NO: 631)

TLGGNSASYTFVSLFSAVTLLLLC (SEQ ID NO: 632)

SGTSPGLSAGATVGIMIGVLVGVALI (SEQ ID NO: 633)

GPI anchor attachment can be achieved by expression of the AFFIMER® fusion protein containing the GPI anchor domain in a eukaryotic system capable of carrying out GPI posttranslational modifications. As with the transmembrane domain fusion proteins, human cells, including lymphocytes and other cells involved in initiating or promoting an antitumor are so capable and can be engineered to express and encoded AFFIMER® construct including a GPI anchor domain in order retain the expressed AFFIMER® polypeptide containing fusion on the surface of the engineered cell.

Still other modifications that can be made to the AFFIMER® polypeptide sequence or to a flanking polypeptide moiety provided as part of a fusion protein is at least one sequence that is a site for post-translational modification by an enzyme. These can include, but are not limited to, glycosylation, acetylation, acylation, lipid-modification, palmitoylation, palmitate addition, phosphorylation, glycolipid-linkage modification, and the like.

B. Multispecific Fusion Proteins

In some embodiments, an AFFIMER® agent is a multispecific polypeptide including, for example, a first CD33 AFFIMER® polypeptide and at least one additional binding domain. The additional binding domain may be a polypeptide sequence selected from amongst, to illustrate, a second AFFIMER® polypeptide (which may be the same or different than the first AFFIMER® polypeptide), an antibody or fragment thereof or other antigen binding polypeptide, a ligand

binding portion of a receptor (such as a receptor trap polypeptide), a receptor-binding ligand (such as a cytokine, growth factor or the like), engineered T-cell receptor, an enzyme or catalytic fragment thereof.

In some embodiments, an AFFIMER® agent includes at least one additional AFFIMER® polypeptide sequence that is also directed to CD33. The additional CD33 AFFIMER® polypeptide(s) may be the same or different (or a mixture thereof) as the first CD33 AFFIMER® polypeptide in order to create a multispecific AFFIMER® fusion protein. The AFFIMER® agents can bind the same or overlapping sites on CD33 or can bind two different sites such that the CD33 AFFIMER® agent can simultaneously bind two sites on the same CD33 protein (biparatopic) or more than two sites (multiparatopic).

In some embodiments, an AFFIMER® agent includes at least one antigen binding site from an antibody. The resulting AFFIMER® agent can be a single chain including both the CD33 AFFIMER® polypeptide and the antigen binding site (such as in the case of an scFv) or can be a multimeric protein complex such as in antibody assembled with heavy and/or light chains to which the sequence of the anti-CD33 antibody has also been fused.

In some embodiments, with respect to a multispecific AFFIMER® agent comprising a full-length immunoglobulin, the fusion of the AFFIMER® polypeptide sequence to the antibody will preserve the Fc function of the Fc region of the immunoglobulin. For example, the AFFIMER® agent may be capable of binding, via its Fc portion, to the Fc receptor of Fc receptor-positive cells. In some further embodiments, the AFFIMER® agent may activate the Fc receptor-positive cell by binding to the Fc receptor-positive cell, thereby initiating or increasing the expression of cytokines and/or co-stimulatory antigens. Furthermore, the AFFIMER® agent may transfer at least a second activation signal required for physiological activation of the T cell to the T cell via the co-stimulatory antigens and/or cytokines.

In some embodiments, resulted from the binding of its Fc portion to other cells that express Fc receptors present on the surface of effector cells from the immune system, such as immune cells, hepatocytes, and endothelial cells, the AFFIMER® agent may possess antibody-dependent cellular cytotoxicity (ADCC) function, a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigen has been bound by an antibody, and therefore, trigger tumor cell death via ADCC. In some further embodiments, the AFFIMER® agent is capable of demonstrating ADCC function.

As described above, apart from the Fc-mediated cytotoxicity, the Fc portion may contribute to maintaining the serum levels of the AFFIMER® agent, critical for its stability and

persistence in the body. For example, when the Fc portion binds to Fc receptors on endothelial cells and on phagocytes, the AFFIMER® agent may become internalized and recycled back to the blood stream, enhancing its half-life within the body.

Exemplary targets of the additional AFFIMER® polypeptides include but are not limited to, another immune checkpoint protein, and immune co-stimulatory receptor (particularly if the additional AFFIMER® polypeptide(s) can agonize the co-stimulatory receptor), a receptor, a cytokine, a growth factor, or a tumor-associated antigen, mere to illustrate.

Where the AFFIMER® agent is an AFFIMER® polypeptide-antibody fusion protein, the immunoglobulin portion, for example, may be an immunoglobulin is a monoclonal antibody against CD20, CD30, CD33, CD38, CD52, VEGF, VEGF receptors, EGFR or Her2/neu. A few illustrative examples for such immunoglobulins include an antibody comprised within any of the following: trastuzumab, panitumumab, cetuximab, obinutuzumab, rituximab, pertuzumab, alemtuzumab, bevacizumab, tositumomab, ibritumomab, ofatumumab, brentuximab and gemtuzumab.

In some embodiments, the CD33 AFFIMER® polypeptide is part of an AFFIMER® agent that includes one more binding domains that inhibit an immune checkpoint molecule, such as those expressed on a T-cell, including but not limited to PD-1, PD-L2, CTLA-4, NKG2A, KIR, LAG-3, TIM-3, CD96, VISTA, or TIGIT.

In some embodiments, the CD33 AFFIMER® polypeptide is part of an AFFIMER® agent that includes one more binding domains that agonizes an immune co-stimulatory molecule, such as expressed on a T-cell, including but not limited to CD28, ICOS, CD137, OX40, GITR, CD27, CD30, HVEM, DNAM-1 or CD28H.

In some embodiments, the CD33 AFFIMER® polypeptide is part of an AFFIMER® agent that includes one more ligand agonists of immune co-stimulatory molecules, such as an agonist ligand for CD28, ICOS, CD137, OX40, GITR, CD27, CD30, HVEM, DNAM-1 or CD28H.

In some embodiments, the CD33 AFFIMER® polypeptide is part of an AFFIMER® agent that includes one more binding domains that bind to a protein upregulated in the tumor microenvironment, e.g., a tumor associated antigen, such as upregulated on tumor cells in the tumor, or macrophage, fibroblasts, T-cells or other immune cells that infiltrate the tumor.

In some embodiments, the CD33 AFFIMER® polypeptide is part of an AFFIMER® agent that includes one more binding domains that bind to a protein selected from the groups consisting of CEACAM-1, CEACAM-5, BTLA, LAIR1, CD160, 2B4, TGFR, B7-H3, B7-H4, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, Galectin-9,

GITRL, HHLA2, ICOS, ICOSL, LIGHT, MHC class I or II, NKG2a, NKG2d, OX4OL, PVR, SIRP α , TCR, CD20, CD30, CD33, CD38, CD52, VEGF, VEGF receptors, EGFR, Her2/neu, ILT1, ILT2, ILT3, ILT4, ILT5, ILT6, ILT7, ILT8, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3, NKG2A, NKG2C, NKG2E or TSLP.

See William BA et al. J. Clin. Med. 2019; 8(8): 1261 for a review of CD33 AFFIMER® agent formats encompassed by the present disclosure.

In some embodiments, a multispecific a CD33 AFFIMER® agent may further comprise a half-life extension moiety, such as any of those described herein. For example, a CD33 AFFIMER® agent may comprise at least one CD33 AFFIMER® polypeptide linked through a peptide linker to a binding domain specific for at least one immune cell (e.g., T cell and/or NK cell) binding domain (e.g., CD3 ϵ chain or CD16) further linked to a half-life extension moiety, such as a fragment crystallizable (Fc) domain, human serum albumin (HSA), or an HSA AFFIMER® polypeptide. In some embodiments, the half-life extension moiety is a fragment crystallizable (Fc) domain. In some embodiments, the half-life extension moiety is a human serum albumin (HSA). In some embodiments, the half-life extension moiety is an HSA AFFIMER® polypeptide.

1. Bispecific Cell Engagers

Provided herein, in some embodiments, are CD33 AFFIMER® agents formatted to bind to two different antigens. Non-limiting examples of such CD33 AFFIMER® agent formats include chemically conjugated antibodies (e.g., a CD33 AFFIMER® polypeptide conjugated to anti-CD16 antibody, antibody fragment, or antibody mimetic), BiTEs®, BiKEs™, and bispecific tandem diabodies.

a) BiTEs®

In some embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide linked to a CD3-specific antibody (e.g., an anti-CD3 ϵ antibody, antibody fragment (e.g., a single variable portion, V_H and V_L, of an antibody), or antibody mimetic). For example, a CD33 AFFIMER® polypeptide conjugated to a CD3-specific antibody forms a bispecific T cell-engager (BiTE®) antibody-AFFIMER® complex. Canonical BiTEs® are recombinant proteins made from two flexibly linked antibody-derived binding domains. These canonical BiTE® molecules typically include a tumor-specific antigen binding domain, a peptide linker, and a T-cell-binding domain (a binding domain specific for the CD3 ϵ chain). The present disclosure provides, in some embodiments, bispecific molecules that comprise a CD33 AFFIMER® polypeptide as the tumor-specific antigen-binding domain, a peptide linker, and a T-cell-binding

domain (e.g., a binding domain specific for the CD3 ϵ chain). Binding of these bispecific molecules to CD3 ϵ chain promotes T cell-mediated anti-tumor activity after engaging with CD33 antigen, directing the activity of CD3 $^{+}$ T cells, such as CD3 $^{+}$ CD8 $^{+}$ T cells, towards CD33 $^{+}$ cells. This allows circulating T cells in a subject to be redirected towards CD33 $^{+}$ cells (e.g., tumor cells) without the need for *ex vivo* expression of a CAR. See, e.g., Aigner M. et al. *Leukemia* 2013; 27: 1107–1115 for a description of a CD33/CD3-bispecific BiTE $^{\circledR}$ antibody construct.

b) BiKEs $^{\text{TM}}$

In some embodiments, a CD33 AFFIMER $^{\circledR}$ agent comprises a CD33 AFFIMER $^{\circledR}$ polypeptide linked to a CD16-specific antibody (e.g., an anti-CD16 antibody, antibody fragment (e.g., a single variable portion, V $_H$ and V $_L$, of an antibody), or antibody mimetic). For example, a CD33 AFFIMER $^{\circledR}$ polypeptide conjugated to a CD16-specific antibody forms a bispecific NK cell-engager (BiKE $^{\text{TM}}$) antibody-AFFIMER $^{\circledR}$ complex. Canonical BiTEs comprise two antibody fragments, a first recognizing a tumor antigen and a second directed against CD16 on NK cells, which together trigger antibody-dependent cell-mediated cytotoxicity.

In some embodiments, a CD33 AFFIMER $^{\circledR}$ agent comprises a CD33 AFFIMER $^{\circledR}$ polypeptide linked to a single-chain variable fragment (scFv) domain specific for CD16 on NK cells.

c) Bispecific Tandem Binders

In some embodiments, a CD33 AFFIMER $^{\circledR}$ agent is formatted as a bispecific tetravalent molecule. For example, a CD33 AFFIMER $^{\circledR}$ agent may be formatted as a single chain construct constructed by linking two CD33 AFFIMER $^{\circledR}$ polypeptides to two antibody variable domains (V $_H$ and V $_L$) with specificities for human CD3 (T cell antigen).

2. Trispecific Cell Engagers

Also provided herein, in some embodiments, are CD33 AFFIMER $^{\circledR}$ agents formatted to bind to associate with three different antigens. Non-limiting examples of such CD33 AFFIMER $^{\circledR}$ agent formats include TriKEs $^{\text{TM}}$, TriNKETs $^{\text{TM}}$ and tandem triple scFvs.

a) TriKEs $^{\text{TM}}$

In some embodiments, a CD33 AFFIMER $^{\circledR}$ agent comprises a CD33 AFFIMER $^{\circledR}$ polypeptide linked to human interleukin (IL)-15 and a CD16-specific antibody (e.g., an anti-CD16 antibody, antibody fragment (e.g., a single variable portion, V $_H$ and V $_L$, of an antibody), or antibody mimetic). For example, a CD33 AFFIMER $^{\circledR}$ agent may comprise a single chain variable fragments (scFv) that is crosslinked with human IL-15 and a CD33 AFFIMER $^{\circledR}$ polypeptide that is crosslinked with the human IL-15 to form a trispecific NK cell-engager (TriKE $^{\text{TM}}$) antibody-AFFIMER $^{\circledR}$ complex. The scFv recognizes the anti-CD16 marker on NK

cells, and the CD33 AFFIMER® polypeptide recognizes CD33 expressed on the tumor cell. The IL-15 component of TriKE provides a self-sustaining signal that activates NK cells and enhances their ability to kill tumor cells. Compared to BiKEs™, TriKEs™ elicit superior NK cytotoxicity and NK cell persistence in a xenograft tumor model *in vivo* and are proposed to be effective adjuncts to existing NK transfer protocols.

In some embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide and a single-chain variable fragment (scFv) domain specific for CD16 on natural killer (NK) cells, each crosslinked to an IL-15 protein. This CD33 AFFIMER® agent is capable of, for example, directing NK cells to tumors by facilitating formation of intracellular synapses, binding CD16 on NK cells to trigger ADC, and driving *in vivo* NK cell expansion. IL-15 promotes NK cell activation, expansion and survival

For a review of BiKE™ and TriKE™ technology, see Felices M et al. *Methods Mol Bio.* 2016; 1441: 333-346, incorporated herein by reference.

b) TriNKETs™

Tri-specific, NK cell Engager Therapies (TriNKETs™) are also encompassed by the present disclosure. In some embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide linked to a domain that binds an NKG2D receptor on NK cells and a domain that binds a CD16 receptor on natural killer cells. Such CD33 AFFIMER® agents can engage more than one kind of NK activating receptor and may block the binding of natural ligands to NKG2D. In some embodiments, these CD33 AFFIMER® agents can agonize NK cells in humans. See International Publication No. WO2019/164930, incorporated herein by reference.

In some embodiments, a CD33 AFFIMER® agent comprises (a) an antibody (e.g., an antibody fragment or antibody mimetic) that binds NKG2D; (b) a CD33 AFFIMER® polypeptide; and (c) an antibody (e.g., an antibody fragment or antibody mimetic) that binds CD16.

In some embodiments, a CD33 AFFIMER® agent comprises (a) an antibody Fab fragment that binds NKG2D; (b) a CD33 AFFIMER® polypeptide; and (c) an antibody Fc domain that binds CD16. In some embodiments, the CD33 AFFIMER® polypeptide is linked to the antibody Fab fragment or the antibody Fc domain via a hinge comprising Ala-Ser or Gly-Ala-Ser.

c) Tandem Triple Binders

In some embodiments, a CD33 AFFIMER® agent is formatted as a tandem triple scFv molecule. For example, a CD33 AFFIMER® agent may comprise two CD33 AFFIMER®

polypeptides linked to an scFv domain specific for CD16. In some embodiments, a CD33 AFFIMER® agent may comprise a CD33 AFFIMER® polypeptide linked to an scFv domain specific for CD16 and an scFv specific for CD123.

3. Tetraspecific Cell Engagers

Also provided herein, in some embodiments, are CD33 AFFIMER® agents formatted to associates with four different antigens. A non-limiting examples of such a CD33 AFFIMER® agent format includes TetraKEs™. In some embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide and a single-chain variable fragment (scFv) domain specific for CD16 on natural killer (NK) cells, each crosslinked to an IL-15 protein, and further comprising an scFv that specifically binds to CD133 on cancer stem cells in order to promote ADCC. In some embodiments, the scFv that specifically binds to CD133 is linked to the CD33 AFFIMER® polypeptide through a hinge region (e.g., mutated IgG/hinge). See, e.g., Schmohl JU et al. *Oncotarget*. 2016; 7(45): 73830.

4. Engineered Cells

CD33 AFFIMER® polypeptides, in some embodiments, are linked to an immune cell. Adoptive cell therapy, also known as cellular immunotherapy, is a form of treatment that uses immune to treat cancer. Non-limiting examples of adoptive cell therapies include tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, and natural killer (NK) cell therapy.

TIL therapy involves naturally occurring T cells that have already infiltrated patients' tumors, and then activating and expands the. Then, large numbers of these activated T cells are re-infused into patients, where they can then seek out and destroy tumors. In some embodiments, TILs are linked to or modified to express a CD33 AFFIMER® polypeptides.

TCR therapy involves taking T cells from patients, but instead of just activating and expanding the available anti-tumor T cells, the T cells can also be equipped with a new T cell receptor that enables them to target specific cancer antigens. In some embodiments, T cells (e.g., CD8+ T cells) obtained from a patient are equipped with a CD33 AFFIMER® polypeptide.

Canonical TIL and TCR therapies can only target and eliminate cancer cells that present their antigens in a certain context (e.g., when the antigens are bound by the major histocompatibility complex, or MHC). To overcome this limitation, a patient's T cells can be equipped with a synthetic receptor known as a CAR. A CAR comprises an antigen-binding fragment, at least one hinge and/or linker domain, a transmembrane domain, and at least one intracellular signaling domain. Binding of the antigen-binding fragment to a target antigen, such as CD33, results in transduction of a signal mediated by the intracellular signaling domains of

the CAR. CARs are typically expressed on the surface of a T cell, such as a CD8⁺ T cell, to direct the activity of the T cell towards cells expressing the antigen recognized by the CAR. CD8⁺ T cells expressing CARs with antigen-binding fragments specific to CD33 are useful for targeted killing of CD33⁺ cells, such as cancer cells. *See, e.g., Dutour A et al., Advances in Hematology 2012 Article ID 683065.*

More recently, adoptive cell therapy strategies have begun to incorporate other immune cells, such as Natural Killer (NK) cells. One application being explored in the clinic involves equipping these NK cells with cancer-targeting CARs. In some embodiments, NK cells are linked to or modified to express an CD33 AFFIMER® polypeptide.

In some embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide, a linker sequence, a transmembrane domain, and a CD3z signaling domain. In other embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide, a linker sequence, a transmembrane domain, a CD28 co-stimulatory domain, and a CD3z signaling domain. In yet other embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide, a linker sequence, a transmembrane domain, a 4-1BB co-stimulatory domain, and a CD3z signaling domain. In still other embodiments, a CD33 AFFIMER® agent comprises a CD33 AFFIMER® polypeptide, a linker sequence, a transmembrane domain, a CD28 co-stimulatory domain and a 4-1BB co-stimulatory domain, and a CD3z signaling domain.

Also provided herein are immune cells, e.g., T cells or NK cells, comprising any one or more of the CD33 AFFIMER® agent described above.

C. Engineering PK and ADME Properties

In some embodiment, the AFFIMER® agent may not have a half-life and/or PK profile that is optimal for the route of administration, such as parenteral therapeutic dosing. A “half-life” is the amount of time it takes for a substance, such as an AFFIMER® agent of the present disclosure, to lose half of its pharmacologic or physiologic activity or concentration. Biological half-life can be affected by elimination, excretion, degradation (e.g., enzymatic) of the substance, or absorption and concentration in certain organs or tissues of the body. In some embodiments, biological half-life can be assessed by determining the time it takes for the blood plasma concentration of the substance to reach half its steady state level (“plasma half-life”). To address this shortcoming, there are a variety of general strategies for prolongation of half-life that have been used in the case of other protein therapeutics, including the incorporation of half-life extending moieties as part of the AFFIMER® agent.

The term “half-life extending moiety” refers to a pharmaceutically acceptable moiety, domain, or molecule covalently linked (chemically conjugated or fused) to an AFFIMER®

polypeptide to form an AFFIMER® agent described herein, optionally via a non-naturally encoded amino acid, directly or via a linker, that prevents or mitigates *in vivo* proteolytic degradation or other activity-diminishing modification of the AFFIMER® polypeptide, increases half-life, and/or improves or alters other pharmacokinetic or biophysical properties including but not limited to increasing the rate of absorption, reducing toxicity, improving solubility, reducing protein aggregation, increasing biological activity and/or target selectivity of the modified AFFIMER® polypeptide, increasing manufacturability, and/or reducing immunogenicity of the modified AFFIMER® polypeptide, compared to a comparator such as an unconjugated form of the modified AFFIMER® polypeptide. The term “half-life extending moiety” includes non-proteinaceous, half-life extending moieties, such as a water soluble polymer such as polyethylene glycol (PEG) or discrete PEG, hydroxyethyl starch (HES), a lipid, a branched or unbranched acyl group, a branched or unbranched C8-C30 acyl group, a branched or unbranched alkyl group, and a branched or unbranched C8-C30 alkyl group; and proteinaceous half-life extending moieties, such as serum albumin, transferrin, adnectins (e.g., albumin-binding or pharmacokinetics extending (PKE) adnectins), Fc domain, and unstructured polypeptide, such as XTEN and PAS polypeptide (e.g. conformationally disordered polypeptide sequences composed of the amino acids Pro, Ala, and/or Ser), and a fragment of any of the foregoing. An examination of the crystal structure of an AFFIMER® polypeptide and its interaction with its target, such as the CD33 AFFIMER® complex with PD-1 shown in the FIGS., can indicate which certain amino acid residues have side chains that are fully or partially accessible to solvent.

In some embodiments, the half-life extending moiety extends the half-life of the resulting AFFIMER® agent circulating in mammalian blood serum compared to the half-life of the protein that is not so conjugated to the moiety (such as relative to the AFFIMER® polypeptide alone). In some embodiments, half-life is extended by greater than or greater than about 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold., 5.0-fold, or 6.0-fold. In some embodiments, half-life is extended by more than 6 hours, more than 12 hours, more than 24 hours, more than 48 hours, more than 72 hours, more than 96 hours or more than 1 week after *in vivo* administration compared to the protein without the half-life extending moiety.

As means for further exemplification, half-life extending moieties that can be used in the generation of AFFIMER® agents of the disclosure include:

- Genetic fusion of the pharmacologically AFFIMER® sequence to a naturally long-half-life protein or protein domain (e.g., Fc fusion, transferrin [Tf] fusion, or albumin fusion. See, for example, Beck et al. (2011) “Therapeutic Fc-fusion proteins and peptides as successful alternatives to antibodies. MAbs. 3:1–2; Czajkowsky et al. (2012) “Fc-fusion

proteins: new developments and future perspectives. *EMBO Mol Med.* 4:1015–28; Huang et al. (2009) “Receptor-Fc fusion therapeutics, traps, and Mimetibody technology” *Curr Opin Biotechnol.* 2009;20:692–9; Keefe et al. (2013) “Transferrin fusion protein therapies: acetylcholine receptor-transferrin fusion protein as a model. In: Schmidt S, editor. *Fusion protein technologies for biopharmaceuticals: applications and challenges.* Hoboken: Wiley; p. 345–56; Weimer et al. (2013) “Recombinant albumin fusion proteins. In: Schmidt S, editor. *Fusion protein technologies for biopharmaceuticals: applications and challenges.* Hoboken: Wiley; 2013. p. 297–323; Walker et al. (2013) “Albumin-binding fusion proteins in the development of novel long-acting therapeutics. In: Schmidt S, editor. *Fusion protein technologies for biopharmaceuticals: applications and challenges.* Hoboken: Wiley; 2013. p. 325–43.

- Genetic fusion of the pharmacologically AFFIMER® sequence to an inert polypeptide, e.g., XTEN (also known as recombinant PEG or “rPEG”), a homoamino acid polymer (HAP; HAPylation), a proline-alanine-serine polymer (PAS; PASylation), or an elastin-like peptide (ELP; ELPylation). See, for example, Schellenberger et al. (2009) “A recombinant polypeptide extends the *in vivo* half-life of peptides and proteins in a tunable manner. *Nat Biotechnol.* 2009;27:1186–90; Schlapschy et al. Fusion of a recombinant antibody fragment with a homo-amino-acid polymer: effects on biophysical properties and prolonged plasma half-life. *Protein Eng Des Sel.* 2007;20:273–84; Schlapschy (2013) PASylation: a biological alternative to PEGylation for extending the plasma halflife of pharmaceutically active proteins. *Protein Eng Des Sel.* 26:489–501. Floss et al. (2012) “Elastin-like polypeptides revolutionize recombinant protein expression and their biomedical application. *Trends Biotechnol.* 28:37–45. Floss et al. “ELP-fusion technology for biopharmaceuticals. In: Schmidt S, editor. *Fusion protein technologies for biopharmaceuticals: application and challenges.* Hoboken: Wiley; 2013. p. 372–98.

- Increasing the hydrodynamic radius by chemical conjugation of the pharmacologically active peptide or protein to repeat chemical moieties, e.g., to PEG (PEGylation) or hyaluronic acid. See, for example, Caliceti et al. (2003) “Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates” *Adv Drug Delivery Rev.* 55:1261–77; Jevsevar et al. (2010) PEGylation of therapeutic proteins. *Biotechnol J* 5:113–28; Kontermann (2009) “Strategies to extend plasma half-lives of recombinant antibodies” *BioDrugs.* 23:93–109; Kang et al. (2009) “Emerging PEGylated drugs” *Expert Opin Emerg Drugs.* 14:363–80; and Mero et al. (2013) “Conjugation of hyaluronan to proteins” *Carb Polymers.* 92:2163–70.

- Significantly increasing the negative charge of fusing the pharmacologically active peptide or protein by polysialylation; or, alternatively, (b) fusing a negatively charged, highly sialylated peptide (e.g., carboxy-terminal peptide [CTP; of chorionic gonadotropin (CG) b-chain]), known to extend the half-life of natural proteins such as human CG b-subunit, to the biological drug candidate. See, for example, Gregoriadis et al. (2005) “Improving the therapeutic efficacy of peptides and proteins: a role for polysialic acids” *Int J Pharm.* 2005; 300:125–30; Duijkers et al. “Single dose pharmacokinetics and effects on follicular growth and serum hormones of a long-acting recombinant FSH preparation (FSHCTP) in healthy pituitary-suppressed females” (2002) *Hum Reprod.* 17:1987–93; and Fares et al. “Design of a longacting follitropin agonist by fusing the C-terminal sequence of the chorionic gonadotropin beta subunit to the follitropin beta subunit” (1992) *Proc Natl Acad Sci USA.* 89:4304–8. 35; and Fares “Half-life extension through O-glycosylation.
- Binding non-covalently, via attachment of a peptide or protein-binding domain to the bioactive protein, to normally long-half-life proteins such as HSA, human IgG, transferrin or fibronectin. See, for example, Andersen et al. (2011) “Extending half-life by indirect targeting of the neonatal Fc receptor (FcRn) using a minimal albumin binding domain” *J Biol Chem.* 286:5234–41; O’Connor-Semmes et al. (2014) “GSK2374697, a novel albumin-binding domain antibody (albudAb), extends systemic exposure of extendin-4: first study in humans—PK/PD and safety” *Clin Pharmacol Ther.* 2014;96:704–12. Sockolosky et al. (2014) “Fusion of a short peptide that binds immunoglobulin G to a recombinant protein substantially increases its plasma half-life in mice” *PLoS One.* 2014;9:e102566.

Classical genetic fusions to long-lived serum proteins offer an alternative method of half-life extension distinct from chemical conjugation to PEG or lipids. Two major proteins have traditionally been used as fusion partners: antibody Fc domains and human serum albumin (HSA). Fc fusions involve the fusion of peptides, proteins or receptor exodomains to the Fc portion of an antibody. Both Fc and albumin fusions achieve extended half-lives not only by increasing the size of the peptide drug, but both also take advantage of the body’s natural recycling mechanism: the neonatal Fc receptor, FcRn. The pH-dependent binding of these proteins to FcRn prevents degradation of the fusion protein in the endosome. Fusions based on these proteins can have half-lives in the range of 3-16 days, much longer than typical PEGylated or lipidated peptides. Fusion to antibody Fc domains can improve the solubility and stability of the peptide or protein drug. An example of a peptide Fc fusion is dulaglutide, a GLP-1 receptor agonist currently in late-stage clinical trials. Human serum albumin, the same protein exploited by the fatty acylated peptides is the other popular fusion partner. Albiglutide is a GLP-1 receptor

agonist based on this platform. A major difference between Fc and albumin is the dimeric nature of Fc versus the monomeric structure of HSA leading to presentation of a fused peptide as a dimer or a monomer depending on the choice of fusion partner. The dimeric nature of an AFFIMER® polypeptide-Fc fusion can produce an avidity effect if the AFFIMER® polypeptide target, such as CD33 on tumor cells, are spaced closely enough together or are themselves dimers. This may be desirable or not depending on the target.

1. Fc Fusions

In some embodiments, the AFFIMER® polypeptide may be part of a fusion protein with an immunoglobulin Fc domain ("Fc domain"), or a fragment or variant thereof, such as a functional Fc region. In this context, an Fc fusion ("Fc-fusion"), such as a CD33 AFFIMER® agent created as an AFFIMER® polypeptide-Fc fusion protein, is a polypeptide comprising at least one CD33 AFFIMER® polypeptide sequence covalently linked through a peptide backbone (directly or indirectly) to an Fc region of an immunoglobulin. An Fc-fusion may comprise, for example, the Fc region of an antibody (which facilitates effector functions and pharmacokinetics) and a CD33 AFFIMER® polypeptide sequence as part of the same polypeptide. An immunoglobulin Fc region may also be linked indirectly to at least one CD33 AFFIMER® polypeptide. Various linkers are known in the art and can optionally be used to link an Fc to a polypeptide including a CD33 AFFIMER® polypeptide sequence to generate an Fc-fusion. In some embodiments, Fc-fusions can be dimerized to form Fc-fusion homodimers, or using non-identical Fc domains, to form Fc-fusion heterodimers.

In some embodiments, an Fc-fusion homodimer comprises a dimer of a CD33 AFFIMER® agent that comprises a CD33 AFFIMER® polypeptide linked to an Fc domain linked to another CD33 AFFIMER® polypeptide (CD33 AFFIMER® polypeptide-Fc domain-CD33 AFFIMER® polypeptide).

There are several reasons for choosing the Fc region of human antibodies for use in generating CD33 AFFIMER® agents as CD33 AFFIMER® fusion proteins. The principle rationale is to produce a stable protein, large enough to demonstrate a similar pharmacokinetic profile compared with those of antibodies, and to take advantage of the properties imparted by the Fc region; this includes the salvage neonatal FcRn receptor pathway involving FcRn-mediated recycling of the fusion protein to the cell surface post endocytosis, avoiding lysosomal degradation and resulting in release back into the bloodstream, thus contributing to an extended serum half-life. Another obvious advantage is the Fc domain's binding to Protein A, which can simplify downstream processing during production of the AFFIMER® agent and permit generation of highly pure preparation of the AFFIMER® agent.

In general, an Fc domain will include the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, Fc domain refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains C γ 2 and C γ 3 and the hinge between C γ 1 and C γ 2. Although the boundaries of the Fc domain may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as set forth in Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, NIH, Bethesda, Md. (1991)). Fc may refer to this region in isolation, or this region in the context of a whole antibody, antibody fragment, or Fc fusion protein. Polymorphisms have been observed at a number of different Fc positions and are also included as Fc domains as used herein.

In some embodiments, the Fc As used herein, a "functional Fc region" refers to an Fc domain or fragment thereof which retains the ability to bind FcRn. A functional Fc region binds to FcRn but does not possess effector function. The ability of the Fc region or fragment thereof to bind to FcRn can be determined by standard binding assays known in the art. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions can be assessed using various assays known in the art for evaluating such antibody effector functions.

In an exemplary embodiment, the Fc domain is derived from an IgG1 subclass, however, other subclasses (e.g., IgG2, IgG3, and IgG4) may also be used. An exemplary sequence of a human IgG1 immunoglobulin Fc domain which can be used is:

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 635)

In some embodiments, the Fc region used in the fusion protein may comprise the hinge region of an Fc molecule. An exemplary hinge region comprises the core hinge residues spanning positions 1-16 (e.g., DKTHTCPPCPAPELLG ((SEQ ID NO: 636)) of the exemplary human IgG1 immunoglobulin Fc domain sequence provided above. In some embodiments, the

AFFIMER® polypeptide-containing fusion protein may adopt a multimeric structure (e.g., dimer) owing, in part, to the cysteine residues at positions 6 and 9 within the hinge region of the exemplary human IgG1 immunoglobulin Fc domain sequence provided above. In other embodiments, the hinge region as used herein, may further include residues derived from the CH1 and CH2 regions that flank the core hinge sequence of the exemplary human IgG1 immunoglobulin Fc domain sequence provided above. In yet other embodiments, the hinge sequence may comprise or consist of GSTHTCPPCPAPELLG (SEQ ID NO: 637) or EPKSCDKTHTCPPCPAPELLG (SEQ ID NO: 638).

In some embodiments, the hinge sequence may include at least one substitution that confer desirable pharmacokinetic, biophysical, and/or biological properties. Some exemplary hinge sequences include:

EPKSCDKTHTCPPCPAPELLGGPS (SEQ ID NO: 639);
 EPKSSDKTHTCPPCPAPELLGGPS (SEQ ID NO: 640);
 EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO: 641);
 EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 642);
 DKTHTCPPCPAPELLGGPS (SEQ ID NO: 643); and
 DKTHTCPPCPAPELLGGSS (SEQ ID NO: 644).

In some embodiments, the residue P at position 18 of the exemplary human IgG1 immunoglobulin Fc domain sequence provided above may be replaced with S to ablate Fc effector function; this replacement is exemplified in hinges having the sequences EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO: 641), EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 642), and DKTHTCPPCPAPELLGGSS (SEQ ID NO: 644). In another embodiment, the residues DK at positions 1-2 of the exemplary human IgG1 immunoglobulin Fc domain sequence provided above may be replaced with GS to remove a potential clip site; this replacement is exemplified in the sequence EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 642). In another embodiment, the C at the position 103 of the heavy chain constant region of human IgG1 (e.g., domains CH₁-CH₃), may be replaced with S to prevent improper cysteine bond formation in the absence of a light chain; this replacement is exemplified by EPKSSDKTHTCPPCPAPELLGGPS (SEQ ID NO: 640), EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO: 641), and EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 642).

In some embodiments, the Fc is a mammalian Fc such as a human Fc, including Fc domains derived from IgG1, IgG2, IgG3 or IgG4. The Fc region may possess at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with a native Fc region and/or with an Fc region of a parent polypeptide. In some embodiments, the Fc region may have at least

about 90% sequence identity with a native Fc region and/or with an Fc region of a parent polypeptide.

In some embodiments, the Fc domain comprises an amino acid sequence selected from SEQ ID NO: 645-658 or an Fc sequence from the examples provided by SEQ ID NOs: 645-658. It should be understood that the C-terminal lysine of an Fc domain is an optional component of a fusion protein comprising an Fc domain. In some embodiments, the Fc domain comprises an amino acid sequence selected from SEQ ID NOs: 645-658, except that the C-terminal lysine thereof is omitted. In some embodiments, the Fc domain comprises the amino acid sequence selected from SEQ ID NO: 645-658. In some embodiments, the Fc domain comprises the amino acid sequence selected from SEQ ID NO: 645-658 except the C-terminal lysine thereof is omitted.

Table 7. Exemplary Immunoglobulin Sequences

Name	Sequence	SEQ ID NO:
hIgG1a_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	645
hIgG1a_189 [hIgG1a_191 sans "GK" on C term; A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSP	646
hIgG1a_191b [A/F subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	647
hIgG1f_1.1_191 [Contains five point- mutations to alter ADCC function, F subtype]	DKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	648
hIgG1f_1.1_186 [Contains five point- mutations to alter ADCC function and C225S (Edlemen numbering); F subtype]	EPKSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG	649

	FYPSPDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	
hIgG1a_(N297G)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGS TYRVVSVLTIVLHQQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	650
hIgG1a_190 [hIgG1a_190 sans "K" on C term; A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTIVLHQQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPG	651
hIgG1a_(N297Q)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQS TYRVVSVLTIVLHQQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	652
hIgG1a_(N297S)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYSS TYRVVSVLTIVLHQQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	653
hIgG1a_(N297A)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTIVLHQQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	654
hIgG1a_(N297H)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYHS TYRVVSVLTIVLHQQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	655
hIgG4	DKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTIVLHQQDWLNGKEYCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK	656
hIgG4_(S241P)	DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTIVLHQQDWLNGKEYCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK	657
hIgG1 (Contain two point-mutations to alter	SEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR	658

ADCC function L20A, L21A)	EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK LTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK	
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“Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) enables these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins.

In some embodiments, the fusion protein includes an Fc domain sequence for which the resulting AFFIMER® agent has no (or reduced) ADCC and/or complement activation or effector functionality. For example, the Fc domain may comprise a naturally disabled constant region of IgG2 or IgG4 isotype or a mutated IgG1 constant region. Examples of suitable modifications are described in EP0307434. One example comprises the substitutions of alanine residues at positions 235 and 237 (EU index numbering).

In other embodiments, the fusion protein includes an Fc domain sequence for which the resulting AFFIMER® agent will retain some or all Fc functionality for example will be capable of one or both of ADCC and CDC activity, as for example if the fusion protein comprises the Fc domain from human IgG1 or IgG3. Levels of effector function can be varied according to known techniques, for example by mutations in the CH2 domain, for example wherein the IgG1 CH2 domain has at least one mutation at positions selected from 239 and 332 and 330, for example the mutations are selected from S239D and I332E and A330L such that the antibody has enhanced effector function, and/or for example altering the glycosylation profile of the antigen-binding protein of the disclosure such that there is a reduction in fucosylation of the Fc region.

2. Albumin Fusions

In some embodiments, the AFFIMER® agent is a fusion protein comprising, in addition to at least one AFFIMER® polypeptide sequence, an albumin sequence or an albumin fragment. In other embodiments, the AFFIMER® agent is conjugated to the albumin sequence or an albumin fragment through chemical linkage other than incorporation into the polypeptide sequence including the AFFIMER® polypeptide. In some embodiments, the albumin, albumin variant, or albumin fragment is human serum albumin (HSA), a human serum albumin variant, or a human serum albumin fragment. Albumin serum proteins comparable to HSA are found in, for example, cynomolgus monkeys, cows, dogs, rabbits and rats. Of the non-human species, bovine serum albumin (BSA) is the most structurally similar to HSA. See, e.g., Kosa et al., (2007) J Pharm Sci. 96(11):3117-24. The present disclosure contemplates the use of albumin

from non-human species, including, but not limited to, albumin sequence derived from cyno serum albumin or bovine serum albumin.

Mature HSA, a 585 amino acid polypeptide (approx. 67 kDa) having a serum half-life of about 20 days, is primarily responsible for the maintenance of colloidal osmotic blood pressure, blood pH, and transport and distribution of numerous endogenous and exogenous ligands. The protein has three structurally homologous domains (domains I, II and III), is almost entirely in the alpha-helical conformation, and is highly stabilized by 17 disulfide bridges. In some embodiments, the AFFIMER® agent can be an albumin fusion protein including at least one AFFIMER® polypeptide sequence and the sequence for mature human serum albumin (SEQ ID NO: 659) or a variant or fragment thereof which maintains the PK and/or biodistribution properties of mature albumin to the extent desired in the fusion protein.

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEF
AKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPER
NECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHP
YFYAPELLFFAKRYKAAFTECCQAADKAAACLLPKLDEL RDEGKASSAQ
RLKCASLQKFGERAFAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTEC
CHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEN
DEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSV
VLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC
LFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPE
AKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSAL
EVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATK
EQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL
(SEQ ID NO: 659)

The albumin sequence can be set off from the AFFIMER® polypeptide sequence or other flanking sequences in the AFFIMER® agent by use of linker sequences as described above.

While unless otherwise indicated, reference herein to “albumin” or to “mature albumin” is meant to refer to HSA. However, it is noted that full-length HSA has a signal peptide of 18 amino acids (MKWVTFISLLFLFSSAYS (SEQ ID NO: 584)) followed by a pro-domain of 6 amino acids (RGVFRR) (SEQ ID NO: 660); this 24 amino acid residue peptide may be referred to as the pre-pro domain. The AFFIMER® polypeptide-HSA fusion proteins can be expressed and secreted using the HSA pre-pro-domain in the recombinant proteins coding sequence. Alternatively, the AFFIMER® polypeptide-HSA fusion can be expressed and secreted through inclusion of other secretion signal sequences, such as described above.

In alternative embodiments, rather than provided as part of a fusion protein with the AFFIMER® polypeptide, the serum albumin polypeptide can be covalently coupled to the AFFIMER® polypeptide-containing polypeptide through a bond other than a backbone amide

bond, such as cross-linked through chemical conjugation between amino acid sidechains on each of the albumin polypeptide and the AFFIMER® polypeptide-containing polypeptide.

3. Serum Binding Domains

In some embodiments, the AFFIMER® agent can include a serum-binding moiety – either as part of a fusion protein (if also a polypeptide) with the AFFIMER® polypeptide sequence or chemically conjugated through a site other than being part of a contiguous polypeptide chain.

In some embodiments, the serum-binding polypeptide is an albumin binding moiety. Albumin contains multiple hydrophobic binding pockets and naturally serves as a transporter of a variety of different ligands such as fatty acids and steroids as well as different drugs. Furthermore, the surface of albumin is negatively charged making it highly water-soluble.

The term “albumin binding moiety” as used herein refers to any chemical group capable of binding to albumin, e.g., has albumin binding affinity. Albumin binds to endogenous ligands such as fatty acids; however, it also interacts with exogenous ligands such as warfarin, penicillin and diazepam. As the binding of these drugs to albumin is reversible the albumin-drug complex serves as a drug reservoir that can enhance the drug biodistribution and bioavailability. Incorporation of components that mimic endogenous albumin-binding ligands, such as fatty acids, has been used to potentiate albumin association and increase drug efficacy.

In some embodiments, a chemical modification method that can be applied in the generation of the subject AFFIMER® agents to increase protein half-life is lipidation, which involves the covalent binding of fatty acids to peptide side chains. Originally conceived of and developed as a method for extending the half-life of insulin, lipidation shares the same basic mechanism of half-life extension as PEGylation, namely increasing the hydrodynamic radius to reduce renal filtration. However, the lipid moiety is itself relatively small and the effect is mediated indirectly through the non-covalent binding of the lipid moiety to circulating albumin. One consequence of lipidation is that it reduces the water-solubility of the peptide but engineering of the linker between the peptide and the fatty acid can modulate this, for example by the use of glutamate or mini PEGs within the linker. Linker engineering and variation of the lipid moiety can affect self-aggregation which can contribute to increased half-life by slowing down biodistribution, independent of albumin. See, for example, Jonassen et al. (2012) *Pharm Res.* 29(8):2104-14.

Other examples of albumin binding moieties for use in the generation of certain AFFIMER® agents include albumin-binding (PKE2) adnectins (See WO2011140086 “Serum Albumin Binding Molecules”, WO2015143199 “Serum albumin-binding Fibronectin Type III

Domains” and WO2017053617 “Fast-off rate serum albumin binding fibronectin type iii domains”), the albumin binding domain 3 (ABD3) of protein G of Streptococcus strain G148, and the albumin binding domain antibody GSK2374697 (“AlbudAb”) or albumin binding nanobody portion of ATN-103 (Ozoralizumab).

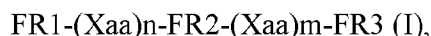
4. AFFIMER® XT

In some embodiments, the molecule that binds a serum protein such as HSA comprises an HSA AFFIMER® polypeptide.

An HSA AFFIMER® polypeptide comprises an AFFIMER® polypeptide in which at least one of the solvent accessible loops is from the wild-type Stefin A protein having amino acid sequences to enable an AFFIMER® polypeptide to bind HSA, selectively, and in some embodiments, with a K_d of 10^{-6} M or less.

In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-9} M to 1×10^{-6} M at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-6} M or less at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-7} M or less at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-8} M or less at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-9} M or less at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-10} M or less at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-11} M or less at pH 7.4 to 7.6. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-9} M to 1×10^{-6} M at pH 7.4. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-6} M or less at pH 7.4. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-7} M or less at pH 7.4. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-8} M or less at pH 7.4. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-9} M or less at pH 7.4. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-10} M or less at pH 7.4. In some embodiments, the AFFIMER® polypeptide binds to HSA with a K_d of 1×10^{-11} M or less at pH 7.4.

In some embodiments, the HSA AFFIMER® polypeptide is derived from the wild-type human Stefin A protein having a backbone sequence and in which one or both of loop 2 (designated (Xaa)_n) and loop 4 (designated (Xaa)_m) are replaced with alternative loop sequences (Xaa)_n and (Xaa)_m, to have the general Formula (I):



wherein FR1 is an amino acid sequence having at least 70% identity to MIPGGLSEAK PATPEIQEIV DKVKPQLEEK TNETYGKLEA VQYKTQVLA (SEQ ID NO: 1); FR2 is an amino acid sequence having at least 70% identity to GTNYYIKVRA GDNKYMHLKV FKSL (SEQ ID NO: 2); FR3 is an amino acid sequence having at least 70% identity to EDLVLTGYQV DKNKDDDELGTG F (SEQ ID NO: 3); Xaa, individually for each occurrence, is an amino acid; and n is an integer from 3 to 20, and m is an integer from 3 to 20.

In some embodiments, FR1 is a polypeptide sequence having 80%-98%, 82%-98%, 84%-98%, 86%-98%, 88%-98%, 90%-98%, 92%-98%, 94%-98%, or 96%-98% homology with SEQ ID NO: 1. In some embodiments, FR1 is a polypeptide sequence having 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, or 95% homology with SEQ ID NO: 1. In some embodiments, FR1 is the polypeptide sequence of SEQ ID NO: 1. In some embodiments, FR2 is a polypeptide sequence having at least 80%-96%, 84%-96%, 88%-96%, or 92%-96% homology with SEQ ID NO: 2. In some embodiments, FR2 is a polypeptide sequence having at least 80%, 84%, 88%, 92%, or 96% homology with SEQ ID NO: 2. In some embodiments, FR2 is a polypeptide sequence having at least 80%, 85%, 90%, 95% or even 98% identity with SEQ ID NO: 2. In some embodiments, FR2 is the polypeptide sequence of SEQ ID NO: 2. In some embodiments, FR3 is a polypeptide sequence having at least 80%-95%, 85%-95%, or 90%-95% homology with SEQ ID NO: 3. In some embodiments, FR3 is a polypeptide sequence having at least 80%, 85%, 90%, or 95% homology with SEQ ID NO: 3. In some embodiments, FR3 is the polypeptide sequence of SEQ ID NO: 3.

In some embodiments, an HSA AFFIMER® polypeptide comprises the amino acid sequence represented in general Formula (II):

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA-(Xaa)_n-Xaa2-TNYYIKVRAGDNKYMHLKVF-Xaa3-Xaa4-Xaa5-(Xaa)_m-Xaa6-D-Xaa7-VLTGYQVDKNKDDDELGTG (SEQ ID NO: 4) (II),

wherein Xaa, individually for each occurrence, is an amino acid; n is an integer from 3 to 20, and m is an integer from 3 to 20; Xaa1 is Gly, Ala, Val, Arg, Lys, Asp, or Glu; Xaa2 is Gly, Ala, Val, Ser or Thr; Xaa3 is Arg, Lys, Asn, Gln, Ser, Thr; Xaa4 is Gly, Ala, Val, Ser or Thr; Xaa5 is Ala, Val, Ile, Leu, Gly or Pro; Xaa6 is Gly, Ala, Val, Asp or Glu; and Xaa7 is Ala, Val, Ile, Leu, Arg or Lys.

In some embodiments, Xaa1 is Gly, Ala, Arg or Lys. In some embodiments, Xaa1 is Gly or Arg. In some embodiments, Xaa2 is Gly, Ala, Val, Ser or Thr. In some embodiments, Xaa2 is Gly or Ser. In some embodiments, Xaa3 is Arg, Lys, Asn, Gln, Ser, Thr. In some embodiments, Xaa3 is Arg, Lys, Asn or Gln. In some embodiments, Xaa3 is Lys or Asn. In some embodiments,

Xaa4 is Gly, Ala, Val, Ser or Thr. In some embodiments, Xaa4 is Gly or Ser. In some embodiments, Xaa5 is Ala, Val, Ile, Leu, Gly or Pro. In some embodiments, Xaa5 is Ile, Leu or Pro. In some embodiments, Xaa5 is Leu or Pro. In some embodiments, Xaa6 is Gly, Ala, Val, Asp or Glu. In some embodiments, Xaa6 is Ala, Val, Asp or Glu. In some embodiments, Xaa6 is Ala or Glu. In some embodiments, Xaa7 is Ala, Val, Ile, Leu, Arg or Lys. In some embodiments, Xaa7 is Ile, Leu or Arg. In some embodiments, Xaa7 is Leu or Arg.

In some embodiments, an HSA AFFIMER® polypeptide comprises the amino acid sequence represented in general Formula (III):

MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKT
QVLA-(Xaa)_n-STNYIYIKVRAGDNKYMHLKVFNGP-(Xaa)_m-ADR
VLTGYQVDKNKDDELTGF (SEQ ID NO: 5) (III),

wherein Xaa, individually for each occurrence, is an amino acid; n is an integer from 3 to 20, and m is an integer from 3 to 20. In some embodiments, n is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, n is 8 to 10, 7 to 11, 6 to 12, 5 to 13, 4 to 14, or 3 to 15. In some embodiments, m is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, m is 8 to 10, 7 to 11, 6 to 12, 5 to 13, 4 to 14, or 3 to 15.

In some embodiments, (Xaa)_n is represented by Formula (IV):

aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8-aa9 (IV),

wherein aa1 is an amino acid with a neutral polar hydrophilic side chain; aa2 is an amino acid with a neutral nonpolar hydrophobic side chain; aa3 is an amino acid with a neutral nonpolar hydrophobic side chain; aa4 is an amino acid with a neutral polar hydrophilic side chain; aa5 is an amino acid with a positively charged polar hydrophilic side chain; aa6 is an amino acid with a positively charged polar hydrophilic side chain; aa7 is an amino acid with a neutral nonpolar hydrophobic side chain; aa8 is an amino acid with a neutral nonpolar hydrophobic side chain; and aa9 is an amino acid with a neutral nonpolar hydrophilic side chain.

In some embodiments, (Xaa)_m is represented by formula (V):

aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8-aa9 (V),

wherein aa1 is an amino acid with a neutral nonpolar hydrophobic side chain; aa2 is an amino acid with a positively charged polar hydrophilic side chain; aa3 is an amino acid with a neutral nonpolar hydrophobic side chain; aa4 is an amino acid with a positively charged polar hydrophilic side chain; aa5 is an amino acid with a neutral polar hydrophilic side chain; aa6 is an amino acid with a neutral polar hydrophilic side chain; aa7 is an amino acid with a negatively charged polar hydrophilic side chain; aa8 is an amino acid with a positively charged polar hydrophilic side chain; and aa9 is an amino acid with a neutral nonpolar hydrophilic side chain.

Examples of amino acids with a neutral nonpolar hydrophilic side chain include cysteine (Cys) and glycine (Gly). In some embodiments, the amino acid with a neutral nonpolar hydrophilic side chain is Cys. In some embodiments, the amino acid with a neutral nonpolar hydrophilic side chain is Gly.

Examples of amino acids with a neutral nonpolar hydrophobic side chain include alanine (Ala), isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), proline (Pro), tryptophan (Trp), and valine (Val). In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Ala. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Ile. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Leu. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Met. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Phe. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Pro. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Trp. In some embodiments, the amino acid with a neutral nonpolar hydrophobic side chain is Val.

Examples of amino acids with a neutral polar hydrophilic side chain include asparagine (Asn), glutamine (Gln), serine (Ser), threonine (Thr), and tyrosine (Tyr). In some embodiments, the amino acid with a neutral polar hydrophilic side chain is Asn. In some embodiments, the amino acid with a neutral polar hydrophilic side chain is Gln. In some embodiments, the amino acid with a neutral polar hydrophilic side chain is Ser. In some embodiments, the amino acid with a neutral polar hydrophilic side chain is Thr. In some embodiments, the amino acid with a neutral polar hydrophilic side chain is Tyr.

Examples of amino acids with a positively charged polar hydrophilic side chain include arginine (Arg), histidine (His), and lysine (Lys). In some embodiments, the amino acid with a positively charged polar hydrophilic side is Arg. In some embodiments, the amino acid with a positively charged polar hydrophilic side is His. In some embodiments, the amino acid with a positively charged polar hydrophilic side is Lys.

Examples of amino acids with a negatively charged polar hydrophilic side chain include aspartate (Asp) and glutamate (Glu). In some embodiments, the amino acid with a negatively charged polar hydrophilic side chain is Asp. In some embodiments, the amino acid with a negatively charged polar hydrophilic side chain is Glu.

In some embodiments, (Xaa)_n is represented by Formula (IV):

aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8-aa9 (IV),

wherein aa1 is an amino acid selected from Asp, Gly, Asn, and Val; aa2 is an amino acid selected from Trp, Tyr, His, and Phe; aa3 is an amino acid selected from Trp, Tyr, Gly, Trp, and Phe; aa4 is an amino acid selected from Gln, Ala, and Pro; aa5 is an amino acid selected from Ala, Gln, Glu, Arg, and Ser; aa6 is an amino acid selected from Lys, Arg, and Tyr; aa7 is an amino acid selected from Trp and Gln; aa8 is an amino acid selected from Pro and His; and/or aa9 is an amino acid selected from His, Gly, and Gln. In some embodiments, aa1 is Asp. In some embodiments, aa1 is Gly. In some embodiments, aa1 is Asn. In some embodiments, aa2 is Trp. In some embodiments, aa2 is Tyr. In some embodiments, aa2 is His. In some embodiments, aa2 is Phe. In some embodiments, aa3 is Trp. In some embodiments, aa3 is Tyr. In some embodiments, aa3 is Gly. In some embodiments, aa3 is Trp. In some embodiments, aa3 is Phe. In some embodiments, aa4 is Gln. In some embodiments, aa4 is Ala. In some embodiments, aa4 is Pro. In some embodiments, aa5 is Ala. In some embodiments, aa5 is Gln. In some embodiments, aa5 is Glu. In some embodiments, aa5 is Arg. In some embodiments, aa5 is Ser. In some embodiments, aa6 is Lys. In some embodiments, aa6 is Arg. In some embodiments, aa6 is Tyr. In some embodiments, aa7 is Trp. In some embodiments, aa7 is Gln. In some embodiments, aa8 is Pro. In some embodiments, aa8 is His. In some embodiments, aa9 is His. In some embodiments, aa9 is Gly. In some embodiments, aa9 is Gln.

In some embodiments, (Xaa)_m is represented by Formula (IV):

aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8-aa9 (IV),

wherein aa1 is an amino acid selected from Tyr, Phe, Trp, and Asn; aa2 is an amino acid selected from Lys, Pro, His, Ala, and Thr; aa3 is an amino acid selected from Val, Asn, Gly, Gln, Ala, and Phe; aa4 is an amino acid selected from His, Thr, Lys, Trp, Lys, Val, and Arg; aa5 is an amino acid selected from Gln, Ser, Gly, Pro, and Asn; aa6 is an amino acid selected from Ser, Tyr, Glu, Leu, Lys, and Thr; aa7 is an amino acid selected from Ser, Asp, Val, and Lys; aa8 is an amino acid selected from Gly, Leu, Ser, Pro, His, Asp, and Arg; and/or aa9 is an amino acid selected from Gly, Gln, Glu, and Ala. In some embodiments, aa1 is Tyr. In some embodiments, aa1 is Phe. In some embodiments, aa1 is Trp. In some embodiments, aa1 is Asn. In some embodiments, aa2 is Lys. In some embodiments, aa2 is Pro. In some embodiments, aa2 is His. In some embodiments, aa2 is Ala. In some embodiments, aa2 is Thr. In some embodiments, aa3 is Val. In some embodiments, aa3 is Asn. In some embodiments, aa3 is Gly. In some embodiments, aa3 is Gln. In some embodiments, aa3 is Ala. In some embodiments, aa3 is Phe. In some embodiments, aa4 is His. In some embodiments, aa4 is Thr. In some embodiments, aa4 is Lys. In some embodiments, aa4 is Trp. In some embodiments, aa4 is Lys. In some embodiments, aa4 is Val. In some embodiments, aa4 is Arg. In some embodiments, aa5 is Gln. In some embodiments,

aa5 is Ser. In some embodiments, aa5 is Gly. In some embodiments, aa5 is Pro. In some embodiments, aa5 is Asn. In some embodiments, aa6 is Ser. In some embodiments, aa6 is Tyr. In some embodiments, aa6 is Glu. In some embodiments, aa6 is Leu. In some embodiments, aa6 is Lys. In some embodiments, aa6 is Thr. In some embodiments, aa7 is Ser. In some embodiments, aa7 is Asp. In some embodiments, aa7 is Val. In some embodiments, aa7 is Lys. In some embodiments, aa8 is Gly. In some embodiments, aa8 is Leu. In some embodiments, aa8 is Ser. In some embodiments, aa8 is Pro. In some embodiments, aa8 is His. In some embodiments, aa8 is Asp. In some embodiments, aa8 is Arg. In some embodiments, aa9 is Gly. In some embodiments, aa9 is Gln. In some embodiments, aa9 is Glu. In some embodiments, aa9 is Ala.

In some embodiments, (Xaa)_n is represented by Formula (V):

Asn-aa1-aa2-Gln-Gln-Arg-Arg-Trp-Pro-Gly (V) (SEQ ID NO: 627),

wherein aa1 is an amino acid selected from Trp and Phe; and aa2 is an amino acid selected from Tyr and Phe. In some embodiments, aa1 is Trp. In some embodiments, aa1 is Phe. In some embodiments, aa2 is Tyr. In some embodiments, aa2 is Phe.

In some embodiments, (Xaa)_n is represented by Formula (VI):

aa1-aa2-Trp-aa3-aa4-Lys-Trp-Pro-aa5 (VI) (SEQ ID NO: 634),

wherein aa1 is an amino acid selected from Asp and Gly; aa2 is an amino acid selected from Trp, Tyr, and Phe; aa3 is an amino acid selected from Gln and Ala; aa4 is an amino acid selected from Ala and Ser; and aa5 is an amino acid selected from His and Gly. In some embodiments, aa1 is Asp. In some embodiments, aa1 is Gly. In some embodiments, aa2 is Trp. In some embodiments, aa2 is Tyr. In some embodiments, aa2 is Phe. In some embodiments, aa3 is Gln. In some embodiments, aa3 is Ala. In some embodiments, aa4 is Ala. In some embodiments, aa4 is Ser. In some embodiments, aa5 is His. In some embodiments, aa5 is Gly.

In some embodiments, (Xaa)_n is represented by Formula (VII):

aa1-aa2-aa3-aa4-aa5-aa6-Trp-Pro-Gly (VII),

wherein aa1 is an amino acid selected from Gly and Asn; aa2 is an amino acid selected from Tyr, Phe, Trp, and His; aa3 is an amino acid selected from Trp, Tyr, and Phe; aa4 is an amino acid selected from Ala and Gln; aa5 is an amino acid selected from Ala, Ser, Gln, and Arg; and aa6 is an amino acid selected from Lys, Arg, and Tyr. In some embodiments, aa1 is Gly. In some embodiments, aa1 is Asn. In some embodiments, aa2 is Tyr. In some embodiments, aa2 is Phe. In some embodiments, aa2 is Trp. In some embodiments, aa2 is His. In some embodiments, aa3 is Trp. In some embodiments, aa3 is Tyr. In some embodiments, aa3 is Phe. In some embodiments, aa4 is Ala. In some embodiments, aa4 is Gln. In some embodiments, aa5 is Ala. In some embodiments, aa5 is Ser. In some embodiments, aa5 is Gln. In some embodiments,

aa5 is Arg. In some embodiments, aa6 is Lys. In some embodiments, aa6 is Arg. In some embodiments, aa6 is Tyr.

In some embodiments, (Xaa)_n is represented by Formula (VIII):

Gly-aa1-aa2-Ala-aa3-aa4-Trp-Pro-Gly (VIII) (SEQ ID NO: 820),

wherein aa1 is an amino acid selected from Tyr, Phe, and His; aa2 is an amino acid selected from Trp and Tyr; aa3 is an amino acid selected from Ala, Ser, and Arg; and aa4 is an amino acid selected from Lys and Tyr. In some embodiments, aa1 is Tyr. In some embodiments, aa1 is Phe His. In some embodiments, aa1 is His. In some embodiments, aa2 is Trp. In some embodiments, aa2 is Tyr. In some embodiments, aa3 is Ala. In some embodiments, aa3 is Ser. In some embodiments, aa3 is Arg. In some embodiments, aa4 is Lys. In some embodiments, aa4 is Tyr.

In some embodiments, (Xaa)_n is represented by Formula (IX):

aa1-aa2-aa3-Gln-aa4-aa5-Trp-Pro-aa6 (IX),

wherein aa1 is an amino acid selected from Asp and Asn; aa2 is an amino acid selected from Trp and Phe; aa3 is an amino acid selected from Trp, Tyr, and Phe; aa4 is an amino acid selected from Ala, Gln, and Arg; aa5 is an amino acid selected from Lys and Arg; and aa6 is an amino acid selected from His and Gly. In some embodiments, aa1 is Asp. In some embodiments, aa1 is Asn. In some embodiments, aa2 is Trp. In some embodiments, aa2 is Phe. In some embodiments, aa3 is Trp. In some embodiments, aa3 is Tyr. In some embodiments, aa3 is Phe. In some embodiments, aa4 is Ala. In some embodiments, aa4 is Gln. In some embodiments, aa4 is Arg. In some embodiments, aa5 is Lys. In some embodiments, aa5 is Arg. In some embodiments, aa6 is His. In some embodiments, aa6 is Gly.

In some embodiments, an HSA AFFIMER® polypeptide comprises a loop 2 amino acid sequence selected from any one of SEQ ID NOs: 661 to 713 (**Table 8**). In some embodiments, an HSA AFFIMER® polypeptide comprises a loop 4 amino acid sequence selected from any one of SEQ ID NOs: 714 to 766 (**Table 8**).

Table 8. Exemplary HSA AFFIMER® Polypeptide Loop Sequences

Name	Loop 2	SEQ ID NO:	Loop 4	SEQ ID NO:
HSA-00	WTQPKNEHH	661	RFKYFAHYQ	714
HSA-01	HLKHTDAQP	662	FHDFWHRRW	715
HSA-02	HDQDVLHAW	663	DWYHYWWEV	716
HSA-03	KFHRQEWAD	664	STRSIHVT	717
HSA-04	PEDFDPEH	665	KQH HHYLDK	718
HSA-05	VVRTTGHVV	666	HSAQDREIP	719
HSA-06	YWWFCTGQS	667	WVQSGYNSQ	720

Name	Loop 2	SEQ ID NO:	Loop 4	SEQ ID NO:
HSA-07	IHHRQARSL	668	AVFWGKWSD	721
HSA-08	SHRRRAYIW	669	QSFDKPWTT	722
HSA-09	WDSHHWRAP	670	HYPLKYSFE	723
HSA-10	DKRVKYGQ	671	WHHPWHRNR	724
HSA-11	SDWVYALQL	672	DPWWAWVVW	725
HSA-12	FWWFWY	673	FDNQDLIQY	726
HSA-13	VRDWPWNTF	674	EKKNWYKWD	727
HSA-14	QKKRDEYI	675	DRHKSRWGI	728
HSA-15	GVHEEPRKL	676	LNPFTPSVT	729
HSA-16	EWQKHWPS	677	YKGALLNHD	730
HSA-17	NFFQRRWPG	678	WKFRNTERG	731
HSA-18	DWWQAKWPH	679	YKVHQSSGG	732
HSA-19	GIWQSRWPG	680	FHPIAGR PW	733
HSA-20	GYWAAKWPG	681	FPNTSYDLQ	734
HSA-21	GFYADHWPG	682	FAHYNLKSG	735
HSA-22	NWYQQRWPG	683	WHNYGESSG	736
HSA-23	GFYARHWPG	684	KFYADHQW	737
HSA-24	DFWKAHWPG	685	YTHADPHSQ	738
HSA-25	DFYSVRWPG	686	FGVPQLGAG	739
HSA-26	YWAANHASK	687	YSGFPFAGF	740
HSA-27	IKRLEHWEY	688	WFSWPYTPL	741
HSA-28	EWDSPWSEN	689	YYHPSIQST	742
HSA-29	KHKNLRWPF	690	FLGWKDTV V	743
HSA-30	RHFPKQTNW	691	DWWKWWWAK	744
HSA-31	VWGPEYQHQ	692	NAGWPLVPE	745
HSA-32	TWKNNGQDV	693	YALDPFGGK	746
HSA-33	ATWLNYYLP	694	GYKFWGVSD	747
HSA-34	DQESLFLNN	695	QKGQYILLR	748
HSA-35	GFYAQHWP D	696	YKRHSAHDY	749
HSA-36	GHYARYWPG	697	WAQSKSVHQ	750
HSA-37	GFWASKWPG	698	FTAVSKKDA	751
HSA-38	GFWQRKWPN	699	WGDKENIWF	752
HSA-39	VVPADNDLK	700	WSGHPWVQK	753
HSA-40	HWAWTSPGY	701	YADYPLSPK	754
HSA-41	NFFQRRWPG	702	WKFRNTDRG	755
HSA-42	HSHRLKGQ	703	QTVATHYHY	756
HSA-43	YQNTIFLSI	704	WHAKHLLSH	757
HSA-44	FQDQFTWSQ	705	SGIKKADSV	758
HSA-45	GEPHWPWQA	706	KANLINVKS	759
HSA-46	ADPRHPWVE	707	WKSHVEVRS	760
HSA-47	FHKRFQSQG	708	WVTQKYIIQ	761
HSA-48	EWQNRWPN	709	WEHAKDWPT	762
HSA-49	EWYQTRWPG	710	FHSKVLDKA	763
HSA-50	EFWQRHWPG	711	YGAQKQAVW	764
HSA-51	KFYERHWPG	712	FSASHFTSQ	765
Consensus	GWWQRRWPG	713	X ₁ X ₂ AX ₃ KX ₄ DX ₅ Q	766

In some embodiments, (Xaa)_n comprises an amino acid sequence having at least 80% or at least 90% identity to the amino acid sequence of any one of SEQ ID NOs: 661 to 713. In some embodiments, (Xaa)_n comprises an amino acid sequence having 80% to 90% identity to the amino acid sequence of any one of SEQ ID NOs: 661 to 713. In some embodiments, (Xaa)_n comprises the amino acid sequence of any one SEQ ID NOs: 661 to 713.

In some embodiments, (Xaa)_m comprises an amino acid sequence having at least 80% or at least 90% identity to the amino acid sequence of any one of SEQ ID NOs: 714 to 766. In some embodiments, (Xaa)_m comprises an amino acid sequence having 80% to 90% identity to the amino acid sequence of any one of SEQ ID NOs: 714 to 766. In some embodiments, (Xaa)_m comprises the amino acid sequence of any one of SEQ ID NOs: 714 to 766.

In some embodiments, an HSA AFFIMER® polypeptide comprises an amino acid sequence selected from any one of SEQ ID NOs: 767 to 773 (**Table 9**).

Table 9. Exemplary HSA AFFIMER® Polypeptide Sequences

Sequence	SEQ ID NO:
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADWWQAKWP HSTNYYIKVRAGDNKYMHLKVFNGPYKVHQSSGGADRVLTGYQVDKNKDDELTGF	767
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGYWAAKWP GSTNYYIKVRAGDNKYMHLKVFNGPFPNTSYDLQADRVLTGYQVDKNKDDELTGF	768
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANWYQQRWP GSTNYYIKVRAGDNKYMHLKVFNGPWHNYGESSGADRVLTGYQVDKNKDDELTGF	769
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVWGPEYQHQ STNYYIKVRAGDNKYMHLKVFNGPNAGWPLVPEADRVLTGYQVDKNKDDELTGF	770
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGHYARYWPG STNYYIKVRAGDNKYMHLKVFNGPWAQSKSVHQADRVLTGYQVDKNKDDELTGF	771
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGFWASKWPG STNYYIKVRAGDNKYMHLKVFNGPFTAVSKKDAADRVLTGYQVDKNKDDELTGF	772
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANFFQRRWPG STNYYIKVRAGDNKYMHLKVFNGPWKFRNTDRGADRVLTGYQVDKNKDDELTGF	773

In some embodiments, an HSA AFFIMER® polypeptide comprises an amino acid sequence having at least 80% or at least 90% identity to the amino acid sequence of any one of SEQ ID NOs: 767 to 773.

In some embodiments, an HSA AFFIMER® polypeptide comprises an amino acid sequence having 80% to 90% identity to the amino acid sequence of any one of SEQ ID NOs: 767 to 773.

An HSA AFFIMER® polypeptide provided herein, in some embodiments, is linked to another molecule and extend the half-life of that molecule (*e.g.*, a therapeutic polypeptide). Provided herein is a range of HSA AFFIMER® polypeptides, with a range of binding affinities, for example, that cross-react with other species such as mouse and cynomolgus (cyno) monkey.

These HSA AFFIMER® polypeptides, in some embodiments, make up what is referred to as the AFFIMER XT™ platform. These HSA AFFIMER® polypeptides have been shown in *in vivo* pharmacokinetic (PK) studies to extend, in a controlled manner, the serum half-life of any other AFFIMER® polypeptide therapeutic to which it is conjugated in a single genetic fusion, for example, that can be made in *E. Coli*. AFFIMER XT™ can also be used to extend the half-life of other peptide or protein therapeutics.

In some embodiments, an HSA AFFIMER® polypeptide extends the serum half-life of a molecule (*e.g.*, a therapeutic polypeptide) *in vivo*. For example, an HSA AFFIMER® polypeptide may extend the half-life of a molecule by at least 2-fold, relative to the half-life of the molecule not linked to an HSA AFFIMER® polypeptide. In some embodiments, an HSA AFFIMER® polypeptide extends the half-life of a molecule by at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 20-fold, or at least 30-fold, relative to the half-life of the molecule not linked to an HSA AFFIMER® polypeptide. In some embodiments, an HSA AFFIMER® polypeptide extends the half-life of a molecule by 2-fold to 5-fold, 2-fold to 10-fold, 3-fold to 5-fold, 3-fold to 10-fold, 15-fold to 5-fold, 4-fold to 10-fold, or 5-fold to 10-fold, relative to the half-life of the molecule not linked to an HSA AFFIMER® polypeptide. In some embodiments, an HSA AFFIMER® polypeptide extends the half-life of a molecule by at least 6 hours, at least 12 hours, at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, for example, at least 1 week after *in vivo* administration, relative to the half-life of the molecule not linked to an HSA AFFIMER® polypeptide.

5. PEGylation, XTEN, PAS and Other Polymers

A wide variety of macromolecular polymers and other molecules can be linked to the AFFIMER® polypeptides of the present disclosure to modulate biological properties of the resulting AFFIMER® agent, and/or provide new biological properties to the AFFIMER® agent. These macromolecular polymers can be linked to the AFFIMER® polypeptide via a naturally encoded amino acid, via a non-naturally encoded amino acid, or any functional substituent of a natural or non-natural amino acid, or any substituent or functional group added to a natural or non-natural amino acid. The molecular weight of the polymer may be of a wide range, including but not limited to, between about 100 Da and about 100,000 Da or more. The molecular weight of the polymer may be between about 100 Da and about 100,000 Da, including but not limited to, 100,000 Da, 95,000 Da, 90,000 Da, 85,000 Da, 80,000 Da, 75,000 Da, 70,000 Da, 65,000 Da, 60,000 Da, 55,000 Da, 50,000 Da, 45,000 Da, 40,000 Da, 35,000 Da, 30,000 Da, 25,000 Da, 20,000 Da, 15,000 Da, 10,000 Da, 9,000 Da, 8,000 Da, 7,000 Da, 6,000 Da, 5,000 Da, 4,000 Da,

3,000 Da, 2,000 Da, 1,000 Da, 900 Da, 800 Da, 700 Da, 600 Da, 500 Da, 400 Da, 300 Da, 200 Da, and 100 Da. In some embodiments, the molecular weight of the polymer is between about 100 Da and about 50,000 Da. In some embodiments, the molecular weight of the polymer is between about 100 Da and about 40,000 Da. In some embodiments, the molecular weight of the polymer is between about 1,000 Da and about 40,000 Da. In some embodiments, the molecular weight of the polymer is between about 5,000 Da and about 40,000 Da. In some embodiments, the molecular weight of the polymer is between about 10,000 Da and about 40,000 Da.

For this purpose, various methods including pegylation, polysialylation, HESylation, glycosylation, or recombinant PEG analogue fused to flexible and hydrophilic amino acid chain (500 to 600 amino acids) have been developed (See Chapman, (2002) *Adv Drug Deliv Rev.* 54, 531-545; Schlapschy et al., (2007) *Prot Eng Des Sel.* 20, 273-283; Contermann (2011) *Curr Op Biotechnol.* 22, 868-876; Jevsevar et al., (2012) *Methods Mol Biol.* 901, 233-246).

Examples of polymers include but are not limited to polyalkyl ethers and alkoxy-capped analogs thereof (e.g., polyoxyethylene glycol, polyoxyethylene/propylene glycol, and methoxy or ethoxy-capped analogs thereof, especially polyoxyethylene glycol, the latter is also known as polyethylene glycol or PEG); discrete PEG (dPEG); polyvinylpyrrolidones; polyvinylalkyl ethers; polyoxazolines, polyalkyl oxazolines and polyhydroxyalkyl oxazolines; polyacrylamides, polyalkyl acrylamides, and polyhydroxyalkyl acrylamides (e.g., polyhydroxypropylmethacrylamide and derivatives thereof); polyhydroxyalkyl acrylates; polysialic acids and analogs thereof; hydrophilic peptide sequences; polysaccharides and their derivatives, including dextran and dextran derivatives, e.g., carboxymethyl dextran, dextran sulfates, aminodextran; cellulose and its derivatives, e.g., carboxymethyl cellulose, hydroxyalkyl celluloses; chitin and its derivatives, e.g., chitosan, succinyl chitosan, carboxymethylchitin, carboxymethylchitosan; hyaluronic acid and its derivatives; starches; alginates; chondroitin sulfate; albumin; pullulan and carboxymethyl pullulan; polyaminoacids and derivatives thereof, e.g., polyglutamic acids, polylysines, polyaspartic acids, polyaspartamides; maleic anhydride copolymers such as: styrene maleic anhydride copolymer, divinylethyl ether maleic anhydride copolymer; polyvinyl alcohols; copolymers thereof; terpolymers thereof; mixtures thereof; and derivatives of the foregoing.

The polymer selected may be water soluble so that the AFFIMER® agent to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. The water-soluble polymer may be any structural form including but not limited to linear, forked or branched. Typically, the water-soluble polymer is a poly(alkylene glycol), such as poly(ethylene glycol) (PEG), but other water-soluble polymers can also be employed. By way of

example, PEG is used to describe some embodiments of this disclosure. For therapeutic use of the AFFIMER® agent, the polymer may be pharmaceutically acceptable.

The term “PEG” is used broadly to encompass any polyethylene glycol molecule, without regard to size or to modification at an end of the PEG, and can be represented as linked to the AFFIMER® polypeptide by the formula:



or



where n is 2 to 10,000 and X is H or a terminal modification, including but not limited to, a C1-4 alkyl, a protecting group, or a terminal functional group. In some cases, a PEG used in the polypeptides of the disclosure terminates on one end with hydroxy or methoxy, e.g., X is H or CH_3 (“methoxy PEG”).

It is noted that the other end of the PEG, which is shown in the above formulas by a terminal “—”, may attach to the AFFIMER® polypeptide via a naturally-occurring or non-naturally encoded amino acid. For instance, the attachment may be through an amide, carbamate or urea linkage to an amine group (including but not limited to, the epsilon amine of lysine or the N-terminus) of the polypeptide. Alternatively, the polymer is linked by a maleimide linkage to a thiol group (including but not limited to, the thiol group of cysteine) – which in the case of attachment to the AFFIMER® polypeptide sequence per se requires altering a residue in the AFFIMER® sequence to a cysteine.

The number of water-soluble polymers linked to the AFFIMER® polypeptide (e.g., the extent of PEGylation or glycosylation) can be adjusted to provide an altered (including but not limited to, increased or decreased) pharmacologic, pharmacokinetic or pharmacodynamic characteristic such as *in vivo* half-life in the resulting AFFIMER® agent. In some embodiments, the half-life of the resulting AFFIMER® agent is increased at least about 10, 20, 30, 40, 50, 60, 70, 80, 90 percent, 2-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 50-fold, or at least about 100-fold over an unmodified polypeptide.

Another variation of polymer system useful to modify the PK or other biological properties of the resulting AFFIMER® agent are the use of unstructured, hydrophilic amino acid polymers that are functional analogs of PEG, particularly as part of a fusion protein with the AFFIMER® polypeptide sequence. The inherent biodegradability of the polypeptide platform makes it attractive as a potentially more benign alternative to PEG. Another advantage is the precise molecular structure of the recombinant molecule in contrast to the polydispersity of PEG.

Unlike HSA and Fc peptide fusions, in which the three-dimensional folding of the fusion partner needs to be maintained, the recombinant fusions to unstructured partners can, in many cases, be subjected to higher temperatures or harsh conditions such as HPLC purification.

One of the more advanced of this class of polypeptides is termed XTEN (Amunix) and is 864 amino acids long and comprised of six amino acids (A, E, G, P, S and T). See Schellenberger et al. "A recombinant polypeptide extends the *in vivo* half-life of peptides and proteins in a tunable manner" 2009 Nat Biotechnol. 27(12):1186-90. Enabled by the biodegradable nature of the polymer, this is much larger than the 40 KDa PEGs typically used and confers a concomitantly greater half-life extension. The fusion of XTEN to the AFFIMER® polypeptide should result in half-life extension of the final AFFIMER® agent by 60- to 130-fold over the unmodified polypeptide.

A second polymer based on similar conceptual considerations is PAS (XL-Protein GmbH). Schlapschky et al. "PASYlation: a biological alternative to PEGylation for extending the plasma half-life of pharmaceutically active proteins" 2013 Protein Eng Des Sel. 26(8):489-501. A random coil polymer comprised of an even more restricted set of only three small uncharged amino acids, proline, alanine and serine. AS with Fc, HAS and XTEN, the PAS modification can be genetically encoded with the AFFIMER® polypeptide sequence to produce an inline fusion protein when expressed.

D. Conjugates

The subject AFFIMER® agents may also include at least one functional moiety intended to impart detectability or additional pharmacologic activity to the AFFIMER® agent. Functional moieties for detection are those which can be employed to detect association of the AFFIMER® agent with a cell or tissue (such as a Tumor cell) *in vivo*. Functional moieties with pharmacologic activity are those agents which are meant to be delivered to the tissue expressing the target of the AFFIMER® agent (CD33 in the case of the CD33 AFFIMER® agents of the present disclosure) and in doing so have a pharmacologic consequence to the targeted tissues or cells.

The present disclosure provides AFFIMER® agents including conjugates of substances having a wide variety of functional groups, substituents or moieties, with those Functional Moieties including but not limited to a label; a dye; an immunoadhesion molecule; a radionuclide; a cytotoxic compound; a drug; an affinity label; a photoaffinity label; a reactive compound; a resin; a second protein or polypeptide or polypeptide analog; an antibody or antibody fragment; a metal chelator; a cofactor; a fatty acid; a carbohydrate; a polynucleotide; a DNA; a RNA; an antisense polynucleotide; a saccharide; a water-soluble dendrimer; a cyclodextrin; an inhibitory ribonucleic acid; a biomaterial; a nanoparticle; a spin label; a

fluorophore, a metal-containing moiety; a radioactive moiety; a novel functional group; a group that covalently or noncovalently interacts with other molecules; a photocaged moiety; an actinic radiation excitable moiety; a photoisomerizable moiety; biotin; a derivative of biotin; a biotin analogue; a moiety incorporating a heavy atom; a chemically cleavable group; a photocleavable group; an elongated side chain; a carbon-linked sugar; a redox-active agent; an amino thioacid; a toxic moiety; an isotopically labeled moiety; a biophysical probe; a phosphorescent group; a chemiluminescent group; an electron dense group; a magnetic group; an intercalating group; a chromophore; an energy transfer agent; a biologically active agent; a detectable label; a small molecule; a quantum dot; a nanotransmitter; a radionucleotide; a radiotransmitter; a neutron-capture agent; or any combination of the above, or any other desirable compound or substance.

1. Labels and Detectable Moieties

Where the moiety is a detectable label, it can be a fluorescent label, radioactive label, enzymatic label or any other label known to the skilled person. In some embodiments, the Functional Moiety is a detectable label that can be included as part of a conjugate to form certain AFFIMER® agents suitable for medical imaging. By "medical imaging" is meant any technique used to visualize an internal region of the human or animal body, for the purposes of diagnosis, research or therapeutic treatment. For instance, the AFFIMER® agent can be detected (and quantitated) by radioscintigraphy, magnetic resonance imaging (MRI), computed tomography (CT scan), nuclear imaging, positron emission comprising a metal tomography (PET) contrast agent, optical imaging (such as fluorescence imaging including near-infrared fluorescence (NIRF) imaging), bioluminescence imaging, or combinations thereof. The Functional Moiety is optionally a contrast agent for X-ray imaging. Agents useful in enhancing such techniques are those materials that enable visualization of a particular locus, organ or disease site within the body, and/or that lead to some improvement in the quality of the images generated by the imaging techniques, providing improved or easier interpretation of those images. Such agents are referred to herein as contrast agents, the use of which facilitates the differentiation of different parts of the image, by increasing the "contrast" between those different regions of the image. The term "contrast agents" thus encompasses agents that are used to enhance the quality of an image that may nonetheless be generated in the absence of such an agent (as is the case, for instance, in MRI), as well as agents that are prerequisites for the generation of an image (as is the case, for instance, in nuclear imaging).

In some embodiments, the detectable label includes a chelate moiety for chelating a metal, e.g., a chelator for a radiometal or paramagnetic ion. In some embodiments, the detectable label is a chelator for a radionuclide useful for radiotherapy or imaging procedures.

Radionuclides useful within the present disclosure include gamma-emitters, positron-emitters, Auger electron-emitters, X-ray emitters and fluorescence-emitters, with beta- or alpha-emitters for therapeutic use. Examples of radionuclides useful as toxins in radiation therapy include: ^{43}K , ^{47}Sc , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{64}Cu , ^{67}Ga , ^{67}Cu , ^{68}Ga , ^{71}Ge , ^{75}Br , ^{76}Br , ^{77}Br , ^{77}As , ^{81}Rb , ^{90}Y , ^{97}Ru , $^{99\text{m}}\text{Tc}$, ^{100}Pd , ^{101}Rh , ^{103}Pb , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{111}In , ^{113}In , ^{119}Sb , ^{121}Sn , ^{123}I , ^{125}I , ^{127}Cs , ^{128}Ba , ^{129}Cs , ^{131}I , ^{131}Cs , ^{143}Pr , ^{153}Sm , ^{161}Tb , ^{166}Ho , ^{169}Eu , ^{177}Lu , ^{186}Re , ^{188}Re , ^{189}Re , ^{191}Os , ^{193}Pt , ^{194}Ir , ^{197}Hg , ^{199}Au , ^{203}Pb , ^{211}At , ^{212}Pb , ^{212}Bi and ^{213}Bi . Conditions under which a chelator will coordinate a metal are described, for example, by Gansow et al., U.S. Pat. NOS: 4,831,175, 4,454,106 and 4,472,509. Examples of chelators includes, merely to illustrate, 1,4,7-triazacyclononane- N,N',N'' -triacetic acid (NOTA) 1,4,7,10-tetraazacyclododecane- N,N',N'',N''' -tetraacetic acid (DOTA) 1,4,8,11-tetraazacyclotetradecane- N,N',N'',N''' -tetraacetic acid (TETA).

Other detectable isotopes that can be incorporated directly into the amino acid residues of the AFFIMER® polypeptide or which otherwise do not require a chelator, include ^3H , ^{14}C , ^{32}P , ^{35}S and ^{36}Cl .

Paramagnetic ions, useful for diagnostic procedures, may also be administered. Examples of paramagnetic ions include chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III), erbium (III), or combinations of these paramagnetic ions.

Examples of fluorescent labels include, but are not restricted to, organic dyes (e.g., cyanine, fluorescein, rhodamine, Alexa Fluors, Dylight fluors, ATTO Dyes, BODIPY Dyes, etc.), biological fluorophores (e.g., green fluorescent protein (GFP), R-Phycoerythrin, etc.), and quantum dots.

Non-limiting fluorescent compound that may be used in the present disclosure include, Cy5, Cy5.5 (also known as Cy5++), Cy2, fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), phycoerythrin, Cy7, fluorescein (FAM), Cy3, Cy3.5 (also known as Cy3++), Texas Red, LightCycler-Red 640, LightCycler Red 705, tetramethylrhodamine (TMR), rhodamine, rhodamine derivative (ROX), hexachlorofluorescein (HEX), rhodamine 6G (R6G), the rhodamine derivative JA133, Alexa Fluorescent Dyes (such as Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 633, Alexa Fluor 555, and Alexa Fluor 647), 4',6-diamidino-2-phenylindole (DAPI), Propidium iodide, AMCA, Spectrum Green, Spectrum Orange, Spectrum Aqua, Lissamine, and fluorescent transition metal complexes, such as europium. Fluorescent compound that can be used also include fluorescent proteins, such as GFP (green fluorescent protein), enhanced GFP (EGFP), blue fluorescent protein and derivatives

(BFP, EBFP, EBFP2, Azurite, mKalamal), cyan fluorescent protein and derivatives (CFP, ECFP, Cerulean, CyPet) and yellow fluorescent protein and derivatives (YFP, Citrine, Venus, YPet). WO2008142571, WO2009056282, WO9922026.

Examples of enzymatic labels include, but are not restricted to, horseradish peroxidase (HRP), alkaline phosphatase (AP), glucose oxidase and β -galactosidase.

Another well-known label is biotin. Biotin labels are typically composed of the biotinyl group, a spacer arm and a reactive group that is responsible for attachment to target functional groups on proteins. Biotin can be useful for attaching the labelled protein to other moieties which comprise an avidin moiety.

2. AFFIMER® Polypeptide-Drug Conjugates

In some embodiments, the AFFIMER® agent includes at least one therapeutic agent, e.g., to form an AFFIMER® polypeptide-drug conjugate. As used herein, the term “therapeutic agent” refers to a substance that may be used in the cure, mitigation, treatment, or prevention of disease in a human or another animal. Such therapeutic agents include substances recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, official National Formulary, or any supplement thereof, and include but are not limited to small molecules, nucleotides, oligopeptides, polypeptides, etc. Therapeutic agents that may be attached to AFFIMER® polypeptides include but are not limited to, cytotoxic agents, anti-metabolites, alkylating agents, antibiotics, growth factor, cytokines, anti-angiogenic agents, anti-mitotic agents, toxins, apoptotic agents or the like, such as DNA alkylating agents, topoisomerase inhibitors, microtubule inhibitors (e.g., DM1, DM4, MMAF and MMAE), endoplasmic reticulum stress inducing agents, platinum compounds, antimetabolites, vincalkaloids, taxanes, epothilones, enzyme inhibitors, receptor antagonists, therapeutic antibodies, tyrosine kinase inhibitors, radiosensitizers, and chemotherapeutic combination therapies, such as illustrations.

Non-limiting examples of DNA alkylating agents are nitrogen mustards, such as Mechlorethamine, Cyclophosphamide (Ifosfamide, Trofosfamide), Chlorambucil (Melphalan, Prednimustine), Bendamustine, Uramustine and Estramustine; nitrosoureas, such as Carmustine (BCNU), Lomustine (Semustine), Fotemustine, Nimustine, Ranimustine and Streptozocin; alkyl sulfonates, such as Busulfan (Mannosulfan, Treosulfan); Aziridines, such as Carboquone, ThioTEPA, Triaziquone, Triethylenemelamine; Hydrazines (Procarbazine); Triazines such as Dacarbazine and Temozolomide; Altretamine and Mitobronitol.

Non-limiting examples of Topoisomerase I inhibitors include Camptothecin derivatives including CPT-11 (irinotecan), SN-38, APC, NPC, camptothecin, topotecan, exatecan mesylate,

9-nitrocamptothecin, 9-aminocamptothecin, lurtotecan, rubitecan, silatecan, gimatecan, diflomotecan, extatecan, BN-80927, DX-8951f, and MAG-CPT as described in Pommier Y. (2006) *Nat. Rev. Cancer* 6(10):789-802 and U.S. Patent Publication No. 200510250854; Protoberberine alkaloids and derivatives thereof including berberrubine and coralyne as described in Li et al. (2000) *Biochemistry* 39(24):7107-7116 and Gatto et al. (1996) *Cancer Res.* 15(12):2795-2800; Phenanthroline derivatives including Benzo[i]phenanthridine, Nitidine, and fagaronine as described in Makhey et al. (2003) *Bioorg. Med. Chem.* 11 (8): 1809-1820; Terbenzimidazole and derivatives thereof as described in Xu (1998) *Biochemistry* 37(10):3558-3566; and Anthracycline derivatives including Doxorubicin, Daunorubicin, and Mitoxantrone as described in Foglesong et al. (1992) *Cancer Chemother. Pharmacol.* 30(2):123-125, Crow et al. (1994) *J. Med. Chem.* 37(19):3191-3194, and Crespi et al. (1986) *Biochem. Biophys. Res. Commun.* 136(2):521-8. Topoisomerase II inhibitors include but are not limited to Etoposide and Teniposide. Dual topoisomerase I and II inhibitors include but are not limited to, Saintopin and other Naphthecenediones, DACA and other Acridine-4-Carboxamides, Intoplicine and other Benzopyridoindoles, TAS-103 and other 7H-indeno[2,1-c]Quinoline-7-ones, Pyrazoloacridine, XR 11576 and other Benzophenazines, XR 5944 and other Dimeric compounds, 7-oxo-7H-dibenz[f,i]Isoquinolines and 7-oxo-7H-benzo[e]Perimidines, and Anthracenyl-amino Acid Conjugates as described in Denny and Baguley (2003) *Curr. Top. Med. Chem.* 3(3):339-353. Some agents inhibit Topoisomerase II and have DNA intercalation activity such as, but not limited to, Anthracyclines (Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Valrubicin, Zorubicin) and Antracenediones (Mitoxantrone and Pixantrone).

Non-limiting examples of DNA synthesis inhibitors include Calicheamicin, Doxorubicin, Duocarmycin, and PBD.

Non-limiting examples of microtubule inhibitors include DM1, DM4, MMAF, and MMAE.

Examples of endoplasmic reticulum stress inducing agents include but are not limited to, dimethyl-celecoxib (DMC), nelfinavir, celecoxib, and boron radiosensitizers (e.g., velcade (Bortezomib)).

Non-limiting examples of platinum-based compound include Carboplatin, Cisplatin, Nedaplatin, Oxaliplatin, Triplatin tetranitrate, Satraplatin, Aroplatin, Lobaplatin, and JM-216. (see McKeage et al. (1997) *J. Clin. Oncol.* 201:1232-1237 and in general, CHEMOTHERAPY FOR GYNECOLOGICAL NEOPLASM, CURRENT THERAPY AND NOVEL APPROACHES, in the Series Basic and Clinical Oncology, Angioli et al. Eds., 2004).

Non-limiting examples of antimetabolite agents include Folic acid based, e.g. dihydrofolate reductase inhibitors, such as Aminopterin, Methotrexate and Pemetrexed; thymidylate synthase inhibitors, such as Raltitrexed, Pemetrexed; Purine based, e.g. an adenosine deaminase inhibitor, such as Pentostatin, a thiopurine, such as Thioguanine and Mercaptopurine, a halogenated/ribonucleotide reductase inhibitor, such as Cladribine, Clofarabine, Fludarabine, or a guanine/guanosine: thiopurine, such as Thioguanine; or Pyrimidine based, e.g. cytosine/cytidine: hypomethylating agent, such as Azacitidine and Decitabine, a DNA polymerase inhibitor, such as Cytarabine, a ribonucleotide reductase inhibitor, such as Gemcitabine, or a thymine/thymidine: thymidylate synthase inhibitor, such as a Fluorouracil (5-FU). Equivalents to 5-FU include prodrugs, analogs and derivative thereof such as 5'-deoxy-5-fluorouridine (doxifluridine), 1-tetrahydrofuryl-5-fluorouracil (ftorafur), Capecitabine (Xeloda), S-I (MBMS-247616, consisting of tegafur and two modulators, a 5-chloro-2,4-dihydroxypyridine and potassium oxonate), raltitrexed (tomudex), nolatrexed (Thymitaq, AG337), LY231514 and ZD9331, as described for example in Papamichael (1999) *The Oncologist* 4:478-487.

Examples of vincalkaloids, include but are not limited to Vinblastine, Vincristine, Vinflunine, Vindesine and Vinorelbine.

Examples of taxanes include but are not limited to docetaxel, Larotaxel, Ortataxel, Paclitaxel and Teseaxel. An example of an epothilone is iabepilone.

Examples of enzyme inhibitors include but are not limited to farnesyltransferase inhibitors (Tipifamib); CDK inhibitor (Alvociclib, Seliciclib); proteasome inhibitor (Bortezomib); phosphodiesterase inhibitor (Anagrelide; rolipram); IMP dehydrogenase inhibitor (Tiazofurine); and lipoxygenase inhibitor (Masoprocol). Examples of receptor antagonists include but are not limited to ERA (Atrasentan); retinoid X receptor (Bexarotene); and a sex steroid (Testolactone).

Examples of therapeutic antibodies include but are not limited to anti-HER1/EGFR (Cetuximab, Panitumumab); Anti-HER2/neu (erbB2) receptor (Trastuzumab); Anti-EpCAM (Catumaxomab, Edrecolomab) Anti-VEGF-A (Bevacizumab); Anti-CD20 (Rituximab, Tositumomab, Ibritumomab); Anti-CD52 (Alemtuzumab); and Anti-CD33 (Gemtuzumab). U.S. Pat. NOS: 5,776,427 and 7,601,355.

Examples of tyrosine kinase inhibitors include but are not limited to inhibitors to ErbB: HER1/EGFR (Erlotinib, Gefitinib, Lapatinib, Vandetanib, Sunitinib, Neratinib); HER2/neu (Lapatinib, Neratinib); RTK class III: C-kit (Axitinib, Sunitinib, Sorafenib), FLT3 (Lestaurtinib), PDGFR (Axitinib, Sunitinib, Sorafenib); and VEGFR (Vandetanib, Semaxanib, Cediranib,

Axitinib, Sorafenib); bcr-abl (Imatinib, Nilotinib, Dasatinib); Src (Bosutinib) and Janus kinase 2 (Lestaurtinib).

Chemotherapeutic agents that can be attached to the present AFFIMER® polypeptides may also include amsacrine, Trabectedin, retinoids (Alitretinoin, Tretinoin), Arsenic trioxide, asparagine depleter Asparaginase/Pegaspargase), Celecoxib, Demecolcine, Elesclomol, Elsamitrucin, Etoglucid, Lonidamine, Lucanthone, Mitoguazone, Mitotane, Oblimersen, Temsirolimus, and Vorinostat.

Examples of specific therapeutic agents that can be linked, ligated, or associated with the AFFIMER® polypeptides of the disclosure are flomoxef; fortimicin(s); gentamicin(s); glucosulfone solasulfone; gramicidin S; gramicidin(s); grepafloxacin; guamecycline; hetacillin; isepamicin; josamycin; kanamycin(s); flomoxef; fortimicin(s); gentamicin(s); glucosulfone solasulfone; gramicidin S; gramicidin(s); grepafloxacin; guamecycline; hetacillin; isepamicin; josamycin; kanamycin(s); bacitracin; bambarmycin(s); biapenem; brodimoprim; butirosin; capreomycin; carbenicillin; carbomycin; carumonam; cefadroxil; cefamandole; cefatrizine; cefbuperazone; cefclidin; cefdinir; cefditoren; cefepime; cefetamet; cefixime; cefinenoxime; cefininox; cladribine; apalcillin; apicycline; apramycin; arbekacin; aspoxicillin; azidamfenicol; aztreonam; cefodizime; cefonicid; cefoperazone; ceforamide; cefotaxime; cefotetan; cefotiam; cefozopran; cefpimizole; cefpiramide; cefpirome; cefprozil; cefroxadine; cefteteram; ceftibuten; cefuzonam; cephalixin; cephaloglycin; cephalosporin C; cephradine; chloramphenicol; chlortetracycline; clinafloxacin; clindamycin; clomocycline; colistin; cyclacillin; dapsone; demeclocycline; diathymosulfone; dibekacin; dihydrostreptomycin; 6-mercaptopurine; thioguanine; capecitabine; docetaxel; etoposide; gemcitabine; topotecan; vinorelbine; vincristine; vinblastine; teniposide; melphalan; methotrexate; 2-p-sulfanilylanilinoethanol; 4,4'-sulfinyldianiline; 4-sulfanilamidosalicylic acid; butorphanol; nalbuphine. streptozocin; doxorubicin; daunorubicin; plicamycin; idarubicin; mitomycin C; pentostatin; mitoxantrone; cytarabine; fludarabine phosphate; butorphanol; nalbuphine. streptozocin; doxorubicin; daunorubicin; plicamycin; idarubicin; mitomycin C; pentostatin; mitoxantrone; cytarabine; fludarabine phosphate; acediasulfone; acetosulfone; amikacin; amphotericin B; ampicillin; atorvastatin; enalapril; ranitidine; ciprofloxacin; pravastatin; clarithromycin; cyclosporin; famotidine; leuprolide; acyclovir; paclitaxel; azithromycin; lamivudine; budesonide; albuterol; indinavir; metformin; alendronate; nizatidine; zidovudine; carboplatin; metoprolol; amoxicillin; diclofenac; lisinopril; ceftriaxone; captopril; salmeterol; xinafoate; imipenem; cilastatin; benazepril; cefaclor; ceftazidime; morphine; dopamine; bialamicol; fluvastatin; phenamidine; podophyllinic acid 2-ethylhydrazine; acriflavine; chloroazodin; arspenamine; amicarbilide;

aminoquinuride; quinapril; oxymorphone; buprenorphine; floxuridine; dirithromycin; doxycycline; enoxacin; enviomycin; epicillin; erythromycin; leucomycin(s); lincomycin; lomefloxacin; lucensomycin; lymecycline; meclocycline; meropenem; methacycline; micronomicin; midecamycin(s); minocycline; moxalactam; mupirocin; nadifloxacin; natamycin; neomycin; netilmicin; norfloxacin; oleandomycin; oxytetracycline; p-sulfanilylbenzylamine; panipenem; paromomycin; pazufloxacin; penicillin N; pipacycline; pipemidic acid; polymyxin; primycin; quinacillin; ribostamycin; rifamide; rifampin; rifamycin SV; rifapentine; rifaximin; ristocetin; ritipenem; rokitamycin; rolitetracycline; rosaramycin; roxithromycin; salazosulfadimidine; sancycline; sisomicin; sparfloxacin; spectinomycin; spiramycin; streptomycin; succisulfone; sulfachrysoidine; sulfaloxic acid; sulfamidochrysoidine; sulfanilic acid; sulfoxone; teicoplanin; temafloxacin; temocillin; tetroxoprim; thiamphenicol; thiazolsulfone; thiostrepton; ticarcillin; tigemonam; tobramycin; tosufloxacin; trimethoprim; trospectomycin; trovafloxacin; tuberactinomycin; vancomycin; azaserine; candididin(s); chlorphenesin; dermostatin(s); filipin; fungichromin; mepartricin; nystatin; oligomycin(s); perimycin A; tubercidin; 6-azauridine; 6-diazo-5-oxo-L-norleucine; aclacinomycin(s); ancitabine; anthramycin; azacitadine; azaserine; bleomycin(s); ethyl biscoumacetate; ethylidene dicoumarol; iloprost; lamifiban; taprostene; tiocloamarol; tirofiban; amiprilose; bucillamine; gusperimus; gentisic acid; glucamethacin; glycol salicylate; meclofenamic acid; mefenamic acid; mesalamine; niflumic acid; olsalazine; oxaceprol; S-enosylmethionine; salicylic acid; salsalate; sulfasalazine; tolfenamic acid; carubicin; carzinophillin A; chlorozotocin; chromomycin(s); denopterin; doxifluridine; edatrexate; eflornithine; elliptinium; enocitabine; epirubicin; mannomustine; menogaril; mitobronitol; mitolactol; mopidamol; mycophenolic acid; nogalamycin; olivomycin(s); peplomycin; pirarubicin; piritrexim; prednimustine; procarbazine; pteropterin; puromycin; ranimustine; streptonigrin; thiamiprine; mycophenolic acid; procodazole; romurtide; sirolimus (rapamycin); tacrolimus; butethamine; fenalcomine; hydroxytetracaine; naepaine; orthocaine; piridocaine; salicyl alcohol; 3-amino-4-hydroxybutyric acid; aceclofenac; alminoprofen; amfenac; bromfenac; bromosaligenin; bumadizon; carprofen; diclofenac; diflunisal; ditazol; enfenamic acid; etodolac; etofenamate; fendosal; fepradinol; flufenamic acid; Tomudex (N-[[5-[[[(1,4-Dihydro-2-methyl-4-oxo-6-quinazoliny]methyl]methylamino]-2-thienyl]carbonyl]-L-glutamic acid), trimetrexate, tubercidin, ubenimex, vindesine, zorubicin; argatroban; coumetarol or dicoumarol.

In some embodiments, the AFFIMER® agent includes a conjugated cytotoxic factor such as diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins and compounds (e.g., fatty acids),

dianthin proteins, Phytoiacca americana proteins PAPI, PAPII, and PAP-S, momordica charantia inhibitor, curcin, croton, saponaria officinalis inhibitor, mitogellin, restrictocin, phenomycin, and enomycin.

Any method known in the art for conjugating to antibodies and other proteins may be employed in generating the conjugates of the present disclosure, including those methods described by Hunter, et al., (1962) Nature 144:945; David, et al., (1974) Biochemistry 13:1014; Pain, et al., (1981) J. Immunol. Meth. 40:219; and Nygren, J., (1982) Histochem. and Cytochem. 30:407. Methods for conjugating peptide, polypeptide and organic and inorganic moieties to antibodies and other proteins are conventional and very well known in the art and readily adapted for generating those versions of the subject AFFIMER® agents.

Where the conjugated moiety is a peptide or polypeptide, that moiety can be chemically cross-linked to the AFFIMER® polypeptide or can be included as part of a fusion protein with the AFFIMER® polypeptide. An illustrative example would be a diphtheria toxin-AFFIMER® fusion protein. In the case of non-peptide entities, the addition to the AFFIMER® polypeptide will generally be by way of chemical conjugation to the AFFIMER® polypeptide – such as through a functional group on an amino acid side chain or the carboxyl group at the C-terminal or amino group at the N-terminal end of the polypeptide. In some embodiment, whether as a fusion protein or chemically cross-linked moiety, the conjugated moiety will include at least one site that can be cleaved by an enzyme or are otherwise sensitive to an environmental condition (such as pH) that permits the conjugated moiety to be released from the AFFIMER® polypeptide, such as in the tumor or other diseased tissue (or tissue to be protected if the conjugated moiety functions to protect healthy tissue).

a) Enzyme-cleavable Linkers

An AFFIMER® polypeptide-drug conjugate, in some embodiments, comprises an enzyme-cleavable linker, which links the half-life extension moiety to a drug moiety. The linker (e.g., the substrate recognition sequence (SRS) of the linker) is selectively cleaved in the vicinity of the target cells so that the free drug moiety is released from the conjugate in the vicinity of the target cells so as to exert its pharmacological activities preferentially on the cells/tissue nearby to the target cells, rather than on wanted (healthy) cells. Thus, in some embodiments, the SRS is selectively cleaved such that the drug moiety is released as the free drug moiety in the vicinity of the target cells at least five times or ten times more than the extent to which the free drug moiety it is released in the vicinity of healthy cells/tissues, and in some embodiments, at least 100 or 500 or 1000 times more.

For a given target cell, the skilled person will be able to identify appropriate SRS that is selectively cleavable in the vicinity of the target cell, using established methods in the art. For example, which proteases cleave which peptides can be assessed by consulting peptide libraries and studying an MS analysis of the fragmentation profile following cleavage. Also, published literature of protease cleavage motifs and peptide cleavage data can be searched as described further below.

In some aspects, the SRS is a protease cleavage site. Thus, when the target cells are tumor cells, the SRS may be cleavable selectively by proteases that reside in the vicinity of the tumor cells. Thus, the SRS may be one that is cleavable by a tumor associated protease. It is well known that during tumor development, tumors aberrantly express proteases which allow them to invade local tissues and eventually metastasize.

For example, the protease may be present extracellularly in the diseased state tissue in a subject at levels at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times greater than the healthy state of the tissue in the subject.

As another example, the protease may be present extracellularly in the diseased state of the tissue in a subject at levels at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 or 100 times greater than other tissue of the subject.

In some embodiments, the protease is a serine protease, metal protease, or cysteine protease.

The protease may be a metalloproteinase (MMP1-28) including both membrane bound (MMP14-17 and MMP24-25) and secreted forms (MMP1-13 and MMP 18-23 and MMP26- 28). The protease may belong to the A Disintegrin and Metalloproteinase (ADAM) and A Disintegrin, or Metalloproteinase with Thrombospondin Motifs (ADAMTS) families of proteases. Other examples include CD 10 (CALLA) and prostate specific antigen (PSA). It is appreciated that the proteases may or may not be membrane bound.

Protease cleavage sites are well known in the scientific literature, and can readily serve as the basis for a given SRS being included in the drug-conjugate moieties using established synthetic techniques known in the art.

To the extent representing a protease whose extracellular concentration is upregulated/increased in the target tissue by changes in expression, cellular trafficking or, in the case of intracellular enzymes that may become extracellular, by cell lysis caused by the disease state, SRS may utilized which are designed to be selectively cleavable by one or a select sub-group of human proteases selected from the group consisting of (MEROPS peptidase database number provided in parentheses; Rawlings N. D., Morton F. R., Kok, C. Y., Kong, J. & Barrett

A. J. (2008) MEROPS: the peptidase database. *Nucleic Acids Res.* 36 Database issue, D320-325): pepsin A (MER000885), gastricsin (MER000894), memapsin-2 (MER005870), renin (MER000917), cathepsin D (MER000911), cathepsin E (MER000944), memapsin-l (MER005534), napsin A (MER004981), Mername-AA034 peptidase (MER014038), pepsin A4 (MER037290), pepsin A5 (Homo sapiens) (MER037291), hCGI733572 (Homo sapiens)-type putative peptidase (MER107386), napsin B pseudogene (MER004982), CYMP g.p. (Homo sapiens) (MER002929), subfamily A1A unassigned peptidases (MER181559), mouse mammary tumor virus retropepsin (MER048030), rabbit endogenous retrovirus endopeptidase (MER043650), S71-related human endogenous retropepsin (MER001812), RTVL-H-type putative peptidase (MER047117), RTVL-H-type putative peptidase (MER047133), RTVL-H-type putative peptidase (MER047160), RTVL- H-type putative peptidase (MER047206), RTVL-H-type putative peptidase (MER047253), RTVL-H-type putative peptidase (MER047260), RTVL-H-type putative peptidase (MER047291), RTVL-H-type putative peptidase (MER047418), RTVL-H-type putative peptidase (MER047440), RTVL-H-type putative peptidase (MER047479), RTVL-H-type putative peptidase (MER047559), RTVL-H-type putative peptidase (MER047583), RTVL- H-type putative peptidase (MERO 15446), human endogenous retrovirus retropepsin homologue 1 (MERO 15479), human endogenous retrovirus retropepsin homologue 2 (MERO 15481), endogenous retrovirus retropepsin pseudogene 1 (Homo sapiens chromosome 14) (MER029977), endogenous retrovirus retropepsin pseudogene 2 (Homo sapiens chromosome 8) (MER029665), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER002660), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER030286), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER047144), endogenous retrovirus retropepsin pseudogene 5 (Homo sapiens chromosome 12) (MER029664), endogenous retrovirus retropepsin pseudogene 6 (Homo sapiens chromosome 7) (MER002094), endogenous retrovirus retropepsin pseudogene 7 (Homo sapiens chromosome 6) (MER029776), endogenous retrovirus retropepsin pseudogene 8 (Homo sapiens chromosome Y) (MER030291), endogenous retrovirus retropepsin pseudogene 9 (Homo sapiens chromosome 19) (MER029680), endogenous retrovirus retropepsin pseudogene 10 (Homo sapiens chromosome 12) (MER002848), endogenous retrovirus retropepsin pseudogene 11 (Homo sapiens chromosome 17) (MER004378), endogenous retrovirus retropepsin pseudogene 12 (Homo sapiens chromosome 11) (MER003344), endogenous retrovirus retropepsin pseudogene 13 (Homo sapiens chromosome 2 and similar) (MER029779), endogenous retrovirus retropepsin pseudogene 14 (Homo sapiens chromosome 2) (MER029778), endogenous retrovirus

retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER047158), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER047332), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER003182), endogenous retrovirus retropepsin pseudogene 16 (MER047165), endogenous retrovirus retropepsin pseudogene 16 (MER047178), endogenous retrovirus retropepsin pseudogene 16 (MER047200), endogenous retrovirus retropepsin pseudogene 16 (MER047315), endogenous retrovirus retropepsin pseudogene 16 (MER047405), endogenous retrovirus retropepsin pseudogene 16 (MER030292), endogenous retrovirus retropepsin pseudogene 17 (Homo sapiens chromosome 8) (MER005305), endogenous retrovirus retropepsin pseudogene 18 (Homo sapiens chromosome 4) (MER030288), endogenous retrovirus retropepsin pseudogene 19 (Homo sapiens chromosome 16) (MER001740), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047222), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047454), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047477), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER004403), endogenous retrovirus retropepsin pseudogene 22 (Homo sapiens chromosome X) (MER030287), subfamily A2A non-peptidase homologues (MER047046), subfamily A2A non-peptidase homologues (MER047052), subfamily A2A non-peptidase homologues (MER047076), subfamily A2A non-peptidase homologues (MER047080), subfamily A2A non-peptidase homologues (MER047088), subfamily A2A non-peptidase homologues (MER047089), subfamily A2A non-peptidase homologues (MER047091), subfamily A2A non-peptidase homologues (MER047092), subfamily A2A non-peptidase homologues (MER047093), subfamily A2A non-peptidase homologues (MER047094), subfamily A2A non-peptidase homologues (MER047097), subfamily A2A non-peptidase homologues (MER047099), subfamily A2A non-peptidase homologues (MER047101), subfamily A2A non-peptidase homologues (MER047102), subfamily A2A non-peptidase homologues (MER047107), subfamily A2A non-peptidase homologues (MER047108), subfamily A2A non-peptidase homologues (MER047109), subfamily A2A non-peptidase homologues (MER047110), subfamily A2A non-peptidase homologues (MER047111), subfamily A2A non-peptidase homologues (MER047114), subfamily A2A non-peptidase homologues (MER047118), subfamily A2A non-peptidase homologues (MER047121), subfamily A2A non-peptidase homologues (MER047122), subfamily A2A non-peptidase homologues (MER047126), subfamily A2A non-peptidase homologues (MER047129), subfamily A2A non-peptidase homologues (MER047130), subfamily A2A non-peptidase homologues (MER047134), subfamily A2A non-peptidase homologues (MER047135),

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hydrolase (animal) (MER002481), tubulointerstitial nephritis antigen (MER016137), tubulointerstitial nephritis antigen-related protein (MER021799), cathepsin L-like pseudogene 1 (Homo sapiens) (MER002789), cathepsin B-like pseudogene (chromosome 4, Homo sapiens) (MER029469), cathepsin B-like pseudogene (chromosome 1, Homo sapiens) (MER029457), CTSL2 g.p. (Homo sapiens) (MER005210), CTSL3 g.p. (Homo sapiens) (MER005209), calpain-1 (MER000770), calpain-2 (MER000964), calpain-3 (MER001446), calpain-9 (MER004042), calpain-8 (MER021474), calpain-1 5 (MER004745), calpain-5 (MER002939), calpain-1 1 (MER005844), calpain-12 (MER029889), calpain-10 (MER013510), calpain-13 (MER020139), calpain-14 (MER029744), Mername-AA253 peptidase (MER005537), calpamodulin (MER000718), hypothetical protein flj40251 (MER003201), ubiquitinyl hydrolase-L1 (MER000832), ubiquitinyl hydrolase-L3 (MER000836), ubiquitinyl hydrolase-BAP1 (MER003989), ubiquitinyl hydrolase-UCH37 (MER005539), ubiquitin-specific peptidase 5 (MER002066), ubiquitin-specific peptidase 6 (MER000863), ubiquitin-specific peptidase 4 (MER001795), ubiquitin-specific peptidase 8 (MER001884), ubiquitin-specific peptidase 13 (MER002627), ubiquitin-specific peptidase 2 (MER004834), ubiquitin-specific peptidase 11 (MER002693), ubiquitin-specific peptidase 14 (MER002667), ubiquitin-specific peptidase 7 (MER002896), ubiquitin-specific peptidase 9X (MER005877), ubiquitin-specific peptidase 10 (MER004439), ubiquitin-specific peptidase 1 (MER004978), ubiquitin-specific peptidase 12 (MER005454), ubiquitin-specific peptidase 16 (MER005493), ubiquitin-specific peptidase 15 (MER005427), ubiquitin-specific peptidase 17 (MER002900), ubiquitin-specific peptidase 19 (MER005428), ubiquitin-specific peptidase 20 (MER005494), ubiquitin-specific peptidase 3 (MER005513), ubiquitin-specific peptidase 9Y (MER004314), ubiquitin-specific peptidase 18 (MER005641), ubiquitin-specific peptidase 21 (MER006258), ubiquitin-specific peptidase 22 (MER012130), ubiquitin-specific peptidase 33 (MER014335), ubiquitin-specific peptidase 29 (MER012093), ubiquitin-specific peptidase 25 (MER011115), ubiquitin-specific peptidase 36 (MER014033), ubiquitin-specific peptidase 32 (MER014290), ubiquitin-specific peptidase 26 (Homo sapiens-type) (MER014292), ubiquitin-specific peptidase 24 (MER005706), ubiquitin-specific peptidase 42 (MER011852), ubiquitin-specific peptidase 46 (MER014629), ubiquitin-specific peptidase 37 (MER014633), ubiquitin-specific peptidase 28 (MER014634), ubiquitin-specific peptidase 47 (MER014636), ubiquitin-specific peptidase 38 (MER014637), ubiquitin-specific peptidase 44 (MER014638), ubiquitin-specific peptidase 50 (MER030315), ubiquitin-specific peptidase 35 (MER014646), ubiquitin-specific peptidase 30 (MER014649), Mername-AA091 peptidase (MER014743), ubiquitin-specific peptidase 45 (MER030314), ubiquitin-specific peptidase 51 (MER014769), ubiquitin-

specific peptidase 34 (MER014780), ubiquitin-specific peptidase 48 (MER064620), ubiquitin-specific peptidase 40 (MERO 15483), ubiquitin-specific peptidase 41 (MER045268), ubiquitin-specific peptidase 31 (MER015493), Mername-AA129 peptidase (MER016485), ubiquitin-specific peptidase 49 (MER016486), Mername-AA187 peptidase (MER052579), ETSP17-like peptidase (MER030192), ubiquitin-specific peptidase 54 (MER028714), ubiquitin-specific peptidase 53 (MER027329), ubiquitin-specific endopeptidase 39 [misleading] (MER064621), Memame-AA090 non-peptidase homologue (MERO 14739), ubiquitin-specific peptidase [misleading] (MER030140), ubiquitin-specific peptidase 52 [misleading] (MER030317), NEK2 pseudogene (MER014736), C19 pseudogene (Homo sapiens: chromosome 5) (MER029972), Memame-AA088 peptidase (MER014750), autophagin-2 (MER013564), autophagin-1 (MER013561), autophagin-3 (MER014316), autophagin-4 (MER064622), Cezanne deubiquitylating peptidase (MER029042), Cezanne-2 peptidase (MER029044), tumor necrosis factor alpha-induced protein 3 (MER029050), traid peptidase (MER029052), VCIP135 deubiquitylating peptidase (MER152304), otubain-1 (MER029056), otubain-2 (MER029061), CylD protein (MER030104), UfSP1 peptidase (MER042724), ETfSP2 peptidase (MER060306), DEIBA deubiquitylating enzyme (MER086098), KIAA0459 (Homo sapiens)-like protein (MER122467), Otudl protein (MER125457), glycosyltransferase 28 domain containing 1, isoform CRA c (Homo sapiens)-like (MER123606), hin1L g.p. (Homo sapiens) (MER139816), ataxin-3 (MER099998), ATXN3L putative peptidase (MER115261), Josephin domain containing 1 (Homo sapiens) (MER125334), Josephin domain containing 2 (Homo sapiens) (MER124068), YOD1 peptidase (MER116559), legumain (plant alpha form) (MER044591), legumain (MER001800), glycosylphosphatidylinositolprotein transamidase (MER002479), legumain pseudogene (Homo sapiens) (MER029741), family C13 unassigned peptidases (MER175813), caspase-1 (MER000850), caspase-3 (MER000853), caspase-7 (MER002705), caspase-6 (MER002708), caspase-2 (MER001644), caspase-4 (MER001938), caspase-5 (MER002240), caspase-8 (MER002849), caspase-9 (MER002707), caspase-10 (MER002579), caspase-14 (MER012083), paracaspase (MER019325), Memame-AA143 peptidase (MER021304), Mername-AA186 peptidase (MER020516), putative caspase (Homo sapiens) (MER021463), FLIP protein (MER003026), Memame-AA142 protein (MER021316), caspase-12 pseudogene (Homo sapiens) (MER019698), Mername-AA093 caspase pseudogene (MER014766), subfamily C14A non-peptidase homologues (MER185329), subfamily C14A non-peptidase homologues (MER179956), separase (Homo sapiens-type) (MER011775), separase-like pseudogene (MER014797), SENP1 peptidase (MER011012), SENP3 peptidase (MER011019), SENP6 peptidase (MER011109),

SENP2 peptidase (MER012183), SENP5 peptidase (MER014032), SENP7 peptidase (MER014095), SENP8 peptidase (MER016161), SENP4 peptidase (MER005557), pyroglutamyl-peptidase I (chordate) (MER011032), Memame-AA073 peptidase (MER029978), Sonic hedgehog protein (MER002539), Indian hedgehog protein (MER002538), Desert hedgehog protein (MER012170), dipeptidyl-peptidase III (MER004252), Mername-AA164 protein (MER020410), LOC138971 g.p. (Homo sapiens) (MER020074), Atp23 peptidase (MER060642), prenyl peptidase 1 (MER004246), aminopeptidase N (MER000997), aminopeptidase A (MER001012), leukotriene A4 hydrolase (MER001013), pyroglutamyl-peptidase II (MER012221), cytosol alanyl aminopeptidase (MER002746), cystinyl aminopeptidase (MER002060), aminopeptidase B (MER001494), aminopeptidase PILS (MER005331), arginyl aminopeptidase-like 1 (MERO 12271), leukocyte-derived arginine aminopeptidase (MER002968), aminopeptidase Q (MER052595), aminopeptidase 0 (MER019730), Tata binding protein associated factor (MER026493), angiotensin-converting enzyme peptidase unit 1 (MER004967), angiotensin-converting enzyme peptidase unit 2 (MER001019), angiotensin-converting enzyme-2 (MER011061), Memame-AA153 protein (MER020514), thimet oligopeptidase (MER001737), neurolysin (MERO 10991), mitochondrial intermediate peptidase (MER003665), Mername-AA154 protein (MER021317), leishmanolysin-2 (MER014492), leishmanolysin-3 (MER180031), matrix metallopeptidase-1 (MER001063), matrix metallopeptidase-8 (MER001084), matrix metallopeptidase-2 (MER001080), matrix metallopeptidase-9 (MER001085), matrix metallopeptidase-3 (MER001068), matrix metallopeptidase-10 (Homo sapiens-type) (MER001072), matrix metallopeptidase-1 1 (MER001075), matrix metallopeptidase-7 (MER001092), matrix metallopeptidase-1 2 (MER001089), matrix metallopeptidase-1 3 (MER001411), membrane-type matrix metallopeptidase-1 (MER001077), membrane-type matrix metallopeptidase-2 (MER002383), membrane-type matrix metallopeptidase-3 (MER002384), membrane-type matrix metallopeptidase-4 (MER002595), matrix metallopeptidase-20 (MER003021), matrix metallopeptidase-1 9 (MER002076), matrix metallopeptidase-23B (MER004766), membrane-type matrix metallopeptidase-5 (MER005638), membrane-type matrix metallopeptidase-6 (MERO 12071), matrix metallopeptidase-21 (MER006101), matrix metallopeptidase-22 (MERO 14098), matrix metallopeptidase-26 (MERO 12072), matrix metallopeptidase-28 (MER013587), matrix metallopeptidase-23A (MER037217), macrophage elastase homologue (chromosome 8, Homo sapiens) (MER030035), Memame-AA156 protein (MER021309), matrix metallopeptidase-like 1 (MER045280), subfamily M10A non-peptidase homologues (MER175912), subfamily M10A non-peptidase homologues (MER187997), subfamily M10A

non-peptidase homologues (MER187998), subfamily M10A non-peptidase homologues (MER180000), meprin alpha subunit (MER001111), meprin beta subunit (MER005213), procollagen C-peptidase (MER001113), mammalian tolloid-like 1 protein (MER005124), mammalian-type tolloid-like 2 protein (MER005866), ADAMTS9 peptidase (MER012092), ADAMTS14 peptidase (MER016700), ADAMTS15 peptidase (MER017029), ADAMTS16 peptidase (MER015689), ADAMTS17 peptidase (MER016302), ADAMTS18 peptidase (MER016090), ADAMTS19 peptidase (MER015663), ADAMS peptidase (MER003902), ADAM9 peptidase (MER001140), ADAM 10 peptidase (MER002382), ADAM 12 peptidase (MER005107), ADAM 19 peptidase (MER012241), ADAM 15 peptidase (MER002386), ADAM 17 peptidase (MER003094), ADAM20 peptidase (MER004725), ADAMDEC1 peptidase (MER000743), ADAMTS3 peptidase (MER005100), ADAMTS4 peptidase (MER005101), ADAMTS1 peptidase (MER005546), ADAM28 peptidase (Homo sapiens-type) (MER005495), ADAMTS5 peptidase (MER005548), ADAMTS8 peptidase (MER005545), ADAMTS6 peptidase (MER005893), ADAMTS7 peptidase (MER005894), ADAM30 peptidase (MER006268), ADAM21 peptidase (Homo sapiens-type) (MER004726), ADAMTS10 peptidase (MER014331), ADAMTS 12 peptidase (MER014337), ADAMTS13 peptidase (MER015450), ADAM33 peptidase (MER015143), ovastacin (MER029996), ADAMTS20 peptidase (Homo sapiens-type) (MER026906), procollagen I N-peptidase (MER004985), ADAM2 protein (MER003090), ADAM6 protein (MER047044), ADAM7 protein (MER005109), ADAM18 protein (MER012230), ADAM32 protein (MER026938), non-peptidase homologue (Homo sapiens chromosome 4) (MER029973), family M12 non-peptidase homologue (Homo sapiens chromosome 16) (MER047654), family M12 non-peptidase homologue (Homo sapiens chromosome 15) (MER047250), ADAM3B protein (Homo sapiens-type) (MER005199), ADAM11 protein (MER001146), ADAM22 protein (MER005102), ADAM23 protein (MER005103), ADAM29 protein (MER006267), protein similar to ADAM21 peptidase preproprotein (Homo sapiens) (MER026944), Memame-AA225 peptidase homologue (Homo sapiens) (MER047474), putative ADAM pseudogene (chromosome 4, Homo sapiens) (MER029975), ADAM3A g.p. (Homo sapiens) (MER005200), ADAM1 g.p. (Homo sapiens) (MER003912), subfamily M12B non peptidase homologues (MER188210), subfamily M12B non-peptidase homologues (MER188211), subfamily M12B non-peptidase homologues (MER188212), subfamily M12B non-peptidase homologues (MER188220), neprilysin (MER001050), endothelin- converting enzyme 1 (MER001057), endothelin-converting enzyme 2 (MER004776), DINE peptidase (MER005197), neprilysin-2 (MER013406), Kell blood-group protein (MER001054), PHEX peptidase (MER002062), i-AAA peptidase

(MEROO 1246), i-AAA peptidase (MER005755), paraplegin (MER004454), Afg3-like protein 2 (MER005496), Afg3-like protein 1A (MER014306), pappalysin-I (MER002217), pappalysin-2 (MER014521), farnesylated-protein converting enzyme 1 (MER002646), metalloprotease-related protein-I (MER030873), aminopeptidase AMZ2 (MER011907), aminopeptidase AMZ1 (MER058242), carboxypeptidase A1 (MER001190), carboxypeptidase A2 (MEROO 1608), carboxypeptidase B (MEROO 1194), carboxypeptidase N (MEROO 1198), carboxypeptidase E (MEROO 1199), carboxypeptidase M (MEROO 1205), carboxypeptidase U (MEROO 1193), carboxypeptidase A3 (MEROO 1187), metallocarboxypeptidase D peptidase unit 1 (MER003781), metallocarboxypeptidase Z (MER003428), metallocarboxypeptidase D peptidase unit 2 (MER004963), carboxypeptidase A4 (MER013421), carboxypeptidase A6 (MER013456), carboxypeptidase A5 (MER017121), metallocarboxypeptidase 0 (MER016044), cytosolic carboxypeptidase-like protein 5 (MER033174), cytosolic carboxypeptidase 3 (MER033176), cytosolic carboxypeptidase 6 (MER033178), cytosolic carboxypeptidase 1 (MER033179), cytosolic carboxypeptidase 2 (MER037713), metallocarboxypeptidase D non-peptidase unit (MER004964), adipocyte-enhancer binding protein 1 (MER003889), carboxypeptidase-like protein XI (MER013404), carboxypeptidase-like protein X2 (MER078764), cytosolic carboxypeptidase (MER026952), family M14 non-peptidase homologues (MER199530), insulysin (MER001214), mitochondrial processing peptidase beta-subunit (MER004497), nardilysin (MER003883), eupitrylsin (MER004877), mitochondrial processing peptidase non-peptidase alpha subunit (MER001413), ubiquinol-cytochrome c reductase core protein I (MER003543), ubiquinol-cytochrome c reductase core protein II (MER003544), ubiquinol-cytochrome c reductase core protein domain 2 (MER043998), insulysin unit 2 (MER046821), nardilysin unit 2 (MER046874), insulysin unit 3 (MER078753), mitochondrial processing peptidase subunit alpha unit 2 (MER124489), nardilysin unit 3 (MER142856), LOC133083 g.p. (Homo sapiens) (MER021876), subfamily M16B non-peptidase homologues (MER188757), leucyl aminopeptidase (animal) (MER003100), Mername-AA040 peptidase (MER003919), leucyl aminopeptidase-I (Caenorhabditis-type) (MER013416), methionyl aminopeptidase 1 (MEROO 1342), methionyl aminopeptidase 2 (MER001728), aminopeptidase P2 (MER004498), Xaa-Pro dipeptidase (eukaryote) (MEROO 1248), aminopeptidase PI (MER004321), mitochondrial intermediate cleaving peptidase 55 kDa (MER013463), mitochondrial methionyl aminopeptidase (MER014055), Mername-AA020 peptidase homologue (MERO 10972), proliferation-association protein 1 (MER005497), chromatin-specific transcription elongation factor 140 kDa subunit (MER026495), proliferation-associated protein 1-like (Homo sapiens chromosome X) (MER029983), Mername-AA226 peptidase homologue

(Homo sapiens) (MER056262), Mername-AA227 peptidase homologue (Homo sapiens) (MER047299), subfamily M24A non-peptidase homologues (MER179893), aspartyl aminopeptidase (MER003373), Gly-Xaa carboxypeptidase (MER033182), carnosine dipeptidase II (MERO 14551), carnosine dipeptidase I (MER015142), Memame-AA161 protein (MER021873), aminoacylase (MER001271), glutamate carboxypeptidase II (MER002104), NAALADASE L peptidase (MER005239), glutamate carboxypeptidase III (MER005238), plasma glutamate carboxypeptidase (MER005244), Mername-AA103 peptidase (MER015091), Fxna peptidase (MER029965), transferrin receptor protein (MER002105), transferrin receptor 2 protein (MER005152), glutaminy cyclise (MERO 15095), glutamate carboxypeptidase II (Homo sapiens)-type non peptidase homologue (MER026971), nicalin (MER044627), membrane dipeptidase (MER001260), membrane-bound dipeptidase-2 (MER013499), membrane-bound dipeptidase-3 (MERO 13496), dihydro-orotase (MER005767), dihydropyrimidinase (MER033266), dihydropyrimidinase related protein-1 (MER030143), dihydropyrimidinase related protein-2 (MER030155), dihydropyrimidinase related protein-3 (MER030151), dihydropyrimidinase related protein-4 (MER030149), dihydropyrimidinase related protein-5 (MER030136), hypothetical protein like 5730457F11RIK (MER033184), 1300019j08rik protein (MER033186)), guanine aminohydrolase (MER037714), Kael putative peptidase (MEROO 1577), OSGEPLI-like protein (MER013498), S2P peptidase (MER004458), subfamily M23B non-peptidase homologues (MER199845), subfamily M23B non-peptidase homologues (MER199846), subfamily M23B non-peptidase homologues (MER199847), subfamily M23B non-peptidase homologues (MER137320), subfamily M23B non-peptidase homologues (MER201557), subfamily M23B non-peptidase homologues (MER199417), subfamily M23B non-peptidase homologues (MER199418), subfamily M23B non-peptidase homologues (MER199419), subfamily M23B non-peptidase homologues (MER199420), subfamily M23B non-peptidase homologues (MER175932), subfamily M23B non-peptidase homologues (MER199665), Pohl peptidase (MER020382), Jab1/MPN domain metalloenzyme (MER022057), Mername-AA165 peptidase (MER021865), Brcc36 isopeptidase (MER021890), histone H2A deubiquitinase MYSM1 (MER021887), AMSH deubiquitinating peptidase (MER030146), putative peptidase (Homo sapiens chromosome 2) (MER029970), Memame-AA168 protein (MER021886), COP9 signalosome subunit 6 (MER030137), 26S proteasome non-ATPase regulatory subunit 7 (MER030134), eukaryotic translation initiation factor 3 subunit 5 (MER030133), 1FP38 peptidase homologue (MER030132), subfamily M67A non-peptidase homologues (MER191181), subfamily M67A unassigned peptidases (MER191144), granzyme B (Homo sapiens-type) (MER000168), testisin (MER005212), tryptase beta (MER000136),

kallikrein-related peptidase 5 (MER005544), conn (MER005881), kallikrein-related peptidase 12 (MER006038), DESC1 peptidase (MER006298), tryptase gamma 1 (MER011036), kallikrein-related peptidase 14 (MER011038), hyaluronan-binding peptidase (MER003612), transmembrane peptidase, serine 4 (MER011104), intestinal serine peptidase (rodent) (MER016130), adrenal secretory serine peptidase (MER003734), tryptase delta 1 (Homo sapiens) (MER005948), matriptase-3 (MER029902), marapsin (MER006119), tryptase-6 (MER006118), ovoidomase-1 domain 1 (MER099182), transmembrane peptidase, serine 3 (MER005926), kallikrein-related peptidase 15 (MER000064), Mernase-AA031 peptidase (MER014054), DMPRSS13 peptidase (MER014226), Mernase-AA038 peptidase (MER062848), Mernase-AA204 peptidase (MER029980), cationic trypsin (Homo sapiens-type) (MER000020), elastase-2 (MER000118), mannan-binding lectin-associated serine peptidase-3 (MER031968), cathepsin G (MER000082), myeloblastin (MER000170), granzyme A (MER001379), granzyme M (MER001541), chymase (Homo sapiens-type) (MER000123), tryptase alpha (MER000135), granzyme K (MER001936), granzyme H (MER000166), chymotrypsin B (MER000001), elastase-1 (MER003733), pancreatic endopeptidase E (MER000149), pancreatic elastase II (MER000146), enteropeptidase (MER002068), chymotrypsin C (MER000761), prostasin (MER002460), kallikrein 1 (MER000093), kallikrein-related peptidase 2 (MER000094), kallikrein-related peptidase 3 (MER000115), mesotrypsin (MER000022), complement component C1r-like peptidase (MER016352), complement factor D (MER000130), complement component activated C1r (MER000238), complement component activated C1s (MER000239), complement component C2a (MER000231), complement factor B (MER000229), mannan-binding lectin-associated serine peptidase 1 (MER000244), complement factor I (MER000228), pancreatic endopeptidase E form B (MER000150), pancreatic elastase IIB (MER000147), coagulation factor XIIa (MER000187), plasma kallikrein (MER000203) coagulation factor XIa (MER000210), coagulation factor IXa (MER000216), coagulation factor VIIa (MER000215), coagulation factor Xa (MER000212), thrombin (MER000188), protein C (activated) (MER000222), acrosin (MER000078), hepsin (MER000156), hepatocyte growth factor activator (MER000186), mannan-binding lectin-associated serine peptidase 2 (MER002758), u-plasminogen activator (MER000195), t-plasminogen activator (MER000192), plasmin (MER000175), kallikrein-related peptidase 6 (MER002580), neurotrypsin (MER004171), kallikrein-related peptidase 8 (MER005400), kallikrein-related peptidase 10 (MER003645), epitheliasin (MER003736), kallikrein-related peptidase 4 (MER005266), proseminal (MER004214), chymopasins (MER001503), kallikrein-related peptidase

11 (MER004861), kallikrein-related peptidase 11 (MER216142), trypsin-2 type A (MER000021), HtrA1 peptidase (Homo sapiens-type) (MER002577), HtrA2 peptidase (MER208413), HtrA2 peptidase (MER004093), HtrA3 peptidase (MER014795), HtrA4 peptidase (MER016351), Tysndl peptidase (MER050461), DMPRSS12 peptidase (MER017085), HAT-like putative peptidase 2 (MER021884), trypsin C (MER021898), kallikrein-related peptidase 7 (MER002001), matriptase (MER003735), kallikrein-related peptidase 13 (MER005269), kallikrein-related peptidase 9 (MER005270), matriptase-2 (MER005278), umbelical vein peptidase (MER005421), LCLP peptidase (MER001900), spinesin (MER014385), marapsin-2 (MER021929), complement factor D-like putative peptidase (MER056164), ovochymase-2 (MER022410), HAT-like 4 peptidase (MER044589), ovochymase 1 domain 1 (MER022412), epidermis-specific SP-like putative peptidase (MER029900), testis serine peptidase 5 (MER029901), Mername-AA258 peptidase (MER000285), polyserase-IA unit 1 (MER030879), polyserase-IA unit 2 (MER030880), testis serine peptidase 2 (human-type) (MER033187), hypothetical acrosin-like peptidase (Homo sapiens) (MER033253), HAT-like 5 peptidase (MER028215), polyserase-3 unit 1 (MER061763), polyserase-3 unit 2 (MER061748), peptidase similar to tryptophan/serine protease (MER056263), polyserase-2 unit 1 (MER061777), Memame-AA123 peptidase (MER021930), HAT-like 2 peptidase (MER099184), hCG204l 452-like protein (MER099172), hCG22067 (Homo sapiens) (MER099169), brain-rescue-factor- 1 (Homo sapiens) (MER098873), hCG204H08 (Homo sapiens) (MER099173), polyserase-2 unit 2 (MER061760), polyserase-2 unit 3 (MER065694), Mername-AA20l (peptidase homologue) MER099175, secreted trypsin-like serine peptidase homologue (MER030000), polyserase-IA unit 3 (MER029880), azurocidin (MER000119), haptoglobin-l (MER000233), haptoglobin-related protein (MER000235), macrophage-stimulating protein (MER001546), hepatocyte growth factor (MER000185), protein Z (MER000227), TESP1 protein (MER047214), LOC136242 protein (MER016132), plasma kallikrein-like protein 4 (MERO 16346), PRSS35 protein (MER016350), DKFZp586H2123 -like protein (MER066474), apolipoprotein (MER000183), psi-KLKI pseudogene (Homo sapiens) (MER033287), tryptase pseudogene I (MER015077), tryptase pseudogene II (MER015078), tryptase pseudogene III (MER015079), subfamily S1A unassigned peptidases (MER216982), subfamily S1A unassigned peptidases (MER216148), amidophosphoribosyltransferase precursor (MER003314), glutamine-fructose-6-phosphate transaminase 1 (MER003322), glutamine:fructose-6-phosphate amidotransferase (MER012158), Mername-AA144 protein (MER021319), asparagine synthetase (MER033254), family C44 non-peptidase homologues (MER159286), family C44 unassigned peptidases (MER185625) family

C44 unassigned peptidases (MER185626), secernin 1 (MER045376), secernin 2 (MER064573), secernin 3 (MER064582), acid ceramidase precursor (MER100794), N-acylethanolamine acid amidase precursor (MER141667), proteasome catalytic subunit 1 (MER000556), proteasome catalytic subunit 2 (MER002625), proteasome catalytic subunit 3 (MER002149), proteasome catalytic subunit li (MER000552), proteasome catalytic subunit 2i (MER001515), proteasome catalytic subunit 3i (MER000555), proteasome catalytic subunit 5t (MER026203), protein serine kinase cl7 (MER026497), proteasome subunit alpha 6 (MER000557), proteasome subunit alpha 2 (MER000550), proteasome subunit alpha 4 (MER000554), proteasome subunit alpha 7 (MER033250), proteasome subunit alpha 5 (MER000558), proteasome subunit alpha 1 (MER000549), proteasome subunit alpha 3 (MER000553), proteasome subunit XAPC7 (MER004372), proteasome subunit beta 3 (MER001710), proteasome subunit beta 2 (MER002676), proteasome subunit beta 1 (MER000551), proteasome subunit beta 4 (MER001711), Mername-AA230 peptidase homologue (Homo sapiens) (MER047329), Memame-AA23 l pseudogene (Homo sapiens) (MER047172), Mername-AA232 pseudogene (Homo sapiens) (MER047316), glycosylasparaginase precursor (MER003299), isoaspartyl dipeptidase (threonine type) (MER031622), taspase-I (MERO 16969), gamma-glutamyltransferase 5 (mammalian-type) (MEROO 1977), gamma-glutamyltransferase 1 (mammalian-type) (MEROO 1629), gamma-glutamyltransferase 2 (Homo sapiens) (MER001976), gamma-glutamyltransferase-like protein 4 (MER002721), gamma-glutamyltransferase-like protein 3 (MERO 16970), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026204), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026205), Memame-AA2l l putative peptidase (MER026207), gamma-glutamyltransferase 6 (MER159283), gamma-glutamyl transpeptidase homologue (chromosome 2, Homo sapiens) (MER037241), polycystin-l (MER126824), KIAA1879 protein (MER159329), polycystic kidney disease l-like 3 (MER172554), gamma-glutamyl hydrolase (MER002963), guanine 5 "-monophosphate synthetase (MER043387), carbamoyl- phosphate synthase (Homo sapiens-type) (MER078640), dihydro-orotase (N-terminal unit) (Homo sapiens-type) (MER060647) DJ-l putative peptidase (MER003390), Mername- AA100 putative peptidase (MER014802), Memame-AA10l non-peptidase homologue (MER014803), KIAA0361 protein (Homo sapiens-type) (MER042827), Fl 134283 protein (Homo sapiens) (MER044553), non-peptidase homologue chromosome 21 open reading frame 33 (Homo sapiens) (MER160094), family C56 non-peptidase homologues (MER177016), family C56 non-peptidase homologues (MER176613), family C56 non peptidase homologues (MER176918), EGF-like module containing mucin-like hormone receptor-like 2 (MER037230), CD97 antigen (human type)

(MER037286), EGF-like module containing mucin-like hormone receptor-like 3 (MER037288), EGF-like module containing mucin-like hormone receptor-like 1 (MER037278), EGF-like module containing mucin-like hormone receptor-like 4 (MER037294), cadherin EGF LAG seven-pass G-type receptor 2 precursor (Homo sapiens) (MER045397), Gpr64 (Mus musculus)-type protein (MER123205), GPR56 (Homo sapiens)-type protein (MER122057), latrophilin 2 (MER122199), latrophilin-1 (MER126380), latrophilin 3 (MER124612), protocadherin Flamingo 2 (MER124239), ETL protein (MER126267), G protein-coupled receptor 112 (MER126114), seven transmembrane helix receptor (MER125448), Gpr114 protein (MER159320), GPR126 vascular inducible G protein-coupled receptor (MER140015), GPR125 (Homo sapiens)-type protein (MER159279), GPR116 (Homo sapiens)-type G-protein coupled receptor (MER159280), GPR128 (Homo sapiens)-type G-protein coupled receptor (MER162015), GPR133 (Homo sapiens)-type protein (MER159334), GPR110 G-protein coupled receptor (MER159277), GPR97 protein (MER159322), KPG 006 protein (MER161773), KPG 008 protein (MER161835), KPG 009 protein (MER159335), unassigned homologue (MER166269), GPR113 protein (MER159352), brain-specific angiogenesis inhibitor 2 (MER159746), PIDD auto-processing protein unit 1 (MER020001), PIDD auto-processing protein unit 2 (MER063690), MUC1 self-cleaving mucin (MER074260), dystroglycan (MER054741), proprotein convertase 9 (MER022416), site-1 peptidase (MER001948), furin (MER000375), proprotein convertase 1 (MER000376), proprotein convertase 2 (MER000377), proprotein convertase 4 (MER028255), PACE4 proprotein convertase (MER000383), proprotein convertase 5 (MER002578), proprotein convertase 7 (MER002984), tripeptidyl-peptidase II (MER000355), subfamily S8A non peptidase homologues (MER201339), subfamily S8A non-peptidase homologues (MER191613), subfamily S8A unassigned peptidases (MER191611), subfamily S8A unassigned peptidases (MER191612), subfamily S8A unassigned peptidases (MER191614), tripeptidyl-peptidase I (MER003575), prolyl oligopeptidase (MER000393), dipeptidyl-peptidase IV (eukaryote) (MER000401), acylaminoacyl-peptidase (MER000408), fibroblast activation protein alpha subunit (MER000399), PREPL A protein (MER004227), dipeptidyl-peptidase 8 (MER013484), dipeptidyl-peptidase 9 (MER004923), FLJ1 putative peptidase (MER017240), Mername-AA194 putative peptidase (MER017353), Mername-AA195 putative peptidase (MER017367), Memame-AA196 putative peptidase (MER017368), Memame-AA197 putative peptidase (MER017371), Cl4orf29 protein (MER033244), hypothetical protein (MER033245), hypothetical esterase/lipase/thioesterase (MER047309), protein bat5 (MER037840), hypothetical protein flj40219 (MER033212), hypothetical protein flj 37464 (MER033240), hypothetical protein flj33678 (MER033241), dipeptidylpeptidase homologue

DPP6 (MER000403), dipeptidylpeptidase homologue DPP 10 (MER005988), protein similar to *Mus musculus* chromosome 20 open reading frame 135 (MER037845), kynurenine formamidase (MER046020), thyroglobulin precursor (MER011604), acetylcholinesterase (MER033188), cholinesterase (MER033198), carboxylesterase D1 (MER033213), liver carboxylesterase (MER033220), carboxylesterase 3 (MER033224), carboxylesterase 2 (MER033226), bile salt-dependent lipase (MER033227), carboxylesterase-related protein (MER033231), neuroligin 3 (MER033232), neuroligin 4, X-linked (MER033235), neuroligin 4, Y-linked (MER033236), esterase D (MER043126), arylacetamide deacetylase (MER033237), KIAA1363-like protein (MER033242), hormone-sensitive lipase (MER033274), neuroligin 1 (MER033280), neuroligin 2 (MER033283), family S9 non-peptidase homologues (MER212939), family S9 non-peptidase homologues (MER211490), subfamily S9C unassigned peptidases (MER192341), family S9 unassigned peptidases (MER209181), family S9 unassigned peptidases (MER200434), family S9 unassigned peptidases (MER209507), family S9 unassigned peptidases (MER209142), serine carboxypeptidase A (MER000430), vitellogenic carboxypeptidase-like protein (MER005492), RISC peptidase (MER010960), family S15 unassigned peptidases (MER199442), family S15 unassigned peptidases (MER200437), family S15 unassigned peptidases (MER212825), lysosomal Pro-Xaa carboxypeptidase (MER000446), dipeptidyl-peptidase II (MER004952), thymus-specific serine peptidase (MER005538), epoxide hydrolase-like putative peptidase (MER031614), Loc3285744like protein (MER033246), abhydrolase domain-containing protein 4 (MER031616), epoxide hydrolase (MER000432), mesoderm specific transcript protein (MER199890), mesoderm specific transcript protein (MER017123), cytosolic epoxide hydrolase (MER029997), cytosolic epoxide hydrolase (MER213866), similar to hypothetical protein FLJ22408 (MER031608), CGI-58 putative peptidase (MER030163), Williams-Beuren syndrome critical region protein 21 epoxide hydrolase (MER031610), epoxide hydrolase (MER031612), hypothetical protein flj22408 (epoxide hydrolase) (MER031617), monoglyceride lipase (MER033247), hypothetical protein (MER033249), valacyclovir hydrolase (MER033259), Ccgl -interacting factor b (MER210738), glycosylasparaginase precursor (MER003299), isoaspartyl dipeptidase (threonine type) (MER031622), tarpase-1 (MER016969), gamma-glutamyltransferase 5 (mammalian-type) (MER001977), gamma-glutamyltransferase 1 (mammalian-type) (MER001629), gamma-glutamyltransferase 2 (*Homo sapiens*) (MER001976), gamma-glutamyltransferase-like protein 4 (MER002721), gamma-glutamyltransferase-like protein 3 (MER016970), similar to gamma-glutamyltransferase 1 precursor (*Homo sapiens*) (MER026204), similar to gamma-glutamyltransferase 1 precursor (*Homo sapiens*) (MER026205), Mername-AA21 1 putative peptidase (MER026207), gamma-glutamyltransferase

6 (MER159283), gamma-glutamyl transpeptidase homologue (chromosome 2, Homo sapiens) (MER037241), polycystin-1 (MER126824), KIAA1879 protein (MER159329), polycystic kidney disease 1-like 3 (MER172554), gamma-glutamyl hydrolase (MER002963), guanine 5'-monophosphate synthetase (MER043387), carbamoyl-phosphate synthase (Homo sapiens-type) (MER078640), dihydro-orotase (N-terminal unit) (Homo sapiens-type) (MER060647), DJ-1 putative peptidase (MER003390), Memame-AAIOO putative peptidase (MER014802), Memame-AAIOI non-peptidase homologue (MER014803), KIAA0361 protein (Homo sapiens-type) (MER042827), FLJ34283 protein (Homo sapiens) (MER044553), non-peptidase homologue chromosome 21 open reading frame 33 (Homo sapiens) (MER160094), family C56 non-peptidase homologues (MER177016), family C56 non-peptidase homologues (MER176613), family C56 non-peptidase homologues (MER176918), EGF-like module containing mucin-like hormone receptor-like 2 (MER037230), CD97 antigen (human type) (MER037286), EGF-like module containing mucin-like hormone receptor-like 3 (MER037288), EGF-like module containing mucin-like hormone receptor-like 1 (MER037278), EGF-like module containing mucin-like hormone receptor-like 4 (MER037294), cadherin EGF LAG seven-pass G-type receptor 2 precursor (Homo sapiens) (MER045397), Gpr64 (Mus musculus)-type protein (MER123205), GPR56 (Homo sapiens)-type protein (MER122057), latrophilin 2 (MER122199), latrophilin-1 (MER126380), latrophilin 3 (MER124612), protocadherin Flamingo 2 (MER124239), ETL protein (MER126267), G protein-coupled receptor 112 (MER126114), seven transmembrane helix receptor (MER125448), Gpr114 protein (MER159320), GPR126 vascular inducible G protein-coupled receptor (MER140015), GPR125 (Homo sapiens)-type protein (MER159279), GPR116 (Homo sapiens)-type G-protein coupled receptor (MER159280), GPR128 (Homo sapiens)-type G-protein coupled receptor (MER162015), GPR133 (Homo sapiens)-type protein (MER159334) GPR110 G-protein coupled receptor (MER159277), GPR97 protein (MER159322), KPG 006 protein (MER161773) KPG 008 protein (MER161835), KPG 009 protein (MER159335), unassigned homologue (MER166269), GPR113 protein (MER159352), brain-specific angiogenesis inhibitor 2 (MER159746), PIDD auto-processing protein unit 1 (MER020001), PIDD auto-processing protein unit 2 (MER063690), MFJC1 self-cleaving mucin (MER074260), dystroglycan (MER054741), proprotein convertase 9 (MER022416), site-1 peptidase (MER001948), furin (MER000375), proprotein convertase 1 (MER000376), proprotein convertase 2 (MER000377), proprotein convertase 4 (MER028255), PACE4 proprotein convertase (MER000383), proprotein convertase 5 (MER002578), proprotein convertase 7 (MER002984), tripeptidyl-peptidase II (MER000355), subfamily S8A non-peptidase homologues (MER201339), subfamily S8A non-peptidase homologues (MER191613),

subfamily S8A unassigned peptidases (MER191611), subfamily S8A unassigned peptidases (MER191612), subfamily S8A unassigned peptidases (MER191614), tripeptidyl-peptidase I (MER003575), prolyl oligopeptidase (MER000393), dipeptidyl-peptidase IV (eukaryote) (MER000401), acylaminoacyl-peptidase (MER000408), fibroblast activation protein alpha subunit (MER000399), PREPL A protein (MER004227), dipeptidyl-peptidase 8 (MER013484), dipeptidyl-peptidase 9 (MER004923), FLJ1 putative peptidase (MERO 17240), Mername-AA194 putative peptidase (MERO 17353), Memame-AA195 putative peptidase (MER017367), Mername-AA196 putative peptidase (MER017368), Mername-AA197 putative peptidase (MER017371), Cl4orf29 protein (MER033244), hypothetical protein (MER033245), hypothetical esterase/lipase/thioesterase (MER047309), protein bat5 (MER037840), hypothetical protein flj40219 (MER033212), hypothetical protein flj 37464 (MER033240), hypothetical protein flj33678 (MER033241), dipeptidylpeptidase homologue DPP6 (MER000403), dipeptidylpeptidase homologue DPP 10 (MER005988), protein similar to Mus musculus chromosome 20 open reading frame 135 (MER037845), kynurenine formamidase (MER046020), thyroglobulin precursor (MERO 11604), acetylcholinesterase (MER033188), cholinesterase (MER033198), carboxylesterase DI (MER033213), liver carboxylesterase (MER033220), carboxylesterase 3 (MER033224), carboxylesterase 2 (MER033226), bile salt-dependent lipase (MER033227), carboxylesterase-related protein (MER033231), neuroligin 3 (MER033232), neuroligin 4, X-linked (MER033235), neuroligin 4, Y-linked (MER033236), esterase D (MER043126), arylacetamide deacetylase (MER033237), KIAA13634like protein (MER033242), hormone-sensitive lipase (MER033274), neuroligin 1 (MER033280), neuroligin 2 (MER033283), family S9 non peptidase homologues (MER212939), family S9 non-peptidase homologues (MER211490), subfamily S9C unassigned peptidases (MER192341), family S9 unassigned peptidases (MER209181), family S9 unassigned peptidases (MER200434), family S9 unassigned peptidases (MER209507), family S9 unassigned peptidases (MER209142), serine carboxypeptidase A (MER000430), vitellogenic carboxypeptidase-like protein (MER005492), RISC peptidase (MERO 10960), family S15 unassigned peptidases (MER199442), family S15 unassigned peptidases (MER200437), family S15 unassigned peptidases (MER212825), lysosomal Pro-Xaa carboxypeptidase (MER000446), dipeptidyl peptidase II (MER004952), thymus-specific serine peptidase (MER005538), epoxide hydrolase-like putative peptidase (MER031614), Loc328574-like protein (MER033246), abhydrolase domain-containing protein 4 (MER031616), epoxide hydrolase (MER000432), mesoderm specific transcript protein (MER199890), mesoderm specific transcript protein (MER017123), cytosolic epoxide hydrolase (MER029997), cytosolic epoxide hydrolase (MER213866), similar to hypothetical protein

FLJ22408 (MER031608), CGI-58 putative peptidase (MER030163), Williams-Beuren syndrome critical region protein 21 epoxide hydrolase (MER031610), epoxide hydrolase (MER031612), hypothetical protein flj22408 (epoxide hydrolase) (MER031617), monoglyceride lipase (MER033247), hypothetical protein (MER033249), valacyclovir hydrolase (MER033259), Ccgl-interacting factor b (MER210738).

In some embodiments, the SRS is a peptide moiety of up to 15 amino acids in length. In some embodiments, the SRS is cleaved by a protease co-localized with the target of the cell binding moiety in a tissue, and the protease cleaves the SRS in the AFFIMER® polypeptide-drug conjugate when the AFFIMER® polypeptide-drug conjugate is exposed to the protease. In some embodiments, the protease is not active or is significantly less active in tissues that do not significantly express the cell surface feature. In some embodiments, the protease is not active or is significantly less active in healthy, e.g., non-diseased tissues.

In some embodiments, the SRS is cleaved by a protease selected from the following:

- ADAMS or ADAMTS, e.g. ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM 17/T ACE, ADAMDEC1, ADAMTS 1, ADAMTS4 or ADAMTS5;
- Aspartate proteases, e.g., BACE or Renin;
- Aspartic cathepsins (to the extent upregulated or released by cell lysis in the extracellular space), e.g., Cathepsin D or Cathepsin E;
- Caspases (to the extent upregulated or released by cell lysis in the extracellular space), e.g., Caspase 1, Caspase 2, Caspase 3, Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10 or Caspase 14;
- Cysteine cathepsins, e.g., Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2, Cathepsin X/Z/P;
- Cysteine proteinases, e.g., Cruzipain, Legumain or Otubain-2;
- KLKs, e.g., KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 or KLK14;
- Metallo-proteinases, e.g., Meprin, Neprilysin, PSMA or BMP-1;
- MMPs, e.g., MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP19, MMP20, MMP23, MMP24, MMP26, MMP27;
- Serine proteases, e.g., activated protein C, Cathepsin A, Cathepsin G, Chymase, coagulation factor proteases (e.g., FVIIa, FIXa, FXa, FXIa, FXIIa), Elastase, Granzyme B, Guanidinobenzoate, HtrA1, Human Neutrophil Elastase, Lactoferrin, Marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA, Thrombin, Trypsin or uPA; and/or

- Type II Transmembrane Serine Proteases (TTSPs), e.g., DESC1, DPP -4, Hepsin, Matriptase-2, MT-SPI/Matriptase, DMPRSS2, DMPRSS3, DMPRSS4.

For example, suitable SRSs that can be included binder-drug conjugate, i.e., SRS is peptide moiety selected from the group consisting of: TGRGPSWV (SEQ ID NO: 777), SARGPSRW (SEQ ID NO: 778), TARGPSFK (SEQ ID NO: 779), LSGRSDNH (SEQ ID NO: 780), GGWHTGRN (SEQ ID NO: 781), HTGRSGAL (SEQ ID NO: 782), PLTGRSGG (SEQ ID NO: 783), AARGPAIH (SEQ ID NO: 784), RGPAFNPM (SEQ ID NO: 785), SSRGPAYL (SEQ ID NO: 786), RGPATPIM (SEQ ID NO: 787), RGPA (SEQ ID NO: 788), GGQPSGMWGW (SEQ ID NO: 789), FPRPLGITGL (SEQ ID NO: 790), VHMPGLGFLGP (SEQ ID NO: 791), SPLTGRSG (SEQ ID NO: 792), SAGFSLPA (SEQ ID NO: 793), LAPLGLQRR (SEQ ID NO: 794), SGGPLGVR (SEQ ID NO: 795), PLGL (SEQ ID NO: 796), GPRSFG (SEQ ID NO: 797), and GPRSFG (SEQ ID NO: 798).

In some embodiments, the SRS is a substrate for an MMP, such as a sequence selected from the group consisting of ISSGLLSS (SEQ ID NO: 799), QNQALRMA (SEQ ID NO: 800), AQNLLGMV (SEQ ID NO: 801), STFPFGMF (SEQ ID NO: 802), PVGYTSSL (SEQ ID NO: 803), DWLYWPGI (SEQ ID NO: 804), MIAPVAYR (SEQ ID NO: 805), RPSPMWAY (SEQ ID NO: 806), WATPRPMR (SEQ ID NO: 807), FRLLDWQW (SEQ ID NO: 808), LKAAPRWA (SEQ ID NO: 809), GPSHLVLT (SEQ ID NO: 810), LPGGLSPW (SEQ ID NO: 811), MGLFSEAG (SEQ ID NO: 812), SPLPLRVP (SEQ ID NO: 813), RMHLRSLG (SEQ ID NO: 814), LAAPLGLL (SEQ ID NO: 815), AVGLLAPP (SEQ ID NO: 816), LLAPSHRA (SEQ ID NO: 817), PAGLWLDP (SEQ ID NO: 818), and ISSGLSS (SEQ ID NO: 819).

In some embodiments, the SRS is a substrate for an MMP, such as a sequence selected from the group consisting of ISSGLSS (SEQ ID NO: 819), QNQALRMA (SEQ ID NO: 800), AQNLLGMV (SEQ ID NO: 801), STFPFGMF (SEQ ID NO: 802), PVGYTSSL (SEQ ID NO: 803), DWLYWPGI (SEQ ID NO: 804), ISSGLLSS (SEQ ID NO: 799), LKAAPRWA (SEQ ID NO: 809), GPSHLVLT (SEQ ID NO: 810), LPGGLSPW (SEQ ID NO: 811), MGLFSEAG (SEQ ID NO: 812), SPLPLRVP (SEQ ID NO: 813), RMHLRSLG (SEQ ID NO: 814), LAAPLGLL (SEQ ID NO: 815), AVGLLAPP (SEQ ID NO: 816), LLAPSHRA (SEQ ID NO: 817), and PAGLWLDP (SEQ ID NO: 818).

In some embodiments, the SRS is a substrate for thrombin, such as GPRSFG (SEQ ID NO: 797) or GPRSFG (SEQ ID NO: 798).

b) Spacers

In some embodiments, an AFFIMER® polypeptide-drug conjugate comprises a spacer or bond (L^1) between the half-life extension moiety and the substrate recognition sequence (SRS) cleavable by the enzyme, e.g., present in a tumor microenvironment.

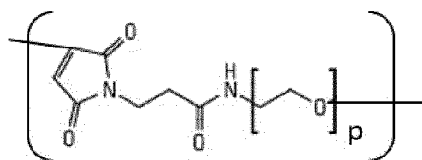
The spacer may be any molecule, for example, one or more nucleotides, amino acids, chemical functional groups. In some embodiments, the spacer is a peptide linker (*e.g.*, two or more amino acids). Spacers should not adversely affect the expression, secretion, or bioactivity of the polypeptides. In some embodiments, spacers are not antigenic and do not elicit an immune response. An immune response includes a response from the innate immune system and/or the adaptive immune system. Thus, an immune response may be a cell-mediate response and/or a humoral immune response. The immune response may be, for example, a T cell response, a B cell response, a natural killer (NK) cell response, a monocyte response, and/or a macrophage response. Other cell response are contemplated herein. In some embodiments, linkers are non-protein-coding.

In some embodiments, L^1 is a hydrocarbon (straight chain or cyclic) such as 6-maleimidocaproyl, maleimidopropanoyl and maleimido ethyl cyclohexane-1-carboxylate, or L^1 is N-Succinimidyl 4-(2-pyridylthio) pentanoate, N-Succinimidyl 4-(N-maleimidoethyl) cyclohexane-1 carboxylate, N-Succinimidyl (4-iodo-acetyl) aminobenzoate.

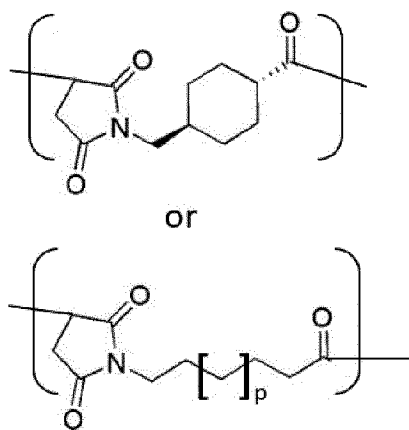
In some embodiments, L^1 is a polyether such as a poly(ethylene glycol) or other hydrophilic linker. For instance, where the CBM includes a thiol (such as a cysteine residue), L^1 can be a polyethylene glycol coupled to the thiol group through a maleimide moiety. Non-limiting examples of linkers for use in accordance with the present disclosure are described in International Publication No. WO 2019/236567, published December 12, 2019, incorporated by reference herein.

c) Self-Immolative Linkers

In some embodiments, an AFFIMER® polypeptide-drug conjugate comprises a self-immolative linker (L^2) between the substrate recognition sequence (SRS) for the enzyme and the drug moiety, such as represented in the formula



wherein, p represents an integer from 1 to 100, preferably 6 to 50, more preferably 6 to 12. In other embodiments, where the CBM includes a thiol and L^1 is a hydrocarbon moiety coupled to the thiol group through a maleimide moiety, L^1 can be represented in the formula



wherein, p represents an integer from 1 to 20, preferably 1 to 4. A self-immolative moiety may be defined as a bifunctional chemical group that is capable of covalently linking together two spaced chemical moieties into a normally stable molecule, releasing one of the spaced chemical moieties from the molecule by means of enzymatic cleavage; and following enzymatic cleavage, spontaneously cleaving from the remainder of the bifunctional chemical group to release the other of said spaced chemical moieties. Therefore, in some embodiments, the self-immolative moiety is covalently linked at one of its ends, directly or indirectly through a spacer unit, to the ligand by an amide bond and covalently linked at its other end to a chemical reactive site (functional group) pending from the drug moiety. The derivatization of the drug moiety with the self-immolative moiety may render the drug less pharmacologically active (*e.g.* less toxic) or not active at all until the drug is cleaved.

An AFFIMER® polypeptide-drug conjugate is generally stable in circulation, or at least that should be the case in the absence of an enzyme capable of cleaving the amide bond between the substrate recognition sequence (enzyme-cleavable linker) and the self-immolative moiety. Upon exposure of an AFFIMER® polypeptide-drug conjugate to a suitable enzyme, the amide bond is cleaved initiating a spontaneous self-immolative reaction resulting in the cleavage of the bond covalently linking the self-immolative moiety to the drug moiety, to thereby effect release of the free drug moiety in its underivatized or pharmacologically active form. The self-immolative moiety in conjugates either incorporate one or more heteroatoms and thereby provides improved solubility, improves the rate of cleavage and decreases propensity for aggregation of the conjugate.

In some embodiments, L^2 is a benzyl oxy carbonyl group. In other embodiments, the self-immolative linker L^2 is $\text{—NH—(CH}_2\text{)}_4\text{—C(=O)—}$ or $\text{—NH—(CH}_2\text{)}_3\text{—C(=O)—}$. In yet other embodiments, the self-immolative linker L^2 is *p*-aminobenzyloxycarbonyl (PABC). In still other embodiments, the self-immolative linker L^2 is 2,4-bis(hydroxymethyl)aniline.

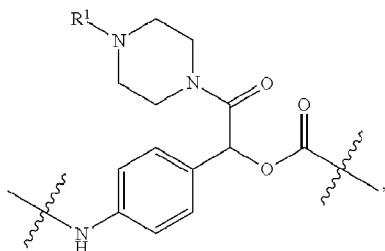
The AFFIMER® polypeptide-drug conjugate of the present disclosure can employ a heterocyclic self-immolative moiety covalently linked to the therapeutic moiety and the cleavable substrate recognition sequence. A self-immolative moiety may be defined as a bifunctional chemical group which is capable of covalently linking together two spaced chemical moieties into a normally stable molecule, releasing one of said spaced chemical moieties from the molecule by means of enzymatic cleavage; and following said enzymatic cleavage, spontaneously cleaving from the remainder of the bifunctional chemical group to release the other of said spaced chemical moieties. In accordance with the present present disclosure, the self-immolative moiety may be covalently linked at one of its ends, directly or indirectly through a spacer unit, to the ligand by an amide bond and covalently linked at its other end to a chemical reactive site (functional group) pending from the drug. The derivatization of the therapeutic moiety with the self-immolative moiety may render the drug less pharmacologically active (e.g. less toxic) or not active at all until the drug is cleaved.

The AFFIMER® polypeptide-drug conjugate is generally stable in circulation, or at least that should be the case in the absence of an enzyme capable of cleaving the amide bond between the substrate recognition sequence and the self-immolative moiety. However, upon exposure of the AFFIMER® polypeptide-drug conjugate to a suitable enzyme, the amide bond is cleaved initiating a spontaneous self-immolative reaction resulting in the cleavage of the bond covalently linking the self-immolative moiety to the drug, to thereby effect release of the free therapeutic moiety in its underivatized or pharmacologically active form.

The self-immolative moiety in conjugates of the present disclosure, in some embodiments, either incorporate one or more heteroatoms and thereby provides improved solubility, improves the rate of cleavage and decreases propensity for aggregation of the conjugate. These improvements of the heterocyclic self-immolative linker constructs of the present disclosure over non-heterocyclic, PAB-type linkers may result in surprising and unexpected biological properties such as increased efficacy, decreased toxicity, and more desirable pharmacokinetics.

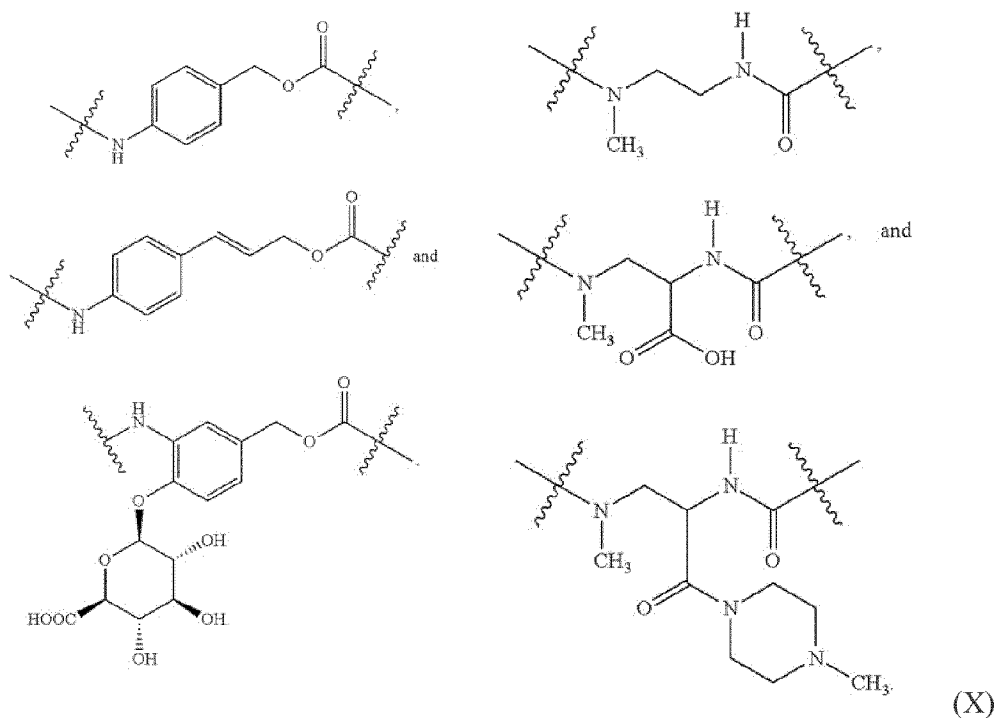
In some embodiments, L^2 is a benzyloxycarbonyl group.

In some embodiments, L^2 is

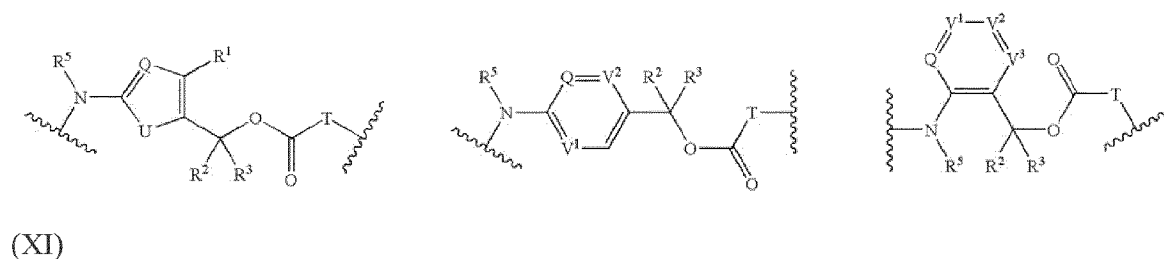


wherein R^1 is hydrogen, unsubstituted or substituted C_{1-3} alkyl, or unsubstituted or substituted heterocyclyl. In some embodiments, R^1 is hydrogen. In some instances, R^1 is methyl.

In some embodiments, L^2 is selected from



In some embodiments, the self-immolative moiety L_2 is selected from



wherein

U is O, S or NR^6 ;

Q is CR^4 or N;

V^1 , V^2 and V^3 are independently CR^4 or N provided that for formula (X) and (XI) at least one of Q, V^1 and V^2 is N;

T is NH, NR^6 , O or S pending from said therapeutic moiety;

R^1 , R^2 , R^3 and R^4 are independently selected from H, F, Cl, Br, I, OH, $-N(R^5)_2$, $-$

$N(R^5)_3^+$, C_{1-8} alkylhalide, carboxylate, sulfate, sulfamate, sulfonate, $-SO_2R^5$, $-$

$S(=O)R^5$, $-SR^5$, $-SO_2N(R^5)_2$, $-C(=O)R^5$, $-CO_2R^5$, $-C(=O)N(R^5)_2$, $-CN$, $-N_3$, $-NO_2$, C_1 - C_8 alkoxy, C_1 - C_8 halosubstituted alkyl, polyethyleneoxy, phosphonate, phosphate, C_1 - C_8 alkyl, C_1 - C_8 substituted alkyl, C_2 - C_8 alkenyl, C_2 - C_8 substituted alkenyl, C_2 - C_8 alkynyl, C_2 - C_8 substituted alkynyl, C_6 - C_{20} aryl, C_6 - C_{20} substituted aryl, C_1 - C_{20} heterocycle, and C_1 - C_{20} substituted heterocycle; or when taken together, R^2 and R^3 form a carbonyl ($=O$), or spiro carbocyclic ring of 3 to 7 carbon atoms; and R^5 and R^6 are independently selected from H, C_1 - C_8 alkyl, C_1 - C_8 substituted alkyl, C_2 - C_8 alkenyl, C_2 - C_8 substituted alkenyl, C_2 - C_8 alkynyl, C_2 - C_8 substituted alkynyl, C_6 - C_{20} aryl, C_6 - C_{20} substituted aryl, C_1 - C_{20} heterocycle, and C_1 - C_{20} substituted heterocycle; where C_1 - C_8 substituted alkyl, C_2 - C_8 substituted alkenyl, C_2 - C_8 substituted alkynyl, C_6 - C_{20} substituted aryl, and C_2 - C_{20} substituted heterocycle are independently substituted with one or more substituents selected from F, Cl, Br, I, OH, $-N(R^5)_2$, $-N(R^5)_3^+$, C_1 - C_8 alkylhalide, carboxylate, sulfate, sulfamate, sulfonate, C_1 - C_8 alkylsulfonate, C_1 - C_8 alkylamino, 4-dialkylaminopyridinium, C_1 - C_8 alkylhydroxyl, C_1 - C_8 alkylthiol, $-SO_2R^5$, $-S(=O)R^5$, $-SR^5$, $-SO_2N(R^5)_2$, $-C(=O)R^5$, $-CO_2R^5$, $-C(=O)N(R^5)_2$, $-CN$, $-N_3$, $-NO_2$, C_1 - C_8 alkoxy, C_1 - C_8 trifluoroalkyl, C_1 - C_8 alkyl, C_3 - C_{12} carbocycle, C_6 - C_{20} aryl, C_2 - C_{20} heterocycle, polyethyleneoxy, phosphonate, and phosphate.

It will be understood that when T is NH, it is derived from a primary amine ($-NH_2$) pending from the therapeutic moiety (prior to coupling to the self-immolative moiety) and when T is N, it is derived from a secondary amine ($-NH-$) from the therapeutic moiety (prior to coupling to the self-immolative moiety). Similarly, when T is O or S, it is derived from a hydroxyl ($-OH$) or sulfhydryl ($-SH$) group respectively pending from the therapeutic moiety prior to coupling to the self-immolative moiety.

In some embodiments, the self-immolative linker L^2 is $-NH-(CH_2)_4-C(=O)-$ or $-NH-(CH_2)_3-C(=O)-$.

In some embodiments, the self-immolative linker L^2 is p-aminobenzyloxycarbonyl (PABC).

In some embodiments, the self-immolative linker L^2 is 2,4-bis(hydroxymethyl)aniline.

Other examples of self-immolative linkers that are readily adapted for use in AFFIMER® polypeptide-drug conjugates described herein are taught in, for example, US Patent 7,754,681; WO 2012/074693A1; US 9,089,614; EP 1,732,607; WO 2015/038426A1 (all of which are incorporated by reference); Walther *et al.* "Prodrugs in medicinal chemistry and enzyme prodrug therapies" *Adv Drug Deliv Rev.* 2017 Sep 1; 118:65-77; and Tranoy-Opalinski et al. "Design of

self-immolative linkers for tumor-activated prodrug therapy”, *Anticancer Agents Med Chem.* 2008 Aug;8(6):618-37; the teachings of each of which are incorporated by reference herein.

Yet other non-limiting examples of self-immolative linkers for use in accordance with the present disclosure are described in International Publication No. WO 2019/236567, published December 12, 2019, incorporated by reference herein.

IV. Encoded AFFIMER® Construct for *In vivo* Delivery

An alternative approach to the delivery of therapeutic AFFIMER® agents, such as a CD33 AFFIMER® agent, would be to leave the production of the therapeutic polypeptide to the body itself. A multitude of clinical studies have illustrated the utility of *in vivo* gene transfer into cells using a variety of different delivery systems. *In vivo* gene transfer seeks to administer to patients the encoded AFFIMER® construct, rather than the AFFIMER® agent. This allows the patient’s body to produce the therapeutic AFFIMER® agent of interest for a prolonged period of time, and secrete it either systemically or locally, depending on the production site. Gene-based encoded AFFIMER® construct can present a labor- and cost-effective alternative to the conventional production, purification and administration of the polypeptide version of the AFFIMER® agent. A number of antibody expression platforms have been pursued *in vivo* to which delivery of encoded AFFIMER® construct can be adapted: these include viral vectors, naked DNA and RNA. encoded AFFIMER® construct gene transfer can not only enable cost-savings by reducing the cost of goods and of production but may also be able to reduce the frequency of drug administration. Overall, a prolonged *in vivo* production of the therapeutic AFFIMER® agent by expression of the encoded AFFIMER® construct can contribute to (i) a broader therapeutic or prophylactic application of AFFIMER® agents in price-sensitive conditions, (ii) an improved accessibility to therapy in both developed and developing countries, and (iii) more effective and affordable treatment modalities. In addition to *in vivo* gene transfer, cells can be harvested from the host (or a donor), engineered with encoded AFFIMER® construct sequences to produce AFFIMER® agents and re-administered to patients.

Intramuscular antibody gene administration has been most widely evaluated (reviewed in Deal et al. (2015) “Engineering humoral immunity as prophylaxis or therapy” *Curr Opin Immunol.* 35:113–22.), and also carries the highest clinical translatability and application when applied to encoded AFFIMER® construct. Indeed, the inherent anatomical, cellular and physiological properties of skeletal muscle make it a stable environment for long-term encoded AFFIMER® construct expression and systemic circulation. Skeletal muscle is easily accessible, allowing multiple or repeated administrations. The abundant blood vascular supply provides an

efficient transport system for secreted therapeutic AFFIMER® agents into the circulation. The syncytial nature of muscle fibers allows dispersal of nucleotides from a limited site of penetration to a large number of neighboring nuclei within the fiber. Skeletal muscle fibers are also terminally differentiated cells, and nuclei within the fibers are post-mitotic. Consequently, integration in the host genome is not a prerequisite to attain prolonged monoclonal antibody (mAb) expression. The liver is another site often used for pre-clinical antibody gene transfer and is typically transfected via intravenous (i.v.) injection and can also be a site of gene transfer for encoded AFFIMER® construct either for local delivery of AFFIMER® agents (such as in the treatment of liver cancer and/or metaplasias) or for the generation of AFFIMER® agents that are secreted into the vascular for systemic circulation. This organ has various physiological functions, including the synthesis of plasma proteins. This organ can be particularly well suited for *in vivo* encoded AFFIMER® construct expression.

The tumor presents another site for encoded AFFIMER® construct transfer, targeted either via i.v. or direct injection/electroporation. Indeed, intratumoral encoded AFFIMER® construct expression can allow for a local production of the therapeutic AFFIMER® agents, waiving the need for high systemic AFFIMER® agent levels that might otherwise be required to penetrate and impact solid tumors. A similar rationale applies for the brain, which is frequently targeted in the context of antibody gene transfer to avoid the difficulties with blood–brain barrier trafficking and would likewise be a target for delivery of encoded AFFIMER® construct. See, for example, Beckman et al. (2015) “Antibody constructs in cancer therapy: protein engineering strategies to improve exposure in solid tumors” *Cancer* 109(2):170–9; Dronca et al. (2015) “Immunomodulatory antibody therapy of cancer: the closer, the better” *Clin Cancer Res.* 21(5):944–6; and Neves et al. (2016) “Antibody approaches to treat brain diseases” *Trends Biotechnol.* 34(1):36–48.

The success of gene therapy has largely been driven by improvements in nonviral and viral gene transfer vectors. An array of physical and chemical nonviral methods have been used to transfer DNA and mRNA to mammalian cells and a substantial number of these have been developed as clinical stage technologies for gene therapy, both *ex vivo* and *in vivo*, and are readily adapted for delivery of the encoded AFFIMER® construct of the present disclosure. To illustrate, cationic liposome technology can be employed, which is based on the ability of amphipathic lipids, possessing a positively charged head group and a hydrophobic lipid tail, to bind to negatively charged DNA or RNA and form particles that generally enter cells by endocytosis. Some cationic liposomes also contain a neutral co-lipid, thought to enhance liposome uptake by mammalian cells. See, for example, Felgner et al. (1987) Lipofection: a

highly efficient, lipid-mediated DNA-transfection procedure. *MNAS* 84:7413–7417; San et al. (1983) “Safety and short-term toxicity of a novel cationic lipid formulation for human gene therapy” *Hum. Gene Ther.* 4:781–788; Xu et al. (1996) “Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection” *Biochemistry* 35:5616–5623; and Legendre et al. (1992) “Delivery of plasmid DNA into mammalian cell lines using pH-sensitive liposomes: comparison with cationic liposomes” *Pharm. Res.* 9, 1235-1242.

Similarly, other polycations, such as poly-L-lysine and polyethylene-imine, can be used to deliver encoded AFFIMER® construct. These polycations complex with nucleic acids via charge interaction and aid in the condensation of DNA or RNA into nanoparticles, which are then substrates for endosome-mediated uptake. Several of these cationic nucleic acid complex technologies have been developed as potential clinical products, including complexes with plasmid DNA, oligodeoxynucleotides, and various forms of synthetic RNA. Modified (and unmodified or “naked”) DNA and RNA have also been shown to mediate successful gene transfer in a number of circumstances and can also be used as systems for delivery of encoded AFFIMER® construct. These include the use of plasmid DNA by direct intramuscular injection, the use of intratumoral injection of plasmid DNA. See, for example, Rodrigo et al. (2012) “De novo automated design of small RNA circuits for engineering synthetic riboregulation in living cells” *PNAS* 109:15271–15276; Oishi et al. (2005) “Smart polyion complex micelles for targeted intracellular delivery of PEGylated antisense oligonucleotides containing acid-labile linkages” *Chembiochem.* 6:718–725; Bhatt et al. (2015) “Microbeads mediated oral plasmid DNA delivery using polymethacrylate vectors: an effectual groundwork for colorectal cancer” *Drug Deliv.* 22:849–861; Ulmer et al. (1994) Protective immunity by intramuscular injection of low doses of influenza virus DNA vaccines” *Vaccine* 12: 1541–1544; and Heinzerling et al. (2005) “Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: clinical efficacy” *Hum. Gene Ther.* 16:35–48.

Viral vectors are currently used as a delivery vehicle in the vast majority of pre-clinical and clinical gene therapy trials and in the first to be approved directed gene therapy. See *Gene Therapy Clinical Trials Worldwide 2017* (abedia.com/wiley/). The main driver thereto is their exceptional gene delivery efficiency, which reflects a natural evolutionary development; viral vector systems are attractive for gene delivery, because viruses have evolved the ability to cross through cellular membranes by infection, thereby delivering nucleic acids such as encoded AFFIMER® construct to target cells. Pioneered by adenoviral systems, the field of viral vector-mediated antibody gene transfer made significant strides in the past decades. The myriad of successfully evaluated administration routes, pre-clinical models and disease indications puts the

capabilities of antibody gene transfer at full display through which the skilled artisan would readily be able to identify and adapt antibody gene transfer systems and techniques for *in vivo* delivery of encoded AFFIMER® polypeptides. Muscle has emerged as the administration site of choice for prolonged mAb expression and would similarly be a suitable target tissue for prolonged AFFIMER® agent expression. In the context of vectored intratumoral encoded AFFIMER® construct gene transfer, oncolytic viruses have a distinct advantage, as they can specifically target tumor cells, boost AFFIMER® agent expression, and amplify therapeutic responses – such as to a CD33 AFFIMER® agent.

In vivo gene transfer of encoded AFFIMER® construct can also be accomplished by use of nonviral vectors, such as expression plasmids. Nonviral vectors are easily produced and do not seem to induce specific immune responses. Muscle tissue is most often used as target tissue for transfection because muscle tissue is well vascularized and easily accessible, and myocytes are long-lived cells. Intramuscular injection of naked plasmid DNA results in transfection of a certain percentage of myocytes. Using this approach, plasmid DNA encoding cytokines and cytokine/IgG1 chimeric proteins has been introduced *in vivo* and has positively influenced (autoimmune) disease outcome.

In some instances, in order to increase transfection efficiency via so-called intravascular delivery in which increased gene delivery and expression levels are achieved by inducing a short-lived transient high pressure in the veins. Special blood-pressure cuffs that may facilitate localized uptake by temporarily increasing vascular pressure and can be adapted for use in human patients for this type of gene delivery. See, for example, Zhang et al. (2001) “Efficient expression of naked DNA delivered intraarterially to limb muscles of nonhuman primates” *Hum. Gene Ther.*, 12:427-438

Increased efficiency can also be gained through other techniques, such as in which delivery of the nucleic acid is improved by use of chemical carriers—cationic polymers or lipids—or via a physical approach—gene gun delivery or electroporation. See Tranchant et al. (2004) “Physicochemical optimization of plasmid delivery by cationic lipids” *J. Gene Med.*, 6 (Suppl. 1):S24-S35; and Niidome et al. (2002) “Gene therapy progress and prospects: nonviral vectors” *Gene Ther.*, 9:1647-1652. Electroporation is especially regarded as an interesting technique for nonviral gene delivery. Somiari, et al. (2000) “Theory and *in vivo* application of electroporative gene delivery” *Mol. Ther.* 2:178-187; and Jaroszeski et al. (1999) “*In vivo* gene delivery by electroporation” *Adv. Drug Delivery Rev.*, 35:131-137. With electroporation, pulsed electrical currents are applied to a local tissue area to enhance cell permeability, resulting in gene transfer across the membrane. Research has shown that *in vivo* gene delivery can be at least 10–

100 times more efficient with electroporation than without. See, for example, Aihara et al. (1998) "Gene transfer into muscle by electroporation *in vivo*" Nat. Biotechnol. 16:867-870; Mir, et al. (1999) "High-efficiency gene transfer into skeletal muscle mediated by electric pulses" PNAS 96:4262-4267; Rizzuto, et al. (1999) "Efficient and regulated erythropoietin production by naked DNA injection and muscle electroporation" PNAS 96: 6417-6422; and Mathiesen (1999) "Electropermeabilization of skeletal muscle enhances gene transfer *in vivo*" Gene Ther., 6:508-514.

Encoded CD33 AFFIMER® polypeptides can be delivered by a wide range of gene delivery system commonly used for gene therapy including viral, non-viral, or physical. See, for example, Rosenberg et al., Science, 242:1575-1578, 1988, and Wolff et al., Proc. Natl. Acad. Sci. USA 86:9011-9014 (1989). Discussion of methods and compositions for use in gene therapy include Eck et al., in Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, Hardman et al., eds., McGraw-Hill, New York, (1996), Chapter 5, pp. 77-101; Wilson, Clin. Exp. Immunol. 107 (Suppl. 1):31-32, 1997; Wivel et al., Hematology/Oncology Clinics of North America, Gene Therapy, S. L. Eck, ed., 12(3):483-501, 1998; Romano et al., Stem Cells, 18:19-39, 2000, and the references cited therein. U.S. Pat. No. 6,080,728 also provides a discussion of a wide variety of gene delivery methods and compositions. The routes of delivery include, for example, systemic administration and administration *in situ*.

An effective encoded AFFIMER® construct gene transfer approach must be directed to the specific tissues/cells where it is needed, and the resulting transgene expression should be at a level that is appropriate to the specific application. Promoters are a major cis-acting element within the vector genome design that can dictate the overall strength of expression as well as cell-specificity.

Table 10. Exemplary Ubiquitous and Cell-specific Promoters.

Promoter	Specificity	Relative Strength	Size (bps)	Reference(s)
CMV	Ubiquitous	+++	750–800	Xu et al. Gene Ther. 2001 8:1323–1332; Gray et al., Hum Gene Ther. 2011 22:1143–1153
CBA (including derivatives: CAG, CBh, etc.)	Ubiquitous	+++	248–1,600	Klein et al. Exp Neurol. 2002 176(1):66–74; Ohlfest et al. Blood. 2005 105:2691–2698; and Gray et al. Hum Gene Ther. 2011 22:1143–1153.
EF-1 α	Ubiquitous	++	2,500	Gill et al. Gene Ther. 2001 8(20):1539–1546; Xu et al. Gene

Promoter	Specificity	Relative Strength	Size (bps)	Reference(s)
				Ther. 2001 8:1323–1332; and Gilham et al. J Gene Med. 2010 12(2):129–136.
PGK	Ubiquitous	++	426	Gilham et al. J Gene Med. 2010 12(2):129–136.
UBC	Ubiquitous	+	403	Gill et al. Gene Ther. 2001 8(20):1539–1546; Qin et al. PLoS One. 2010 5(5):e10611.
GUSB (hGBp)	Ubiquitous	+	378	Husain et al. Gene Ther. 2009 16:927–932.
UCOE (Promoter of HNRPA2B1-CBX3)	Ubiquitous	++	600–2,500	Antoniou et al. Hum Gene Ther. 2013 24(4):363–374.
hAAT	Liver	++	347–1,500	Van Linthout et al. Hum Gene Ther. 2002 13(7):829–840; Cunningham et al. Mol Ther. 2008 16(6):1081–1088
TBG	Liver	++	400	Yan et al. Gene. 2012 506(2):289–294.
Desmin	Skeletal muscle	+++	1,700	Talbot et al. Mol Ther. 2010 18:601–608.
MCK	Skeletal muscle	++	595–1,089	Talbot et al. Mol Ther. 2010 18:601–608; Wang et al. Gene Ther. 2008 15:1489–1499; Katwal et al. Gene Ther. 2013 20(9):930–938.
C5-12	Skeletal, cardiac, and diaphragm	++	312	Wang et al. Gene Ther. 2008 15:1489–1499
NSE	Neuron	+++	300–2,200	Xu et al. Gene Ther. 2001 8:1323–1332
Synapsin	Neuron	+	470	Kügler et al. Virology. 2003 311:89–95; Hioki et al. Gene Ther. 2007 14:872–882; Kuroda et al. J Gene Med. 2008 10:1163–1175.
PDGF	Neuron	+++	1,400	Patterna et al. Gene Ther. 2000 7(15):1304–1311; Hioki et al. Gene Ther. 2007 14:872–882
MecP2	Neuron	+	229	Rastegar et al. LoS One. 2009 4:e6810; Gray et al., Hum Gene Ther. 2011 22:1143–1153

Promoter	Specificity	Relative Strength	Size (bps)	Reference(s)
CaMKII	Neuron	++	364–2,300	Hioki et al. Gene Ther. 2007 14:872–882; Kuroda et al. J Gene Med. 2008 10:1163–1175
mGluR2	Neuron	+	1,400	Brené et al. Eur J Neurosci. 2000 12:1525–1533; Kuroda et al. J Gene Med. 2008 10:1163–1175
NFL	Neuron	+	650	Xu et al. Gene Ther. 2001 8:1323–1332
NFH	Neuron	+	920	Xu et al. Gene Ther. 2001 8:1323–1332
nβ2	Neuron	+	650	Xu et al. Gene Ther. 2001 8:1323–1332
PPE	Neuron	+	2700	Xu et al. Gene Ther. 2001 8:1323–1332
Enk	Neuron	+	412	Xu et al. Gene Ther. 2001 8:1323–1332
EAAT2	Neuron and astrocyte	++	966	Su et al. Proc Natl Acad Sci U S A. 2003 100:1955–1960; Kuroda et al. J Gene Med. 2008 10:1163–1175
GFAP	Astrocyte	++	681–2,200	Brenner et al. J Neurosci. 1994 14:1030–1037; Xu et al. Gene Ther. 2001 8:1323–1332; Lee et al. Glia. 2008 56:481–493; Dirren et al. Hum Gene Ther. 2014 25:109–120
MBP	Oligodendrocytes	++	1,900	Chen et al. Gene Ther. 1998 5(1):50–58

In some cases, ubiquitous expression of the encoded AFFIMER® construct in all cell types is desired. Constitutive promoters such as the human elongation factor 1 α -subunit (EF1 α), immediate-early cytomegalovirus (CMV), chicken β -actin (CBA) and its derivative CAG, the β glucuronidase (GUSB), or ubiquitin C (UBC) can be used to promote expression of the encoded AFFIMER® construct in most tissues. Generally, CBA and CAG promote the larger expression among the constitutive promoters; however, their size of ~1.7 kbs in comparison to CMV (~0.8 kbs) or EF1 α (~1.2 kbs) may limit use in vectors with packaging constraints such as AAV, particularly where AFFIMER® agent produced by expression of the encoded AFFIMER® construct is large. The GUSB or UBC promoters can provide ubiquitous gene expression with a smaller size of 378 bps and 403 bps, respectively, but they are considerably weaker than the CMV or CBA promoter. Thus, modifications to constitutive promoters in order to reduce the size

without affecting its expression have been pursued and examples such as the CBh (~800 bps) and the miniCBA (~800 bps) can promote expression comparable and even higher in selected tissues (Gray et al., Hum Gene Ther. 2011 22:1143–1153).

When expression of the encoded AFFIMER® construct should be restricted to certain cell types within an organ, promoters can be used to mediate this specificity. For example, within the nervous system promoters have been used to restrict expression to neurons, astrocytes, or oligodendrocytes. In neurons, the neuron-specific enolase (NSE) promoter drives stronger expression than ubiquitous promoters. Additionally, the platelet-derived growth factor B-chain (PDGF- β), the synapsin (Syn), and the methyl-CpG binding protein 2 (MeCP2) promoters can drive neuron-specific expression at lower levels than NSE. In astrocytes, the 680 bps-long shortened version [gfaABC(1)D] of the glial fibrillary acidic protein (GFAP, 2.2 kbs) promoter can confer higher levels of expression with the same astrocyte-specificity as the GFAP promoter. Targeting oligodendrocytes can also be accomplished by the selection of the myelin basic protein (MBP) promoter, whose expression is restricted to this glial cell; however, its size of 1.9 kbs and low expression levels limit its use.

In the case of expressing the encoded AFFIMER® construct in skeletal muscle cells, exemplary promoters based on muscle creatine kinase (MCK) and desmin (1.7 kbs) have showed a high rate of specificity (with minimal expression in the liver if desired). The promoter of the α -myosin heavy chain (α -MHC; 1.2 kbs) has shown significant cardiac specificity in comparison with other muscle promoters (Lee et al., 2011 J Cardiol. 57(1):115-22). In hematopoietic stem cells the synthetic MND promoter (Li et al., 2010 J Neurosci Methods. 189(1):56-64) and the promoter contained in the 2AUCOE (ubiquitous chromatin opening element) have shown to drive a higher transgene expression in all cell lineages when compared to the EF1 α and CMV promoters, respectively (Zhang et al., 2007 Blood. 110(5):1448-57; Koldej 2013 Hum Gene Ther Clin Dev. 24(2):77-85; Dighe et al., 2014 PLoS One. 9(8):e104805.). Conversely, using promoters to restrict expression to only liver hepatocytes after vector-mediated gene transfer has been shown to reduce transgene-specific immune responses in systems where that is a risk, and to even induce immune tolerance to the expressed protein (Zhang et al., 2012 Hum Gene Ther. 23(5):460-72), which for certain AFFIMER® agents may be beneficial. The α 1-antitrypsin (hAAT; 347 bps) and the thyroxine binding globulin (TBG; ~400 bps) promoters drive gene expression restricted to the liver with minimal invasion to other tissues (Yan et al., 2012 Gene. 506(2):289-94; Cunningham et al., 2008 Mol Ther. 16(6):1081-8).

In some embodiments, a mechanism to control the duration and amount of *in vivo* encoded AFFIMER® construct expression will typically be desired. There are a variety of

inducible promoters which can be adapted for use with viral vectored- and plasmid DNA-based encoded AFFIMER® construct gene transfer. See Fang et al. (2007) “An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies *in vivo*” *Mol Ther.* 5(6):1153–9; and Perez et al. (2004) “Regulatable systemic production of monoclonal antibodies by *in vivo* muscle electroporation” *Genet Vaccines Ther.* 2(1):2. An exemplary a regulatable mechanism currently under clinical evaluation is an ecdysone-based gene switch activated by a small molecule ligand. Cai et al. (2016) “Plasma pharmacokinetics of veledimex, a small-molecule activator ligand for a proprietary gene therapy promoter system, in healthy subjects” *Clin Pharmacol Drug Dev.* 2016.

In some embodiments of an encoded AFFIMER® construct, viral post-transcriptional regulatory elements (PREs) may be used; these cis-acting elements are required for nuclear export of intronless viral RNA (Huang and Yen, 1994 *J Virol.* 68(5):3193-9; and 1995 *Mol Cell Biol.* 15(7):3864-9). Examples include HPRE (Hepatitis B Virus PRE, 533 bps) and WPRE (Woodchuck Hepatitis Virus PRE, 600 bps), which can increase the level of transgene expression by almost 10-fold in certain instances (Donello et al., 1998 *J Virol.* 72(6):5085-92). To further illustrate, using lentiviral and AAV vectors, WPRE was found to increase CMV promoter driven transgene expression, as well as increase PPE, PDGF and NSE promoter-driven transgene expression. Another effect of the WPRE can be to protect encoded AFFIMER® transgenes from silencing (Paterna et al., 2000 *Gene Ther.* 7(15):1304-11; Xia et al., 2007 *Stem Cells Dev.* 2007 Feb; 16(1):167-76).

The polyadenylation of a transcribed encoded AFFIMER® construct transcript can also be important for nuclear export, translation, and mRNA stability. Therefore, in some embodiments, the encoded AFFIMER® construct will include a polyadenylation signal sequence. A variety of studies are available that have determined the effects of different polyA signals on gene expression and mRNA stability. Exemplary polyadenylation signal sequences include SV40 late or bovine growth hormone polyA (bGHpA) signal sequences, as well as minimal synthetic polyA (SPA) signal (Levitt et al., 1989 *Genes Dev.* 3(7):1019-25; Yew et al., 1997 *Hum Gene Ther.* 1997 8(5):575-84). The efficiency of polyadenylation is increased by the SV40 late polyA signal upstream enhancer (USE) placed upstream of other polyA signals (Schek et al., 1992 *Mol Cell Biol.* 12(12):5386-93). In some embodiments, merely to illustrate, the encoded AFFIMER® construct will include an SV40 late + 2xUSE polyA signal.

Table 11. Exemplary Polyadenylation Signals

PolyA Signal and USE	Relative Strength	Size (bps)	Source	Reference(s)
hGH	+	624	Human growth hormone	Ostedgaard et al. Proc Natl Acad Sci U S A. 2005 102(8):2952–2957
SV40 late	+++	135	Simian virus 40	Choi et al. Mol Brain. 2014 7:17
SPA (synthetic polyA)	+	49	Rabbit β -globin	Levitt et al. Genes Dev. 3(7):1019–1025; Yew et al. Hum Gene Ther. 1997 8(5):575–584; Ostedgaard et al. Proc Natl Acad Sci U S A. 2005 102(8):2952–2957; Choi et al. Mol Brain. 2014 7:17
bGH	++	250	Bovine growth hormone	Yew et al. Hum Gene Ther. 1997 8(5):575–584; Xu et al. Gene Ther. 2001 8:1323–1332; Wu et al. Mol Ther. 2008 16(2):280–289; Gray et al., Hum Gene Ther. 2011 22:1143–1153; Choi et al. Mol Brain. 2014 7:17
SV40 late 2xUSE	++	100	Simian virus 40	Schambach et al. Mol Ther. 2007 15(6):1167–1173; Choi et al. Mol Brain. 2014 7:17
HIV-1 USE	+	35	Human immunodeficiency virus 1	Schambach et al. Mol Ther. 2007 15(6):1167–1173
GHV USE	+	39	Ground squirrel hepatitis virus	Schambach et al. Mol Ther. 2007 15(6):1167–1173
Adenovirus (L3) USE	+	21	Adenovirus	Schambach et al. Mol Ther. 2007 15(6):1167–1173
hTHGB USE	+	21	Human prothrombin	Schambach et al. Mol Ther. 2007 15(6):1167–1173
hC2 USE	+	53	Human C2 complement gene	Schambach et al. Mol Ther. 2007 15(6):1167–1173

In some embodiments, it may be desirable for the encoded AFFIMER® construct to include at least one regulatory enhancers, e.g., in addition to any promoter sequences. The CMV enhancer is upstream of the CMV promoter at –598 to –68 (Boshart et al., 1985 Cell. 41(2):521–30) (~600 bps) and contains transcription binding sites. In some embodiments, a CMV enhancer can be included in the construct to increase tissue-specific promoter-driven transgene expression, such as using the ANF (atrial natriuretic factor) promoter, the CC10 (club cell 10) promoter, SP-C (surfactant protein C) promoter, or the PDGF- β (platelet-derived growth factor- β) promoter (merely as examples). Altogether, the CMV enhancer increases transgene expression under different cell-specific promoters and different cell types making it a broadly applicable tool to

increase transgene expression levels. In muscle, for example, in AAV expression systems transgene expression using the CMV enhancer with a muscle-specific promoter can increase expression levels of the protein encoded by the transgene, so would be particularly useful in the current disclosure for expressing AFFIMER® agents from encoded AFFIMER® constructs introduced into muscle cells of a patient.

The encoded AFFIMER® agents may also include at least one intronic sequence. The presence of an intron or intervening sequence in mRNA was first described, *in vitro*, to be important for mRNA processing and increased transgene expression (Huang and Gorman, 1990 *Mol Cell Biol.* 10(4):1805-10; Niwa et al., 1990 *Genes Dev.* 4(9):1552-9). The intron(s) can be placed within the coding sequence for the AFFIMER® agent and/or can be placed between the promoter and transgene. A variety of introns (**Table 12**) placed between the promoter and transgene were compared, in mice using AAV2, for liver transgene expression (Wu et al., 2008). The MVM (minute virus of mice) intron increased transgene expression more than any other intron tested and more than 80-fold over no intron (Wu et al., 2008). However, in cultured neurons using AAV expression cassettes, transgene expression was less under a CaMPKII promoter with a chimeric intron (human β -globin donor and immunoglobulin heavy chain acceptor) between the transgene and polyA signal compared to a WPRE (Choi et al., 2014). Together, an intron can be a valuable element to include in an expression cassette to increase transgene expression.

Table 12. Exemplary Introns

Ittron	Relative Strength	Size (bps)	Source	Reference(s)
MVM	+++	67–97	Minute virus of mice	Wu et al. <i>Mol Ther.</i> 2008 16(2):280–289
F.IX truncated intron 1	+	300	Human factor IX	Wu et al. <i>Mol Ther.</i> 2008 16(2):280–289; Kurachi et al. <i>J Biol Chem.</i> 1995 270(10):5276–5281
β -globin SD / immunoglobulin heavy chain SA	+	250	Human, pZac2.1	Wu et al. <i>Mol Ther.</i> 2008 16(2):280–289; Choi et al. <i>Mol Brain.</i> 2014;7:17
Adenovirus SD [#] / immunoglobulin SA*	++	500	pAd β	Wong et al. <i>Chromosoma.</i> 1985 92(2):124–135; Yew et al. <i>Hum Gene Ther.</i> 1997 8(5):575–584
SV40 late SD [#] / SA* (19S/16S)	+	180	pCMV β	Yew et al. <i>Hum Gene Ther.</i> 1997 8(5):575–584

Itron	Relative Strength	Size (bps)	Source	Reference(s)
Hybrid adenovirus SD [#] / IgG SA [*]	+++	230	Adenovirus	Choi et al. Mol Brain. 2014;7:17; Huang et al. Mol Cell Biol. 1990 10(4):1805–1810

In the case of episomal vectors, the encoded AFFIMER® constructs may also include at least one origin of replication, minichromosome maintenance elements (MME) and/or nuclear localization elements. Episomal vectors of the disclosure comprise a portion of a virus genomic DNA that encodes an origin of replication (ori), which is required for such vectors to be self-replicating and, thus, to persist in a host cell over several generations. In addition, an episomal vector of the disclosure also may contain at least one gene encoding at least one viral protein required for replication, e.g., replicator protein (s). Optionally, the replicator protein(s) which help initiate replication may be expressed in trans on another DNA molecule, such as on another vector or on the host genomic DNA, in the host cell containing a self-replicating episomal expression vector of this disclosure. Preferred self-replicating episomal LCR-containing expression vectors of the disclosure do not contain viral sequences that are not required for long-term stable maintenance in a eukaryotic host cell such as regions of a viral genome DNA encoding core or capsid proteins that would produce infectious viral particles or viral oncogenic sequences which may be present in the full-length viral genomic DNA molecule. The term "stable maintenance" herein, refers to the ability of a self-replicating episomal expression vector of this disclosure to persist or be maintained in non-dividing cells or in progeny cells of dividing cells in the absence of continuous selection without a significant loss (e.g., >50%) in copy number of the vector for two, three, four, or five or more generations. In some embodiments, the vectors will be maintained over 10-15 or more cell generations. In contrast, "transient" or "short-term" persistence of a plasmid in a host cell refers to the inability of a vector to replicate and segregate in a host cell in a stable manner; that is, the vector will be lost after one or two generations or will undergo a loss of >51% of its copy number between successive generations.

Several representative self-replicating, LCR-containing, episomal vectors useful in the context of the present disclosure are described further below. The self-replicating function may alternatively be provided by at least one mammalian sequence such as described by Wohlgeuth et al., 1996, Gene Therapy 3:503; Vos et al., 1995, Jour. Cell. Biol., Supp. 21A, 433; and Sun et al., 1994, Nature Genetics 8:33, optionally in combination with at least one sequence that may be required for nuclear retention. The advantage of using mammalian, especially human sequences for providing the self-replicating function is that no extraneous activation factors are required which could have toxic or oncogenic properties. It will be understood by one of skill in the art

that the disclosure is not limited to any one origin of replication or any one episomal vector but encompasses the combination of the tissue-restricted control of an LCR in an episomal vector. See also WO1998007876 "Self-replicating episomal expression vectors conferring tissue-specific gene expression" and US Patent 7790446 "Vectors, cell lines and their use in obtaining extended episomal maintenance replication of hybrid plasmids and expression of gene products"

Epstein-Barr Virus-Based Self-Replicating Episomal Expression Vectors. The latent origin oriP from Epstein-Barr Virus (EBV) is described in Yates et. al., Proc. Natl. Acad. Sci. USA 81:3806-3810 (1984); Yates et al., Nature 313:812-815 (1985); Krysan et al., Mol. Cell. Biol. 9:1026-1033 (1989); James et al. Gene 86: 233-239 (1990), Peterson and Legerski, Gene 107:279-284 (1991); and Pan et al., Som. Cell Molec. Genet. 18:163-177 (1992)). An EBV-based episomal vector useful according to the disclosure can contain the oriP region of EBV which is carried on a 2.61 kb fragment of EBV and the EBNA-1 gene which is carried on a 2.18 kb fragment of EBV. The EBNA-1 protein, which is the only viral gene product required to support in trans episomal replication of vectors containing oriP, may be provided on the same episomal expression vector containing oriP. It is also understood, that as with any protein such as EBNA-1 known to be required to support replication of viral plasmid in trans, the gene also may be expressed on another DNA molecule, such as a different DNA vector.

Papilloma Virus-Based, Self-Replicating, Episomal Expression Vectors. The episomal expression vectors of the disclosure also may be based on replication functions of the papilloma family of virus, including but not limited to Bovine Papilloma Virus (BPV) and Human Papilloma Viruses (HPVs). BPV and HPVs persist as stably maintained plasmids in mammalian cells. -S trans-acting factors encoded by BPV and HPVs, namely E1 and E2, have also been identified which are necessary and sufficient for mediate replication in many cell types via minimal origin of replication (Ustav et al., EMBO J. 10: 449-457 (1991); Ustav et al., EMBO J. 10:4231-4329, (1991); Ustav et al., Proc. Natl. Acad. Sci. USA 90: 898-902 (1993)).

An episomal vector useful according to the disclosure is the BPV-I vector system described in Piirsoo et al., EMBO J. , 15:1 (1996) and in WO 94/12629. The BPV-1 vector system described in Piirsoo et al. comprises a plasmid harboring the BPV-1 origin of replication (minimal origin plus extrachromosomal maintenance element) and optionally the E1 and E2 genes. The BPV-1 E1 and E2 genes are required for stable maintenance of a BPV episomal vector. These factors ensure that the plasmid is replicated to a stable copy number of up to thirty copies per cell independent of cell cycle status. The gene construct therefore persists stably in both dividing and non-dividing cells. This allows the maintenance of the gene construct in cells such as hemopoietic stem cells and more committed precursor cells.

The BPV origin of replication has been located at the 3' end of the upstream regulatory region within a 60-base pair (bp) DNA fragment (nucleotides (nt) 7914 - 7927) which includes binding sites for the E1 and E2 replication factors. The minimal origin of replication of HPV has also been characterized and located in the URR fragment (nt 7022- 7927) of HPV (see, for example, Chiang et al., Proc . Natl . Acad. Sci . USA 89:5799-5803 (1992)). As used herein, "E1" refers to the protein encoded by nucleotides (nt) 849-2663 of BPV subtype 1 or by nt 832- 2779 of HPV of subtype 11, to equivalent E1 proteins of other papilloma viruses, or to functional fragments or mutants of a papilloma virus E1 protein, e.g., fragments or mutants of E1 which possess the replicating properties of E1.

As used herein, "E2H refers to the protein encoded by nt 2594-3837 of BPV subtype 1 or by nt 2723-3823 of HPV subtype 11, to equivalent E2 proteins of other papilloma viruses, or to functional fragments or mutants of a papilloma virus E2 protein, e.g., fragments or mutants of E2 which possess the replicating properties of E2. "Minichromosomal maintenance element" (MME) refers to the extrachromosomal maintenance element of the papilloma viral genome to which viral or human proteins essential for papilloma viral replication bind, which region is essential for stable episomal maintenance of the papilloma viral MO in a host cell, as described in Piirsoo et al. (supra). Preferably, the MME is a sequence containing multiple binding sites for the transcriptional activator E2. The MME in BPV is herein defined as the region of BPV located within the upstream regulatory region which includes a minimum of about six sequential E2 binding sites, and which gives optimum stable maintenance with about ten sequential E2 binding sites. E2 binding site 9 is an example sequence for this site, as described hereinbelow, wherein the sequential sites are separated by a spacer of about 4-10 nucleotides, and optimally 6 nucleotides. E1 and E2 can be provided to the plasmid either in cis or in trans, also as described in WO 94/12629 and in Piirsoo et al. (supra).

"E2 binding site" refers to the minimum sequence of papillomavirus double-stranded DNA to which the E2 protein binds. An E2 binding site may include the sequence 5' ACCGTTGCCGGT 3' (SEQ ID NO: 774), which is high affinity E2 binding site 9 of the BPV-1 URR; alternatively, an E2 binding site may include permutations of binding site 9, which permutations are found within the URR, and fall within the generic E2 binding sequence 5' ACCN₆GGT 3' (SEQ ID NO: 775). Transcriptional activator E2 binding sites are, in most papillomaviruses, located in the upstream regulatory region, as in BPV and HPV. A vector which also is useful according to the disclosure may include a region of BPV between 6959 - 7945/1 - 470 on the BPV genetic map (as described in WO 94/12629) , which region includes an origin of replication, a first promoter operatively associated with a gene of interest, the BPV E1 gene

operatively associated with a second promoter to drive transcription of the E1 gene; and the BPV E2 gene operatively associated with a third promoter to drive transcription of the E2 gene.

E1 and E2 from BPV will replicate vectors containing the BPV origin or the origin of many HPV subtypes (Chiang et al., supra). E1 and E2 from HPV will replicate vectors via the BPV origin and via the origin of many HPV subtypes (Chiang et al., supra). As with all vectors of the disclosure, the BPV-based episomal expression vectors of the disclosure must persist through 2-5 or more divisions of the host cell.

See also US Patent 7790446 and Abroi et al. (2004) "Analysis of chromatin attachment and partitioning functions of bovine papillomavirus type 1 E2 protein. *Journal of Virology* 78:2100-13 which have shown that the BPV1 E2 protein dependent MME and EBV EBNA1 dependent FR segregation/partitioning activities function independently from replication of the plasmids. The stable-maintenance function of EBNA1/FR and E2/MME can be used to ensure long-time episomal maintenance for cellular replication origins.

Papovavirus-Based, Self-Replicating, Episomal Expression Vectors. The vectors of the disclosure also may be derived from a human papovavirus BK genomic DNA molecule. For example, the BK viral genome can be digested with restriction enzymes EcoRI and BamHI to produce a 5 kilobase (kb) fragment that contains the BK viral origin of replication sequences that can confer stable maintenance on vectors (see, for example, De Benedetti and Rhoads, *Nucleic Acids Res.* 19:1925 (1991), as can a 3.2 kb fragment of the BK virus (Cooper and Miron, *Human Gene Therapy* 4:557 (1993)).

The encoded AFFIMER® constructs of the present disclosure can be provided as circular or linear nucleic acids. The circular and linear nucleic acids are capable of directing expression of the AFFIMER® agent coding sequence in an appropriate subject cell. The at least one nucleic acid system for expressing an AFFIMER® agent may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components.

A. Viral Vectors

Exemplary viral gene therapy system that are readily adapted for use in the present disclosure include plasmid, adenovirus, adeno-associated virus (AAV), retrovirus, lentivirus, herpes simplex virus, vaccinia virus, poxvirus, reovirus, measles virus, Semliki Forest virus, and the like. Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the nucleic acid construct carrying the nucleic acid sequences encoding the epitopes and targeting sequences of interest.

To further illustrate, encoded AFFIMER® constructs can be delivered *in vivo* using adenoviruses and adeno-associated (AAV) viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy.

1. Adenovirus Vectors

One illustrative method for *in vivo* delivery of at least one nucleic acid sequence involves the use of an adenovirus (“AdV”) expression vector. AdVs are non-enveloped, double-stranded DNA viruses that neither integrate in the host genome nor replicate during cell division. AdV-mediated antibody gene transfer has shown therapeutic efficacy in a variety of different disease models advancing towards the clinic. Systemic mAb expression has mostly been pursued, via s.c. and especially i.v. and intramuscular AdV injection. See Wold et al. (2013) “Adenovirus vectors for gene therapy, vaccination and cancer gene therapy” *Curr Gene Ther.* 13(6):421–33; and Deal et al. “Engineering humoral immunity as prophylaxis or therapy” 2015 *Curr Opin Immunol.* 35:113–22. Other routes of delivery have focused on more local mAb production, such as via intranasal, intratracheal or intrapleural administration of the encoding AdV. The use of AdVs as oncolytic vectors is a popular approach particularly for generation of encoded antibodies at the site of tumors. Foreign genes delivered by current adenoviral gene delivery system are episomal, and therefore, have low genotoxicity to host cells. Therefore, gene therapy using adenoviral gene delivery systems may be considerably safe. The present disclosure specifically contemplates the delivery of AFFIMER® agents by expression of encoded AFFIMER® constructs delivered in the form of an adenoviral vector and delivery system.

Adenovirus has been usually employed as a gene delivery vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contains 100-200 bp ITRs (inverted terminal repeats), which are cis elements necessary for viral DNA replication and packaging. The E1 region (E1A and E1B) of genome encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The E2 region (E2A and E2B) encodes proteins responsible for viral DNA replication. Of adenoviral vectors developed so far, the replication incompetent adenovirus having the deleted E1 region is usually used and represent one exemplary choice of AdV for generating the encoded AFFIMER® constructs of the present disclosure. The deleted E3 region in adenoviral vectors may provide an insertion site for transgenes (Thimmappaya, B. et al., *Cell*, 31:543-551(1982); and Riordan, J. R. et al., *Science*, 245:1066-1073(1989)).

An “adenovirus expression vector” is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that encodes a polypeptide including an AFFIMER® agent such as a CD33

AFFIMER® polypeptide (the encoded AFFIMER® construct sequence). In some embodiments, the sequence for an encoded AFFIMER® construct may be inserted into the DA promoter region. According to an exemplary embodiment, the recombinant adenovirus comprises deleted E1B and E3 region and the nucleotide sequence for an encoded AFFIMER® construct is inserted into the deleted E1B and E3 region.

2. Adeno-Associated Virus Vectors (AAV)

AAVs (or “rAAV” for recombinant AAV) are non-enveloped small, single-stranded DNA viruses capable of infecting both dividing and non-dividing cells. Similar to AdV, AAV-based vectors remain in an episomal state in the nucleus and display a limited risk of integration. In contrast to the generally limited durability of AdV-mediated gene transfer, transgene expression can persist for years following intramuscular recombinant AAV (rAAV) vector delivery.

Alipogene tiparvovec (Glybera™), an rAAV encoding the human lipoprotein lipase gene, was approved in 2012 as the first gene therapy product in Europe. Since then, various rAAV-based gene therapy products are currently under clinical evaluation. In the context of antibody gene transfer, a variety of reports have demonstrated *in vivo* production of an anti-human immune deficiency virus (HIV) mAb in mice following intramuscular injection of the mAb-encoding rAAV. The rAAV vector's potential for combination therapy has also been demonstrated, e.g., by expressing two mAbs. Similar to AdV, intramuscular and i.v. rAAV administration have been most often pursued. Reviewed in Deal et al. “Engineering humoral immunity as prophylaxis or therapy” 2015 Curr Opin Immunol. 35:113–22. A variety of additional delivery sites have also been demonstrated to achieve more local therapeutic effects, including intracranial, intranasal, intravitreal, intrathecal, intrapleural, and intraperitoneal routes. With the utility of rAAV demonstrated for antibody gene transfer, the present disclosure also specifically contemplates the use of rAAV systems for the delivery of encoded AFFIMER® construct sequences *in vivo* and the production of AFFIMER® agents in the body of a patient as a consequence to expression of the rAAV construct.

One important feature to AAV is that these gene transfer viruses are capable of infecting non-dividing cells and various types of cells, making them useful in constructing the encoded AFFIMER® construct delivery system of this disclosure. The detailed descriptions for use and preparation of exemplary AAV vectors are found in, for example, U.S. Pat. NOS. 5,139,941 and 4,797,368, as well as LaFace et al, Virology, 162:483486 (1988), Zhou et al., Exp. Hematol. (NY), 21:928-933 (1993), Walsh et al, J. Clin. Invest., 94:1440-1448(1994) and Flotte et al., Gene Therapy, 2:29-37(1995). AAV is a good choice of delivery vehicles due to its safety, e.g.,

genetically engineered (recombinant) does not integrate into the host genome. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, recombinant AAV does not evoke an inflammatory response.

Typically, a recombinant AAV virus is made by co-transfecting a plasmid containing the gene of interest (e.g., the coding sequence for an AFFIMER® agent) flanked by the two AAV terminal repeats (McLaughlin et al., J. Virol., 62:1963-1973(1988); Samulski et al., J. Virol., 63:3822-3828(1989)) and an expression plasmid containing the wild type AAV coding sequences without the terminal repeats (McCarty et al., J. Virol., 65:2936-2945(1991)). Typically, viral vectors containing an encoded AFFIMER® construct are assembled from polynucleotides encoding the AFFIMER® polypeptide, suitable regulatory elements and elements necessary for expression of the encoded AFFIMER® construct which mediate cell transduction. In some embodiments, adeno-associated viral (AAV) vectors are employed. In a more specific embodiment, the AAV vector is an AAV1, AAV6, or AAV8.

The AAV expression vector which harbors the encoded AFFIMER® construct sequence bounded by AAV ITRs, can be constructed by directly inserting the selected sequence(s) into an AAV genome which has had the major AAV open reading frames ("ORFs") excised therefrom.

For eukaryotic cells, expression control sequences typically include a promoter, an enhancer, such as one derived from an immunoglobulin gene, SV40, cytomegalovirus, etc. (see above), and a polyadenylation sequence which may include splice donor and acceptor sites. The polyadenylation sequence generally is inserted following the transgene sequences and before the 3' ITR sequence.

Selection of these and other common vector and regulatory elements are conventional, and many such sequences are available. See, e.g., Sambrook et al., and references cited therein at, for example, pages 3.18-3.26 and 16.17-16.27 and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989). Of course, not all vectors and expression control sequences will function equally well to express all of the transgenes of this disclosure. However, one of skill in the art may make a selection among these expression control sequences without departing from the scope of this disclosure. Suitable promoter/enhancer sequences may be selected by one of skill in the art using the guidance provided by this application. Such selection is a routine matter and is not a limitation of the molecule or construct.

3. Retrovirus Vectors

Non-cytopathic viruses useful in the context of delivery of encoded AFFIMER® constructs include retroviruses, the life cycle of which involves reverse transcription of genomic

viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (e.g., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are known to those of skill in the art.

In order to construct a retroviral vector, the AFFIMER® agent coding sequence is inserted into the viral genome in the place of certain viral sequences to produce a replication-defective virus. To produce virions, a packaging cell line containing the gag, pol and env genes but without the LTR (long terminal repeat) and psi (□) components is constructed (Mann et al., Cell, 33:153-159(1983)). When a recombinant plasmid containing the cytokine gene, LTR and psi is introduced into this cell line, the psi sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubinstein "Retroviral vectors," In: Vectors: A survey of molecular cloning vectors and their uses, Rodriguez and Denhardt (eds.), Stoneham: Butterworth, 494-513(1988)). The media containing the recombinant retroviruses is then collected, optionally concentrated and used for gene delivery system.

Successful gene transfer using such second-generation retroviral vectors has been reported. Kasahara et al. (Science, 266:1373-1376(1994)) prepared variants of moloney murine leukemia virus in which the EPO (erythropoietin) sequence is inserted in the place of the envelope region, consequently, producing chimeric proteins having novel binding properties. Likely, the present gene delivery system can be constructed in accordance with the construction strategies for the second-generation retroviral vector.

In some embodiments, the retrovirus is a "gammaretroviruses", which refers to a genus of the retroviridae family. Exemplary gammaretroviruses include mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses.

In some embodiments, the retroviral vector for use in the present disclosure is a lentiviral vector, which refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells and typically produce high viral titers. Several examples of lentiviruses include

HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

Another class of widely used retroviral vectors that can be used for the delivery and expression of an encoded AFFIMER® construct include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV) and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739, 1992; Johann et al., J. Virol. 66: 1635-1640, 1992; Sommerfelt et al., Virol. 176:58-59, 1990; Wilson et al., J. Virol. 63:2374-2378, 1989; Miller et al., J. Virol. 65:2220-2224, 1991 ; and PCT/US94/05700).

Still other retroviral vectors that can be also be used in the present disclosure include, e.g., vectors based on human foamy virus (HFV) or other viruses in the Spumavirus genera. Foamy viruses (FVes) are the largest retroviruses known today and are widespread among different mammals, including all non-human primate species, however, are absent in humans. This complete apathogenicity qualifies FV vectors as ideal gene transfer vehicles for genetic therapies in humans and clearly distinguishes FV vectors as gene delivery system from HIV-derived and also gammaretrovirus-derived vectors.

Suitable retroviral vectors for use herein are described, for example, in U.S. Pat. NOS: 5,399,346 and 5,252,479; and in WIPO publications WO 92/07573, WO 90/06997, WO 89/05345, WO 92/05266 and WO 92/14829, which provide a description of methods for efficiently introducing nucleic acids into human cells using such retroviral vectors. Other retroviral vectors include, for example, mouse mammary tumor virus vectors (e.g., Shackleford et al., Proc. Natl. Acad. Sci. U.S.A. 85:9655-9659, 1998), lentiviruses, and the like.

Additional retroviral viral delivery systems that can be readily adapted for delivery of a transgene encoding a CD33 AFFIMER® agent include, merely to illustrate Published PCT Applications WO/2010/045002, WO/2010/148203, WO/2011/126864, WO/2012/058673, WO/2014/066700, WO/2015/021077, WO/2015/148683, WO/2017/040815 - the specifications and FIGS. of each of which are incorporated by reference herein.

In some embodiments, a retroviral vector contains all of the cis-acting sequences necessary for the packaging and integration of the viral genome, e.g., (a) a long terminal repeat (LTR), or portions thereof, at each end of the vector; (b) primer binding sites for negative and positive strand DNA synthesis; and (c) a packaging signal, necessary for the incorporation of genomic RNA into virions. More detail regarding retroviral vectors can be found in Boesen, et al., 1994, Biotherapy 6:291-302; Clowes, et al., 1994, J. Clin. Invest. 93:644-651 ; Kiem, et al., 1994, Blood 83: 1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4: 129-141 ;

Miller, et al., 1993, *Meth. Enzymol.* 217:581- 599; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3: 110-1 14.

In some embodiments, the retrovirus is a recombinant replication competent retrovirus comprising: a nucleic acid sequence encoding a retroviral GAG protein; a nucleic acid sequence encoding a retroviral POL protein; a nucleic acid sequence encoding a retroviral envelope; an oncoretroviral polynucleotide sequence comprising Long-Terminal Repeat (LTR) sequences at the 5' and 3' end of the oncoretroviral polynucleotide sequence; a cassette comprising an internal ribosome entry site (IRES) operably linked to a coding sequence for an AFFIMER® agent, such as for a CD33 AFFIMER® agent, wherein the cassette is positioned 5' to the U3 region of the 3' LTR and 3' to the sequence encoding the retroviral envelope; and cis-acting sequences for reverse transcription, packaging and integration in a target cell.

In some embodiments, the retrovirus is a recombinant replication competent retrovirus comprising: a retroviral GAG protein; a retroviral POL protein; a retroviral envelope; a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, the promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain; a cassette comprising an encoded AFFIMER® construct sequence, wherein the cassette is positioned 5' to the 3' LTR and is operably linked and 3' to the env nucleic acid domain encoding the retroviral envelope; and cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell.

In some embodiments of the recombinant replication competent retrovirus, the envelope is chosen from one of amphotropic, polytropic, xenotropic, 10A1, GALV, Baboon endogenous virus, RD114, rhabdovirus, alphavirus, measles or influenza virus envelopes.

In some embodiments of the recombinant replication competent retrovirus, the retroviral polynucleotide sequence is engineered from a virus selected from the group consisting of murine leukemia virus (MLV) , Moloney murine leukemia virus (MoMLV) , Feline leukemia virus (FeLV) , Baboon endogenous retrovirus (BEV) , porcine endogenous virus (PERV) , the cat derived retrovirus RD114, squirrel monkey retrovirus, Xenotropic murine leukemia virus-related virus (XMRV) , avian reticuloendotheliosis virus (REV) , or Gibbon ape leukemia virus (GALV).

In some embodiments of the recombinant replication competent retrovirus, retrovirus is a gammaretrovirus.

In some embodiments of the recombinant replication competent retrovirus, there is a second cassette comprising a coding sequence for a second therapeutic protein, such as another

checkpoint inhibitor polypeptide, a co-stimulatory polypeptide and/or an immunostimulatory cytokine (merely as examples), e.g., downstream of the cassette. In certain instances, the second cassette can include an internal ribosome entry site (IRES) or a minipromoter or a polIII promoter operably linked to the coding sequence for the second therapeutic protein.

In some embodiments of the recombinant replication competent retrovirus, it is a nonlytic, amphotropic retroviral replicating vector which, preferably, selectively infects and replicates in the cells of the tumor microenvironment.

4. Other Viral Vectors as Expression Constructs

In the context of vectored intratumoral encoded AFFIMER® construct gene transfer, oncolytic viruses have a distinct advantage, as they can specifically target tumor cells, boost therapeutic AFFIMER® agent expression, and amplify antitumor therapeutic responses. Oncolytic viruses, which overlap with certain viral systems described above, promote anti-tumor responses through selective tumor cell killing and induction of systemic anti-tumor immunity. The mechanisms of action are not fully elucidated but are likely to depend on viral replication within transformed cells, induction of primary cell death, interaction with tumor cell anti-viral elements and initiation of innate and adaptive anti-tumor immunity. Reviewed in Kaufman et al. 2015 “Oncolytic viruses: a new class of immunotherapy drugs” *Nat Rev Drug Discov.* 14(9):642–62. Many of the oncolytic viruses that are currently in the clinic have a natural tropism for cell surface proteins that are aberrantly expressed by cancer cells. To date, AdV, poxviruses, coxsackieviruses, poliovirus, measles virus, Newcastle disease virus, reovirus, and others have entered into early-phase clinical trials. In 2015, the FDA and EMA approved talimogene laherparepvec (T-VEC, Imlygic™), an oncolytic herpes virus armed with the gene for granulocyte–macrophage colony-stimulating factor (GM-CSF). The self-perpetuating nature of oncolytic viruses makes them an appealing platform for encoded AFFIMER® construct gene transfer of the present disclosure, as transgene products can be amplified along with viral replication, thereby maximizing therapeutic effect. Liu et al. 2008 “Oncolytic adenoviruses for cancer gene therapy” *Methods Mol Biol.* 433:243–58.

In the case of AFFIMER® agents that are large fusion proteins, e.g., which comprise other protein domains beyond a single AFFIMER® domain, local intratumoral expression can present an appealing strategy to overcome poor penetration in solid tumors if and where that might be an issue. Beckman et al. (2007) “Antibody constructs in cancer therapy: protein engineering strategies to improve exposure in solid tumors” *Cancer* 109(2):170–9; and Dronca et al. 2015 “Immunomodulatory antibody therapy of cancer: the closer, the better” *Clin Cancer Res.* 21(5):944–6. Likewise, intratumoral delivery of the encoded AFFIMER® construct and

concomitant local expression of the AFFIMER® agent can create a better therapeutic index where dose-limiting toxicities might otherwise prevent reaching the effective intratumoral concentration for efficacy when the AFFIMER® agent is delivered (or expressed) systemically.

In the case of the CD33 AFFIMER® agents of the present disclosure, the immunomodulatory nature of these AFFIMER® agents are very relevant to the use of oncolytic viruses. Indeed, for oncolytic virus therapy, it is desirable to override immune checkpoint inhibitor networks and thereby create a pro-inflammatory environment within the cancer. Numerous clinical trials are currently underway to evaluate the combination of oncolytic viruses and conventional immunomodulatory mAb administration. Kaufman et al. 2015 “Oncolytic viruses: a new class of immunotherapy drugs” *Nat Rev Drug Discov.* 14(9):642–62; and Lichty et al. 2014 “Going viral with cancer immunotherapy” *Nat Rev Cancer.* 14(8):559–67. However, systemic treatment with checkpoint-blocking mAbs can lead to severe immune-related adverse effects, which may also be an issue for some embodiments of the subject CD33 AFFIMER® agents, highlighting the opportunity for local therapies, e.g., via encoded AFFIMER® construct-armed oncolytic viruses. Different studies have pursued this approach and can be readily adapted for use with the encoded AFFIMER® construct. Dias et al. armed a replication-deficient and -competent oncolytic AdV with an anti-human CTLA-4 mAb. Dias et al. 2012 “Targeted cancer immunotherapy with oncolytic adenovirus coding for a fully human monoclonal antibody specific for CTLA-4” *Gene Ther.* 19(10):988–98. Another system recently described (and that can be adapted for use with the encoded AFFIMER® construct of the present disclosure) involved armed oncolytic vaccinia viruses with anti-murine programmed cell death protein 1 (PD-1) Fab, scFv or full-length mAb. Reflecting virus replication, mAb levels in the tumor peaked 3–5 days after intratumoral injection at 9 or 30 µg/ml, depending on the tumor model. Serum mAb levels followed the same trend, albeit threefold, or more, lower, although mAb detection was lost after 5 days. Intratumorally expressed mAbs lasted longer compared to intratumoral injection of anti-PD-1 mAb protein, with follow-up limited to 11 days after injection. Fab and scFv expression were not reported. Anti-tumor responses of the virus armed with either the anti-PD-1 scFv or mAb were superior to the unarmed virus and as effective as the combination of the unarmed virus and systemic anti-PD-1 mAb protein injections. Kleinpeter et al. 2016 “Vectorization in an oncolytic vaccinia virus of an antibody, a Fab and a scFv against programmed cell death-1 (PD-1) allows their intratumoral delivery and an improved tumor-growth inhibition” *Oncoimmunology.* 5(10):e1220467 (online).

Other viral vectors may be employed as a gene delivery system in the present disclosure. Vectors derived from viruses such as vaccinia virus (Puhlmann M. et al., *Human Gene Therapy*,

10:649-657(1999); Ridgeway, "Mammalian expression vectors," In: Vectors: A survey of molecular cloning vectors and their uses. Rodriguez and Denhardt, eds. Stoneham: Butterworth, 467-492(1988); Baichwal and Sugden, "Vectors for gene transfer derived from animal DNA viruses: Transient and stable expression of transferred genes," In: Kucherlapati R, ed. Gene transfer. New York: Plenum Press, 117-148(1986) and Coupar et al., Gene, 68:1-10(1988)), lentivirus (Wang G. et al., J. Clin. Invest., 104(11):R55-62(1999)), herpes simplex virus (Chamber R., et al., Proc. Natl. Acad. Sci USA, 92:1411-1415(1995)), poxvirus (GCE, NJL, Krupa M, Esteban M., The poxvirus vectors MVA and NYVAC as gene delivery systems for vaccination against infectious diseases and cancer Curr Gene Ther 8(2):97-120(2008)), reovirus, measles virus, Semliki Forest virus, and polioviruses may be used in the present delivery systems for transferring the gene of interest into cells. They offer several attractive features for various mammalian cells. Also included are hepatitis B viruses.

B. Non-Viral Vectors

In 1990, Wolff et al. showed how injection of naked plasmid DNA (pDNA) into the skeletal muscle of mice led to the local expression of the encoded protein, kick-starting the field of DNA-based therapeutics. See Wolff et al. 1990 "Direct gene transfer into mouse muscle *in vivo*" Science. 247(4949 Pt 1):1465–8. The use of "pDNA" for delivering encoded AFFIMER® construct of the present disclosure waives the need for a virus as biological vector and presents an appealing platform for encoded AFFIMER® construct gene transfer. Compared to viral vectors, pDNA is considered low-immunogenic (allowing e.g., repeated dosing), is cheaper to produce, ship, and store, and has a much longer shelf-life. After entry in the nucleus, pDNA remains in a non-replicating non-integrating episomal state and is lost during the breakdown of the nuclear envelope at mitosis. pDNA has no defined restrictions regarding the size of the transgene compared to viral vectors, and its modular nature allows for straightforward molecular cloning, making them easy to manipulate and design for therapeutic use. Hardee et al. 2017 "Advances in non-viral DNA vectors for gene therapy" Genes. 8(2):65. Plasmids are used in about 17% of the ongoing or completed gene therapy clinical trials and showed to be well-tolerated and safe.

The method of DNA administration can greatly impact transgene expression. *In vivo* DNA-mediated encoded AFFIMER® construct gene transfer can utilize such physical methods of transfection used for antibody gene transfer, such as electroporation or hydrodynamic injection. Electroporation presents the propagation of electrical fields within tissues, which induces a transient increase in cell membrane permeability. Electrotransfer of DNA is a multistep process, involving (i) electrophoretic migration of DNA towards the plasma

membrane, (ii) DNA accumulation and interaction with the plasma membrane, and (iii) intracellular trafficking of the DNA to the nucleus, after which gene expression can commence. Heller LC. 2015 “Gene electrotransfer clinical trials” *Adv Genet.* 89:235–62. Intramuscular, intratumoral and intradermal administration have been evaluated in clinical trials and are also suitable target tissues for electroporation of encoded AFFIMER® constructs.

Hydrodynamic-based transfection utilizes the i.v. injection of high volumes of pDNA, driving DNA molecules out of the blood circulation and into tissue. Other potentially less invasive physical delivery methods include sonoporation and magnetofection. DNA uptake can also be improved by complexing the molecules with chemical delivery vehicles (e.g., cationic lipids or polymers and lipid nanoparticles). Such techniques can also be applied to *in vivo* DNA-mediated encoded AFFIMER® construct gene transfer.

In addition to the choice of delivery method, encoded AFFIMER® construct transgene expression can be improved by modifying the make-up of pDNA constructs. See, for example, Hardee et al. 2017 “Advances in non-viral DNA vectors for gene therapy” *Genes* 8(2):65; and Simcikova et al. 2015 “Towards effective non-viral gene delivery vector” *Biotechnol Genet Eng Rev.* 31(1–2):82–107. Conventional pDNA consists of a transcription unit and bacterial backbone. The transcription unit carries the encoded AFFIMER® construct sequence along with regulatory elements. The bacterial backbone includes elements like an antibiotic resistance gene, an origin of replication, unmethylated CpG motifs, and potentially cryptic expression signals. Some of these sequences are required for the production of plasmid DNA. However, in general, for therapeutic encoded AFFIMER® construct gene therapy the presence of a bacterial backbone will likely be counterproductive. However, there are a variety of different types of available minimal vectors that can be selected, including minicircle DNA (mcDNA) which already been used for antibody gene transfer and can be readily adapted for encoded AFFIMER® construct gene transfer. Minicircles are plasmid molecules devoid of bacterial sequences, generated via a process of recombination, restriction and/or purification. Simcikova et al. 2015 *supra*. Elimination of the bacterial backbone has shown higher transfection efficiency and prolonged transgene expression in a variety of tissues.

Also provided herein is a linear nucleic acid, or linear expression cassette (“LEC”), that is capable of being efficiently delivered to a subject via electroporation and expressing the encoded AFFIMER® construct sequence included therein. The LEC may be any linear DNA devoid of any phosphate backbone. The LEC may contain a promoter, an intron, a stop codon, and/or a polyadenylation signal. The expression of the encoded AFFIMER® construct coding sequence may be controlled by the promoter.

1. Plasmid Vectors

In some embodiments, the encoded AFFIMER® constructs are delivered as plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g., Sambrook et al., 1989, cited above. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells *in vivo*. They are particularly advantageous for this because they reduced safety concerns relative to other vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide epitope encoded by nucleic acid within the plasmid. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

To expand the application and efficiency of using plasmid DNA to deliver an encoded AFFIMER® construct to tissue *in vivo*, different approaches can be pursued based on principles producing higher mAb expression or overall efficacy in prior art reports. A first strategy simply relies on giving multiple or repeated pDNA doses. Kitaguchi et al. 2005 “Immune deficiency enhances expression of recombinant human antibody in mice after nonviral *in vivo* gene transfer” *Int J Mol Med* 16(4):683–8; and Yamazaki et al. 2011 “Passive immune-prophylaxis against influenza virus infection by the expression of neutralizing anti-hemagglutinin monoclonal antibodies from plasmids” *Jpn J Infect Dis.* 64(1):40–9. Another approach relates to the use of a delivery adjuvant. pDNA electrotransfer can be enhanced by pre-treating the muscle with hyaluronidase, an enzyme that transiently breaks down hyaluronic acid, decreasing the viscosity of the extracellular matrix and facilitating DNA diffusion. Yamazaki et al. 2011, *supra*; and McMahon et al. 2001 “Optimisation of electrotransfer of plasmid into skeletal muscle by pretreatment with hyaluronidase: increased expression with reduced muscle damage” *Gene Ther.* 8(16):1264–70. For antibody gene transfer, this led to an increase in mAb expression by approximately 3.5-fold, achieving plasma peak titers of 3.5 µg/ml with 30 µg pDNA, and can be adapted by one skilled in the art for encoded AFFIMER® construct gene transfer. Still another strategy focuses on antibody or cassette engineering. Following codon-, RNA- and leader

sequence-optimization, peak serum mAb or Fab titers have been attained with intramuscular electrotransfer of 'optimized' pDNA. See, for example, Flingai et al. 2015 "Protection against dengue disease by synthetic nucleic acid antibody prophylaxis/immunotherapy" Sci Rep. 5:12616.

The purpose of the plasmid is the efficient delivery of nucleic acid sequences to and expression of therapeutic AFFIMER® agents in a cell or tissue. In particular, the purpose of the plasmid may be to achieve high copy number, avoid potential causes of plasmid instability and provide a means for plasmid selection. As for expression, the nucleic acid cassette contains the necessary elements for expression of the encoded AFFIMER® construct within the cassette. Expression includes the efficient transcription of an inserted gene, nucleic acid sequence, or nucleic acid cassette with the plasmid. Thus, in some aspects, a plasmid is provided for expression of encoded AFFIMER® construct which includes an expression cassette comprising the coding sequence for the AFFIMER® agent; also referred to as a transcription unit. When a plasmid is placed in an environment suitable for epitope expression, the transcriptional unit will express the AFFIMER® agent and anything else encoded in the construct. The transcription unit includes a transcriptional control sequence, which is transcriptionally linked with a cellular immune response element coding sequence. Transcriptional control sequence may include promoter/enhancer sequences such as cytomegalovirus (CMV) promoter/enhancer sequences, such as described above. However, those skilled in the art will recognize that a variety of other promoter sequences suitable for expression in mammalian cells, including human patient cells, are known and can similarly be used in the constructs disclosed herein. The level of expression of the AFFIMER® agent will depend on the associated promoter and the presence and activation of an associated enhancer element.

In some embodiments, the encoded AFFIMER® construct sequence (encoding the desired AFFIMER® agent) can be cloned into an expression plasmid which contains the regulatory elements for transcription, translation, RNA stability and replication (e.g., including a transcriptional control sequence). Such expression plasmids are well known in the art and one of ordinary skill would be capable of designing an appropriate expression construct for producing a recombinant AFFIMER® agent *in vivo*.

2. Minicircle

Minicircle (mcDNA) -based antibody gene transfer can also be adapted for delivery of encoded AFFIMER® construct to tissues *in vivo*. Under certain circumstances, plasmid DNA used for non-viral gene delivery can cause unacceptable inflammatory responses. Where this happens, immunotoxic responses are largely due to the presence of unmethylated CpG motifs

and their associated stimulatory sequences on plasmids following bacterial propagation of plasmid DNA. Simple methylation of DNA in vitro may be enough to reduce an inflammatory response but can result in reduced gene expression. The removal of CpG islands by cloning out, or elimination of non-essential sequences has been a successful technique for reducing inflammatory responses. Yew et al. 2000 "Reduced inflammatory response to plasmid DNA vectors by elimination and inhibition of immunostimulatory CpG motifs" *Mol Ther* 1(3), 255-62.

Since bacterial DNA contains on average 4 times more CpG islands than mammalian DNA, a good solution is to eliminate entirely the bacterial control regions, such as the origin of replication and antibiotic resistance genes, from gene delivery vectors during the process of plasmid production. Thus, the "parent" plasmid is recombined into a "minicircle" which generally comprises the gene to be delivered (in this case, the encoded AFFIMER® construct coding sequence) and suitable control regions for its expression, and a miniplasmid which generally comprises the remainder of the parent plasmid.

Removal of bacterial sequences needs to be efficient, using the smallest possible excision site, whilst creating supercoiled DNA minicircles which consist solely of gene expression elements under appropriate--preferably mammalian--control regions. Some techniques for minicircle production use bacterial phage lambda (λ) integrase mediated recombination to produce minicircle DNA. See, for example, Darquet, et al. 1997 *Gene Ther* 4(12): 1341-9; Darquet et al. 1999 *Gene Ther* 6(2): 209-18; and Kreiss, et al. 1998 *Appl Microbiol Biotechnol* 49(5):560-7).

Therefore, embodiments of nucleic acid constructs described herein may be processed in the form of minicircle DNA. Minicircle DNA pertains to small (2-4 kb) circular plasmid derivatives that have been freed from all prokaryotic vector parts. Since minicircle DNA vectors contain no bacterial DNA sequences, they are less likely to be perceived as foreign and destroyed. As a result, these vectors can be expressed for longer periods of time compared to certain conventional plasmids. The smaller size of minicircles also extends their cloning capacity and facilitates their delivery into cells. Kits for producing minicircle DNA are known in the art and are commercially available (System Biosciences, Inc., Palo Alto, Calif.). Information on minicircle DNA is provided in Dietz et al., *Vector Engineering and Delivery Molecular Therapy* (2013); 21 8, 1526-1535 and Hou et al., *Molecular Therapy—Methods & Clinical Development*, Article number: 14062 (2015) doi:10.1038/mtm.2014.62. More information on Minicircles is provided in Chen Z Y, He C Y, Ehrhardt A, Kay M A. *Mol Ther*. 2003 September; 8(3):495-500 and Minicircle DNA vectors achieve sustained expression reflected by active chromatin and

transcriptional level. Gracey Maniar L E, Maniar J M, Chen Z Y, Lu J, Fire A Z, Kay M A. Mol Ther. 2013 January; 21(1):131-8

As a nonlimiting example, a minicircle DNA vector may be produced as follows. An expression cassette, which comprises the encoded AFFIMER® construct coding sequence along with regulatory elements for its expression, is flanked by attachment sites for a recombinase. A sequence encoding the recombinase is located outside of the expression cassette and includes elements for inducible expression (such as, for example, an inducible promoter). Upon induction of recombinase expression, the vector DNA is recombined, resulting in two distinct circular DNA molecules. One of the circular DNA molecules is relatively small, forming a minicircle that comprises the expression cassette for the encoded AFFIMER® construct; this minicircle DNA vector is devoid of any bacterial DNA sequences. The second circular DNA sequence contains the remaining vector sequence, including the bacterial sequences and the sequence encoding the recombinase. The minicircle DNA containing the encoded AFFIMER® construct sequence can then be separately isolated and purified. In some embodiments, a minicircle DNA vector may be produced using plasmids similar to pBAD.φ.C31.hFIX and pBAD.φ.C31.RHB. See, e.g., Chen et al. (2003) Mol. Ther. 8:495-500.

Exemplary recombinases that may be used for creating a minicircle DNA vector include but are not limited to, Streptomyces bacteriophage φ31 integrase, Cre recombinase, and the λ integrase/DNA topoisomerase IV complex. Each of these recombinases catalyzes recombination between distinct sites. For example, φ31 integrase catalyzes recombination between corresponding attP and attB sites, Cre recombinase catalyzes recombination between loxP sites, and the λ integrase/DNA topoisomerase IV complex catalyzes recombination between bacteriophage λ attP and attB sites. In some embodiments, such as, for example, with φ31 integrase or with λ integrase in the absence of the λ protein, the recombinase mediates an irreversible reaction to yield a unique population of circular products and thus high yields. In other embodiments, such as, for example, with Cre recombinase or with λ integrase in the presence of the λ protein, the recombinase mediates a reversible reaction to yield a mixture of circular products and thus lower yields. The reversible reaction by Cre recombinase can be manipulated by employing mutant loxP71 and loxP66 sites, which recombine with high efficiency to yield a functionally impaired P71/66 site on the minicircle molecule and a wild-type loxP site on the minicircle molecule, thereby shifting the equilibrium towards the production of the minicircle DNA product.

Published US Application 20170342424 also describes a system making use of a parent plasmid which is exposed to an enzyme which causes recombination at recombination sites,

thereby forming a (i) minicircle including the encoded AFFIMER® construct sequence and (ii) a miniplasmid comprising the remainder of the parent plasmid. One recombination site is modified at the 5' end such that its reaction with the enzyme is less efficient than the wild type site, and the other recombination site is modified at the 3' end such that its reaction with the enzyme is less efficient than the wild type site, and the other recombination site is modified at the 3' end such that its reaction with the enzyme is less efficient than the wild type site, both modified sites being located in the minicircle after recombination. This favors the formation of minicircle.

C. RNA-Mediated Encoded AFFIMER® Construct Gene Transfer

Exemplary nucleic acids or polynucleotides for the encoded CD33 AFFIMER® constructs of the present disclosure include but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β -D-ribo configuration, a-LNA having an α -L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α -LNA having a 2'-amino functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) or hybrids or combinations thereof.

mRNA presents an emerging platform for antibody gene transfer that can be adapted by those skilled in the art for delivery of encoded AFFIMER® constructs of the present disclosure. Although current results differ considerably, in certain instances the mRNA constructs appear to be able to rival viral vectors in terms of generated serum mAb titers. Levels were in therapeutically relevant ranges within hours after mRNA administration, a marked shift in speed compared to DNA. The use of lipid nanoparticles (LNP) for mRNA transfection, rather than the physical methods typically required for DNA, can provide significant advantages in some embodiments towards application range.

In their 1990 study, Wolff et al. (1990, *supra*) found that, in addition to pDNA, intramuscular injection of in vitro transcribed (IVT) mRNA also led to local expression of the encoded protein. mRNA was not pursued as actively as DNA at that time because of its low stability. Progress over the past years allowed mRNA to catch up with DNA and viral vectors as a tool for gene transfer. Reviewed in Sahin et al. (2014) "mRNA-based therapeutics: developing a new class of drugs" Nat Rev Drug Discov. 13(10):759–80. Conceptually, there are several differences with these expression platforms. mRNA does not need to enter into the nucleus to be functional. Once it reaches the cytoplasm, mRNA is translated instantly. mRNA-based therapeutics are expressed more transiently compared to DNA- or viral vector-mediated gene transfer, and do not pose the risk of insertional mutagenesis in the host genome. mRNA

production is relatively simple and inexpensive. In terms of administration, mRNA uptake can be enhanced using electroporation. Broderick et al. 2017 “Enhanced delivery of DNA or RNA vaccines by electroporation” *Methods Mol Biol.* 2017;1499:193–200. Most focus, however, has gone to non-physical transfection methods. Indeed, a variety of mRNA complexing formulations have been developed, including lipid nanoparticles (LNP), which have proven to be safe and very efficient mRNA carriers for administration in a variety of tissues and i.v. Pardi et al. 2015 “Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes” *J Control Release* 217:345–51. In line with this progress, IVT mRNA has reached the stage of clinical evaluation.

Beissert et al. WO2017162266 “RNA Replicon for Versatile and Efficient Gene Expression” describes agents and methods suitable for efficient expression of AFFIMER® polypeptides of the present disclosure, such as suitable for immunotherapeutic treatment for the prevention and therapy of tumors. For instance, the AFFIMER® agent coding sequence can be provided as an RNA replicon comprising a 5' replication recognition sequence such as from an alphavirus 5' replication recognition sequence. In some embodiments, the RNA replicon comprises a (modified) 5' replication recognition sequence and an open reading frame encoding the AFFIMER® agent, in particular located downstream from the 5' replication recognition sequence such as that the 5' replication recognition sequence and the open reading frame do not overlap, e.g., the 5' replication recognition sequence does not contain a functional initiation codon and in some embodiments does not contain any initiation codon. Most preferably, the initiation codon of the open reading frame encoding the AFFIMER® agent is in the 5'→3' direction of the RNA replicon.

In some embodiments, to prevent immune activation, modified nucleosides can be incorporated into the in vitro–transcribed mRNA. In some embodiments, the IVT RNA can be 5' capped, such as an m7GpppG-capped or m7G5'ppp5'G2'-O-Met-capped IVT. Efficient translation of the modified mRNA can be ensured by removing double-stranded RNA. Moreover, the 5' and 3' UTRs and the poly(A) tail can be optimized for improved intracellular stability and translational efficiency. See, for example, Stadler et al. (2017) *Nature Medicine* 23:815–817 and Kariko et al. WO/2017/036889 “Method for Reducing Immunogenicity of RNA”.

In some embodiments, the mRNA that encodes the CD33 AFFIMER® agent may include at least one chemical modification described herein. As a non-limiting example, the chemical modification may be 1-methylpseudouridine, 5-methylcytosine or 1-methylpseudouridine and 5-methylcytosine. In some embodiments, the chemical modification is a pseudouridine or a modified 5 nucleoside, wherein said modified nucleoside is m⁵C, m⁵U, m₆A, s2U, Ψ, or 2'-O-

methyl-U. In some embodiments, linear polynucleotides encoding at least one CD33 AFFIMER® agent that are made using only in vitro transcription (IVT) enzymatic synthesis methods are referred to as "IVT polynucleotides." Methods of making IVT polynucleotides are known in the art and are described in International Publication Nos. WO 2007/024708A2 and WO 2013/151666, the contents of which are incorporated herein by reference in their entirety.

In another embodiment, the polynucleotides that encode the CD33 AFFIMER® agent of the present disclosure have portions or regions which differ in size and/or chemical modification pattern, chemical modification position, chemical modification percent or chemical modification population and combinations of the foregoing are known as "chimeric polynucleotides." A "chimera" according to the present disclosure is an entity having two or more incongruous or heterogeneous parts or regions. As used herein a "part" or "region" of a polynucleotide is defined as any portion of the polynucleotide which is less than the entire length of the polynucleotide. Such constructs are taught in for example International Publication No. WO2015/034928.

In yet another embodiment, the polynucleotides of the present disclosure that are circular are known as "circular polynucleotides" or "circP." As used herein, "circular polynucleotides" or "circP" means a single stranded circular polynucleotide which acts substantially like, and has the properties of, an RNA. The term "circular" is also meant to encompass any secondary or tertiary configuration of the circP. Such constructs are taught in for example International Publication Nos. WO2015/034925 and WO2015/034928, the contents of each of which are incorporated herein by reference in their entirety.

Exemplary mRNA (and other polynucleotides) that can be used to encode CD33 AFFIMER® agents of the present disclosure include those which can be adapted from the specifications and FIGS. of, for example, International Publication No.s WO2017/049275, WO2016/118724, WO2016/118725, WO2016/011226, WO2015/196128, WO/2015/196130, WO/2015/196118, WO/2015/089511, and WO2015/105926 (the later titled "Polynucleotides for the *In vivo* Production of Antibodies"), each of which is incorporated by reference herein.

Electroporation, as described below, is one exemplary method for introducing mRNA or other polynucleotides into a cell.

Lipid-containing nanoparticle compositions have proven effective as transport vehicles into cells and/or intracellular compartments for a variety of RNAs (and related polynucleotides described herein). These compositions generally include at least one "cationic" and/or ionizable lipids, phospholipids including polyunsaturated lipids, structural lipids (e.g., sterols), and lipids containing polyethylene glycol (PEG lipids). Cationic and/or ionizable lipids include, for example, amine-containing lipids that can be readily protonated.

D. Other Methods of Delivery of Encoded AFFIMER® Construct into Target Cells

The introduction into host cell of the gene delivery system can be performed through various methods known to those skilled in the art.

Where the present gene delivery system is constructed on the basis of viral vector construction, delivery can be performed as conventional infection methods known in the art.

Physical methods to enhance delivery both viral and non-viral encoded AFFIMER® constructs include electroporation (Neumann, E. et al., EMBO J., 1:841(1982); and Tur-Kaspa et al., Mol. Cell Biol., 6:716-718(1986)), gene bombardment (Yang et al., Proc. Natl. Acad. Sci., 87:9568-9572 (1990) where DNA is loaded onto (e.g., gold) particles and forced to achieve penetration of the DNA into the cells, sonoporation, magnetofection, hydrodynamic delivery and the like, all of which are known to those of skill in the art.

1. Electroporation

In the past several years, there has been a great advance in the plasmid DNA delivery technology that is utilized for *in vivo* production of proteins. This included codon optimization for expression in human cells, RNA optimization to improve mRNA stability as well as more efficient translation at the ribosomal level, the addition of specific leader sequences to enhance translation efficiency, the creation of synthetic inserts to further enhance production *in vivo* and the use of improved adaptive electroporation (EP) delivery protocols to improve *in vivo* delivery. EP assists in the delivery of plasmid DNA by generating an electrical field that allows the DNA to pass into the cell more efficiently. *In vivo* electroporation is a gene delivery technique that has been used successfully for efficient delivery of plasmid DNA to many different tissues. Kim et al. "Gene therapy using plasmid DNA-encoded anti-HER2 antibody for cancers that overexpress HER2" (2016) Cancer Gene Ther. 23(10): 341–347 teaches a vector and electroporation system for intramuscular injection and *in vivo* electroporation of the plasmids that results in high and sustained antibody expression in sera; the plasmid and electroporation system of Kim et al. can be readily adapted for the *in vivo* delivery of a plasmid for expressing an encoded CD33 AFFIMER® construct of the present disclosure.

Accordingly, in certain some embodiments of the present disclosure, the encoded AFFIMER® construct is introduced into target cells via electroporation.

Administration of the composition via electroporation may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal, a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation

device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate at least one of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an *in vivo* electroporation device, for example CELLECTRA EP system (VGX Pharmaceuticals, Blue Bell, Pa.) or Elgen electroporator (Genetronics, San Diego, Calif.) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not be limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 μ s, 20 μ s, 10 μ s or 1 μ s, but in some embodiments is a real-time feedback or instantaneous (e.g., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the encoded AFFIMER® constructs of the present disclosure, include those described in U.S. Pat. NOS: 7,245,963; 6,302,874; 5,676,646; 6,241,701; 6,233,482; 6,216,034; 6,208,893; 6,192,270; 6,181,964; 6,150,148; 6,120,493; 6,096,020; 6,068,650; and 5,702,359, the contents of which are incorporated herein by reference in their entirety. The electroporation may be carried out via a minimally invasive device.

In some embodiments, the electroporation is carried using a minimally invasive electroporation device ("MID"). The device may comprise a hollow needle, DNA cassette, and fluid delivery means, wherein the device is adapted to actuate the fluid delivery means in use so as to concurrently (for example, automatically) inject the encoded AFFIMER® construct into body tissue during insertion of the needle into the body tissue. This has the advantage that the ability to inject the DNA and associated fluid gradually while the needle is being inserted leads to a more even distribution of the fluid through the body tissue. The pain experienced during injection may be reduced due to the distribution of the DNA being injected over a larger area.

A desired encoded AFFIMER® construct in a form suitable for direct or indirect electrotransport may be introduced (e.g., injected) using a needle-free injector into the tissue to be treated, usually by contacting the tissue surface with the injector so as to actuate delivery of a jet of the agent, with sufficient force to cause penetration of the nucleic acid into the tissue. For example, if the tissue to be treated is a mucosa, skin or muscle, the agent is projected towards the mucosal or skin surface with sufficient force to cause the agent to penetrate through the stratum corneum and into dermal layers, or into underlying tissue and muscle, respectively. Needle-free injectors are well suited to deliver encoded AFFIMER® constructs to all types of tissues, including into tumors (intratumoral delivery).

In addition, the automatic injection of fluid facilitates automatic monitoring and registration of an actual dose of fluid injected. This data can be stored by a control unit for documentation purposes if desired.

It will be appreciated that the rate of injection could be either linear or non-linear and that the injection may be carried out after the needles have been inserted through the skin of the subject to be treated and while they are inserted further into the body tissue.

Suitable tissues into which fluid may be injected by the apparatus of the present disclosure include tumor tissue, skin and other epithelial tissues, liver tissue and muscle tissue, merely as examples.

The apparatus further comprises needle insertion means for guiding insertion of the needle into the body tissue. The rate of fluid injection is controlled by the rate of needle insertion. This has the advantage that both the needle insertion and injection of fluid can be controlled such that the rate of insertion can be matched to the rate of injection as desired. It also makes the apparatus easier for a user to operate. If desired means for automatically inserting the needle into body tissue could be provided.

Use of *in vivo* electroporation enhances plasmid DNA uptake in tumor tissue, resulting in expression within the tumor, and delivers plasmids to muscle tissue, resulting in systemic expression of secreted proteins, such as cytokines (see, e.g., US8026223). Additional exemplary techniques, vectors and devices for electroporating CD33 AFFIMER® agent transgenes into cells *in vivo* include PCT Publications WO/2017/106795, WO/2016/161201, WO/2016/154473, WO/2016/112359 and WO/2014/066655.

Typically, the electric fields needed for *in vivo* cell electroporation are generally similar in magnitude to the fields required for cells *in vitro*. In some embodiments, the magnitude of the electric field ranges from approximately, 10 V/cm to about 1500 V/cm, 300 V/cm to 1500 V/cm, or 1000 V/cm to 1500 V/cm. Alternatively, lower field strengths (from about 10 V/cm to 100 V/cm, and more preferably from about 25 V/cm to 75 V/cm) the pulse length is long. For example, when the nominal electric field is about 25-75 V/cm, it is preferred that the pulse length is about 10 msec.

The pulse length can be about 10 s to about 100 ms. There can be any desired number of pulses, typically one to 100 pulses per second. The delay between pulses sets can be any desired time, such as one second. The waveform, electric field strength and pulse duration may also depend upon the type of cells and the type of molecules that are to enter the cells via electroporation.

Also encompassed are electroporation devices incorporating electrochemical impedance spectroscopy ("EIS"). Such devices provide real-time information on *in vivo*, in particular, intratumoral electroporation efficiency, allowing for the optimization of conditions. Examples of electroporation devices incorporating EIS can be found, e.g., in WO2016/161201, which is hereby incorporated by reference.

Uptake of the encoded AFFIMER® constructs of the present disclosure may also be enhanced by plasma electroporation also termed avalanche transfection. Briefly, microsecond discharges create cavitation microbubbles at electrode surface. The mechanical force created by the collapsing microbubbles combined with the magnetic field serve to increase transport efficiency across the cell membrane as compared with the diffusion mediated transport associated with conventional electroporation. The technique of plasma electroporation is described in United States Patent NOS: 7,923,251 and 8,283,171. This technique may also be employed *in vivo* for the transformation of cells. Chaiberg, et al (2006) Investigative Ophthalmology & Visual Science 47:4083-4090; Chaiberg, et al United States Patent No 8, 101 169 Issued January 24, 2012.

Other alternative electroporation technologies are also contemplated. *In vivo* nucleic acid delivery can also be performed using cold plasma. Plasma is one of the four fundamental states of matter, the others being solid, liquid, and gas. Plasma is an electrically neutral medium of unbound positive and negative particles (e.g., the overall charge of a plasma is roughly zero). A plasma can be created by heating a gas or subjecting it to a strong electromagnetic field, applied with a laser or microwave generator. This decreases or increases the number of electrons, creating positive or negative charged particles called ions (Luo, et al. (1998) Phys. Plasma 5:2868-2870) and is accompanied by the dissociation of molecular bonds, if present.

Cold plasmas (e.g., non-thermal plasmas) are produced by the delivery of pulsed high voltage signals to a suitable electrode. Cold plasma devices may take the form of a gas jet device or a dielectric barrier discharge (DBD) device. Cold temperature plasmas have attracted a great deal of enthusiasm and interest by virtue of their provision of plasmas at relatively low gas temperatures. The provision of plasmas at such a temperature is of interest to a variety of applications, including wound healing, anti-bacterial processes, various other medical therapies and sterilization. As noted earlier, cold plasmas (e.g., non-thermal plasmas) are produced by the delivery of pulsed high voltage signals to a suitable electrode. Cold plasma devices may take the form of a gas jet device, a dielectric barrier discharge (DBD) device or multi-frequency harmonic-rich power supply.

In some embodiments, the present disclosure provides a method for treating a subject having a tumor, the method comprising: injecting the tumor with an effective dose of plasmid coding for a CD33 AFFIMER® agent; and administering electroporation therapy to the tumor. In some embodiments, the electroporation therapy further comprises the administration of at least one voltage pulse of about 200 V/cm to about 1500 V/cm over a pulse width of about 100 microseconds to about 20 milliseconds.

In some embodiments, the plasmid (or a second electroporated plasmid) further encodes at least one immunostimulatory cytokine, such as selected from the group encoding IL-12, IL-15, and a combination of IL-12 and IL-15.

2. Transfection Enhancing Formulations

Encoded AFFIMER® constructs can also be encapsulated in liposomes, preferably cationic liposomes (Wong, T. K. et al., *Gene*, 10:87(1980); Nicolau and Sene, *Biochim. Biophys. Acta*, 721:185-190 (1982); and Nicolau et al., *Methods Enzymol.*, 149:157-176 (1987)) or polymersomes (synthetic liposomes) which can interact with the cell membrane and fuse or undergo endocytosis to effect nucleic acid transfer into the cell. The DNA also can be formed into complexes with polymers (polyplexes) or with dendrimers which can directly release their load into the cytoplasm of a cell.

Illustrative carriers useful in this regard include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other illustrative carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (see e.g., U.S. Pat. No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active agent contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Biodegradable microspheres (e.g., polylactate polyglycolate) may be employed as carriers for compositions. Suitable biodegradable microspheres are disclosed, for example, in U.S. Pat. NOS: 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344, 5,407,609 and 5,942,252. Modified hepatitis B core protein carrier systems such as described in WO/99 40934, and references cited therein, will also be useful for many applications. Another illustrative carrier/delivery system employs a carrier comprising particulate-protein complexes, such as those described in U.S. Pat. No. 5,928,647, which can have the added benefit when used intratumorally to deliver the coding sequence for a CD33 AFFIMER® polypeptide.

Biodegradable polymeric nanoparticles facilitate nonviral nucleic acid transfer to cells. Small (approximately 200 nm), positively charged (approximately 10 mV) particles are formed by the self-assembly of cationic, hydrolytically degradable poly(beta-amino esters) and plasmid DNA.

Polynucleotides may also be administered to cells by direct microinjection, temporary cell permeabilizations (e.g., co-administration of repressor and/or activator with a cell permeabilizing agent), fusion to membrane translocating peptides, and the like.

Lipid-mediated nucleic acid delivery and expression of foreign nucleic acids, including mRNA, *in vitro* and *in vivo* has been very successful. Lipid based non-viral formulations provide an alternative to viral gene therapies. Current *in vivo* lipid delivery methods use subcutaneous, intradermal, intratumoral, or intracranial injection. Advances in lipid formulations have improved the efficiency of gene transfer *in vivo* (see PCT Application WO 98/07408). For instance, a lipid formulation composed of an equimolar ratio of 1,2-bis(oleoyloxy)-3-(trimethyl ammonio)propane (DOTAP) and cholesterol can significantly enhance systemic *in vivo* gene transfer. The DOTAP:cholesterol lipid formulation forms unique structure termed a "sandwich liposome". This formulation is reported to "sandwich" DNA between an invaginated bi-layer or 'vase' structure. Beneficial characteristics of these lipid structures include a positive p, colloidal stabilization by cholesterol, two-dimensional nucleic acid packing and increased serum stability.

Cationic liposome technology is based on the ability of amphipathic lipids, possessing a positively charged head group and a hydrophobic lipid tail, to bind to negatively charged DNA or RNA and form particles that generally enter cells by endocytosis. Some cationic liposomes also contain a neutral co-lipid, thought to enhance liposome uptake by mammalian cells. Similarly, other polycations, such as poly-l-lysine and polyethylene-imine, complex with nucleic acids via charge interaction and aid in the condensation of DNA or RNA into nanoparticles, which are then substrates for endosome-mediated uptake. Several of these cationic-nucleic acid complex technologies have been developed as potential clinical products, including complexes with plasmid DNA (pDNA), oligodeoxynucleotides, and various forms of synthetic RNA, and be used as part of the delivery system for the encoded AFFIMER® construct of the present disclosure.

The encoded AFFIMER® construct disclosed herein may be associated with polycationic molecules that serve to enhance uptake into cells. Complexing the nucleic acid construct with polycationic molecules also helps in packaging the construct such their size is reduced, which is believed to assist with cellular uptake. Once in the endosome, the complex dissociates due to the lower pH, and the polycationic molecules can disrupt the endosome's membrane to facilitate

DNA escape into the cytoplasm before it can be degraded. Preliminary data shows that the nucleic acid construct embodiments had enhanced uptake into SCs over DCs when complexed with the polycationic molecules polylysine or polyethyleneimine.

One example of polycationic molecules useful for complexing with nucleic acid constructs includes cell penetrating peptides (CPP), examples include polylysine (described above), polyarginine and Tat peptides. Cell penetrating peptides (CPP) are small peptides which can bind to DNA and once released penetrate cell membranes to facilitate escape of the DNA from the endosome to the cytoplasm. Another example of a CPP pertains to a 27-residue chimeric peptide, termed MPG, was shown some time ago to bind ss- and ds-oligonucleotides in a stable manner, resulting in a non-covalent complex that protected the nucleic acids from degradation by DNase and effectively delivered oligonucleotides to cells *in vitro* (Mahapatro A, et al., J Nanobiotechnol, 2011, 9:55). The complex formed small particles of approximately 150 nm to 1 μ m when different peptide:DNA ratios were examined, and the 10:1 and 5:1 ratios (150 nm and 1 μ m respectively). Another CPP pertains to a modified tetrapeptide [tetralysine containing guanidinocarbonylpyrrole (GCP) groups (TL-GCP)], which was reported to bind with high affinity to a 6.2 kb plasmid DNA resulting in a positive charged aggregate of 700-900 nm Li et al., Agnew Chem Int Ed Engl 2015; 54(10):2941-4). RNA can also be complexed by such polycationic molecules for *in vivo* delivery.

Other examples of polycationic molecules that may be complexed with the nucleic acid constructs described herein include polycationic polymers commercially available as JETPRIME® and *In vivo* JET (Polypus-transfection, S.A., Illkirch, France).

In some embodiments, the present disclosure contemplates a method of delivering an mRNA (or other polynucleotide) encoding a CD33 AFFIMER® agent to a patient's cells by administering a nanoparticle composition comprising (i) a lipid component, a phospholipid, a structural lipid, and a PEG lipid; and (ii) an mRNA (or other polynucleotide), said administering comprising contacting said mammalian cell with said nanoparticle composition, whereby said mRNA (or other polynucleotide) is delivered to said cell.

In exemplary embodiments, the PEG lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatide acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol and a PEG-modified dialkylglycerol. In exemplary embodiments, the structural lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, and alphatocopherol. In some embodiments, the structural lipid is cholesterol.

In exemplary embodiments, the phospholipid includes a moiety selected from the group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and a sphingomyelin. In some embodiments, the phospholipid includes at least one fatty acid moiety selected from the group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, arachidic acid, arachidonic acid, phytanoic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid. In some embodiments, the phospholipid is selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-*l*-octadecenyl-sn-glycero-3-phosphocholine (1,3-bis(sn)-3'-phosphatidyl)-sn-glycero-3-phosphocholine (1,3-bis(sn)-3'-phosphatidyl PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 1,6-bis(sn)-3'-phosphatidyl)-sn-glycero-3-phosphoethanolamine (ME 1,6-bis(sn)-3'-phosphatidyl PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1,3-bis(sn)-3'-phosphatidyl)-sn-glycerol sodium salt (DOPG), and sphingomyelin. In some embodiments, the phospholipid is DOPE or DSPC.

To further illustrate, the phospholipid can be DOPE and said the component can comprise about 35 mol % to about 45 mol % said compound, about 10 mol % to about 20 mol % DOPE, about 38.5 mol % to about 48.5 mol % structural lipid, and about 1.5 mol % PEG lipid. The lipid component can be about 40 mol % said compound, about 15 mol % phospholipid, about 43.5 mol % structural lipid, and about 1.5 mol % PEG lipid.

In some embodiments, the wt/wt ratio of lipid component to CD33 AFFIMER® agent encoding mRNA (or other polynucleotide) is from about 5:1 to about 50:1, or about 10:1 to about 40:1.

In some embodiments, the mean size of said nanoparticle composition is from about 50 nm to about 150 nm, or from about 80 nm to about 120 nm.

In some embodiments, the polydispersity index of said nanoparticle composition is from about 0 to about 0.18, or from about 0.13 to about 0.17.

In some embodiments, the nanoparticle composition has a zeta potential of about -10 to about +20 mV.

In some embodiments, the nanoparticle composition further comprises a cationic and/or ionizable lipid selected from the group consisting of 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinolexyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-diolexyloxy-N,N-dimethylaminopropane (DODMA), and (2R)-2-({8-[(3P)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yl oxy]propan-1-amine (Octyl-CLinDMA (2R)).

For exemplary lipid nanoparticle compositions and other polymeric carrier compositions, see International Publication Nos. WO 2016/118724A1, WO 2017/112865A1, WO 2017/049245A2, and WO2012013326A1.

V. Expression Methods and Systems

CD33 AFFIMER® agent proteins described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. For those recombinant AFFIMER® agent proteins including further modifications, such as a chemical modifications or conjugation, the recombinant AFFIMER® agent protein can be further manipulated chemically or enzymatically after isolation from the host cell or chemical synthesis.

The present disclosure includes recombinant methods and nucleic acids for recombinantly expressing the recombinant AFFIMER® agent proteins of the present disclosure comprising (i) introducing into a host cell a polynucleotide encoding the amino acid sequence of said AFFIMER® agent, for example, wherein the polynucleotide is in a vector and/or is operably linked to a promoter; (ii) culturing the host cell (e.g., eukaryotic or prokaryotic) under condition favorable to expression of the polynucleotide and, (iii) optionally, isolating the AFFIMER® agent from the host cell and/or medium in which the host cell is grown. See e.g., WO 04/041862, WO 2006/122786, WO 2008/020079, WO 2008/142164 or WO 2009/068627.

In some embodiments, a DNA sequence encoding a recombinant AFFIMER® agent protein of interest may be constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Once a nucleic acid sequence encoding a recombinant AFFIMER® agent protein of the disclosure has been obtained, the vector for the production of the recombinant AFFIMER® agent protein may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the recombinant AFFIMER® agent coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (See, for example, the techniques described in Sambrook et al, 1990, MOLECULAR CLONING, A LABORATORY MANUAL, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al. eds., 1998, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY).

An expression vector comprising the nucleotide sequence of a recombinant AFFIMER® agent protein can be transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation) and the transfected cells are then cultured by conventional techniques to produce the recombinant AFFIMER® agent protein of the disclosure. In specific embodiments, the expression of the recombinant AFFIMER® agent protein is regulated by a constitutive, an inducible or a tissue, specific promoter.

The expression vector may include an origin of replication, such as may be selected based upon the type of host cell being used for expression. By way of example, the origin of replication from the plasmid pBR322 (Product No. 303-3s, New England Biolabs, Beverly, Mass.) is useful for most Gram- negative bacteria while various origins from SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV) or papillomaviruses (such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is

not needed for mammalian expression vectors (for example, the SV40 origin is often used because it contains the early promoter).

The vector may include at least one selectable marker gene, e.g., genetic elements that encode a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells. Other selection genes may be used to amplify the gene which will be expressed. Amplification is a process where genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the marker present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the recombinant AFFIMER® agent protein. As a result, increased quantities of the recombinant AFFIMER® agent protein are synthesized from the amplified DNA.

The vector may also include at least one ribosome binding site, which will be transcribed into the mRNA including the coding sequence for the recombinant AFFIMER® agent protein. For example, such a site is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of the polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth above and used in a prokaryotic vector.

The expression vectors will typically contain a promoter that is recognized by the host organism and operably linked to a nucleic acid molecule encoding the recombinant AFFIMER® agent protein. Either a native or heterologous promoter may be used depending on the host cell used for expression and the yield desired.

Promoters for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, and they can be ligated to a desired nucleic acid sequence(s), using linkers or adapters as desired to supply restriction sites.

Promoters for use with yeast hosts are also known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, e.g., heat-shock promoters and the actin promoter.

Additional promoters which may be used for expressing the selective binding agents of the disclosure include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature*, 290:304-310, 1981); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al. (1980), *Cell* 22: 787-97); the herpes thymidine kinase promoter (Wagner et al. (1981), *Proc. Natl. Acad. Sci. U.S.A.* 78: 1444-5); the regulatory sequences of the metallothionein gene (Brinster et al, *Nature*, 296; 39-42, 1982); prokaryotic expression vectors such as the beta- lactamase promoter (Villa-Kamaroff, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 75; 3727-3731, 1978); or the tac promoter (DeBoer, et al. (1983), *Proc. Natl. Acad. Sci. U.S.A.*, 80: 21-5). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift et al. (1984), *Cell* 38: 639-46; Ornitz et al. (1986), *Cold Spring Harbor Symp. Quant. Biol.* 50: 399-409; MacDonald (1987), *Hepatology* 7: 425-515); the insulin gene control region which is active in pancreatic beta cells (Hanahan (1985), *Nature* 315: 115-22); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al. (1984), *Cell* 38; 647-58; Adames et al. (1985), *Nature* 318; 533-8; Alexander et al. (1987), *Mol. Cell. Biol.* 7: 1436-44); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al. (1986), *Cell* 45: 485-95), albumin gene control region which is active in liver (Pinkert et al. (1987), *Genes and Devel.* 1: 268-76); the alphafetoprotein gene control region which is active in liver (Krumlauf et al. (1985), *Mol. Cell. Biol.* 5: 1639-48; Hammer et al. (1987), *Science*, 235: 53-8); the alpha 1- antitrypsin gene control region which is active in the liver (Kelsey et al. (1987), *Genes and Devel.* 1: 161-71); the

beta-globin gene control region which is active in myeloid cells (Mogram et al., *Nature*, 315: 338-340, 1985; Kollias et al. (1986), *Cell* 46: 89-94); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al. (1987), *Cell*, 48: 703-12); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani (1985), *Nature*, 314: 283-6); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al. (1986), *Science* 234: 1372-8).

An enhancer sequence may be inserted into the vector to increase transcription in eukaryotic host cells. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters.

While an enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide coding region, it is typically located at a site 5' from the promoter.

Vectors for expressing nucleic acids include those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, inter alia, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, Calif.), pBSII (Stratagene Company, La Jolla, Calif.), pET15 (Novagen, Madison, Wis.), pGEX (Pharmacia Biotech, Piscataway, N.J.), pEGFP-N2 (Clontech, Palo Alto, Calif.), pETL (BlueBacII; Invitrogen), pDSR- alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, N.Y.).

Additional possible vectors include but are not limited to, cosmids, plasmids or modified viruses, but the vector system must be compatible with the selected host cell. Such vectors include but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColEI-based phagemid, Stratagene Cloning Systems Inc., La Jolla Calif.), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO™. TA Cloning® Kit, PCR2.1 plasmid derivatives, Invitrogen, Carlsbad, Calif.), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, Calif.). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, or other known techniques

Eukaryotic and prokaryotic host cells, including mammalian cells as hosts for expression of the recombinant AFFIMER® agent protein disclosed herein are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, inter alia, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular

carcinoma cells (e.g., Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. Fungal cells include yeast and filamentous fungus cells including, for example, *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium* sp., *Fusarium gramineum*, *Fusarium venenatum*, *Physcomitrella patens* and *Neurospora crassa*. *Pichia* sp., any *Saccharomyces* sp., *Hansenula polymorpha*, any *Kluyveromyces* sp., *Candida albicans*, any *Aspergillus* sp., *Trichoderma reesei*, *Chrysosporium lucknowense*, any *Fusarium* sp., *Yarrowia lipolytica*, and *Neurospora crassa*.

A variety of host-expression vector systems may be utilized to express the recombinant AFFIMER® agent protein of the disclosure. Such host-expression systems represent vehicles by which the coding sequences of the recombinant AFFIMER® agent protein may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the recombinant AFFIMER® agent protein of the disclosure in situ. These include but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing AFFIMER® agent protein coding sequences; yeast (e.g., *Saccharomyces pichia*) transformed with recombinant yeast expression vectors containing AFFIMER® agent protein coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the AFFIMER® agent protein coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (C μ MV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing AFFIMER® agent protein coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 293T, 3T3 cells, lymphatic cells (see U.S. Pat. No. 5,807,715), Per C.6 cells (rat retinal cells developed by Crucell)) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the recombinant AFFIMER® agent protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of the recombinant AFFIMER® agent protein, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al. (1983) "Easy Identification Of cDNA Clones," EMBO J. 2:1791-1794), in which the AFFIMER® agent protein coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye et al. (1985) "Up-Promoter Mutations In The Lpp Gene Of Escherichia coli," Nucleic Acids Res. 13:3101-3110; Van Heeke et al. (1989) "Expression Of Human Asparagine Synthetase In Escherichia coli," J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The AFFIMER® agent protein coding sequence may be cloned individually into non-essential regions (e.g., the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (e.g., the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the AFFIMER® agent protein coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts. (see e.g., see Logan et al. (1984) "Adenovirus Tripartite Leader Sequence Enhances Translation Of mRNAs Late After Infection," Proc. Natl. Acad. Sci. (U.S.A.) 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted AFFIMER® agent protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure

translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bitter et al. (1987) "Expression and Secretion Vectors For Yeast," *Methods in Enzymol.* 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 293T, 3T3, WI38, BT483, Hs578T, HTB2, BT20 and T47D, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is contemplated. For example, cell lines which stably express an antibody of the disclosure may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the recombinant AFFIMER® agent proteins of the disclosure. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the recombinant AFFIMER® agent proteins.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al. (1977) "Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells," *Cell* 11:223-232), hypoxanthine-guanine phosphoribosyltransferase (Szybalska et al. (1962) "Genetics Of Human Cess Line. IV. DNA-Mediated Heritable Transformation of a Biochemical Trait," *Proc. Natl. Acad. Sci. (U.S.A.)*

48:2026-2034), and adenine phosphoribosyltransferase (Lowy et al. (1980) "Isolation Of Transforming DNA: Cloning The Hamster Aprt Gene," Cell 22:817-823) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al. (1980) "Transformation Of Mammalian Cells With An Amplifiable Dominant-Acting Gene," Proc. Natl. Acad. Sci. (U.S.A.) 77:3567-3570; O'Hare et al. (1981) "Transformation Of Mouse Fibroblasts To Methotrexate Resistance By A Recombinant Plasmid Expressing A Prokaryotic Dihydrofolate Reductase," Proc. Natl. Acad. Sci. (U.S.A.) 78:1527-1531); *gpt*, which confers resistance to mycophenolic acid (Mulligan et al. (1981) "Selection For Animal Cells That Express The Escherichia coli Gene Coding For Xanthine-Guanine Phosphoribosyltransferase," Proc. Natl. Acad. Sci. (U.S.A.) 78:2072-2076); *neo*, which confers resistance to the aminoglycoside G-418 (Tachibana et al. (1991) "Altered Reactivity Of Immunoglobulin Produced By Human-Human Hybridoma Cells Transfected By pSV.2-Neo Gene," Cytotechnology 6(3):219-226; Tolstoshev (1993) "Gene Therapy, Concepts, Current Trials And Future Directions," Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan (1993) "The Basic Science of Gene Therapy," Science 260:926-932; and Morgan et al. (1993) "Human gene therapy," Ann. Rev. Biochem. 62:191-217). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY; Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, CURRENT PROTOCOLS IN HUMAN GENETICS, John Wiley & Sons, NY.; Colbere-Garapin et al. (1981) "A New Dominant Hybrid Selective Marker For Higher Eukaryotic Cells," J. Mol. Biol. 150:1-14; and hyg^r, which confers resistance to hygromycin (Santerre et al. (1984) "Expression Of Prokaryotic Genes For Hygromycin B And G418 Resistance As Dominant-Selection Markers In Mouse L Cells," Gene 30:147-156).

The expression levels of a recombinant AFFIMER® agent protein can be increased by vector amplification (for a review, see Bebbington and Hentschel, "The Use of Vectors Based On Gene Amplification For The Expression Of Cloned Genes In Mammalian Cells," in DNA CLONING, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing a recombinant AFFIMER® agent protein is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the recombinant AFFIMER® agent protein, production of the recombinant AFFIMER® agent protein will also

increase (Crouse et al. (1983) "Expression and Amplification of Engineered Mouse Dihydrofolate Reductase Minigenes," Mol. Cell. Biol. 3:257-266).

Where the AFFIMER® agent is an AFFIMER® polypeptide-antibody fusion or other multiprotein complex, the host cell may be co-transfected with two expression vectors, for instance the first vector encoding a heavy chain and the second vector encoding a light chain derived polypeptide, one or both of which includes an AFFIMER® polypeptide coding sequence. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot (1986) "Expression and Amplification Of Engineered Mouse Dihydrofolate Reductase Minigenes," Nature 322:562-565; Kohler (1980) "Immunoglobulin Chain Loss In Hybridoma Lines," Proc. Natl. Acad. Sci. (U.S.A.) 77:2197-2199). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

In general, glycoproteins produced in a particular cell line or transgenic animal will have a glycosylation pattern that is characteristic for glycoproteins produced in the cell line or transgenic animal. Therefore, the particular glycosylation pattern of the recombinant AFFIMER® agent protein will depend on the particular cell line or transgenic animal used to produce the protein. In some embodiments of AFFIMER® polypeptide-antibody fusions, a glycosylation pattern comprising only non-fucosylated N-glycans may be advantageous, because in the case of antibodies this has been shown to typically exhibit more potent efficacy than fucosylated counterparts both *in vitro* and *in vivo* (See for example, Shinkawa et al., J. Biol. Chem. 278: 3466-3473 (2003); U.S. Pat. NOS: 6,946,292 and 7,214,775).

Further, expression of an AFFIMER® agent from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent NOS: 0216846, 0256055, and 0323997 and European Patent Application No. 89303964.4. Thus, in some embodiments of the disclosure, the mammalian host cells (e.g., CHO) lack a glutamine synthetase gene and are grown in the absence of glutamine in the medium wherein, however, the polynucleotide encoding the immunoglobulin chain comprises a glutamine synthetase gene which complements the lack of the gene in the host cell. Such host cells containing the binder or polynucleotide or vector as discussed herein as well as expression methods, as discussed herein, for making the binder using such a host cell are part of the present disclosure.

Expression of recombinant proteins in insect cell culture systems (e.g., baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art.

The recombinant AFFIMER® agent proteins produced by a transformed host can be purified according to any suitable method. Standard methods include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine, maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, mass spectrometry (MS), nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), and x-ray crystallography.

In some embodiments, recombinant AFFIMER® agent proteins produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by at least one concentration, salting-out, aqueous ion exchange, or size exclusion chromatography steps. HPLC can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

VI. Methods of Use and Pharmaceutical Compositions

The AFFIMER® agents of the disclosure are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as immunotherapy for cancer. In some embodiments, AFFIMER® agents described herein are useful for activating, promoting, increasing, and/or enhancing an immune response, inhibiting tumor growth, reducing tumor volume, inducing tumor regression, increasing tumor cell apoptosis, and/or reducing the tumorigenicity of a tumor. In some embodiments, the polypeptides or agents of the disclosure are also useful for immunotherapy against pathogens, such as viruses. In some embodiments, the AFFIMER® agents described herein are useful for inhibiting viral infection, reducing viral infection, increasing virally-infected cell apoptosis, and/or increasing killing of virus-infected cells. The methods of use may be *in vitro*, *ex vivo*, or *in vivo* methods.

CD33 expression is associated with acute myeloid leukemia (AML). CD33 AFFIMER® polypeptides may thus be used to direct anti-cancer therapies, such as T cells, antibodies, viral therapies, nucleic acids, and other small molecules or effector proteins, to CD33+ cells for the

treatment of cancer. Therefore, in some embodiments, the subject has a CD33+ cancer, such as AML.

CD33 is expressed by microglia, brain-resident macrophages, and the expression of CD33 on these cells is associated with development of Alzheimer's disease. Specifically, CD33 is believed to interfere with the phagocytosis and clearance of A β plaques, resulting in plaque accumulation and the development of Alzheimer's disease. Mouse models using genetic approaches to reduce or eliminate CD33 expression in microglia demonstrate a reduction in A β plaque deposition and inhibition of Alzheimer's disease progression. CD33 AFFIMER® polypeptides may thus be useful in treating or delaying the progression of Alzheimer's disease, and in some embodiments, the subject has Alzheimer's disease. (10.1159/000492596)

A challenge to targeting CD33 in the brain is the blood-brain barrier, which prevents the entry of many molecules, including antibodies, into the brain. However, engineered antibodies, such as multispecific antibodies that bind a target antigen and have low affinity for transferrin receptor can enter the brain by binding transferrin receptor, crossing the blood-brain barrier, releasing from transferrin receptor, and then binding the target antigen. Without wishing to be bound by theory it is thought that linking a CD33 AFFIMER® polypeptide to an antibody or antigen-binding fragment that binds to transferrin receptor may allow CD33 AFFIMER® polypeptides to target CD33 activity in the brain. (10.1126/scitranslmed.3002230)

CD33 is frequently expressed on innate immune cells, such as monocytes. Cross-linking of CD33 on monocytes, such as through the action of antibodies with multiple antigen-binding fragments, has a pro-inflammatory effect, resulting in production of IL-1 β . Thus, a molecule with multiple CD33 AFFIMER® polypeptides motifs may exhibit a similar inflammatory effect by cross-linking CD33. (10.1002/eji.200425273)

Conversely, sialic acid motifs on the surface of CD33+ cells act as cis ligands for CD33, which has an anti-inflammatory effect, inhibiting IL-1 β production in monocytes. Thus, CD33 AFFIMER® polypeptides may exhibit a similar anti-inflammatory effect by inhibiting CD33 cross-linking. Furthermore, the lack of antibody constant (Fc) domains allows CD33 AFFIMER® polypeptides to exert such activity without triggering an immune response towards the engineered polypeptide-bound CD33+ cells.

The present disclosure provides methods for activating an immune response in a subject using an AFFIMER® agent. In some embodiments, the disclosure provides methods for promoting an immune response in a subject using an AFFIMER® agent described herein. In some embodiments, the disclosure provides methods for increasing an immune response in a subject using an AFFIMER® agent. In some embodiments, the disclosure provides methods for

enhancing an immune response in a subject using an AFFIMER® agent. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing cell-mediated immunity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing Th1-type responses. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing T-cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CD4+ T-cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CD8+ T-cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CTL activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing T-cell activity and increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CU activity and increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises inhibiting or decreasing the suppressive activity of Treg cells. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises inhibiting or decreasing the suppressive activity of MDSCs. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing the number of the percentage of memory T-cells. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing long-term immune memory function. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing long-term memory. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises no evidence of substantial side effects and/or immune-based toxicities. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises no evidence of cytokine release syndrome (CRS) or a cytokine storm. In some embodiments, the immune response is a result of antigenic stimulation. In some embodiments, the antigenic stimulation is a tumor cell. In some embodiments, the antigenic stimulation is cancer. In some embodiments, the antigenic stimulation is a pathogen. In some embodiments, the antigenic stimulation is a virally-infected cell.

In vivo and *in vitro* assays for determining whether an AFFIMER® agent activates, or inhibits an immune response are known in the art.

In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of an AFFIMER® agent described herein, wherein an AFFIMER® agent binds human CD33. In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of an AFFIMER® agent described herein, wherein the AFFIMER® agent is an AFFIMER®-containing antibody or receptor trap fusion polypeptide including an AFFIMER® polypeptide that specifically binds to CD33. In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of an encoded AFFIMER® construct, wherein the encoded AFFIMER® construct, when expressed in the patient, produces a recombinant AFFIMER® agent including an CD33 AFFIMER® polypeptide.

In some embodiments of the methods described herein, a method of activating or enhancing a persistent or long-term immune response to a tumor comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent which binds human CD33. In some embodiments, a method of activating or enhancing a persistent immune response to a tumor comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent described herein, wherein the AFFIMER® agent is an AFFIMER®-containing antibody or receptor trap fusion polypeptide including an AFFIMER® polypeptide that specifically binds to CD33. In some embodiments, a method of activating or enhancing a persistent immune response to a tumor comprises administering to a subject a therapeutically effective amount of an encoded AFFIMER® construct, wherein the encoded AFFIMER® construct, when expressed in the patient, produces a recombinant AFFIMER® agent including an CD33 AFFIMER® polypeptide.

In some embodiments of the methods described herein, a method of inducing a persistent or long-term immunity which inhibits tumor relapse or tumor regrowth comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent which binds human CD33. In some embodiments, a method of inducing a persistent immunity which inhibits tumor relapse or tumor regrowth comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent described herein, wherein the AFFIMER® agent is an AFFIMER® polypeptide-containing antibody or receptor trap fusion polypeptide including an AFFIMER® polypeptide that specifically binds to CD33. In some embodiments, a method of inducing a persistent immunity which inhibits tumor relapse or tumor regrowth comprises administering to a subject a therapeutically effective amount of an encoded AFFIMER®

construct, wherein the encoded AFFIMER® construct, when expressed in the patient, produces a recombinant AFFIMER® agent including an CD33 AFFIMER® polypeptide.

In some embodiments of the methods described herein, a method of inhibiting tumor relapse or tumor regrowth comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent which binds human CD33. In some embodiments, a method of inhibiting tumor relapse or tumor regrowth comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent described herein, wherein the AFFIMER® agent is an AFFIMER®-containing antibody or receptor trap fusion polypeptide including an AFFIMER® polypeptide that specifically binds to CD33. In some embodiments, a method of inhibiting tumor relapse or tumor regrowth comprises administering to a subject a therapeutically effective amount of an encoded AFFIMER® construct, wherein the encoded AFFIMER® construct, when expressed in the patient, produces a recombinant AFFIMER® agent including CD33 AFFIMER® polypeptide.

In some embodiments, the tumor expresses or overexpresses a tumor antigen that is targeted by an additional binding entity provided in the AFFIMER® agent along with the CD33 AFFIMER® polypeptide, e.g., where the AFFIMER® agent is a bispecific or multispecific agent.

In some embodiments, the method of inhibiting growth of a tumor comprises administering to a subject a therapeutically effective amount of an AFFIMER® agent described herein. In some embodiments, the subject is a human. In some embodiments, the subject has a tumor, or the subject had a tumor which was removed.

In some embodiments, the tumor is a solid tumor. In some embodiments, the tumor is a tumor selected from the group consisting of: colorectal tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, neuroendocrine tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor. In some embodiments, the tumor is a colorectal tumor. In some embodiments, the tumor is an ovarian tumor. In some embodiments, the tumor is a lung tumor. In some embodiments, the tumor is a pancreatic tumor. In some embodiments, the tumor is a melanoma tumor. In some embodiments, the tumor is a bladder tumor.

To further illustrate, the subject AFFIMER® agents can be used to treat patients suffering from cancer, such as osteosarcoma, rhabdomyosarcoma, neuroblastoma, kidney cancer, leukemia, renal transitional cell cancer, bladder cancer, Wilm's cancer, ovarian cancer, pancreatic cancer, breast cancer (including triple negative breast cancer), prostate cancer, bone cancer, lung cancer (e.g., small cell or non-small cell lung cancer), gastric cancer, colorectal

cancer, cervical cancer, synovial sarcoma, head and neck cancer, squamous cell carcinoma, multiple myeloma, renal cell cancer, retinoblastoma, hepatoblastoma, hepatocellular carcinoma, melanoma, rhabdoid tumor of the kidney, Ewing's sarcoma, chondrosarcoma, brain cancer, glioblastoma, meningioma, pituitary adenoma, vestibular schwannoma, a primitive neuroectodermal tumor, medulloblastoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, idiopathic myelofibrosis, soft tissue sarcoma, thyroid cancer, endometrial cancer, carcinoid cancer or liver cancer, breast cancer or gastric cancer. In some embodiments of the disclosure, the cancer is metastatic cancer, e.g., of the varieties described above.

In some embodiments, the cancer is a hematologic cancer. In some embodiment, the cancer is selected from the group consisting of: acute myelogenous leukemia (AML), Hodgkin lymphoma, multiple myeloma, T-cell acute lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML), non-Hodgkin lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), and cutaneous T-cell lymphoma (CTCL).

The present disclosure also provides pharmaceutical compositions comprising an AFFIMER® agent described herein and a pharmaceutically acceptable vehicle. In some embodiments, the pharmaceutical compositions find use in immunotherapy. In some embodiments, the pharmaceutical compositions find use in immuno-oncology. In some embodiments, the compositions find use in inhibiting tumor growth. In some embodiments, the pharmaceutical compositions find use in inhibiting tumor growth in a subject (e.g., a human patient). In some embodiments, the compositions find use in treating cancer. In some embodiments, the pharmaceutical compositions find use in treating cancer in a subject (e.g., a human patient).

Formulations are prepared for storage and use by combining a purified AFFIMER® agent of the present disclosure with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Those of skill in the art generally consider pharmaceutically acceptable carriers, excipients, and/or stabilizers to be inactive ingredients of a formulation or pharmaceutical composition.

In some embodiments, an AFFIMER® agent described herein is lyophilized and/or stored in a lyophilized form. In some embodiments, a formulation comprising an AFFIMER® agent described herein is lyophilized.

Suitable pharmaceutically acceptable vehicles include but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride;

antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (Remington: The Science and Practice of Pharmacy, 22.sup.nd Edition, 2012, Pharmaceutical Press, London.).

The pharmaceutical compositions of the present disclosure can be administered in any number of ways for either local or systemic treatment. Administration can be topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal; oral; or parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular).

The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and diluents (e.g., water). These can be used to form a solid preformulation composition containing a homogeneous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of a type described above. The tablets, pills, etc. of the formulation or composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used

for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

The AFFIMER® agents described herein can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions as described in Remington: The Science and Practice of Pharmacy, 22nd Edition, 2012, Pharmaceutical Press, London.

In some embodiments, pharmaceutical formulations include an AFFIMER® agent of the present disclosure complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes can be extruded through filters of defined pore size to yield liposomes with the desired diameter.

In some embodiments, sustained-release preparations comprising AFFIMER® agents described herein can be produced. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing an AFFIMER® agent, where the matrices are in the form of shaped articles (e.g., films or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM. (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

In some embodiments, in addition to administering an AFFIMER® agent described herein, the method or treatment further comprises administering at least one additional immune response stimulating agent. In some embodiments, the additional immune response stimulating agent includes, but is not limited to, a colony stimulating factor (e.g., granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), stem cell factor (SCF)), an interleukin (e.g., IL-1, IL-2, IL-3, IL-7, IL-12, IL-15, IL-18), a checkpoint inhibitor, an antibody that blocks immunosuppressive functions (e.g., an anti-CTLA-4 antibody, anti-CD28 antibody, anti-CD3 antibody), a toll-like receptor (e.g., TLR4, TLR7, TLR9), or a member of the B7 family (e.g.,

CD80, CD86). An additional immune response stimulating agent can be administered prior to, concurrently with, and/or subsequently to, administration of the AFFIMER® agent.

Pharmaceutical compositions comprising an AFFIMER® agent and the immune response stimulating agent(s) are also provided. In some embodiments, the immune response stimulating agent comprises 1, 2, 3, or more immune response stimulating agents.

In some embodiments, in addition to administering an AFFIMER® agent described herein, the method or treatment further comprises administering at least one additional therapeutic agent. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the AFFIMER® agent. Pharmaceutical compositions comprising an AFFIMER® agent and the additional therapeutic agent(s) are also provided. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

Combination therapy with two or more therapeutic agents often uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action may result in additive or synergetic effects. Combination therapy may allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the AFFIMER® agent. Combination therapy may decrease the likelihood that resistant cancer cells will develop. In some embodiments, combination therapy comprises a therapeutic agent that affects the immune response (e.g., enhances or activates the response) and a therapeutic agent that affects (e.g., inhibits or kills) the tumor/cancer cells.

In some embodiments of the methods described herein, the combination of an AFFIMER® agent described herein and at least one additional therapeutic agent results in additive or synergistic results. In some embodiments, the combination therapy results in an increase in the therapeutic index of the AFFIMER® agent. In some embodiments, the combination therapy results in an increase in the therapeutic index of the additional therapeutic agent(s). In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the AFFIMER® agent. In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the additional therapeutic agent(s).

Useful classes of therapeutic agents include, for example, anti-tubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, anti-folates, anti-metabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins,

nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In some embodiments, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

Therapeutic agents that may be administered in combination with the AFFIMER® agent described herein include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the administration of an AFFIMER® agent of the present disclosure in combination with a chemotherapeutic agent or in combination with a cocktail of chemotherapeutic agents. Treatment with an AFFIMER® agent can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *The Chemotherapy Source Book*, 4^{sup}.th Edition, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, Pa.

Chemotherapeutic agents useful in the present disclosure include but are not limited to, alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-

azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diazi quone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel (TAXOL) and docetaxel (TAXOTERE); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine (XELODA); and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In some embodiments, the additional therapeutic agent is cisplatin. In some embodiments, the additional therapeutic agent is carboplatin.

In some embodiments of the methods described herein, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include but are not limited to, doxorubicin HCl, daunorubicin citrate, mitoxantrone HCl, actinomycin D, etoposide, topotecan HCl, teniposide (VM-26), and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In some embodiments, the additional therapeutic agent is irinotecan.

In some embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with at least one normal function of cells, such as cell division. Anti-metabolites include but are not limited to, gemcitabine, fluorouracil,

capecitabine, methotrexate sodium, raltitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In some embodiments, the additional therapeutic agent is gemcitabine.

In some embodiments of the methods described herein, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some embodiments, the agent is a taxane. In some embodiments, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In some embodiments, the agent is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (nab-paclitaxel; ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative embodiments, the antimitotic agent comprises a vinca alkaloid, such as vincristine, vinblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some embodiments, the antimitotic agent is an inhibitor of kinesin Eg5 or an inhibitor of a mitotic kinase such as Aurora A or Plk1. In some embodiments, the additional therapeutic agent is paclitaxel. In some embodiments, the additional therapeutic agent is nab-paclitaxel.

In some embodiments of the methods described herein, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of an AFFIMER® agent of the present disclosure with a small molecule that acts as an inhibitor against tumor-associated antigens including, but not limited to, EGFR, HER2 (ErbB2), and/or VEGF. In some embodiments, an AFFIMER® agent of the present disclosure is administered in combination with a protein kinase inhibitor selected from the group consisting of: gefitinib (IRESSA), erlotinib (TARCEVA), sunitinib (SUTENT), lapatanib, vandetanib (ZACTIMA), AEE788, CI-1033, cediranib (RECENTIN), sorafenib (NEXAVAR), and pazopanib (GW786034B). In some embodiments, an additional therapeutic agent comprises an mTOR inhibitor.

In some embodiments of the methods described herein, the additional therapeutic agent is a small molecule that inhibits a cancer stem cell pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the BMP pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Hippo pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the mTOR/AKR pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the RSPO/LGR pathway.

In some embodiments of the methods described herein, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of an AFFIMER® agent of the present disclosure with antibodies against tumor-associated antigens including, but not limited to, antibodies that bind EGFR, HER2/ErbB2, and/or VEGF. In some embodiments, the additional therapeutic agent is an antibody specific for a cancer stem cell marker. In some embodiments, the additional therapeutic agent is an antibody that binds a component of the Notch pathway. In some embodiments, the additional therapeutic agent is an antibody that binds a component of the Wnt pathway. In some embodiments, the additional therapeutic agent is an antibody that inhibits a cancer stem cell pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the BMP pathway. In some embodiments, the additional therapeutic agent is an antibody that inhibits β -catenin signaling. In some embodiments, the additional therapeutic agent is an antibody that is an angiogenesis inhibitor (e.g., an anti-VEGF or VEGF receptor antibody). In some embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), ramucirumab, trastuzumab (HERCEPTIN), pertuzumab (OMNITARG), panitumumab (VECTIBIX), nimotuzumab, zalutumumab, or cetuximab (ERBITUX).

In some embodiments of the methods described herein, the additional therapeutic agent is an antibody that modulates the immune response. In some embodiments, the additional therapeutic agent is an anti-PD-1 antibody, an anti-LAG-3 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody, or an anti-TIGIT antibody.

Furthermore, treatment with an AFFIMER® agent described herein can include combination treatment with other biologic molecules, such as at least one cytokine (e.g., lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of tumors, removal of cancer cells, or any other therapy deemed necessary by a treating physician. In some embodiments, the additional therapeutic agent is an immune response stimulating agent.

In some embodiments of the methods described herein, the AFFIMER® agent can be combined with a growth factor selected from the group consisting of: adrenomedullin (AM), angiopoietin (Ang), BMPs, BDNF, EGF, erythropoietin (EPO), FGF, GDNF, G-CSF, GM-CSF, GDF9, HGF, HDGF, IGF, migration-stimulating factor, myostatin (GDF-8), NGF, neurotrophins, PDGF, thrombopoietin, TGF- α , TGF- β , TNF- α , VEGF, PlGF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, and IL-18.

In some embodiments of the methods described herein, the additional therapeutic agent is an immune response stimulating agent. In some embodiments, the immune response stimulating agent is selected from the group consisting of granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), interleukin 3 (IL-3), interleukin 12 (IL-12), interleukin 1 (IL-1), interleukin 2 (IL-2), B7-1 (CD80), B7-2 (CD86), 4-1BB ligand, anti-CD3 antibody, anti-CTLA-4 antibody, anti-TIGIT antibody, anti-PD-1 antibody, anti-LAG-3 antibody, and anti-TIM-3 antibody.

In some embodiments of the methods described herein, an immune response stimulating agent is selected from the group consisting of: a modulator of PD-1 activity, a modulator of PD-L2 activity, a modulator of CTLA-4 activity, a modulator of CD28 activity, a modulator of CD80 activity, a modulator of CD86 activity, a modulator of 4-1BB activity, an modulator of OX40 activity, a modulator of KIR activity, a modulator of Tim-3 activity, a modulator of LAG3 activity, a modulator of CD27 activity, a modulator of CD40 activity, a modulator of GITR activity, a modulator of TIGIT activity, a modulator of CD20 activity, a modulator of CD96 activity, a modulator of IDO1 activity, a cytokine, a chemokine, an interferon, an interleukin, a lymphokine, a member of the tumor necrosis factor (TNF) family, and an immunostimulatory oligonucleotide.

In some embodiments of the methods described herein, an immune response stimulating agent is selected from the group consisting of: a PD-1 antagonist, a PD-L2 antagonist, a CTLA-4 antagonist, a CD80 antagonist, a CD86 antagonist, a KIR antagonist, a Tim-3 antagonist, a LAG3 antagonist, a TIGIT antagonist, a CD20 antagonist, a CD96 antagonist, and/or an IDO1 antagonist.

In some embodiments of the methods described herein, the PD-1 antagonist is an antibody that specifically binds PD-1. In some embodiments, the antibody that binds PD-1 is KEYTRUDA (MK-3475), pidilizumab (CT-011), nivolumab (OPDIVO, BMS-936558, MDX-1106), MEDI0680 (AMP-514), REGN2810, BGB-A317, PDR-001, or STI-A1110. In some embodiments, the antibody that binds PD-1 is described in PCT Publication WO 2014/179664, for example, an antibody identified as APE2058, APE1922, APE1923, APE1924, APE 1950, or APE1963, or an antibody containing the CDR regions of any of these antibodies. In other embodiments, the PD-1 antagonist is a fusion protein that includes PD-L2, for example, AMP-224. In other embodiments, the PD-1 antagonist is a peptide inhibitor, for example, AUNP-12.

In some embodiments, the CTLA-4 antagonist is an antibody that specifically binds CTLA-4. In some embodiments, the antibody that binds CTLA-4 is ipilimumab (YERVOY) or

tremelimumab (CP-675,206). In some embodiments, the CTLA-4 antagonist is a CTLA-4 fusion protein, for example, KHR-102.

In some embodiments, the LAG3 antagonist is an antibody that specifically binds LAG3. In some embodiments, the antibody that binds LAG3 is IMP701, IMP731, BMS-986016, LAG525, and GSK2831781. In some embodiments, the LAG3 antagonist includes a soluble LAG3 receptor, for example, IMP321.

In some embodiments, the KIR antagonist is an antibody that specifically binds KIR. In some embodiments, the antibody that binds KIR is lirilumab.

In some embodiments, an immune response stimulating agent is selected from the group consisting of: a CD28 agonist, a 4-1BB agonist, an OX40 agonist, a CD27 agonist, a CD80 agonist, a CD86 agonist, a CD40 agonist, and a GITR agonist. In some embodiments, the OX40 agonist includes OX40 ligand, or an OX40-binding portion thereof. For example, the OX40 agonist may be MEDI6383. In some embodiments, the OX40 agonist is an antibody that specifically binds OX40. In some embodiments, the antibody that binds OX40 is MEDI6469, MEDI0562, or MOXR0916 (RG7888). In some embodiments, the OX40 agonist is a vector (e.g., an expression vector or virus, such as an adenovirus) capable of expressing OX40 ligand. In some embodiments the OX40-expressing vector is Delta-24-RGDOX or DNX2401.

In some embodiments, the 4-1BB (CD137) agonist is a binding molecule, such as an anticalin. In some embodiments, the anticalin is PRS-343. In some embodiments, the 4-1BB agonist is an antibody that specifically binds 4-1BB. In some embodiments, antibody that binds 4-1BB is PF-2566 (PF-05082566) or urelumab (BMS-663513).

In some embodiments, the CD27 agonist is an antibody that specifically binds CD27. In some embodiments, the antibody that binds CD27 is varlilumab (CDX-1127).

In some embodiments, the GITR agonist comprises GITR ligand or a GITR-binding portion thereof. In some embodiments, the GITR agonist is an antibody that specifically binds GITR. In some embodiments, the antibody that binds GITR is TRX518, MK-4166, or INBRX-110.

In some embodiments, immune response stimulating agents include but are not limited to, cytokines such as chemokines, interferons, interleukins, lymphokines, and members of the tumor necrosis factor (TNF) family. In some embodiments, immune response stimulating agents include immunostimulatory oligonucleotides, such as CpG dinucleotides.

In some embodiments, an immune response stimulating agent includes, but is not limited to, anti-PD-1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-CD28 antibodies, anti-CD80 antibodies, anti-CD86 antibodies, anti-4-1BB antibodies, anti-OX40 antibodies, anti-

KIR antibodies, anti-Tim-3 antibodies, anti-LAG3 antibodies, anti-CD27 antibodies, anti-CD40 antibodies, anti-GITR antibodies, anti-TIGIT antibodies, anti-CD20 antibodies, anti-CD96 antibodies, or anti-IDO1 antibodies.

In some embodiments, the AFFIMER® agents disclosed herein may be used alone, or in association with radiation therapy.

In some embodiments, the AFFIMER® agents disclosed herein may be used alone, or in association with targeted therapies. Examples of targeted therapies include: hormone therapies, signal transduction inhibitors (e.g., EGFR inhibitors, such as cetuximab (Erbix) and erlotinib (Tarceva)); HER2 inhibitors (e.g., trastuzumab (Herceptin) and pertuzumab (Perjeta)); BCR-ABL inhibitors (such as imatinib (Gleevec) and dasatinib (Sprycel)); ALK inhibitors (such as crizotinib (Xalkori) and ceritinib (Zykadia)); BRAF inhibitors (such as vemurafenib (Zelboraf) and dabrafenib (Tafinlar)), gene expression modulators, apoptosis inducers (e.g., bortezomib (Velcade) and carfilzomib (Kyprolis)), angiogenesis inhibitors (e.g., bevacizumab (Avastin) and ramucirumab (Cyramza), monoclonal antibodies attached to toxins (e.g., brentuximab vedotin (Adcetris) and ado-trastuzumab emtansine (Kadcyla)).

In some embodiments, the AFFIMER® agents of the disclosure may be used in combination with an anti-cancer therapeutic agent or immunomodulatory drug such as an immunomodulatory receptor inhibitor, e.g., an antibody or antigen-binding fragment thereof that specifically binds to the receptor.

In some embodiments of the disclosure, an AFFIMER® agent is administered in with a STING agonist, for example, as part of a pharmaceutical composition. The cyclic-di-nucleotides (CDNs) cyclic-di-AMP (produced by *Listeria monocytogenes* and other bacteria) and its analogs cyclic-di-GMP and cyclic-GMP-AMP are recognized by the host cell as a pathogen associated molecular pattern (PAMP), which bind to the pathogen recognition receptor (PRR) known as Stimulator of Interferon Genes (STING). STING is an adaptor protein in the cytoplasm of host mammalian cells which activates the TANK binding kinase (TBK1)-IRF3 and the NF- κ B signaling axis, resulting in the induction of IFN- β and other gene products that strongly activate innate immunity. It is now recognized that STING is a component of the host cytosolic surveillance pathway, that senses infection with intracellular pathogens and in response induces the production of IFN- α , leading to the development of an adaptive protective pathogen-specific immune response consisting of both antigen-specific CD4⁺ and CD8⁺ T cells as well as pathogen-specific antibodies. U.S. Pat. NOS: 7,709,458 and 7,592,326; PCT Publication NOS: WO2007/054279, WO2014/093936, WO2014/179335, WO2014/189805, WO2015/185565,

WO2016/096174, WO2016/145102, WO2017/027645, WO2017/027646, and WO2017/075477; and Yan et al., Bioorg. Med. Chem Lett. 18:5631-4, 2008.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an Akt inhibitor. Exemplary AKT inhibitors include GDC0068 (also known as GDC-0068, ipatasertib and RG7440), MK-2206, perifosine (also known as KRX-0401), GSK690693, AT7867, triciribine, CCT128930, A-674563, PHT-427, Akti-1/2, afuresertib (also known as GSK2110183), AT13148, GSK2141795, BAY1125976, uprosertib (aka GSK2141795), Akt Inhibitor VIII (1,3-dihydro-1-[1-[[4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl]m-ethyl]-4-piperidiny]-2H-benzimidazol-2-one), Akt Inhibitor X (2-chloro-N,N-diethyl-10H-phenoxazine-10-butanamine, monohydrochloride), MK-2206 (8-(4-(1-aminocyclobutyl)phenyl)-9-phenyl-[1,2,4]triazolo[3,4-f][1,6]naphthyridin-3(2H)-one), uprosertib (N-((S)-1-amino-3-(3,4-difluorophenyl)propan-2-yl)-5-chloro-4-(4-chloro-1-methyl-1H-pyrazol-5-yl)furan-2-carboxamide), ipatasertib ((S)-2-(4-chlorophenyl)-1-(4-((5R,7R)-7-hydroxy-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)-3-(isopropylamino)propan-1-one)-, AZD 5363 (4-Piperidinecarboxamide, 4-amino-N-[(1S)-1-(4-chlorophenyl)-3-hydroxypropyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)), perifosine, GSK690693, GDC-0068, triciribine, CCT128930, A-674563, PF-04691502, AT7867, miltefosine, PHT-427, honokiol, triciribine phosphate, and KP372-1A (10H-indeno[2,1-e]tetrazolo[1,5-b][1,2,4]triazin-10-one), Akt Inhibitor IX (CAS 98510-80-6). Additional Akt inhibitors include: ATP-competitive inhibitors, e.g. isoquinoline-5-sulfonamides (e.g., H-8, H-89, NL-71-101), azepane derivatives (e.g., (-)-balanol derivatives), aminofurazans (e.g., GSK690693), heterocyclic rings (e.g., 7-azaindole, 6-phenylpurine derivatives, pyrrolo[2,3-d]pyrimidine derivatives, CCT128930, 3-aminopyrrolidine, anilintriazole derivatives, spiroindoline derivatives, AZD5363, A-674563, A-443654), phenylpyrazole derivatives (e.g., AT7867, AT13148), thiophenecarboxamide derivatives (e.g., Afuresertib (GSK2110183), 2-pyrimidyl-5-amidothiophene derivative (DC120), uprosertib (GSK2141795); Allosteric inhibitors, e.g., 2,3-diphenylquinoxaline analogues (e.g., 2,3-diphenylquinoxaline derivatives, triazolo[3,4-f][1,6]naphthyridin-3(2H)-one derivative (MK-2206)), alkylphospholipids (e.g., Edelfosine (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, ET-18-OCH3) ilmofofosine (BM 41.440), miltefosine (hexadecylphosphocholine, HePC), perifosine (D-21266), erucylphosphocholine (ErPC), erufosine (ErPC3, erucylphosphohomocholine), indole-3-carbinol analogues (e.g., indole-3-carbinol, 3-chloroacetylindole, diindolylmethane, diethyl 6-methoxy-5,7-dihydroindolo [2,3-b]carbazole-2,10-dicarboxylate (SR13668), OSU-A9), Sulfonamide derivatives (e.g., PH-316, PHT-427), thiourea derivatives (e.g., PIT-1, PIT-2, DM-PIT-1, N-[(1-

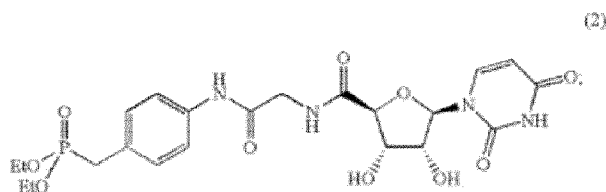
methyl-1H-pyrazol-4-yl)carbonyl]-N'-(3-bromophenyl)-thiourea), purine derivatives (e.g., Triciribine (TCN, NSC 154020), triciribine mono-phosphate active analogue (TCN-P), 4-amino-pyrido[2,3-d]pyrimidine derivative API-1, 3-phenyl-3H-imidazo[4,5-b]pyridine derivatives, ARQ 092), BAY 1125976, 3-methyl-xanthine, quinoline-4-carboxamide, 2-[4-(cyclohexa-1,3-dien-1-yl)-1H-pyrazol-3-yl]phenol, 3-oxo-tirucallic acid, 3.alpha.- and 3.beta.-acetoxy-tirucallic acids, acetoxy-tirucallic acid; and irreversible inhibitors, e.g., natural products, antibiotics, Lactoquinomycin, Frenolicin B, kalafungin, medermycin, Boc-Phe-vinyl ketone, 4-hydroxynonenal (4-HNE), 1,6-naphthyridinone derivatives, and imidazo-1,2-pyridine derivatives.

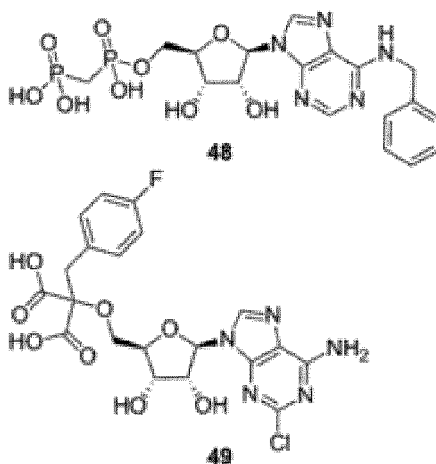
In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with a MEK inhibitor. Exemplary MEK inhibitors include AZD6244 (Selumetinib), PD0325901, GSK1120212 (Trametinib), U0126-EtOH, PD184352, RDEA119 (Rafametinib), PD98059, BIX 02189, MEK162 (Binimetinib), AS-703026 (Pimasertib), SL-327, BIX02188, AZD8330, TAK-733, cobimetinib and PD318088.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with both an anthracycline such as doxorubicin and cyclophosphamide, including pegylated liposomal doxorubicin .

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with both an anti-CD20 antibody and an anti-CD3 antibody, or a bispecific CD20/CD3 binder (including a CD20/CD3 BiTE).

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with a CD73 inhibitor, a CD39 inhibitor or both. These inhibitors can be CD73 binders or CD39 binders (such as antibody, antibody fragments or antibody mimetics) that inhibit the ectonucleosidase activity. The inhibitor may be a small molecule inhibitor of the ectonucleosidase activity, such as 6-N,N-Diethyl-β-γ-dibromomethylene-D-adenosine-5'-triphosphate trisodium salt hydrate, PSB069, PSB 06126,





In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an inhibitor poly ADP ribose polymerase (PARP). Exemplary PARP inhibitors include Olaparib, Niraparib, Rucaparib, Talazoparib, Veliparib, CEP9722, MK4827 and BGB-290.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an oncolytic virus. An exemplary oncolytic virus is Talimogene Laherparepvec.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an CSF-1 antagonist, such as an agent that binds to CSF-1 or CSF1R and inhibits the interaction of CSF-1 with CSF1R on macrophage. Exemplary CSF-1 antagonists include Emactuzumab and FPA008.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an anti-CD38 antibody. Exemplary anti-CD39 antibodies include Daratumumab and Isatuximab.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an anti-CD40 antibody. Exemplary anti-CD40 antibodies include Selicrelumab and Dacetuzumab.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with an inhibitor of anaplastic lymphoma kinase (ALK). Exemplary ALK inhibitors include Alectinib, Crizotinib and Ceritinib.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with multikinase inhibitor that inhibits at least one selected from the group consisting of the family members of VEGFR, PDGFR and FGFR, or an anti-angiogenesis inhibitor. Exemplary inhibitors include Axitinib, Cediranib, Linifanib, Motesanib, Nintedanib,

Pazopanib, Ponatinib, Regorafenib, Sorafenib, Sunitinib, Tivozanib, Vatalanib, LY2874455, or SU5402.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in conjunction with at least one vaccine intended to stimulate an immune response to at least one predetermined antigen. The antigen(s) may be administered directly to the individual, or may be expressed within the individual from, for example, a tumor cell vaccine (e.g., GVAX) which may be autologous or allogenic, a dendritic cell vaccine, a DNA vaccine, an RNA vaccine, a viral-based vaccine, a bacterial or yeast vaccine (e.g., a *Listeria monocytogenes* or *Saccharomyces cerevisiae*), etc. See, e.g., Guo et al., *Adv. Cancer Res.* 2013; 119: 421-475; Obeid et al., *Semin Oncol.* 2015 August; 42(4): 549-561. The target antigen may also be a fragment or fusion polypeptide comprising an immunologically active portion of the antigens listed in the table.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with at least one antiemetic including, but not limited to: casopitant (GlaxoSmithKline), Netupitant (MGI-Helsinn) and other NK-1 receptor antagonists, palonosetron (sold as Aloxi by MGI Pharma), aprepitant (sold as Emend by Merck and Co.; Rahway, N.J.), diphenhydramine (sold as Benadryl by Pfizer; New York, N.Y.), hydroxyzine (sold as Atarax by Pfizer; New York, N.Y.), metoclopramide (sold as Reglan by AH Robins Co.; Richmond, Va.), lorazepam (sold as Ativan by Wyeth; Madison, N.J.), alprazolam (sold as Xanax by Pfizer; New York, N.Y.), haloperidol (sold as Haldol by Ortho-McNeil; Raritan, N.J.), droperidol (Inapsine), dronabinol (sold as Marinol by Solvay Pharmaceuticals, Inc.; Marietta, Ga.), dexamethasone (sold as Decadron by Merck and Co.; Rahway, N.J.), methylprednisolone (sold as Medrol by Pfizer; New York, N.Y.), prochlorperazine (sold as Compazine by Glaxosmithkline; Research Triangle Park, N.C.), granisetron (sold as Kytril by Hoffmann-La Roche Inc.; Nutley, N.J.), ondansetron (sold as Zofran by Glaxosmithkline; Research Triangle Park, N.C.), dolasetron (sold as Anzemet by Sanofi-Aventis; New York, N.Y.), tropisetron (sold as Navoban by Novartis; East Hanover, N.J.).

Other side effects of cancer treatment include red and white blood cell deficiency. Accordingly, in some embodiments of the disclosure, an AFFIMER® agent is administered in association with an agent which treats or prevents such a deficiency, such as, e.g., filgrastim, PEG-filgrastim, erythropoietin, epoetin alfa or darbepoetin alfa.

In some embodiments of the disclosure, an AFFIMER® agent of the disclosure is administered in association with anti-cancer radiation therapy. For example, in some embodiments of the disclosure, the radiation therapy is external beam therapy (EBT): a method

for delivering a beam of high-energy X-rays to the location of the tumor. The beam is generated outside the patient (e.g., by a linear accelerator) and is targeted at the tumor site. These X-rays can destroy the cancer cells and careful treatment planning allows the surrounding normal tissues to be spared. No radioactive sources are placed inside the patient's body. In some embodiments of the disclosure, the radiation therapy is proton beam therapy: a type of conformal therapy that bombards the diseased tissue with protons instead of X-rays. In some embodiments of the disclosure, the radiation therapy is conformal external beam radiation therapy: a procedure that uses advanced technology to tailor the radiation therapy to an individual's body structures. In some embodiments of the disclosure, the radiation therapy is brachytherapy: the temporary placement of radioactive materials within the body, usually employed to give an extra dose--or boost--of radiation to an area.

In some embodiments of the methods described herein, the treatment involves the administration of an AFFIMER® agent of the present disclosure in combination with anti-viral therapy. Treatment with an AFFIMER® agent can occur prior to, concurrently with, or subsequent to administration of antiviral therapy. The anti-viral drug used in combination therapy will depend upon the virus the subject is infected with.

Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously.

It will be appreciated that the combination of an AFFIMER® agent described herein and at least one additional therapeutic agent may be administered in any order or concurrently. In some embodiments, the AFFIMER® agent will be administered to patients that have previously undergone treatment with a second therapeutic agent. In certain other embodiments, the AFFIMER® agent and a second therapeutic agent will be administered substantially simultaneously or concurrently. For example, a subject may be given an AFFIMER® agent while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In some embodiments, an AFFIMER® agent will be administered within 1 year of the treatment with a second therapeutic agent. In certain alternative embodiments, an AFFIMER® agent will be administered within 10, 8, 6, 4, or 2 months of any treatment with a second therapeutic agent. In certain other embodiments, an AFFIMER® agent will be administered within 4, 3, 2, or 1 weeks of any treatment with a second therapeutic agent. In some embodiments, an AFFIMER® agent will be administered within 5, 4, 3, 2, or 1 days of any treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments may be

administered to the subject within a matter of hours or minutes (e.g., substantially simultaneously).

For the treatment of a disease, the appropriate dosage of an AFFIMER® agent of the present disclosure depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the AFFIMER® agent is administered for therapeutic or preventative purposes, previous therapy, the patient's clinical history, and so on, all at the discretion of the treating physician. The AFFIMER® agent can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is affected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual agent. The administering physician can determine optimum dosages, dosing methodologies, and repetition rates. In some embodiments, dosage is from 0.01 µg to 100 mg/kg of body weight, from 0.1 µg to 100 mg/kg of body weight, from 1 µg to 100 mg/kg of body weight, from 1 mg to 100 mg/kg of body weight, 1 mg to 80 mg/kg of body weight from 10 mg to 100 mg/kg of body weight, from 10 mg to 75 mg/kg of body weight, or from 10 mg to 50 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is from about 0.1 mg to about 20 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 0.1 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 0.25 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 0.5 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 1 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 1.5 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 2 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 2.5 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 5 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 7.5 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 10 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 12.5 mg/kg of body weight. In some embodiments, the dosage of the AFFIMER® agent is about 15 mg/kg of body weight. In some embodiments, the dosage can be given once or more daily, weekly, monthly, or yearly. In some embodiments, the AFFIMER® agent is given once every week, once every two weeks, once every three weeks, or once every four weeks.

In some embodiments, an AFFIMER® agent may be administered at an initial higher "loading" dose, followed by at least one lower dose. In some embodiments, the frequency of

administration may also change. In some embodiments, a dosing regimen may comprise administering an initial dose, followed by additional doses (or "maintenance" doses) once a week, once every two weeks, once every three weeks, or once every month. For example, a dosing regimen may comprise administering an initial loading dose, followed by a weekly maintenance dose of, for example, one-half of the initial dose. In some embodiments, a dosing regimen comprises administering an initial loading dose, followed by maintenance doses of, for example one-half of the initial dose every other week. In some embodiments, a dosing regimen comprises administering three initial doses for 3 weeks, followed by maintenance doses of, for example, the same amount every other week.

As is known to those of skill in the art, administration of any therapeutic agent may lead to side effects and/or toxicities. In some cases, the side effects and/or toxicities are so severe as to preclude administration of the particular agent at a therapeutically effective dose. In some cases, drug therapy must be discontinued, and other agents may be tried. However, many agents in the same therapeutic class often display similar side effects and/or toxicities, meaning that the patient either has to stop therapy, or if possible, suffer from the unpleasant side effects associated with the therapeutic agent.

In some embodiments, the dosing schedule may be limited to a specific number of administrations or "cycles". In some embodiments, the AFFIMER® agent is administered for 3, 4, 5, 6, 7, 8, or more cycles. For example, the AFFIMER® agent is administered every 2 weeks for 6 cycles, the AFFIMER® agent is administered every 3 weeks for 6 cycles, the AFFIMER® agent is administered every 2 weeks for 4 cycles, the AFFIMER® agent is administered every 3 weeks for 4 cycles, etc. Dosing schedules can be decided upon and subsequently modified by those skilled in the art.

Thus, the present disclosure provides methods of administering to a subject the polypeptides or agents described herein comprising using an intermittent dosing strategy for administering at least one agent (e.g., two or three agents), which may reduce side effects and/or toxicities associated with administration of an AFFIMER® agent, chemotherapeutic agent, etc. In some embodiments, a method for treating cancer in a human subject comprises administering to the subject a therapeutically effective dose of an AFFIMER® agent in combination with a therapeutically effective dose of a chemotherapeutic agent, wherein one or both of the agents are administered according to an intermittent dosing strategy. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of an AFFIMER® agent to the subject and administering subsequent doses of the AFFIMER® agent about once every 2 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of an

AFFIMER® agent to the subject and administering subsequent doses of the AFFIMER® agent about once every 3 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of an AFFIMER® agent to the subject and administering subsequent doses of the AFFIMER® agent about once every 4 weeks. In some embodiments, the AFFIMER® agent is administered using an intermittent dosing strategy and the chemotherapeutic agent is administered weekly.

In some embodiments, the disclosure also provides methods for treating subjects using an AFFIMER® agent of the disclosure, wherein the subject suffers from a viral infection. In some embodiments, the viral infection is infection with a virus selected from the group consisting of human immunodeficiency virus (HIV), hepatitis virus (A, B, or C), herpes virus (e.g., VZV, HSV-I, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus or arboviral encephalitis virus.

In some embodiments, the disclosure provides methods for treating subjects using an AFFIMER® agent thereof of the disclosure, wherein the subject suffers from a bacterial infection. In some embodiments, the bacterial infection is infection with a bacterium selected from the group consisting of Chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumococci, meningococci and gonococci, klebsiella, proteus, serratia, pseudomonas, Legionella, Corynebacterium diphtheriae, Salmonella, bacilli, Vibrio cholerae, Clostridium tetan, Clostridium botulinum, Bacillus anthracis, Yersinia pestis, Mycobacterium leprae, Mycobacterium lepromatosis, and Borriella.

In some embodiments, the disclosure provides methods for treating subjects using an AFFIMER® agent of the disclosure, wherein the subject suffers from a fungal infection. In some embodiments, the fungal infection is infection with a fungus selected from the group consisting of Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizopus), Sporothrix schenckii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

In some embodiments, the disclosure provides methods for treating subjects using an AFFIMER® agent of the disclosure, wherein the subject suffers from a parasitic infection. In some embodiments, the parasitic infection is infection with a parasite selected from the group consisting of Entamoeba histolytica, Balantidium coli, Naegleria fowleri, Acanthamoeba,

Giardia lamblia, Cryptosporidium, Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondii and Nippostrongylus brasiliensis.

EXAMPLES

Example 1: Selection of CD33 AFFIMER® polypeptides from a phage display library.

Identification of candidate clones

CD33 AFFIMER® polypeptides of the present disclosure were identified by selection from a library of AFFIMER® polypeptides with two random loop sequences, each loop having a length of about 9 amino acids displayed in a constant AFFIMER® polypeptide framework backbone based on the amino acid sequence of Stefin A. Such selection procedures have been described (see, e.g., Tiede et al. *Protein Eng Des Sel.* 2014. 27(5): 145–155 and Hughes et al. *Sci Signal.* 2018. 10(505): eaaj2005). According to such procedures, suspensions of phage were incubated with human CD33, which was biotinylated and captured on alternating streptavidin and neutravidin beads. Unbound phage particles were then washed away and, following washing, bound phage were eluted. Elution of bound phage was accomplished by incubating the antigen with low pH solution, followed by high pH solution and exposure to trypsin. Eluted phage particles were then used to infect *Escherichia coli* (*E. coli*), and infected bacteria were incubated under conditions suitable for replication of the bacteriophage. Following release of bacteriophage particles from these infected bacteria, the cycle of allowing phage particles to bind to the target antigen, eluting bound phage particles, propagating eluted phage particles in bacteria, and isolating released phage particles from infected bacteria was repeated to enrich the bacteriophage population for phage particles displaying proteins that bind the target antigen. Specific conditions were modified in these cycles, such as increasing the number of wash steps, reducing the amount of available antigen, or adding a blocking reagent, to select for phage particles displaying proteins that bind more tightly or specifically to the target antigen.

Following multiple rounds of phage display library selection and amplification, proteins expressed by phages were expressed and screened by enzyme-linked immunosorbent assay (ELISA). Briefly, AFFIMER® polypeptides were overexpressed from phagemid vectors, the bacterial cells were lysed, and lysates were used as substrates in ELISAs. In these ELISAs, human CD33 was immobilized on a plate, lysates were added, and the amount of CD33-binding AFFIMER® polypeptide in each plate was measured using a detector antibody specific to the 6xMyc tag expressed on the candidate AFFIMER® polypeptides. The phagemid vectors encoding AFFIMER® polypeptides with the best human CD33-binding activity were sequenced

to identify DNA sequences of candidate clones for further development. The loop 2 and loop 4 amino acid sequences of each of these candidate clones are shown in **Table 1** and **Table 2**.

Affinity maturation

Following the identification of candidate AFFIMER® clones with human CD33-binding activity, affinity maturation of the candidate clones was conducted to generate additional AFFIMER® polypeptides with increased affinity for human CD33. Briefly, loop mutagenesis was performed by circular polymerase extension cloning using degenerated oligonucleotides to encode for loop 2, such that libraries of amplicons, each encoding an amino acid sequence derived from loop 2 of the candidate clone, were produced. These amplicon libraries were cloned into the phagemid expression vector at the appropriate position to generate a library of phagemids encoding diverse AFFIMER® polypeptides with affinity-matured loop 2 sequences, and the resulting plasmid libraries were used to generate phage libraries encoding these diverse AFFIMER® polypeptides. These phage libraries were screened as described in the preceding paragraphs to identify affinity-matured loop 2 amino acid sequences that resulted in improved CD33-binding activity. Bacterial cell lysates were prepared and screened as in the preceding paragraphs to verify CD33-binding activity, and the phagemid vectors encoding AFFIMER® polypeptides with improved CD33-binding activity were sequenced to identify DNA sequences encoding affinity-matured loop 2 sequences. A similar approach was used for affinity maturation of loop 4 amino acid sequences and identification of the DNA sequences encoding these affinity matured loop 4 amino acid sequences.

Loop combinations

Following identification of affinity-matured loop 2 and loop 4 amino acid sequences that improved CD33-binding activity of AFFIMER® polypeptides, additional AFFIMER® polypeptides were generated by combining an affinity-matured loop 2 sequence and an affinity-matured loop 4 sequence. Briefly, PCR of the loop 2 region was used to generate a DNA sequences of the loop 2 region. This loop 2 DNA sequence was used as the megaprimer in combination with the phagemid expression vector containing the loop 4 DNA sequence at the appropriate positions to generate the final phagemid expression vector encoding an AFFIMER® scaffold with the affinity-matured loop 2 and loop 4 sequences.

AFFIMER® production

To express an AFFIMER® polypeptide with a given amino acid sequence, 200 ng of expression plasmid pD86l encoding the amino acid sequence of the AFFIMER® polypeptide was transformed into BL21 *E. coli* cells using the manufacturer's protocol. Total transformed cell mixture was plated onto LB agar plates containing 50 µg/ml kanamycin (AppliChem) and incubated at 37°C overnight. Next, the lawn of transformed *E. coli* was transferred to a sterile flask of 2x Terrific Broth (TB) media (Melford) and 50 µg/ml kanamycin and incubated at 30 °C with shaking at 250 rpm. Expression was induced with 10 mM rhamnose once the cells reached an OD₆₀₀ of 0.8–1.0, after which the culture was incubated for 5 hours at 37 °C. Cells were harvested by centrifuging at 4,500 rpm for 1 h.

For culture volumes < 500 ml, the *E. coli* cell pellet was lysed by resuspending in 1:10 NPI20 buffer (50mM Sodium phosphate, 0.5M NaCl, 20mM Imidazole) supplemented with 0.5 mL 10x BugBuster per gram of wet cell paste, lysozyme, and Benzonase. Cells were lysed for 1 hour at room temperature on a bottle roller. For culture volumes > 500 ml, the cell pellet was resuspended in 1:10 supplemented NPI20 and sonicated for 2 minutes (10 second on/off cycles). Following lysing the solution was centrifuged at 20,000 xg for 1 hour at 4°C.

An appropriate volume of NiNTA resin (Super-NiNTA500; binding capacity 1 mL per 20 mg protein) was washed with 5 column volumes (CV) water to remove storage solution, followed by equilibration with 5 CV NPI20 buffer using gravity flow in a StEP™ column (Thompson). Resin was incubated with the clarified *E. coli* solution for 1 hour at room temperature. Then, the solution was passed through a StEP™ column by gravity flow and the resin was washed with 5CV NPI20 buffer. Bound protein was eluted off the resin with 5 CV of NPI400 (50mM Sodium phosphate, 0.5M NaCl, 0.4M Imidazole). Eluted protein was desalted into 1x PBS using Centripure columns. The protein yield was estimated using Nanodrop A280 reading. Preparative SEC was performed using HiLoad 26/600 Superdex 200pg run in PBS 1x at 2.6ml/min flow rate on an AKTA Xpress. Eluted protein samples were run on SDS-PAGE Bolt Bis Tris plus 4–12% gel in Novex™ 20X Bolt™ MES SDS running buffer at 200V with reducing sample buffer for 10 minutes at 95°C. Protein bands on gel were stained with Quick Coomassie. PageRuler prestained protein molecular weight marker was run on the gel to estimate the molecular weight of the AFFIMER® polypeptides.

Example 2. Measurement of AFFIMER® affinity for CD33 by Octet analysis.

The affinity of the AFFIMER® polypeptides described herein for human CD33 was measured using Octet analysis. Octet analysis uses bio-layer interferometry (BLI) to measure the

association between two molecules in real time, which allows for the measurement of interaction kinetics and estimation of parameters such as K_D , K_{on} , and K_{dis} .

Solid-supported sensors containing streptavidin were incubated with 0.1 $\mu\text{g/mL}$ biotinylated human CD33 containing a polyhistidine tag, forming a solid-supported CD33 sensor that could be bound by CD33 AFFIMER® polypeptides. For single-point analysis, AFFIMER® polypeptides were added to sensors as a concentration of 100 nM, and the optical shift was measured over time to quantify the apparent K_D of each AFFIMER® polypeptide. After 3 minutes, buffer was aspirated from the sensors and replaced with 0.5 M MgCl_2 to allow the AFFIMER® polypeptides to dissociate from CD33, and the rate of dissociation of each AFFIMER® polypeptide was measured. Candidate clones were chosen for further analysis based on apparent K_D and dissociation rates. AFFIMER® polypeptides of candidate clones were diluted from 200nM (3 $\mu\text{g/mL}$) to 3.125 nM (0.47 $\mu\text{g/mL}$) using serial 2-fold dilutions. Each dilution was added to a CD33 sensor to measure the optical shift over time as AFFIMER® polypeptides associated with CD33, with buffer being replaced after 3 minutes to allow for measurement of dissociation. For each candidate clone, regression of Octet binding and dissociation curves was used to estimate the K_D , K_{on} , and K_{off} of each AFFIMER® polypeptide.

Estimated K_D , K_{on} , and K_{dis} constants of Clone 13-derived and Clone 21-derived AFFIMER® polypeptides with affinity-matured loop 2 or loop 4 sequences from multiple rounds of affinity maturation, and combinations of affinity-matured loop 2 and loop 4 sequences, are shown in **Table 13**.

Table 13. Structure and affinity of AFFIMER® polypeptides with affinity-matured loops and combinations.

Clone type/ parent	Clone #	K_D	K_{on}	K_{dis}	R^2	+ cell binding
Loop combo (80 + 115)	208	2.07E-08	3.41E+05	7.05E-03	0.9931	0.096
Loop combo (80 + 114)	209	2.21E-08	8.57E+05	1.89E-02	0.9936	0.67
Loop combo (80 + 128)	207	3.98E-08	5.15E+05	2.05E-02	0.9958	0.76
Loop combo (82 + 115)	211	3.88E-08	3.29E+05	1.28E-02	0.9918	0.35
Loop combo (82 + 114)	212	5.30E-08	6.51E+05	3.45E-02	0.9970	1.99
Loop combo (82 + 128)	210	9.51E-08	2.82E+05	2.69E-02	0.9933	3.2
21	115	2.30E-08	6.06E+05	1.40E-02	0.9879	4.08
21	128	3.44E-08	8.35E+05	2.87E-02	0.9738	0.1644

21	114	4.08E-08	9.95E+05	4.06E-02	0.9842	15.27
21	80	7.45E-08	9.08E+05	6.77E-02	0.9874	18.93
21	82	5.08E-08	1.70E+06	8.61E-02	0.98	157.1
Parental	21	2.02E-07	5.00E+05	1.01E-01	0.9726	30.2
Parental	13	1.35E-06	5.68E+05	7.66E-01	0.9633	723.95

Example 3. Measurement of AFFIMER® polypeptides binding to CD33⁺ cells by flow cytometry.

The ability of the AFFIMER® polypeptides described herein to bind to CD33 on the surface of cells, was measured using flow cytometry. U937 cells (ATCC CRL-1593.2), a human myeloid cell line that expresses human CD33. Similarly, to assess non-specific binding of the AFFIMER® polypeptides a negative cell line for CD33 (Jurkat E6.1, ATCC® TIB-152™) was used using the same protocol as described for the positive cell line U937.

Cells were incubated with an AFFIMER® polypeptide, then washed with buffer, pelleted by centrifugation, and buffer was removed. AFFIMER® polypeptide-bound cells were then stained by incubation one hour with mouse anti-His antibody, to bind the histidine tag of the AFFIMER® polypeptide cells were washed with FACS buffer (PBS + 2% fetal calf serum and 20 mM EDTA). After the final wash the supernatant was removed and the cells were incubated with anti-mouse IgG Alexa Fluor 488 antibody, to bind the mouse anti-His antibody, such that AFFIMER® polypeptide-bound cells would be stained with Alexa Fluor 488. Cells were washed with FACS buffer (PBS + 2% fetal calf serum and 20 mM EDTA), pelleting cells by centrifugation, and removing buffer. After the final wash, cells were resuspended in FACS buffer, and the percentage of AFFIMER® polypeptide-bound cells, defined as Alexa Fluor 488 cells, was measured by flow cytometry. Separate cell preparations were stained with a range of concentrations of each AFFIMER® polypeptide. Dose-response curves of the percentage of cells stained were regressed for the dilution series of each AFFIMER® polypeptide using the logistic regression function of GraphPad Prism.

The results of these flow cytometry analyses are shown in **FIGs. 1-3**. Each of the AFFIMER® polypeptide with affinity-matured loop sequences exhibited improved binding to human CD33 on cells, as a lower concentration of each AFFIMER® polypeptide was required to stain a given proportion of cells relative to the concentration of AFFIMER® polypeptide Clone 21 (parent) required to label an equivalent proportion of cells (**FIG. 1**). In addition, the AFFIMER® polypeptides described herein did not exhibit binding to negative cells.

Two AFFIMER® polypeptides derived from Clone 21, Clones 80 and 115, each having one loop sequence of parental Clone 21 and one affinity-matured loop sequence exhibited improved binding, relative to AFFIMER® Clone 21, to human CD33 on U937 cells (**FIG. 2**).

Furthermore, AFFIMER® Clone 208, which comprised the affinity-matured loop 2 amino acid sequence of Clone 80 and the affinity-matured loop 4 amino acid sequence of Clone 115, exhibited improved binding, relative to AFFIMER® Clones 21, 80, and 115, to human CD33 on U937 cells (**FIG. 2**).

Two AFFIMER® polypeptides derived from Clone 21, Clones 82 and 115, each having one loop sequence of parental Clone 21 and one affinity-matured loop sequence, exhibited improved binding, relative to AFFIMER® Clone 21, to human CD33 on cells (**FIG. 3**). Furthermore, AFFIMER® Clone 211, which comprised the affinity-matured loop 2 amino acid sequence of Clone 82 and the affinity-matured loop 4 amino acid sequence of Clone 115, exhibited improved binding, relative to AFFIMERS® Clones 21, 82, and 115, to human CD33 on cells (**FIG. 3**).

Estimated K_D , K_{on} , and K_{dis} , and EC_{50} (the concentration of AFFIMER® polypeptide required to bind 50% of CD33⁺ cells) values of these and other AFFIMER® polypeptides is shown in **Table 14**.

Table 14. Structure and affinity of AFFIMER® polypeptide with affinity-matured loops and combinations.

Parental clone	Clone #	K_D [M]	K_{on}	K_{dis}	R^2	EC_{50} (nM) in CD33 ⁺ cell-binding assay
Parental	13	4.58E-07	1.12E+06	5.13E-01	0.9446	723.95
Parental	13	1.35E-06	5.68E+05	7.66E-01	0.9633	723.95
Parental	21	1.96E-07	6.07E+05	1.19E-01	0.9712	30.2
21	123	5.34E-08	6.48E+05	3.46E-02	0.9776	52.42
21	124	6.66E-08	5.67E+05	3.78E-02	0.9877	24.89
21	125	6.35E-08	7.01E+05	4.45E-02	0.9752	21.64
13	88	3.95E-08	1.18E+06	4.67E-02	0.9686	21.66
13	92	3.14E-08	2.07E+06	6.50E-02	0.9834	23.42
13	94	2.36E-08	2.99E+06	7.05E-02	0.9819	71.89
21	119	4.86E-08	4.12E+05	2.00E-02	0.9926	35.94
21	128	3.44E-08	8.35E+05	2.87E-02	0.9738	0.1644
21	114	4.08E-08	9.95E+05	4.06E-02	0.9842	11.69
21	120	5.89E-08	7.56E+05	4.45E-02	0.9925	17.69
21	111	6.53E-08	7.00E+05	4.57E-02	0.97	61.15
21	112	5.77E-08	8.76E+05	5.05E-02	0.9913	27.74
21	113	5.26E-08	9.04E+05	4.75E-02	0.9939	27.17
21	131	1.03E-07	5.59E+05	5.77E-02	0.9581	50.85
21	80	7.45E-08	9.08E+05	6.77E-02	0.9874	19.44
21	86	1.22E-07	7.21E+05	8.81E-02	0.9539	164.3
21	117	1.03E-07	9.31E+05	9.59E-02	0.9576	70.94

Parental clone	Clone #	KD [M]	K _{on}	K _{dis}	R ²	EC50 (nM) in CD33+ cell-binding assay
21	84	9.54E-08	1.04E+06	9.88E-02	0.9816	85.83
21	122	7.76E-08	1.30E+06	1.01E-01	0.9736	110.2
21	121	8.32E-08	8.02E+05	6.67E-02	0.953	112.5
13	90	1.67E-08	2.43E+06	4.04E-02	0.9868	50.4
13	105	1.40E-07	1.05E+06	1.47E-01	0.8917	73.4
21	115	2.30E-08	6.06E+05	1.40E-02	0.9879	2.4
21	82	5.08E-08	1.70E+06	8.61E-02	0.98	93.84
13	126	3.91E-06	3.12E+05	1.22E+00	0.8991	>1000
13	89	3.19E-08	2.16E+06	6.90E-02	0.9622	13.13
13	90	1.67E-08	2.43E+06	4.04E-02	0.9868	7.98
13	96	1.08E-07	3.34E+06	3.60E-01	0.9593	146.4
21	113	5.26E-08	9.04E+05	4.75E-02	0.9939	39.12
21	128	3.44E-08	8.35E+05	2.87E-02	0.9738	2.15
21	162	3.04E-08	5.90E+05	1.79E-02	0.9945	0.47
21	165	4.48E-08	3.88E+05	1.74E-02	0.9944	1.1
21	168	3.96E-08	5.51E+05	2.18E-02	0.9838	4.8
21	174	5.05E-08	5.56E+05	2.81E-02	0.9933	3.2
21	182	7.87E-08	4.73E+05	3.73E-02	0.9935	7
21	183	5.79E-08	5.45E+05	3.15E-02	0.991	6

Example 4: Epitope binning of AFFIMER® polypeptides by Octet analysis.

Epitope binning of pairs of AFFIMER® polypeptides described herein was conducted by measuring the degree of interference between two AFFIMERS® in Octet analyses of CD33 binding. The concentration of all AFFIMER® polypeptide in the epitope region binning experiments was 400 nM. In epitope binning, a first reagent, such as a buffer or AFFIMER® polypeptide, is added to a sensor, and a signal of ligand binding (optical shift, in Octet analysis) is measured for period of time known as the first association phase. Next, a second reagent, such as a buffer or second AFFIMER® polypeptide, is added, and the signal of ligand-binding is measured for an additional period of time known as the second association phase. If the same second AFFIMER® polypeptide is added to two distinct sensors, then a difference between the signals observed during the second association phase can be attributed to the first reagents that were added to the sensors. For example, if the first reagent added to a first sensor was a buffer, and the first reagent added to a second sensor was a first AFFIMER® polypeptide, and the same second AFFIMER® polypeptide was then added to both sensors, observation of a lower signal from the second sensor compared to the first sensor would indicate that the first AFFIMER® polypeptide interfered with binding by the second AFFIMER® polypeptide. Such interference occurs because if a first AFFIMER® polypeptide is already bound to an epitope, then that

epitope is unavailable to be bound by the second AFFIMER® polypeptide. Thus, the observation that a first AFFIMER® polypeptide interferes with the ability of a second AFFIMER® polypeptide to bind to an antigen in this manner indicates that the two AFFIMER® polypeptides bind to the same epitope of the antigen and are said to belong to the same epitope bin.

Epitope binning analyses were conducted for AFFIMER® polypeptides of Clone 155 and Clone 208, using the Octet system and CD33-coated streptavidin sensors described in Example 2. Clone 155 was derived from Clone 13, having a modified loop 2 with the amino acid sequence GLEAYTALE (SEQ ID NO: 46) and the same loop 4 amino acid sequence as Clone 13, YGDDWGVKW (SEQ ID NO: 113). Clone 208 was derived from Clone 21, having a modified loop 2 with the amino acid sequence FQWGSSYHE (SEQ ID NO: 64) and modified loop 4 with the amino acid sequence VEYDYNLIW (SEQ ID NO: 118). In a first epitope binning experiment, a first reagent, either buffer or AFFIMER® Clone 208, was added to sensors, and optical shift was measured for 15 min during the first association phase (**FIG. 4A**). Next, the first reagent was removed, and a second reagent, either buffer, AFFIMER® Clone 155, or AFFIMER® Clone 208, was added to the sensors, and the optical shift was measured for 3 min (**FIG. 4B**). Finally, the second reagent was removed, and replaced with 0.5 M MgCl₂ to allow bound AFFIMER® polypeptide to dissociate from the sensors. The first and second reagents added to each sensor are shown in **Table 15**, and the results of this epitope binning analysis are shown in **FIGs. 4A–4B**.

Table 15. Reagent combinations tested in first epitope binning analysis.

Sensor	First Reagent	Second Reagent
1	Buffer	Clone 155
2	Clone 208	Clone 208
3	Clone 208	Buffer
4	Clone 208	Clone 155

As shown in **FIG. 4B**, AFFIMER® Clone 155 was added to sensor 1 and sensor 4, but a lower optical shift was observed in sensor 4, to which AFFIMER® Clone 208 had previously been added, indicating that AFFIMER® Clone 208 interfered with the ability of AFFIMER® Clone 155 to bind CD33. Thus, AFFIMER® Clones 208 and 155 bind to the same epitope region on human CD33 and belong to the same epitope bin.

A second epitope binning analysis was conducted using the approach described in the preceding paragraphs, but in which AFFIMER® Clone 155 was added during the first association phase, and AFFIMER® Clones 115 or 208 were added during the second association

phase. The first and second reagents added to each sensor in this second analysis are shown in **Table 16**, and the results of this epitope binning analysis are shown in **FIGs. 5A–5B**.

Table 16. Reagent combinations tested in second epitope binning analysis.

Sensor	First Reagent	Second Reagent
1	Buffer	Clone 208
2	Clone 155	Clone 155
3	Clone 155	Buffer
4	Clone 155	Clone 208

As shown in **FIG. 5B**, AFFIMER® Clone 208 was added to sensor 1 and sensor 4, but a lower optical shift was observed in sensor 4, to which AFFIMER® Clone 155 had previously been added, indicating that AFFIMER® Clone 155 interfered with the ability of AFFIMER® Clone 208 to bind CD33. Thus, AFFIMER® Clones 155 and 208 bind to the same epitope on human CD33 and belong to the same epitope bin.

What is claimed is:

CLAIMS

1. An engineered polypeptide that binds specifically to CD33 with a K_d of 1×10^{-6} M or less, wherein the engineered polypeptide is a variant of a Stefin A protein.
2. The engineered polypeptide of claim 1 comprising an amino acid sequence having at least 80%, at least 90%, at least 95, or at least 98% identity to an amino acid sequence of IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA-(Xaa)_n-STNYYIKVRAGDNKYMHLKVFNGP-(Xaa)_m-ADR VLTGYQVDKNKDDELTF (SEQ ID NO: 5), wherein Xaa, individually for each occurrence, is an amino acid residue; and n and m are each, independently, an integer from 3 to 20.
3. The engineered polypeptide of claim 2 comprising the amino acid sequence of SEQ ID NO: 5.
4. The engineered polypeptide of any one of the preceding claims that binds to CD33 with a K_d of 1×10^{-7} M.
5. The engineered polypeptide of claim 4 that binds to CD33 with a K_d of 1×10^{-8} M.
6. The engineered polypeptide of any one of the preceding claims, wherein (Xaa)_n is represented by formula (IV)

$$\text{aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8-aa9} \quad (\text{IV}),$$
 wherein aa1 is F; aa2 is Q, R, or T; aa3 is W; aa4 is G; aa5 is S; aa6 is S; aa7 is Y; aa8 is H; and aa9 is E or Q.
7. The engineered polypeptide of any one of the preceding claims, wherein (Xaa)_m is represented by formula (IV)

$$\text{aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8-aa9} \quad (\text{IV}),$$
 wherein aa1 is I, K, V, or Y; aa2 is D, E, or Q; aa3 is D, N, S or Y; aa4 is D, E, N, or Q; aa5 is F, K, N, V or Y; aa6 is A or N; aa7 is L; aa8 is I or T; and aa9 is W.
8. The engineered polypeptide of any one of claims 1-7 comprising the amino acid sequence of

IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FRWGSSYHQST**
 NYIYIKVRAGDNKYMHLKVFNGP**VQDEFALI**WADRVLTGYQVDKNKDDELTGF (SEQ
 ID NO: 150)

9. The engineered polypeptide of any one of claims 1-7 comprising the amino acid sequence of

IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FRWGSSYHQST**
 NYIYIKVRAGDNKYMHLKVFNGP**YDNDVALT**WADRVLTGYQVDKNKDDELTGF (SEQ
 ID NO: 300)

10. The engineered polypeptide of any one of claims 1-7 comprising the amino acid sequence of

IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FRWGSSYHQST**
 NYIYIKVRAGDNKYMHLKVFNGP**IESDFNLI**WADRVLTGYQVDKNKDDELTGF (SEQ
 ID NO: 297)

11. The engineered polypeptide of any one of claims 1-7 comprising the amino acid sequence of

IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FQWGSSYHEST**
 NYIYIKVRAGDNKYMHLKVFNGP**VEYDYNLI**WADRVLTGYQVDKNKDDELTGF (SEQ
 ID NO: 343)

12. The engineered polypeptide of any one of claims 1-7 comprising the amino acid sequence of

IPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLA**FTWGSSYHEST**
 NYIYIKVRAGDNKYMHLKVFNGP**VEYDYNLI**WADRVLTGYQVDKNKDDELTGF (SEQ
 ID NO: 346)

13. The engineered polypeptide of any one of claims 1-5, wherein (Xaa)_n is an amino acid sequence having at least 75% or at least 85% identity to the amino acid sequence of any one of SEQ ID NOs: 6-65.

14. The engineered polypeptide of any one of claims 1-5, wherein (Xaa)_n is the amino acid sequence of any one of SEQ ID NOs: 6-65.

15. The engineered polypeptide of any one of claims 1-5, wherein (Xaa)_m is an amino acid sequence having at least 75% or at least 85% identity to the amino acid sequence of any one of SEQ ID NOs: 66-129.
16. The engineered polypeptide of any one of claims 1-5, wherein (Xaa)_m is the amino acid sequence of any one of SEQ ID NOs: 66-129.
17. The engineered polypeptide of any one of claims 1-16 further comprising a signal peptide.
18. A fusion protein comprising the engineered polypeptide of any one of claims 1-16 linked to a therapeutic or diagnostic moiety.
19. The fusion protein of claim 18, wherein the therapeutic moiety is a therapeutic protein or peptide, optionally an antibody.
20. The fusion protein of claim 18, wherein the diagnostic moiety is a fluorescent protein.
21. The fusion protein of any one of claims 18-20, wherein the engineered polypeptide is linked to the therapeutic or diagnostic moiety through a linker.
22. A multispecific fusion protein comprising:
 - (a) the engineered polypeptide of any one of claims 1-17; and
 - (b) an immune cell binding domain.
23. The multispecific fusion protein of claim 22, wherein the immune cell binding domain is a T cell binding domain.
24. The multispecific fusion protein of claim 23, wherein the T cell binding domain specifically binds to CD3, optionally a CD3 ϵ chain.

25. The multispecific fusion protein of claim 23 or 24, wherein the immune cell binding domain is an antibody.
26. The multispecific fusion protein of claim 25, wherein the antibody is selected from whole antibodies, antibody fragments, and antibody mimetics.
27. The multispecific fusion protein of claim 26, comprising a peptide linker.
28. The multispecific fusion protein of claim 27 comprising:
(a) the engineered polypeptide of any one of claims 1-17;
(b) the peptide linker; and
(b) an antibody, optionally single-chain variable fragment (scFv), that specifically binds to a CD3ε chain.
29. The multispecific fusion protein of claim 22, wherein the immune cell binding domain is a Natural Killer (NK) cell binding domain.
30. The multispecific fusion protein of claim 29, wherein the NK cell binding domain specifically binds to CD16.
31. The multispecific fusion protein of claim 29 or 30, wherein the immune cell binding domain is an antibody.
32. The multispecific fusion protein of claim 31, wherein the antibody is selected from whole antibodies, antibody fragments, and antibody mimetics.
33. The multispecific fusion protein of claim 32, further comprising a peptide linker.
34. The multispecific fusion protein of claim 33 comprising:
(a) the engineered polypeptide of any one of claims 1-17;
(b) the peptide linker; and
(b) an antibody, optionally single-chain variable fragment (scFv), that specifically binds to a CD16.

35. The multispecific fusion protein of any one of claims 29-34, wherein the engineered polypeptide and the NK cell binding domain are crosslinked to an interleukin-15 (IL-15) protein.
36. The multispecific fusion protein of any one of claims 29-34 comprising:
- (a) the engineered polypeptide of any one of claims 1-17;
 - (b) IL-15 protein; and
 - (c) an antibody, optionally single-chain variable fragment (scFv), that specifically binds to CD16, wherein the engineered polypeptide and the antibody are crosslinked to the IL-15 protein.
37. The multispecific fusion protein of claim 35 or 36, further comprising an antibody, optionally scFv, that specifically binds to CD133.
38. The multispecific fusion protein of any one of claims 29-32, further comprising an immune cell binding domain that specifically binds to NKGD2.
39. The multispecific fusion protein of claim 38, comprising:
- (a) the engineered polypeptide of any one of claims 1-17;
 - (b) an antibody, optionally a Fab fragment, that specifically binds NKGD2; and
 - (c) an antibody, optionally an Fc domain, that specifically binds to a CD16, optionally wherein the engineered polypeptide is linked to the antibody of (b) or (c) through a linker.
40. The multispecific fusion protein of claim 39, wherein the linker comprises Ala-Ser or Gly-Ala-Ser.
41. A T cell comprising the multispecific fusion protein of any one of claims 23-28.
42. An NK cell comprising the multispecific fusion protein of any one of claims 29-41.
43. A chimeric antigen receptor, comprising:
- (a) the engineered polypeptide of any one of claims 1-17;
 - (b) a linker sequence;
 - (c) a transmembrane domain; and
 - (d) a CD3z signaling domain.

44. The chimeric antigen receptor of claim 43, further comprising a CD28 co-stimulatory domain, a 4-1BB co-stimulatory domain, or both a CD28 co-stimulatory domain and a 4-1BB co-stimulatory domain.
45. A T cell comprising the chimeric antigen receptor of claim 43 or 44.
46. A fusion protein comprising:
- (a) a first engineered polypeptide of any one of claims 1-17;
 - (b) an antibody Fc domain; and
 - (c) a second engineered polypeptide of any one of claims 1-17.
47. The engineered polypeptide of any one of claims 1-17 or the fusion protein of any one of claim 18-40 further comprising a half-life extension moiety.
48. The engineered polypeptide or fusion protein of claim 47, wherein the half-life extension moiety is selected from antibody Fc domains, human serum albumin, serum binding proteins.
49. The engineered polypeptide or fusion protein of claim 47, wherein the half-life extension moiety is an engineered polypeptide is a variant of a Stefin A protein that binds specifically to human serum albumin with a K_d of $1 \times 10^{-6} \text{M}$ or less.
50. A polynucleotide or set of polynucleotides encoding the engineered polypeptide of any one of claims 1-17, the fusion protein of any one of claim 18-40, or the chimeric antigen receptor of any one of claims 43 or 44.
51. The polynucleotide of claim 50, wherein the polynucleotide is a DNA or an RNA.
52. The polynucleotide of claim 51, wherein the RNA is an mRNA.
53. A delivery vehicle comprising the polynucleotide of any one of claims 50-52.
54. The delivery vehicle of claim 53, which is a viral delivery vehicle.

55. The delivery vehicle of claim 54, wherein the viral delivery vehicle is an adenoviral vector, a retroviral vector, a lentiviral vector, or an adeno-associated viral (AAV) vector.
56. The delivery vehicle of claim 53, which is a non-viral delivery vehicle.
57. The delivery vehicle of claim 56, wherein the non-viral delivery vehicle is a liposome or a lipid nanoparticle.
58. A plasmid or minicircle comprising the polynucleotide of any one of claims 50-52.
59. A messenger RNA (mRNA) comprising an open reading frame encoding the engineered polypeptide of any one of claims 1-17.
60. The mRNA of claim 59 further comprising an open reading frame encoding an immune cell binding.
61. A lipid nanoparticle, optionally a cationic lipid nanoparticle, comprising the mRNA of claim 59 or 60.
62. A conjugate comprising:
- (a) the engineered polypeptide of any one of claims 1-17; and
 - (b) a chemotherapeutic agent.
63. A pharmaceutical composition comprising the engineered polypeptide, the fusion protein, the cell, or the delivery vehicle of any one of preceding claims, and optionally a pharmaceutically acceptable excipient.
64. A method comprising administering to a subject the pharmaceutical composition of claim 63.
65. The method of claim 64, wherein the subject has a CD33⁺ cancer.
66. The method of claim 65, wherein the CD33⁺ cancer is acute myeloid leukemia.

67. The method of claim 64, wherein the subject has Alzheimer's disease.
68. The method of any one of claims 64-67, wherein the pharmaceutical composition is administered subcutaneously, intravenously, or intramuscularly.

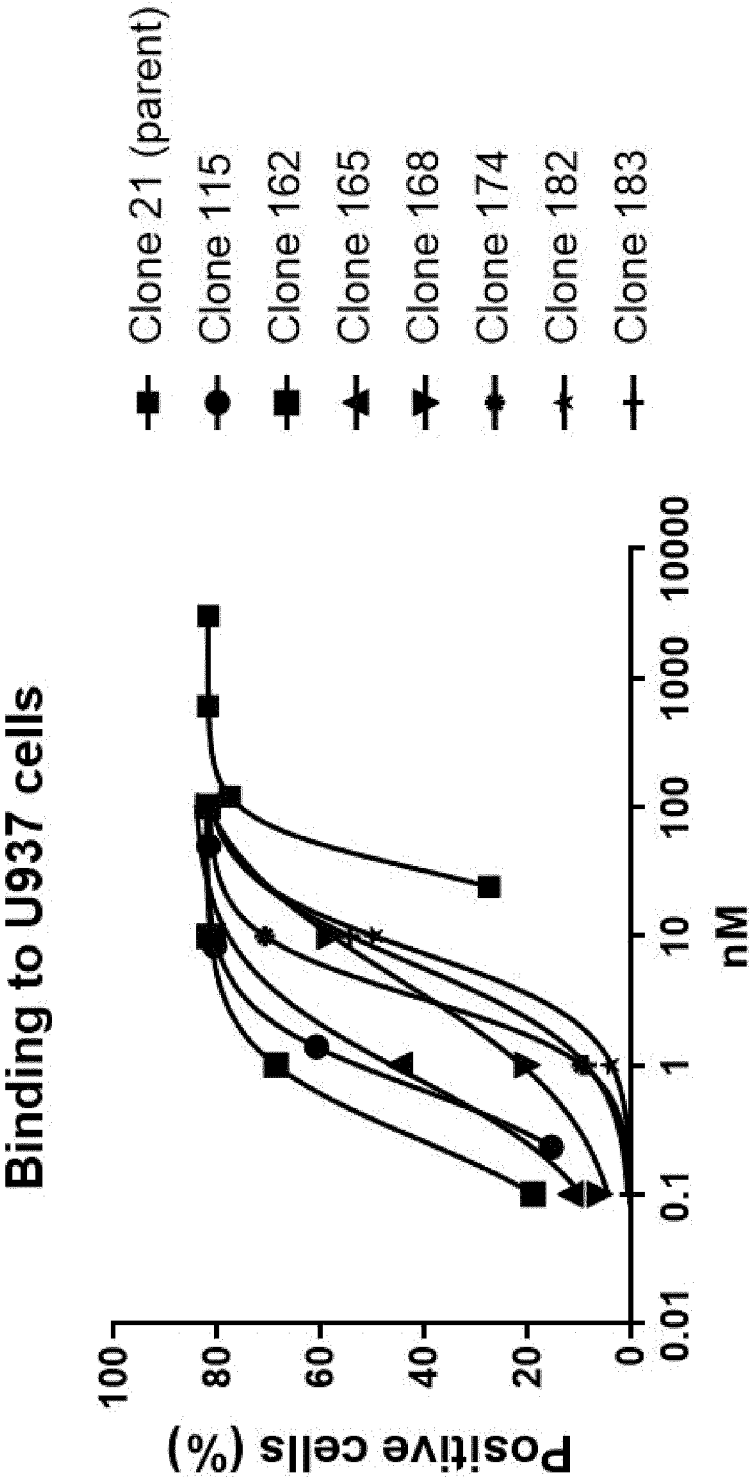


FIG. 1

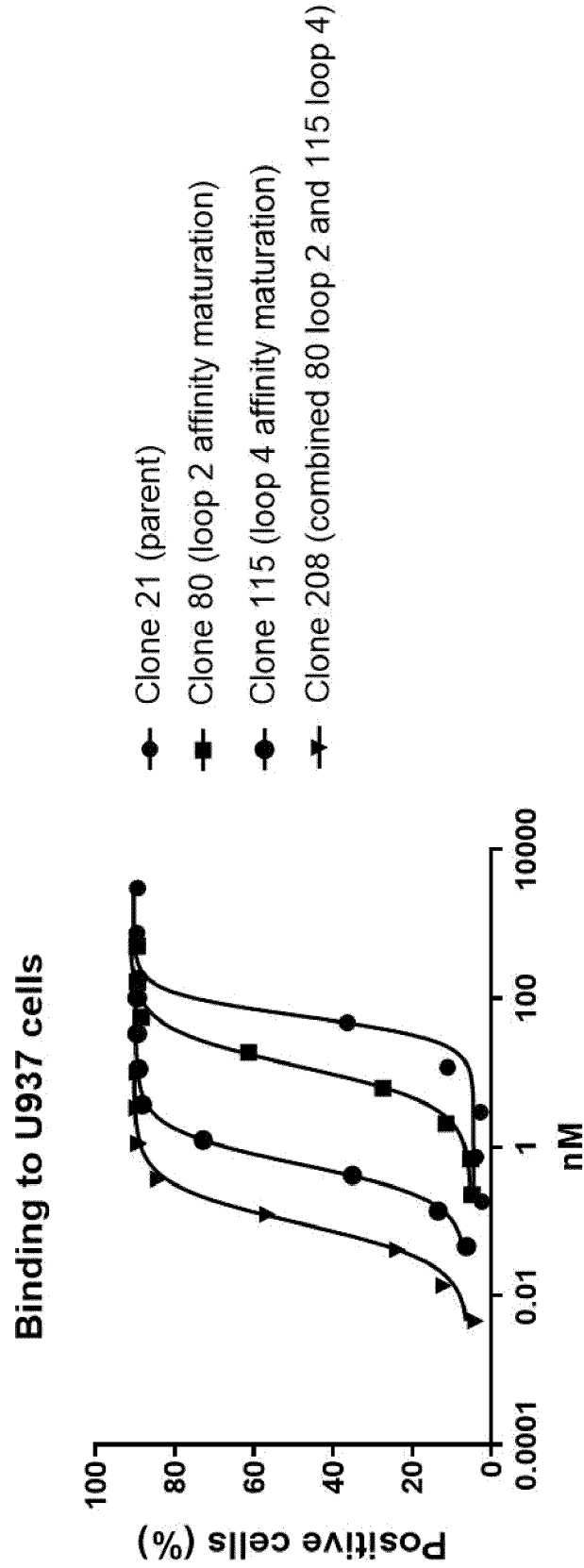


FIG. 2

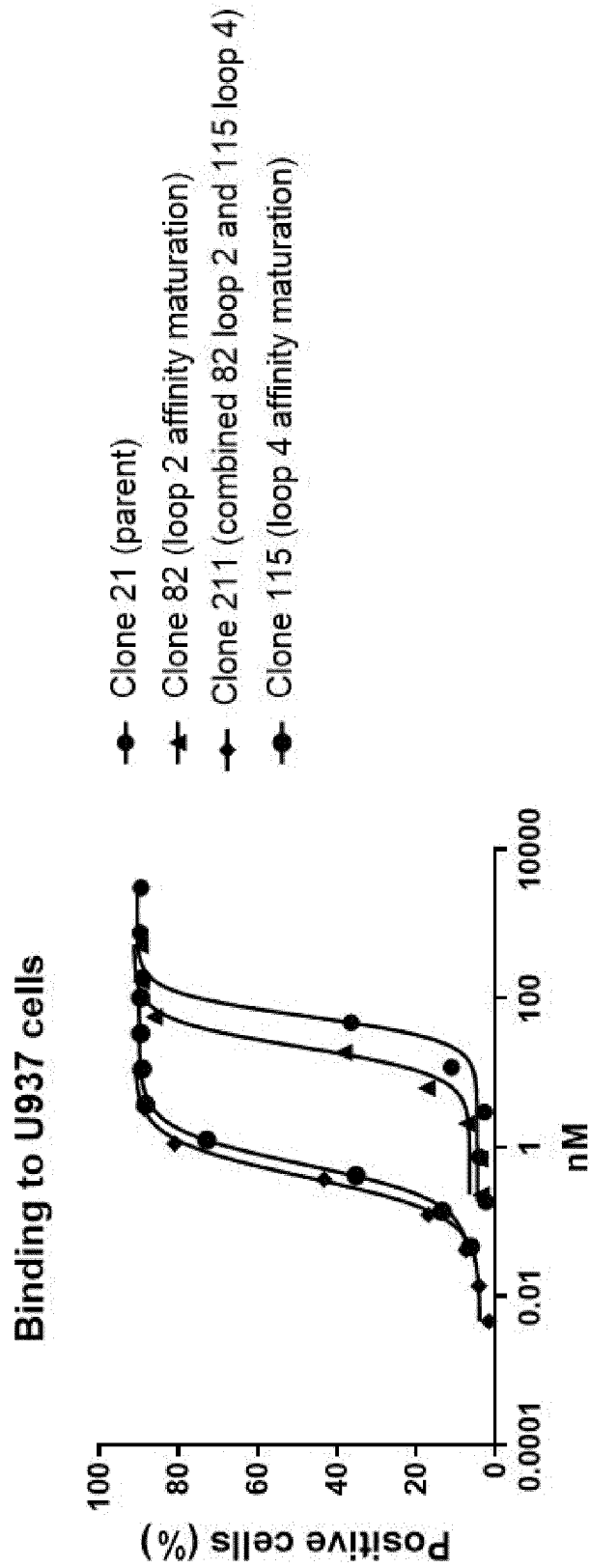


FIG. 3

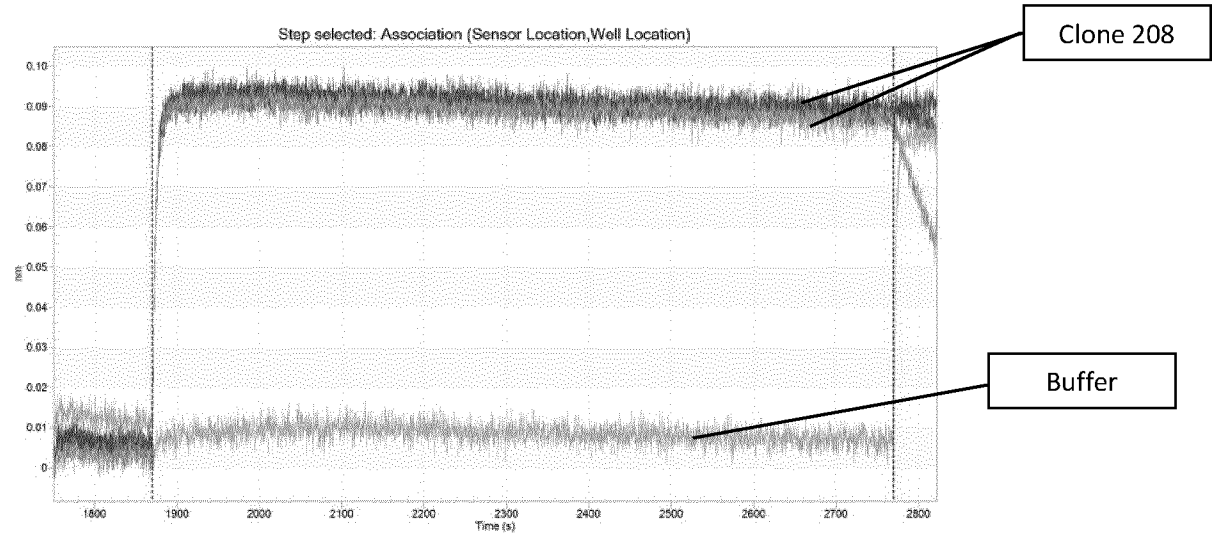


FIG. 4A

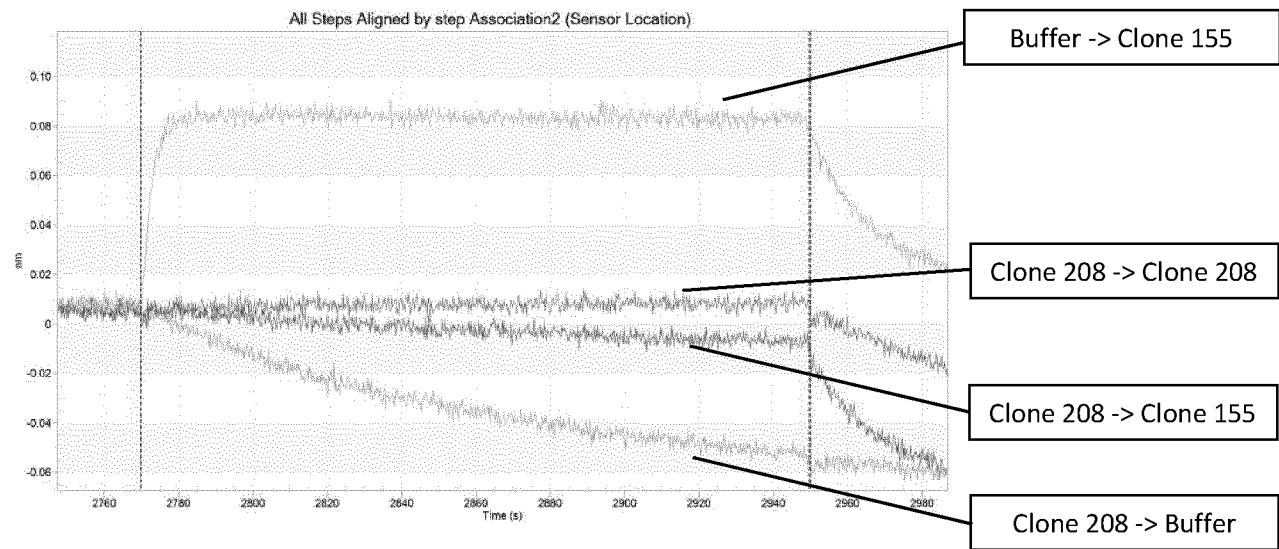


FIG. 4B

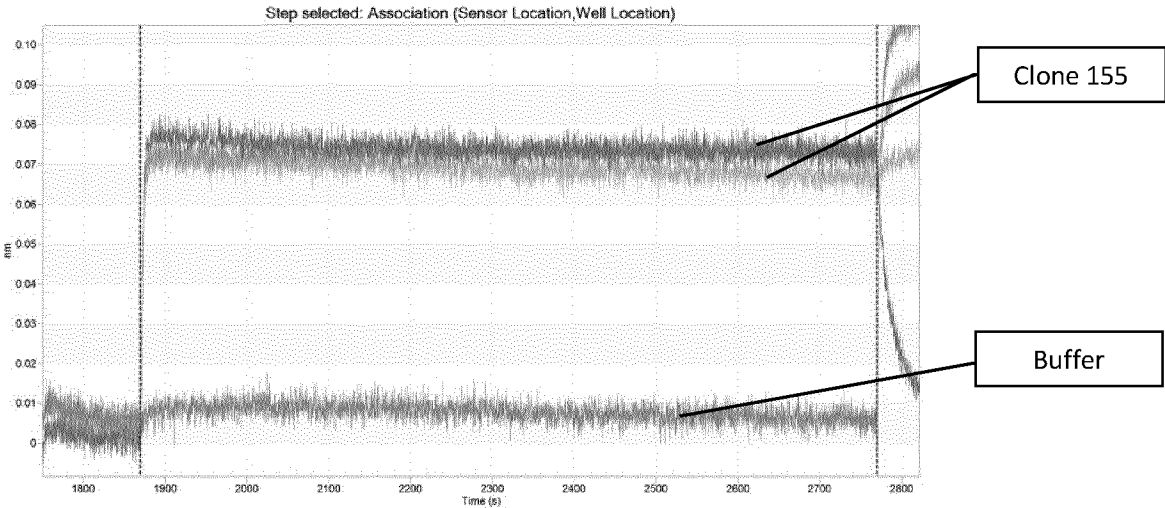


FIG. 5A

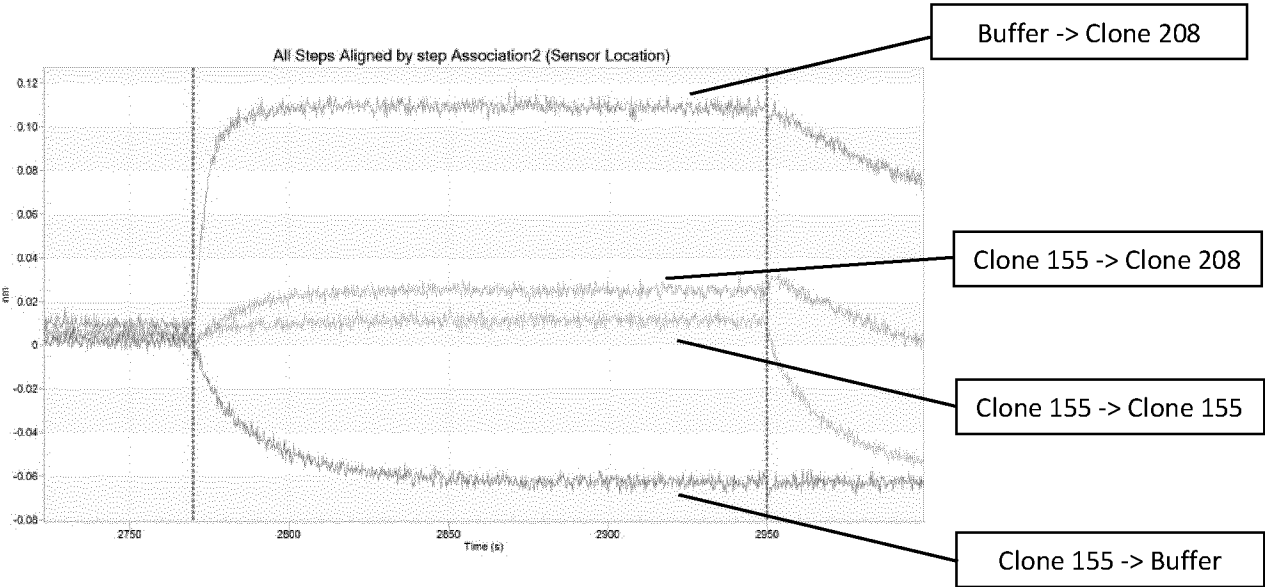


FIG. 5B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/062125

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K14/705
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)
C07K

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/201388 A2 (ALECTOR LLC [US]) 15 December 2016 (2016-12-15) the whole document -----	1-68
Y	WO 2019/197583 A1 (AVACTA LIFE SCIENCES LTD [GB]) 17 October 2019 (2019-10-17) over 86% sequence identity with SEQ ID NOS 5, 150, 300, 297, 343, 346 -----	1-68
Y	WO 2021/074695 A1 (AVACTA LIFE SCIENCES LTD [GB]) 22 April 2021 (2021-04-22) over 86% sequence identity with SEQ ID NOS 5, 150, 300, 297, 343, 346 ----- --/--	1-68

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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"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

12 September 2022

Date of mailing of the international search report

21/09/2022

Name and mailing address of the ISA/
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/062125

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Dr Alastair Smith ET AL: "Non-confidential Technical Introduction to the Affimer® Technology for Therapeutics and Reagents", / 1 November 2017 (2017-11-01), XP055770316, Retrieved from the Internet: URL:https://www.cosmobio.co.jp/product/upl oads/document/ALS_Affimer_document2.pdf [retrieved on 2021-01-29] the whole document -----	1-68

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2022/062125

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