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(54) Title: TNFR2 BINDING POLYPEPTIDES AND METHODS OF USE

(57) Abstract: The present disclosure relates to engineered TNFR2-binding Stefin A polypeptide variants, polynucleotides encoding the engineered TNFR2-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 inflammatory, autoimmunity and cancer.

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TNFR2 BINDING POLYPEPTIDES AND METHODS OF USE

Maintaining control of the cell-mediated and humoral immune responses is an important dimension of healthy immune system activity. The abnormal regulation of T cell and B cell driven immune reactions has been associated with a wide array of human diseases, as the inappropriate increase of an immune response against various self and foreign antigens plays a causal role in such pathologies as autoimmune disorders, asthma, allergic reactions, graft versus host disease (GvHD), transplantation graft rejection, and a variety of other immunological disorders including cancer.

These diseases are mediated by T and B lymphocytes that exhibit reactivity against self antigens and those derived from non-threatening sources, such as allergens or transplantation allografts. Regulatory T cells (Tregs) have evolved to inhibit the activity of immune cells that are cross reactive with "self" major histocompatibility complex (MHC) proteins and other benign antigens. The most well understood populations of Tregs are CD4+, CD25+, FoxP3+ Tregs and CD17+ Tregs.

Tumor necrosis factor receptor (TNFR) subtypes 1 and 2 have been identified specifically on the Tregs surface. The activation of TNFR1 by soluble TNF-alpha, enhances the caspase signaling cascade leading to Treg apoptosis. On the other hand, activation of TNFR2 by mainly membrane bound TNF-alpha induces signaling through the mitogen activated protein kinase (MAPK) signaling pathway, which orchestrates the TRAF2/3 and NF- κ B mediated transcription of genes that promote cell proliferation and escape from apoptosis.

Importance of TNFR2's role in Treg proliferation and function was shown in various studies. TNFR2 deficient mice had reduced numbers of thymic and peripheral Tregs (2013_Chen X, et al. PMID: 23277487), and TNFR2 -/- Tregs were not able to control inflammatory responses in vivo (2008_Van Mierlo GJ, et al. PMID: 18292492). In humans, Tregs were shown to express a higher level of TNFR2 than T effector cells (2013_Okubo Y, et al. PMID: 24193319 & 2002_Annunziato F, et al. PMID: 12163566) and TNFR2+ Tregs exhibited the most potent suppression of proliferation and cytokine production of co-cultured T-responder cells (2010_Chen X, et al. PMID: 20127680). Furthermore, TNFR2 has been shown to play a role in carcinogenesis and tumour growth by mediating TNF responses in immunosuppressive cells, allowing for immune escape and tumour development (Sheng Y., et al. doi: 10.3389/fimmu.2018.01170).

Moreover, in autoimmune diseases affecting the central nervous system (CNS), specifically Multiple sclerosis (MS), pathogenic lymphocytes are triggered in the periphery to infiltrate the CNS and cause local inflammation and demyelination. Demyelination is the damage to the protective covering (myelin sheath) that surrounds nerve fibers in the brain. Protecting against demyelination or revert it are strategies explored to tackle MS. Interestingly, several studies have reported TNFR2 involvement in neuroprotection. For example, in a cuprizone induced mouse model of demyelination, it was shown that TNFR2 is critical for regeneration of oligodendrocyte the cells primarily responsible for maintenance and generation of the myelin sheath that surrounds axons, whereas TNF signaling via TNFR1 promoted nerve demyelination (2001_HA Arnett, et al. PMID: 11600888).

Due to its role in directing cell survival and growth, TNFR2 represents an attractive target for treating diseases such as autoimmune disorders, GvHD, allograft rejection, allergic reactions, asthma and cancer.

AFFIMER®, developed by Avacta Life Sciences Limited, is a small stable protein molecule engineered based on a stefin A protein, which is an in-vivo protein. AFFIMER® includes two short peptide sequences having a random sequence and an N-terminal sequence, and is able to bind to a target material with high affinity and specificity in a manner similar to a monoclonal antibody. AFFIMER® shows remarkably improved binding affinity and specificity compared to the free peptide library, and has a very small size and high stability compared to antibodies, and is therefore receiving great attention as a next-generation alternative pharmaceutical platform to replace antibodies (U.S. Patent Nos. 9447170, 8853131, etc.).

The present invention relates to polypeptides that specifically bind to TNFR2 by engineering the natural stefin A protein, resulting in the development of AFFIMER® polypeptides capable of binding to TNFR2 with excellent affinity and specificity.

SUMMARY

In one aspect there is provided an engineered polypeptide that binds specifically to TNFR2 with a K_d of $1 \times 10^{-6}M$ or less, wherein the engineered polypeptide is a variant of a Stefin A protein.

In a further aspect there is provided a fusion protein comprising a dimer, a trimer or a tetramer of the engineered polypeptides of the invention, optionally comprising one or more linkers.

In another aspect there is provided a fusion protein comprising one or more of the engineered polypeptides of the invention linked to a therapeutic or diagnostic moiety.

In a further aspect there is provided a polynucleotide or set of polynucleotides encoding the engineered polypeptide or the fusion protein of the invention.

5 In one aspect there is provided a delivery vehicle comprising the polynucleotide of the invention.

In a further aspect there is provided a plasmid or minicircle comprising the polynucleotide of the invention.

10 In another aspect there is provided a messenger RNA (mRNA) comprising an open reading frame encoding the engineered polypeptide of the invention.

In one aspect there is provided a lipid nanoparticle, optionally a cationic lipid nanoparticle, comprising the mRNA of the invention.

15 In another aspect there is provided a pharmaceutical composition comprising the engineered polypeptide, the fusion protein, or the delivery vehicle of the invention, and optionally a pharmaceutically acceptable excipient.

In one aspect there is provided a conjugate comprising the engineered polypeptide or the fusion protein of the invention linked to a pharmacologically active moiety.

In a further aspect there is provided a method comprising administering to a subject the pharmaceutical composition of the invention.

20 In one aspect there is provided an engineered polypeptide, fusion protein, or delivery vehicle of the invention for use as a medicament.

In another aspect there is provided an engineered polypeptide that competes for binding to TNFR2 with an engineered polypeptide according to the invention.

25 In a further aspect there is provided an engineered polypeptide which binds the same epitope on TNFR2 as that of an engineered polypeptide according to the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic of the selection strategy for the human TNFR2 selection campaign. Both libraries underwent solution and passive selections. Stringency was introduced by decreasing
30 hTNFR2 concentration as the rounds progressed and increasing the number of wash steps between round 1 (5x PBS/0.1% Tween 20, 2x PBS) and round 2 (15x PBS/0.1% Tween 20, 2x PBS). For the passive selections using Avacta's proprietary Type 3 AFFIMER® phage library (US Pat. No. 9.932.575), the deselection and competition approaches were simultaneously

explored. For the deselection approach, the Fc concentration in round n+1 was the concentration of hTNFR2 in round n. For the competition approach, the Fc concentration was 10x molar excess of the hTNFR2 concentration specified in the given round. For the passive selections using Avacta's proprietary Type 1 AFFIMER® phage library (US Pat. No. 8,841,491), the deselection approach was exclusively performed and a round 4 was introduced since early-stage screening of the round 3 outputs indicated a high diversity and hit rate. For the solution selections, both libraries underwent the competition approach for reducing enrichment of Fc-binding phage-displayed AFFIMER® polypeptides. Streptavidin- and neutravidin-coated beads were alternated between rounds to reduce enrichment for streptavidin and/or neutravidin-binding phage-displayed peptides.

Figure 2. AFFIMER® polypeptide binding to Expi293F™ cells overexpressing human TNFR2.

Figure 3. Titration experiments data compilation. % inhibition at 3 µM and IC50 for best blockers.

Figure 4. SEAP results in duplicate, ranking of 20 clones.

Figure 5. Cross-reactivity of hTNFR2 AFFIMER® clones with cynomolgus TNFR2, in triplicate

DETAILED DESCRIPTION

AFFIMER®

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 herein 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 TFNR2), 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®-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® polynucleotide” refers to a nucleic acid construct which, when introduced into and expressed by cells, produces an intended AFFIMER® agent.

TNFR2

Tumour necrosis factor receptor 2 (“TNFR2”), along with tumour necrosis factor receptor 1 (TNFR1), is a Type 1 membrane bound receptors that binds tumour necrosis factor alpha (“TNF α ”). TNFR2, also known as p75, TNF Receptor Superfamily Member 1B or CD120b, is encoded in humans as *TNFRSF1B* which expresses a 48 kDa protein of 461 amino acids in length (UniProt P20333). In mice, the TNFR2 protein is 474 amino acids long and has a 50 kDa mass.

A “TFNR2 AFFIMER® agent” refers to an AFFIMER® agent that comprises at least one AFFIMER® polypeptide that binds to TFNR2, particularly human TFNR2 and optionally cynomolgus TNFR2, with a dissociation constant (Kd) of at least 10⁻⁶M. In some embodiments, the TFNR2 AFFIMER® agent binds TFNR2 with a Kd of 1×10⁻⁷M or less, Kd of 1×10⁻⁸M or less, Kd of 1×10⁻⁹M or less, or a Kd of 1×10⁻¹⁰M or less. It should be understood that the terms “TFNR2 AFFIMER® polypeptide”, “TFNR2 AFFIMER® protein” and “engineered TFNR2-binding Stefin A polypeptide variant” are used interchangeably herein. Thus, a “TFNR2 AFFIMER® polypeptide” is an engineered polypeptide that binds specifically to TFNR2 with a Kd of 1×10⁻⁶M or less, wherein the engineered polypeptide is a variant of a Stefin A protein.

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 $--CH(NH_2)COOH$ 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 peptidylglycan 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® polypeptide (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, diaminopimelic 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.

When calculating percent identity, any residues defined as 'Xaa' or 'X' in a reference sequence herein are included in the percentage identity calculation, i.e. any amino acid in this position in a comparison sequence matches the reference sequence.

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 60%, at least 70%, at least 80%, 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 TFNR2 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.

A transmembrane domain (also called a TM domain) is a sequence that can be added to an AFFIMER® polypeptide, such as through the addition of a polynucleotide sequence encoding the TM domain to the polynucleotide sequence encoding the AFFIMER® polypeptide, to ensure the AFFIMER® polypeptide is retained on the surface of the cell that is expressing it. Various TM domains are available and readily usable with the AFFIMER® polypeptide.

An “AFFIMER®-antibody fusion” is a fusion protein that includes an AFFIMER® polypeptide portion and a variable region of an antibody. AFFIMER®-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®-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.

The antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., 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. In some embodiments, the Fc is null binding (does not bind) to FcγR2b. In some embodiments, the antibody is not an IgG1 Fc.

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 is "specific for" refers to measurable and reproducible interactions such as binding between a target (e.g., TFNR2) 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 TFNR2 is an AFFIMER® polypeptide that binds TFNR2 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 TFNR2 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.

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.

Treatments

"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.

“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.

“Sustained response” refers to the sustained effect on reducing inflammation after cessation of a treatment. 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 “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., inflammatory or autoimmune disease) 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.

“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.

5 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.

10 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.

15 "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
20 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
25 an amount of an agent, such as a TFNR2 AFFIMER® agent, effective to treat a disease or disorder in a subject such as, a mammal. In the case of inflammatory or autoimmune disease, the therapeutically effective amount of an TFNR2 AFFIMER® agent has a therapeutic effect and as such can reduce inflammation; relieve to some extent at least one of the symptoms associated with the inflammatory or autoimmune disease; reduce morbidity and mortality;
30 improve quality of life; or a combination of such effects.

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."

TFNR2 AFFIMER® Polypeptide

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 TFNR2 (also referred to as "TFNR2 AFFIMER® polypeptides") in which at least one of the solvent accessible loops from the wild-type Stefin A protein having the ability to bind TFNR2, preferably selectively, and preferably with K_d of 10⁻⁶M or less.

In some embodiments, a TFNR2 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 TGETYGGLEA VQYKTQVX (SEQ ID NO: 1) or a polypeptide sequence having at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%,
5 or 100%) identity to the amino acid sequence of SEQ ID NO: 1, wherein X is any number of independently selected amino acids, more suitably three or fewer independently selected amino acids, or more suitably X is V; and/or

FR2 is a polypeptide sequence comprising the amino acid sequence of GTNYYIKVRA GDNKYMHLKV FKSL (SEQ ID NO: 2) or a polypeptide sequence having at least 70% (e.g.,
10 at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) identity to the amino acid sequence of SEQ ID NO: 2;

FR3 is a polypeptide sequence comprising the amino acid sequence of EDLVLTGYQV DKNKDELDTG F (SEQ ID NO: 3) or a polypeptide sequence having at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) identity to the amino
15 acid sequence of SEQ ID NO: 3; and

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, 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
20 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
25 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 TNFR2 AFFIMER® polypeptide has an amino acid sequence represented in the general Formula (II):

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

wherein

5 Xaa, individually for each occurrence, is an amino acid residue, and

n and m are each, independently, an integer from 3- 20.

In some embodiments, the TNFR2 AFFIMER® polypeptide comprises an amino acid sequence having at least 90% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identity to the amino acid sequence of:

10 MIPGGLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVD-(Xaa)_n-
GTNYYIKVRAGDNKYMHLKVFKSL-(Xaa)_m-EDLVLTGYQVDKNKDDELDTGF (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- 20.

15 In some embodiments, 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,
20 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.

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.

25 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 further embodiments, the TNFR2 AFFIMER® polypeptide has an amino acid sequence represented in the general Formula (IIb):

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQV (SEQ ID NO: 484) Xaa2-(Xaa)_n-Xaa3-TNYYIKVRAGDNKYMHLKVF (SEQ ID NO: 485)-Xaa4-Xaa5-Xaa6-(Xaa)_m-Xaa7-D-Xaa8-VLTGYQVDKNKDDELDTGF (SEQ ID NO: 486)

wherein

5 Xaa, individually for each occurrence, is any number of amino acid residues, more suitably three or fewer (preferably, one or two) independently selected amino acids, and

n and m are each, independently, an integer from 3- 20.

In some embodiments, the TNFR2 AFFIMER® polypeptide comprises an amino acid sequence having at least 90% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, at
10 least 99%, or 100%) identity to the amino acid sequence of:

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQV (SEQ ID NO: 484) Xaa2-(Xaa)_n-Xaa3-TNYYIKVRAGDNKYMHLKVF (SEQ ID NO: 485)-Xaa4-Xaa5-Xaa6-(Xaa)_m-Xaa7-D-Xaa8-VLTGYQVDKNKDDELDTGF (SEQ ID NO: 486),

or

15 MIPGGLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVD (SEQ ID NO: 487)-(Xaa)_n-GTNYYIKVRAGDNKYMHLKVFKSL (SEQ ID NO: 488)-(Xaa)_m-EDLVLTGYQVDKNKDDELDTGF (SEQ ID NO: 489), wherein

Xaa, individually for each occurrence, is any number of amino acid residues, more suitably three or fewer (preferably, one or two) independently selected amino acids, and

20 n and m are each, independently, an integer from 3- 20.

In some embodiments, 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 Val, Asp or 'Leu-Ala'; Xaa3 is Gly, Ala, Val, Ser or Thr, more preferably Gly or Ser; Xaa4 is Arg, Lys, Asn, Gln, Ser, Thr, more preferably Arg, Lys, Asn or Gln, and even more preferably Lys or Asn;
25 Xaa5 is Gly, Ala, Val, Ser or Thr, more preferably Gly or Ser; Xaa6 is Ala, Val, Ile, Leu, Gly or Pro, more preferably Ile, Leu or Pro, and even more preferably Leu or Pro; Xaa7 is Gly, Ala, Val, Asp or Glu, more preferably Ala, Val, Asp or Glu, and even more preferably Ala or Glu; and Xaa8 is Ala, Val, Ile, Leu, Arg or Lys, more preferably Ile, Leu or Arg, and even more preferably Leu or Arg.

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.

- 5 In some embodiments, Xaa, independently 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 102, 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 102. 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 102.

In some embodiments of the above sequences and formulas, (Xaa)_m is an amino acid sequence selected from SEQ ID NOs: 103 to 199, or an amino acid sequence having at least 80%, 85%, 90%, 95% or even 98% homology with a sequence selected from SEQ ID NOs: 103 to 199. 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: 103 to 199.

In some embodiments, the TNFR2 AFFIMER® polypeptide has an amino acid sequence selected from SEQ ID NOs: 200 to 296, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues. In some embodiments, the TNFR2 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: 200 to 296, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues.

Table 1. TNFR2 AFFIMER® polypeptide sequences. The C-terminal 21 amino acids are extra sequences added for cloning and assay purposes: 3xAla linker, ten amino acid Myc tag, 2xAla linker and 6xHis tag, all of which are optional and not required for the present invention.

Clone Name	Loop 2	Loop 2 SEQ ID NO:	Loop 4	Loop 4 SEQ ID NO:	Protein sequence	Protein SEQ ID NO:
CLONE 001	GQDQWGNYA	6	PDIPHNHWH	103	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVGQD QWGNYAGTNYIYKVRAG DNKYMHLKVFKSLPDI P HNHWHEDLVLTGYQVDK	200

					NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	
CLONE 003	GEDKWLNYA	7	ESEDHGQNT	104	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVGED KWLNYAGTNYIYIKVRAG DNKYMHLKVFKSLESED HGQNTEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	201
CLONE 004	RNLGNIYQW	8	SLNARYALD	105	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVRNL GNIYQWGTNYIYIKVRAG DNKYMHLKVFKSLSLNA RYALDEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	202
CLONE 005	KLSYDLPPV	9	SLNKESIIV	106	MIPGGLSEAKPATPEIQ EI VDKVKPQLEEKGETY KLEAVQYKTQVVKLSYD LPPVGTNYIYIKVRAGDN KYMHLKVFKSLSLNKES IIVEDLVLTYGYQVDK DDELDTGF AAAEQKLI SEEDLAAHHHHHH	203
CLONE 006	VYWDILVEL	10	ISGGYSAQT	107	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVY DILVELGTNYIYIKVRAG DNKYMHLKVFKSLSISG YSAQTEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	204
CLONE 007	GLDIWGNNA	11	ADTARTEEA	108	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVGLD IWGNAGTNYIYIKVRAG DNKYMHLKVFKSADTA RTEEAEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	205
CLONE 008	FVLDTNLWQ	12	RGFGNVKKV	109	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVFVL DTNLWQGTNYIYIKVRAG DNKYMHLKVFKSRLGFG NVKKVEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	206
CLONE 009	RLFHIWRWW	13	IHIYDSIQ	110	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVRFL HIWRWGTNYIYIKVRAG DNKYMHLKVFKSLSIHIY TDSIQEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	207
CLONE 010	QEPGVWWWW	14	REGDFDYDI	111	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVQEP GVWWWGTNYIYIKVRAG DNKYMHLKVFKSRLREGD FDYDIEDLVLTYGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	208

CLONE 011	LNWNDIYEH	15	SLTDQYKIS	112	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVLNW NDIYEHGTNYIYIKVRAG DNKYMHLKVFKSLSLTD QYKISEDVLVTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHH	209
CLONE 012	YWGVQGALD	16	NWNHEFDLV	113	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVYWG VQGALDGTNYIYIKVRAG DNKYMHLKVFKSLSNWNH EFDLVEDLVTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHH	210
CLONE 013	AHDYDGYKW	17	LLSNRASEH	114	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVAHD YDGYKWGTNYIYIKVRAG DNKYMHLKVFKSLSLLSN RASEHEDLVTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHH	211
CLONE 014	WQDYQWRGQ	18	YQHIDPPYS	115	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVWQD YQWRGQGTNYIYIKVRAG DNKYMHLKVFKSLSYQHI DPPYSEDLVTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHH	212
CLONE 015	EWEVGDVWG	19	WWVRGFFEP	116	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVEWE VGDVWGGTNYIYIKVRAG DNKYMHLKVFKSLSWVR GFFEPEDLVTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHH	213
CLONE 016	AETAKWFFY	20	VSIEGAWK	117	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAAE TAKWFFYSTNYIYIKVRA GDNKYMHLKFVNGPVS IEGAWKADRVLTGYQVD KNKDDELDTGF AAAEQKL I SEEDLAAHHHHHH	214
CLONE 021	GELGEWQWY	21	QPPDWGWT F	118	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAGE LGEWQWYSTNYIYIKVRA GDNKYMHLKFVNGPQPP DWGWTFADRVLTGYQVD KNKDDELDTGF AAAEQKL I SEEDLAAHHHHHH	215
CLONE 022	GHTDHYWYL	22	THERETGFT	119	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAGE TDHYWYSTNYIYIKVRA GDNKYMHLKFVNGP THERETGFTADRVLTGYQVD KNKDDELDTGF AAAEQKL I SEEDLAAHHHHHH	216
CLONE 023	GHTDQFWLT	23	IWQGDYDRY	120	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAGE	217

					TDQFWLTSTNYYIKVRA GDNKYMHLKVFN GPIWQ GDYDRYADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLI SEEDLAAHHHHH H	
CLONE 026	LEIPSAIYY	24	YFWGDQEH W	121	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLAL E IPSAIYSTNYYIKVRA GDNKYMHLKVFN GPYFW GDQEHWADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLI SEEDLAAHHHHH H	218
CLONE 027	NQLGNGWDA	25	YQAKPNWRH	122	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLANQ LGNGWDASTNYYIKVRA GDNKYMHLKVFN GPYQA KPNWRHADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLI SEEDLAAHHHHH H	219
CLONE 032	DSNGVSAVI	26	TWNIKPQWP	123	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLADS NGVSAVISTNYYIKVRA GDNKYMHLKVFN GPWTN IKPQWPADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLI SEEDLAAHHHHH H	220
CLONE 035	KWHQKQLPL	27	SLNNNFQIQ	124	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVKWH QKQLPLGTNYYIKVRAG DNKYMHLKVFKSLSLNN NFKIQEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHHAAA QKLI SEEDLAAHHHHHH	221
CLONE 036	HDEHISHPV	28	DWWANING T	125	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVHDE HISHPVGTNYYIKVRAG DNKYMHLKVFKSLDWWA NINGTEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHHAAA QKLI SEEDLAAHHHHHH	222
CLONE 037	AVNDFEPSL	29	FQLPDYSVN	126	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVAVN DFEPSLGTNYYIKVRAG DNKYMHLKVFKSLFQLP DYSVNEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHHAAA QKLI SEEDLAAHHHHHH	223
CLONE 038	QHAFDGYAW	30	LQSAIKGIF	127	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVQHA FDGYAWGTNYYIKVRAG	224

					DNKYMHLKVFKSLLQSA IKGIFEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	
CLONE 042	VGDETFDAQ	31	AIQVIGYGT	128	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVVD ETFDAQGTNYIKVRAG DNKYMHLKVFKS LAIQV IGYGTEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	225
CLONE 043	HYHERHRIQ	32	SLDKQYNII	129	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVHYH ERHRIQGTNYIKVRAG DNKYMHLKVFKSLSLDK QYNIIEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	226
CLONE 044	LFPEAVYWV	33	IWNDNAAQH	130	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVLFP EAVYWGTNYIKVRAG DNKYMHLKVFKS LIWND NAAQHEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	227
CLONE 045	VNDEGDHIA	34	YNVTGYVHW	131	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVND EGDHIAGTNYIKVRAG DNKYMHLKVFKS LYNVT GYVHWEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	228
CLONE 049	YDTDWQQDA	35	AYWYDVQGW	132	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVYDT DWQQDAGTNYIKVRAG DNKYMHLKVFKS LAYWY DVQGWEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	229
CLONE 051	WLVHAKTWL	36	LEPQYNVAN	133	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVWL VHAKTWLGTNYIKVRAG DNKYMHLKVFKS LLEPQ YNVANEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	230
CLONE 052	KRELHYWW	37	DEDVSYAYE	134	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVKRE LVHYWWGTNYIKVRAG DNKYMHLKVFKS LDEDV SYAYEEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHAAAE QKLI SEEDLAAHHHHHH	231

CLONE 056	EWNQHAVWL	38	LEHPYDQYG	135	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVEWN QHAVWLGTNYIYIKVRAG DNKYMHLKVFKSLLLEHP YDQYGEDLVLTGYQVQDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHHAAAE QKLI SEEDLAAHHHHHH	232
CLONE 057	KGWGEKTWE	39	QLDGWAEYF G	136	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVKGW GEKTWEGTNYIYIKVRAG DNKYMHLKVFKSLLQLDG WAEYFGEDLVLTGYQVD KNKDELDTGF AAAEQKLI I SEEDLAAHHHHHHHAAA EQKLI SEEDLAAHHHHH H	233
CLONE 058	EWGFGLSIS	40	EYQRYWFEF	137	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVEWG FGLSISGTNYIYIKVRAG DNKYMHLKVFKSLEYQR YWFEDLVLTGYQVQDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHHAAAE QKLI SEEDLAAHHHHHH	234
CLONE 059	FNVQDIWNH	41	ALNHQYTIQ	138	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVFNV QDIWNHGTNYIYIKVRAG DNKYMHLKVFKSLLALNH QYTIQEDLVLTGYQVQDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHHAAAE QKLI SEEDLAAHHHHHH	235
CLONE 060	TESFEQLGY	42	STGGFSWKV	139	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLATE SFEQLGYSTNYIYIKVRA GDNKYMHLKVFNPGSTG GFSWKVADRVLTYGYQVD KNKDELDTGF AAAEQKLI I SEEDLAAHHHHHHHAAA EQKLI SEEDLAAHHHHH H	236
CLONE 063	EETGYHVSY	43	SLNRQYAVI	140	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVEET GYHVSYGTNYIYIKVRAG DNKYMHLKVFKSLSLNR QYAVIEDLVLTGYQVQDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHHAAAE QKLI SEEDLAAHHHHHH	237
CLONE 064	HQPEVKHAW	44	PINPWNLPP	141	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVHQP EVKHAWGTNYIYIKVRAG DNKYMHLKVFKSLLPINP WNLLPEDLVLTGYQVQDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHHAAAE QKLI SEEDLAAHHHHHH	238
CLONE 065	EQWDKSVDV	45	RQYYDEET	142	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVEQW	239

					DKSVDVGTNYIYIKVRAG DNKYMHLKVFKSLRQYY DEETEDLVLTGYQVDKN KDDELDTGF AAAEQKLIS EEDLAAHHHHHHAAAEQ KLISEEDLAAHHHHHH	
CLONE 067	VEGHEHWDW	46	DDWQQQTE V	143	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAVE GHEHWDWSTNYIYIKVRA GDNKYMHLKVFNPGDDW QQQTEVADRVLTYGYQVD KNKDDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLISEEDLAAHHHHH H	240
CLONE 069	GPFQLQLED	47	WELVNAYFP	144	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAGE FQLQLEDSTNYIYIKVRA GDNKYMHLKVFNPGWEL VNAYFPADRVLTYGYQVD KNKDDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLISEEDLAAHHHHH H	241
CLONE 071	EPYNRLSVH	48	SLNKDYFIE	145	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVPEY NRLSVHGTNYIYIKVRAG DNKYMHLKVFKSLSLNK DYFIEEDLVLTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLISEEDLAAHHHHHH	242
CLONE 072	KSVHDVQQH	49	TLTRGYAVE	146	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLKSV HDVQQHGTNYIYIKVRAG DNKYMHLKVFKSLTLTR GYAVEEDLVLTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLISEEDLAAHHHHHH	243
CLONE 073	DDYDDLHWW	50	TSVEYWWDA	147	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVDDY DDLHWWGTNYIYIKVRAG DNKYMHLKVFKSLTSVE YWWDAEDLVLTGYQVDK NKDDELDTGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLISEEDLAAHHHHHH	244
CLONE 076	AWHDDDSLQ	51	NWPEVDIRW	148	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAAW HDDDSLQSTNYIYIKVRA GDNKYMHLKVFNPGNWP EVDIRWADRVLTYGYQVD KNKDDELDTGF AAAEQKL I SEEDLAAHHHHHHAAA EQKLISEEDLAAHHHHH H	245
CLONE 077	TEEQVLGIS	52	TLTRHYLIH	149	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVTEE QVLGISGTNYIYIKVRAG DNKYMHLKVFKSLTLTR	246

					HYLIHEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	
CLONE 078	SIYDDEVND	53	FLNNAYQIF	150	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVSII DDEVNDGTNYIIVKVRAD DNKYMHLKVFKSFLNN AYQIFEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	247
CLONE 079	RWWDEEHPI	54	SLDSLYHKV	151	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVRWW DEEHPIGTNYIIVKVRAG DNKYMHLKVFKSLSLDS LYHKVEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	248
CLONE 080	WWVEWEPEI	55	FQDNDYHIF	152	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVWVWV EWEPEIGTNYIIVKVRAG DNKYMHLKVFKSFLQDN DYHIFEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	249
CLONE 081	FWEHTWVEQ	56	SLSEETII	153	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVFWE HTWVEQGTNYIIVKVRAG DNKYMHLKVFKSLSLSE EYTIIEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	250
CLONE 085	HVWSEKHHV	57	SLTAKYEIL	154	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVHVW SEKHHVGTNYIIVKVRAG DNKYMHLKVFKSLSLTA KYEILEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	251
CLONE 087	QHQSLSHAI	58	NLNRDYVII	155	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVQHQ KSLHAIGTNYIIVKVRAG DNKYMHLKVFKSLSLNR DYVIIEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	252
CLONE 088	YFHYLGYEW	59	GPPWQYQKG	156	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVYFH YLGYEWTNYIIVKVRAG DNKYMHLKVFKSLSGPPW QYQKGEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	253

CLONE 089	YHDVDP TTT	60	NKLPTGYGV	157	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVVYHD VDPTTGTNYI KVRAG DNKYMHLKVFKSLNKL P TGYGVEDLVLTGYQV DK NKDDEL TGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	254
CLONE 093	WIDDEQFPV	61	TLDFDYVIQ	158	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVVWID DEQFPVGTNYI KVRAG DNKYMHLKVFKSLT LDF DYVIQEDLVLTGYQV DK NKDDEL TGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	255
CLONE 095	IKQYPAELAWW	62	FTDKNPASV	159	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVVIKQ YPAELAWGTNYI KVR AGDNKYMHLKVFKSL FT DKNPASVEDLVLTGYQV DKNKDDEL TGF AAAEQK LISEEDLAAHHHHHHAA AEQKLI SEEDLAAHHHH HH	256
CLONE 096	DAHLDIHWY	63	DRIEWFGN	160	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVVD A LDIHWYGTNYI KVRAG DNKYMHLKVFKSLDR IE YWFGNEDLVLTGYQV DK NKDDEL TGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	257
CLONE 097	WDNPKELWE	64	KEHPYDEKF	161	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVVDN PKELWEGTNYI KVRAG DNKYMHLKVFKSLKEHP YDEKFEDLVLTGYQV DK NKDDEL TGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	258
CLONE 100	QSWDKHYQLRE	65	EVPHHAHGV	162	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVLAQS WDKHYQLRESTNYI KV RAGDNKYMHLKVFN GPE VPHHAHGVADRVLTGYQ VDKNKDDEL TGF AAAEQ KLI SEEDLAAHHHHHHAA AAEQKLI SEEDLAAHHH HHH	259
CLONE 102	WAEVAWVED	66	WETYDSYGF	163	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVWAE VAWVEDGTNYI KVRAG DNKYMHLKVFKSLWET Y DSYGFEDLVLTGYQV DK NKDDEL TGF AAAEQKLI SEEDLAAHHHHHHAAAE QKLI SEEDLAAHHHHHH	260
CLONE 103	DYHWLWVVG	67	SLTREYTTQ	164	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEK TGET YGKLEAVQYKTQVVDYH	261

					WLWVVGGTNYIYIKVRAG DNKYMHLKVFKSLSLTR EYTTQEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLISEEDLAAHHHHHH	
CLONE 105	KWSQNSVTQ	68	TLERTFRVV	165	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVKWS QNSVTQGTNYIYIKVRAG DNKYMHLKVFKSLTLER TFRVVEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLISEEDLAAHHHHHH	262
CLONE 108	YWSDLWVY	69	WKINLFAED	166	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAIW SDLWFVYSTNYIYIKVRA GDNKYMHLKVFNPGWKI NLFAEDADRVLTGYQVD KNKDELTFG AAAEQKLI SEEDLAAHHHHHHAAAE QKLISEEDLAAHHHHHH	263
CLONE 109	DQIRFQAHH	70	SLDKLYQVI	167	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVDQI RFQAHGNTNYIYIKVRAG DNKYMHLKVFKSLSLDK LYQVIEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	264
CLONE 110	YPGEGDFAV	71	ALNRDYYII	168	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVYPG EGDFAVGNTNYIYIKVRAG DNKYMHLKVFKSLALNR DYYIIEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	265
CLONE 111	LWLESLINQ	72	NLSRNYQLW	169	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVLWL ESLINQGTNYIYIKVRAG NNKYMHLKVFKSLNLSR NYQLWEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	266
CLONE 112	YFRKHVHPV	73	SIDKYYQII	170	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVYFR KHVHPVGTNYIYIKVRAG DNKYMHLKVFKSLSIDK YYQIIEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	267
CLONE 113	DEDEYLLVS	74	FLDYFYNIG	171	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVDDE EYLLVSGTNYIYIKVRAG DNKYMHLKVFKSLFLDY FYNIGEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	268
CLONE 115	DHYAEHWDD	75	YKADIQWKH	172	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET	269

					YGKLEAVQYKTQVLADH YAEHWDDSTNYIYIKVRA GDNKYMHLKVFNPGPYKA DIQWKHADRVLTGYQVD KNKDDDELTF AAAEQKL I SEEDLAAHHHHHH	
CLONE 116	WPQGQDKTH	76	DDGSLEWLH G	173	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLAWP QGQDKTHSTNYIYIKVRA GDNKYMHLKVFNPGPDDG SLEWLHGADRVLTGYQV DKNKDDDELTF AAAEQK LI SEEDLAAHHHHHH	270
CLONE 117	WLLDGLLWK	77	LEHQYDEFL	174	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVWLL DGLLWKGTNYIYIKVRAG DNKYMHLKVFKSLLEHQ YDEFLEDVLTGYQVDK NKDDDELTF AAAEQKLI SEEDLAAHHHHHH	271
CLONE 118	KWQQLGPIQ	78	ALAKDYTLI	175	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVKWQ QLGPIQGTNYIYIKVRAG DNKYMHLKVFKSLALAK DYTLIEDVLTGYQVDK NKDDDELTF AAAEQKLI SEEDLAAHHHHHH	272
CLONE 119	KDHQASHTL	79	SLSGEYIVI	176	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVKDH QASHTLGTNYIYIKVRAG DNKYMHLKVFKSLSLSG EYIVIEDVLTGYQVDK NKDDDELTF AAAEQKLI SEEDLAAHHHHHH	273
CLONE 120	HYLYSLPVL	80	QDVKYWVIE	177	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVHYL YSLPVLGTNYIYIKVRAG DNKYMHLKVFKSLQDVK YWVIEEDVLTGYQVDK NKDDDELTF AAAEQKLI SEEDLAAHHHHHH	274
CLONE 124	YWAGKQLVL	81	NLALQFDTV	178	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLAYW AGKQLVLTSTNYIYIKVRA GDNKYMHLKVFNGNPLA LQFDTVADRVLTGYQVD KNKDDDELTF AAAEQKL I SEEDLAAHHHHHH	275
CLONE 125	FWIEPGYIY	82	IHLDPELTW	179	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLAFW IEPGYIYSTNYIYIKVRA GDNKYMHLKVFNGP IHL DPELTWADRVLTGYQVD KNKDDDELTF AAAEQKL I SEEDLAAHHHHHH	276
CLONE 129	LEGAQYYIS	83	FEQLYFSPK	180	MIPRGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVLALE GAQYYISSTNYIYIKVRA GDNKYMHLKVFNPGFEQ	277

					LYFSPKADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHH	
CLONE 130	DWAGLSFNE	84	IFPDVWEWG	181	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLADW AGLSFNESTNYIYIKVRA GDNKYMHLKVFNGLPIFP DVWEWGADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHH	278
CLONE 133	ADHGQGIW	85	AQLWVSPD W	182	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAAD HGQGIWSTNYIYIKVRA GDNKYMHLKVFNGLPAQL WVSPDWADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHH	279
CLONE 139	HQLGDYVRI	86	WLILINND	183	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLAHQ LGDYVRISTNYIYIKVRA GDNKYMHLKVFNGLPWL ILINNDADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHH	280
CLONE 141	NEEPWGEWY	87	WDPYQLIS	184	MIPRGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVLANE EPWGEWYSTNYIYIKVRA GDNKYMHLKVFNGLPWP YTQLISADRVLTGYQVD KNKDELDTGF AAAEQKL I SEEDLAAHHHHHH	281
CLONE 145	VYEKWLAVV	88	IPNNDILA	185	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVYE KWLAVVGTNYIYIKVRAG DNKYMHLKVFKSLIPNN DII LAEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	282
CLONE 157	DHRVPDIGI	89	GPSLYFNHF	186	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVDHR VPDIGIGTNYIYIKVRAG DNKYMHLKVFKSLGPSL YFNHFEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	283
CLONE 160	VSLHYVSAV	90	SEWAFFND	187	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVSL HYVSAVGTNYIYIKVRAG DNKYMHLKVFKSLSEWA FFNDEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	284
CLONE 162	VWRVSWAVE	91	WGVYEESKW	188	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVWR VSWAVEGTNYIYIKVRAG DNKYMHLKVFKSLWGVY EESKWEDLVLTGYQVDK NKDELDTGF AAAEQKLI SEEDLAAHHHHHH	285

CLONE 163	PRVEYVNE	92	GGPNGWLYA	189	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVPV EYVNEGNTNYYIKVRAG DNKYMHLKVFKS LGGP NWLYAEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	286
CLONE 164	IDRQSFSVV	93	WFFPPKYHP	190	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVIDR QSFSVVGNTNYYIKVRAG DNKYMHLKVFKS LWFPP PKYHPEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	287
CLONE 165	VDLQYVQVI	94	FNDANQIHW	191	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVDL QYVQVIGNTNYYIKVRAG DNKYMHLKVFKS LFND NQIHWEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	288
CLONE 166	VGWEGDIEE	95	SDQWPYVAI	192	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVGW EGDIEEGNTNYYIKVRAG DNKYMHLKVFKS LSDQW PYVAIEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	289
CLONE 171	LQWHGYLNG	96	FPIQEGIVH	193	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVLQW HGYLNGGNTNYYIKVRAG DNKYMHLKVFKS LFPIQ EGIVHEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	290
CLONE 172	WAEQAPYVHHF	97	LTFPVREAW	194	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVWAE QAPYVHHFGNTNYYIKVR AGDNKYMHLKVFKS LLT FPVREAWEDLVLTGYQV DKNKDDELTFG AAAEQK LISEEDLAAHHHHHH	291
CLONE 174	AHGQYLIVW	98	SNEVHAGDG	195	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVAHG QYLIVWGTNYYIKVRAG DNKYMHLKVFKS LSNEV HAGDGEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	292
CLONE 175	IDPIPAVY	99	DYFKFNHEH	196	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVIDP IPFAVYGTNYYIKVRAG DNKYMHLKVFKS LDYFK FNHEHEDLVLTGYQVDK NKDDELTFG AAAEQKLI SEEDLAAHHHHHH	293
CLONE 176	WDYWRELHA	100	RYLGQAADD	197	MIPGGLSEAKPATPEIQ EIVDKVKPQLEEKGET YGKLEAVQYKTQVVVDY	294

					WRELHAGTNYIYIKVRAG DNKYMHLKVFKSRLYLG QAADEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHH	
CLONE 177	YHLEWLQQW	101	YFDSLQWDP	198	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVYHL EWLQQWGTNYIYIKVRAG DNKYMHLKVFKSRLYFDS LGWDPEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHH	295
CLONE 179	PYERWLVT	102	VDIANLFEY	199	MIPGGLSEAKPATPEIQ EIVDKVKPQLEKTGET YGKLEAVQYKTQVVPYE RWLVSTGTNYIYIKVRAG DNKYMHLKVFKSRLVDIA NLFEYEDLVLTGYQVDK NKDDELTGF AAAEQKLI SEEDLAAHHHHHH	296

In some embodiments, the TNFR2 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: 297 to 393, optionally
5 excluding the nucleotides encoding the twenty one carboxy terminal amino acids. In some embodiments, the TNFR2 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: 297 to 393 optionally excluding the nucleotides encoding the twenty one carboxy
10 terminal amino acids 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 2. Nucleic acid sequences of the present invention. The 5' 63 nucleotides encoding C-terminal 21 amino acids are extra sequences added for cloning and assay purposes: 3xAla linker, ten amino acid Myc tag, 2xAla linker and 6xHis tag, all of which are optional and not required for the present invention.

Clone Name	DNA sequence	DNA SEQ ID NO:
CLONE 001	ATGATTCCCGGCGGACTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCCGGTCAGGATCAGTGGGGTAACTAC GCAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGCCAGATATCCACATAACCATTGGCATGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATACCA CCAC	297
CLONE 003	ATGATTCCCGGCGGACTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCCGGTGAAGATAAATGGCTGAACCTAC GCAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT	298

	GAAAGTGTTTAAGAGCCTGGAATCTGAAGATCATGGTCAGAACACCGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	
CLONE 004	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCGTAACCTGGGTAACATCTACCAG TGGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTCTGAACGCAAGATACGCACTGGATGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	299
CLONE 005	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCAAACGTCTTACGATCTGCCACCA GTTGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTCTGAACAAAGAATCTATCATCGTTGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	300
CLONE 006	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGTTTACTGGGATATCCTGGTTGAA CTGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGATCTCTGGTGGTTACTCTGCACAGACCGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	301
CLONE 007	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGTCTGGATATCTGGGGTAACAAC GCAGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGGCAGATACCGCAAGAACCGAAGAAGCAGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	302
CLONE 008	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTTCTGGTATACCAACCTGTGG CAGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGAGAGGTTTCGGTAACGTTAAAAAAGTTGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	303
CLONE 009	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCGTCTGTTCCATATCTGGCGTTGG TGGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGATCCATATCTACACCGATTCTATCCAGGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	304
CLONE 010	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCAGGAACCAGGTGTTTGGTGGTGG TGGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGAGAGAAGGTGATTTTCGATTACGATATCGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	305

CLONE 011	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGAACTGGAACGATATCTACGAA CATGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGACCGATCAGTACAAAATCTCTGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	306
CLONE 012	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTACTGGGGTGTTTCAGGGTGCACTG GATGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGAACTGGAACCATGAATTCGATCTGGTTGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	307
CLONE 013	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGCACATGATTACGATGGTTACAAA TGGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTGTCTAACAGAGCATCTGAACATGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	308
CLONE 014	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGCAGGATTACAGTGCGGTGGT CAGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTACCAGCATATCGATCCACCATACTCTGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	309
CLONE 015	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGAATGGGAAGTTGGTGATGTTTGG GGTGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTGGTGGGTTAGAGGTTTCTTGAACACAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	310
CLONE 016	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGCAGAAACCGCAAATGGTTT TTTTACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGGTTTCTTACATCGAAGGTGCATGGAAAGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	311
CLONE 021	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTGAACTGGGTGAATGGCAG TGGTACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAAGTGTTCAACGGCCCCGAGCCACCAGATTGGGGTTGGACCTTTGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	312
CLONE 022	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTGATACCGATCATTACTGG TACCTGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGACCCATGAACGTGAAACCGGTTTTACCGCGGACC	313

	GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	
CLONE 023	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGT CATACCGATCAGTTTTGG CTGACCTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGATCTGGCAGGGTGATTACGATCGTTACGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	314
CLONE 026	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACTGGAAATCCCATCTGCAATC ATCTACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGTACTTTTGGGGTGATCAGGAACATTGGGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	315
CLONE 027	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACAGCTGGGTAACGGTTGG GATGCATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGTACCAGGCAAAACCAAACCTGGCGTCATGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	316
CLONE 032	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATTCTAACGGTGTTTCTGCA GTTATCTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGACCTGGAACATCAAACCACAGTGGCCAGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	317
CLONE 035	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATT CAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCAAATGGCATCAGAAACAGCTGCCA CTGGGTACGAAC TACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTCTGAACAACAACCTCAAATCCAGGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	318
CLONE 036	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATT CAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCATGATGAACATATCTCTCATCCA GTTGGTACGAAC TACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGGATTGGTGGGCAAACATCAACGGTACC GAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	319
CLONE 037	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATT CAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTGCGAGTTAACGATTTTGAACCATCT CTGGGTACGAAC TACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTTCCAGCTGCCAGATTACTCTGTTAACGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	320
CLONE 038	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATT CAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC	321

	TGGAAGCCGTCAGTATAAGACTCAAGTCGTCCAGCATGCATTGATGGTTACGCA TGGGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGCTGCAAGTCTGCAATCAAAGGTATCTTCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	
CLONE 042	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTGTTGGTGATGAAACCTTCGATGCA CAGGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGCAATCCAGGTTATCGGTTACGGTACCAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	322
CLONE 043	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCCATTACCATGAACGTCATCGTATC CAGGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGGATAAACAGTACAACATCATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	323
CLONE 044	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTCTGTTCCAGAAGCAGTTTACTGG GTTGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGATCTGGAACGATAACGCAGCACAGCATGAAGATTTGG TGCTGACGGGCTACCAAGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	324
CLONE 045	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTGTTAACGATGAAGGTGATCATATC GCAGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTACAACGTTACCGGTTACGTTTATTGGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	325
CLONE 049	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTACGATACCGATTGGCAGCAGGAT GCAGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGCATACTGGTACGATGTTTACGGGTTGGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	326
CLONE 051	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTGGCTGGTTCATGCAAAAACCTGG CTGGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGCTGGAACCACAGTACAACGTTGCAAACGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	327
CLONE 052	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCAAACGTGAACGTTGTTTACTTGG TGGGGTACGAACACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGATGAAGATGTTTCTTACGCATACGAAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG	328

	GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	
CLONE 056	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTGCGGGTGACAACAAGTATATGCACCT CTGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGCTGGAACATCCATACGATCAGTACGGTGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	329
CLONE 057	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCAAAGGTTGGGGTGAAAAACCTGG GAAGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGCAGCTGGATGGTTGGGCAGAATACTTCGGTGAAGATT TGGTGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	330
CLONE 058	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTGCGGGTGACAACAAGTATATGCACCT TCTGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGAATACCAGAGATACTGGTTCGAATTTCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	331
CLONE 059	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTTCAACGTTTCAGGATATCTGGAAC CATGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGCACTGAACCATCAGTACACCATCCAGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	332
CLONE 060	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAGTCAAGCCGCGAGCTGGAAGAGAAAACCGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAACCGAATCTTTTGAACAGCTG GGTTACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCGTCTACCGGTGGTTTTTTCTTGAAAGTTGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	333
CLONE 063	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTGCGGGTGACAACAAGTATATGCACCT TACGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGAACAGACAGTACGCAGTTATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	334
CLONE 064	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCCATCAGCCAGAAGTTAAACATGCA TGGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGCCAATCAACCCATGGAACCTGCTGCCAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	335
CLONE 065	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTGCGGGTGAGGATAAATCTGTTGAT	336

	GTTGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGAGACAGTACTACGATGAAGAAACCGAAGATTTGGTGC TGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCC GCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCACCA C	
CLONE 067	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGTTGAAGGTCATGAACATTGG GATTGGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGATGATTGGCAGCAGCAGACCGAAGTTGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	337
CLONE 069	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGGTCCATTTAGCTGCAGCTG GAAGATTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGTGGGAAGTTGTTAACGCATACTTTCCAGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	338
CLONE 071	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCTGTCGAACCATACAACCGTCTGTCTGTT CATGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTCTGAACAAAGATTACTTCATCGAAGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	339
CLONE 072	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCTCAAATCTGTTTATGATGTTTACGAG CATGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGACCCTGACCAGAGGTTACGCAGTTGAAGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	340
CLONE 073	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCTGTCGATGATTACGATGATCTGCATTGG TGGGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGACCTCTGTTGAATACTGGTGGGATGCAGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	341
CLONE 076	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGCATGGCATGATGATGATTCT CTGCAGTCCACCAACTATTACATTAAGGTTCTGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGAAGTTGGCCAGAAGTTGATATCCGTTGGGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	342
CLONE 077	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCTGCACCGAAGAACAGGTTCTGGGTATC TCTGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGACCCTGACCAGACATTACCTGATCCATGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACC CCAC	343

CLONE 078	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTCTATCTACGATGATGAAGTTAAC GATGGTACGAACACTACTACATCAAGGTTTCGTGCGGATGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTTCCCTGAACAACGCATACCAGATCTTCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	344
CLONE 079	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCGTTGGTGGGATGAAGAACATCCA ATCGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGGATTCTCTGTACCATAAAGTTGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	345
CLONE 080	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGTGGGTTGAATGGGAACCAGAA ATCGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTTCCAGGATAACGATTACCATATCTTCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	346
CLONE 081	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTTCTGGGAACATACCTGGGTTGAA CAGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGTCTGAAGAATACACCATCATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	347
CLONE 085	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCATGTTTGGTCTGAAAAACATCAT GTTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGACCGCAAATACGAAATCCTGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	348
CLONE 087	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCAGCATCAGAAATCTCTGCATGCA ATCGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGAACCTGAACAGAGATTACGTTATCATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	349
CLONE 088	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTACTTCCATTACCTGGGTTACGAA TGGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGGTCCACCATGGCAGTACCAGAAAGGTGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	350
CLONE 089	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTACCATGATGTTGATCCAACCACC ACCGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGAACAAACTGCCAACCAGTTACGGTGTTGAAGATTTGG	351

	TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	
CLONE 093	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGATCGATGATGAACAGTTCCCA GTTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGACCCTGGATTTCGATTACGTTATCCAGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	352
CLONE 095	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTCATCAAACAGTACCCAGCTGAACTG GCATGGTGGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATAT GCACCTGAAAGTGTTTAAAGAGCCTGTTCCACCGATAAAAACCCAGCATCTGTTGAAG ATTTGGTGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGT TTCGCGGGCCGTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACC TCACCACCAC	353
CLONE 096	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGATGCACATCTGGATATCCATTGG TACGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGATAGAATCGAATACTGGTTTCGGTAACGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	354
CLONE 097	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGGATAACCCAAAAGAACTGTGG GAAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGAAAGAACATCCATACGATGAAAAATTTCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	355
CLONE 100	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAGTCAAGCCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACAGTCTTGGGATAAACATTAC CAGCTGCGTGAATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTA TATGCACCTGAAAGTGTTCAACGGCCCGGAAGTTCCACATCATGCACATGGTGTTG CGGACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACG GGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCA CCATCACCACCAC	356
CLONE 102	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGGCAGAAGTTGCATGGGTGAA GATGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTGGGAAACCTACGATTCTTACGGTTTCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	357
CLONE 103	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGATTACCATTTGGCTGTGGGTTGTT GGTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTCTGACCAGAGAATACACCACCCAGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	358
CLONE 105	ATGATTCCCGGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC	359

	TGGAAGCCGTCAGTATAAGACTCAAGTCGTCAAATGGTCTCAGAACTCTGTTACC CAGGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGACCCTGGAAAGAACCTTCAGAGTTGTTGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	
CLONE 108	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATACTGGTCTGATCTGTGGTTT GTTTACTCCACCAACTATTACATTAAGGTTCTGTCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCACGGCCCGTGGAAAATCAACCTGTTTGCAGAAGATGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	360
CLONE 109	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTGATCAGATCCGTTTCCAGGCACAT CATGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTCTGGATAAACTGTACCAGGTTATCGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	361
CLONE 110	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTACCCAGGTGAAGGTGATTTTCGCA GTTGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGGCACTGAACAGAGATTACTACATCATCGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	362
CLONE 111	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTGTGGCTGGAATCTCTGATCAAC CAGGGTACGAACTACTACATCAAGGTTCTGTGCGGGTAACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGAACCTGTCTAGAACTACCAGCTGTGGGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	363
CLONE 112	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTACTTCCGTAAACATGTTTCATCCA GTTGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTATCGATAAATACTACCAGATCATCGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	364
CLONE 113	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCAGTATAAGACTCAAGTCGTCTGATGAAGATGAATACCTGCTGGTT TCTGGTACGAACTACTACATCAAGGTTCTGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTTCTTGATTACTTCTACAACATCGGTGAAGATTTGG TGCTGACGGGTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	365
CLONE 115	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATCATTACGCAGAACATTGG GATGATTCACCAACTATTACATTAAGGTTCTGTCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCACGGCCCGTACAAAGCAGATATCCAGTGGAACATGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC	366

	GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	
CLONE 116	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATGGCCACAGGGTCAGGATAAA ACCCATTCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGATGATGGTTCTCTGGAATGGCTGCATGGTGCGG ACCGTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGT TTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCA TCACCACCAC	367
CLONE 117	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGCTGGATGGTCTGCTGTGG AAAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGCTGGAACATCAGTACGATGAATTCCTGGAAGATTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	368
CLONE 118	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCAAATGGCAGCAGCTGGGTCCAATC CAGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGGCACTGGCAAAAGATTACACCCTGATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	369
CLONE 119	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCAAAGATCATCAGGCATCTCATACC CTGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGTCTCTGTCTGGTGAATACATCGTTATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	370
CLONE 120	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTACAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCATTACCTGTACTCTCTGCCAGTT CTGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAGAGCCTGCAGGATGTTAAATACTGGGTTATCGAAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	371
CLONE 124	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATACTGGGCAGGTAAACAGCTG GTTCTGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCAACCCTGGCACTGCAGTTTGATACCGTTGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	372
CLONE 125	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCATTTTGGATCGAACCAGGTTAC ATCTACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCA CCTGAAAGTGTTCAACGGCCCCGATCCATCTGGATCCAGAACTGACCTGGGCGGACC GTGTTCTGACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTC GCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCA CCACCAC	373
CLONE 129	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAAT TGTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGC TGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCACTGGAAGGTGCACAGTACTAC	374

	ATCTCTTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCCGTTTGAACAGCTGTACTTTTCTCCAAAAGCGGACCGTGTTCGTACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACACCAC	
CLONE 130	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAATGTTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATTGGGCAGGTCTGTCTTTTAAACGAATCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCCGATCTTTCCAGATGTTTGGGAATGGGGTGCGGACCGTGTTCGTACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACACCAC	375
CLONE 133	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAATGTTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGCAGATCATGGTCAGGGTATCATCTGGTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCCGGCACAGCTGTGGGTTTCTCCAGATTGGGCGGACCGTGTTCGTACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACACCAC	376
CLONE 139	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAATGTTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAGATCAGCTGGGTGATTACGTTCTATCTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAGTGTTCAACGGCCCCGTGGCTGATCATCCTGATCAACAACGATGCGGACCGTGTTCGTACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACACCAC	377
CLONE 141	ATGATCCCGAGGGGACTGTCCGAGGCCAAGCCTGCCACCCCTGAGATCCAGGAAATGTTCGACAAAAGTCAAGCCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGTAAGCTGGAAGCGGTCCAGTACAAAACCCAAGTGCTAGCAAACGAAGAACCATGGGTGAAATGGTACTCCACCAACTATTACATTAAGGTTTCGTGCCGGTGACAATAAGTATATGCACCTGAAAAGTGTTCAACGGCCCCGTGGGATCCATACACCCAGCTGATCTCTGCGGACCGTGTTCGTACCGGTTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACACCAC	378
CLONE 145	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAATCGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAACCTGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGTTTACGAAAAATGGCTGGCAGTTGTTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCTGAAAGTGTTTAAAGAGCCTGATCCCAAACAACGATATCATCCTGGCAGAAGATTTGGTGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCAACAC	379
CLONE 157	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAATCGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAACCTGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGATCATCGTGTTCAGATATCGGTATCCGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCTGAAAGTGTTTAAAGAGCCTGGGTCCATCTCTGTACTTCAACCATTTCGAAGATTTGGTGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCAACAC	380
CLONE 160	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAATCGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACGGGCGAAACCTACGGCAAACCTGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGTTTCTCTGCATTACGTTTCTGCAGTTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCTGAAAGTGTTTAAAGAGCCTGTCTGAATGGGCATTCTTCTTCAACGATGAAGATTTGGTGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCAACAC	381

CLONE 162	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCGTTTGGCGTGTTTCTTGGGCAGTT GAAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTGGGGTGTTTACGAAGAATCTAAATGGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	382
CLONE 163	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCACGTTGAATACGTTAACAAC GAAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGGTGGTCCAAACGGTTGGCTGTACGCAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	383
CLONE 164	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCATCGATCGTCAGTCTTTCTCTGTT GTTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTGGTTCTTCCACCAAATAACCATCCAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	384
CLONE 165	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCGTTGATCTGCAGTACGTTTCAGGTT ATCGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTTCAACGATGCAAACAGATCCATTGGGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	385
CLONE 166	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCGTTGGTTGGGAAGGTGATATCGAA GAAGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTGATCAGTGGCCATACGTTGCAATCGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	386
CLONE 171	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCTGCAGTGGCATGGTTACCTGAAC GGTGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTTCCCAATCCAGGAAGGTATCGTTTCATGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	387
CLONE 172	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCGTTGGCAGAACAGGCACCATACGTT CATCATTTTCGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATAT GCACCTGAAAGTGTTTAAAGAGCCTGCTGACCTTCCAGTTAGAGAAGCATGGGAAG ATTTGGTGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGT TTCGCGGCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACC TCACCACCAC	388
CLONE 174	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCGCACATGGTCAGTACCTGATCGTT TGGGGTACGAACACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTCTAACGAAGTTTCATGCAGGTGATGGTGAAGATTTGG	389

	TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	
CLONE 175	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTCGATCCAATCCCATTTCGCAAGTT TACGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGATTACTTCAAATTC AACCATGAACATGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	390
CLONE 176	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTGGGATTACTGGCGGGAAGTGCAT GCAGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGAGATACCTGGGTGAGGCAGCAGATGATGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	391
CLONE 177	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCTACCATCTGGAATGGCTGCAGCAG TGGGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGTACTTTCGATTCTCTGGGTGGGATCCAGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	392
CLONE 179	ATGATTCCCGGCGGACTTTTCGGAAGCCAAGCCAGCCACTCCGGAGATTTCAGGAAAT CGTGGACAAGGTCAAACCGCAGCTGGAAGAGAAAACCGGCGAAACCTACGGCAAAC TGGAAGCCGTCCAGTATAAGACTCAAGTCGTCCCATACGAACGTTGGCTGGTTTCT ACCGGTACGAACTACTACATCAAGGTTTCGTGCGGGTGACAACAAGTATATGCACCT GAAAGTGTTTAAAGAGCCTGGTTGATATCGCAAACCTGTTTCAATACGAAGATTTGG TGCTGACGGGCTACCAGGTTGACAAGAACAAGATGACGAGCTGACGGGTTTCGCG GCCGCTGAACAGAAGCTGATCTCCGAAGAAGATCTGGCCGCGCATCACCATCACCA CCAC	393

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 TNFR2 AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human TNFR2 as a monomer with a dissociation constant (KD) 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 TNFR2 AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human TNFR2 as a monomer with an off-rate constant (Koff), such as measured by BIACORE™ assay, 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 TNFR2 AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human TNFR2 as a monomer with an association constant (K_{on}), such as measured by BIACORE™ assay, of at least about 10^3 M⁻¹s⁻¹ or faster; at least about 10^4 M⁻¹s⁻¹ or faster; at least about 10^5 M⁻¹s⁻¹ or faster; or even at least about 10^6 M⁻¹s⁻¹ or faster.

In some embodiments, the AFFIMER® agent is a TNFR2 AFFIMER® agent comprising an AFFIMER® polypeptide portion that binds human TNFR2 as a monomer with an IC₅₀ in a competitive binding assay with human TNFR2 of 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 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).

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® polypeptides. For example, the additions, substitutions or deletions may modulate affinity for the AFFIMER® polypeptide, e.g., for binding to and inhibiting TNFR2, 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® polynucleotide), 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. Many 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
 5 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
 10 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:

15 Table 3. Signal peptide sequences

Native Protein	Signal Sequence	SEQ ID NO:
Human Serum Albumin (HSA)	MKWVTFISLLFLFSSAYS	483
Ig kappa light chain	MDMRAPAGIFGFLVLFPGYRS	394
Human azurocidin preprotein	MTRLTVLALLAGLLASSRA	395
IgG heavy chain	MELGLSWIFLLAILKGVQC	396
IgG heavy chain	MELGLRWVFLVAILEGVQC	397
IgG heavy chain	MKHLWFFLLLVAAPRWVLS	398
IgG heavy chain	MDWTWRILFLVAAATGAHS	399
IgG heavy chain	MDWTWRFLFVAAATGVQS	400
IgG heavy chain	MEFGLSWLFLVAILKGVQC	401
IgG heavy chain	MEFGLSWVFLVALFRGVQC	402
IgG heavy chain	MDLLHKNMKHLWFFLLLVAAPRWVLS	403
IgG Kappa light	MDMRVPAQLLGLLLWLSGARC	404
IgG Kappa light	MKYLLPTAAAGLLLLAAQPAMA	405
Gaussia luciferase	MGVKVLFALICIAVAEA	406
Human chymotrypsinogen	MAFLWLLSCWALLGTTFG	407
sHuman interleukin-2	MQLLSCIALILALV	408
Human trypsinogen-2	MNLLLILTFVAAAVA	409
Human CD33	MPLLLLLPLWAGALA	410
Prolactin	MDSKGSSQKGSRLLLL VVSNLLLCQGVVS	411

Native Protein	Signal Sequence	SEQ ID NO:
Human tPA	MDAMKRGLCCVLLLCGAVFVSPS	412
Synthetic/Consensus	MLLLLLLLLLLALALA	413
Synthetic/Consensus	MWWRLWWLLLLLLLLWPMVWA	414

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: 421) 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 GRA, poly(Gly), poly(Ala) and those provided in Table 4.

Table 4. Linker sequences.

Sequence	SEQ ID NO:
(GGGGS) _n (e.g., n = 1-6)	415
(Gly) ₈	416
(Gly) ₆	417
KESGSVSSEQLAQFRSLD	418
EGKSSGSGSESKST	419
GSAGSAAGSGEF	420
(EAAAK) _n (e.g., n = 1-6)	421
A(EAAAK) ₄ ALEA(EAAAK) ₄ A	422
PAPAP	423
AEAAAKEAAKA	424
(Ala-Pro) _n (10 to 34 aa)	425
GGSG	426
GSGS	427
GGGS	428
S(GGS) _n (n=1-7)	429

Sequence	SEQ ID NO:
GGGSGGG	430
ESGGGGVT	431
LESGGGGT	432
GRAQVT	433
WRAQVT	434
ARGRAQVT	435
SGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT	436
SGTSPGLSAGATVGIMIGVLVGVALI	437
SAPVLSAVATVGITIGVLARVALI	438
SSPDL SAGTAVSIMIGVLGAMALI	439
TLGGNSASYTFVSLLFSAVTLLLLC	440
SGTSPGLSAGATVGIMIGVLVGVALI	441

The AFFIMER® polypeptide may further comprise, for example be fused to, a transmembrane domain (TM domain). Such TM domains ensure that the AFFIMER® polypeptide remains on the surface of the cell expressing the AFFIMER®. Such TM domains are readily available and include CD3, CD8, CD28, PDGFR TMD, and others.

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.

Multispecific Fusion Proteins

In some embodiments, an AFFIMER® agent is a multispecific polypeptide including, for example, a first TNFR2 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 TNFR2. The additional TNFR2 AFFIMER® polypeptide(s) may be the same or different (or a mixture thereof) as the first TNFR2 AFFIMER® polypeptide in order to create a multispecific AFFIMER® fusion protein.

The AFFIMER® agents can bind the same or overlapping sites on TNFR2 or can bind two different sites such that the TNFR2 AFFIMER® agent can simultaneously bind two sites on the same TNFR2 protein (biparatopic) or more than two sites (multiparatopic).

5 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 TNFR2 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-TNFR2 antibody has also been fused.

10 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
15 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
20 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
25 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
30 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®(s) can agonize the co-stimulatory receptor), a receptor, a cytokine, a growth factor, or a tumor-associated antigen, merely to illustrate. In some embodiments, the immunoglobulin portion may be a monoclonal antibody against at least one autoimmune target (e.g., TNFR2 or IL6-R). In some embodiments, the TNFR2 AFFIMER® polypeptide is part of an AFFIMER® agent that includes one or more binding domains that bind to a protein upregulated in autoimmune conditions (e.g., TNFR2 or IL6-R).

See William BA et al. J. Clin. Med. 2019; 8(8): 1261 for a review of TNFR2 AFFIMER® agent formats encompassed by the present disclosure.

In some embodiments, a multispecific a TNFR2 AFFIMER® agent may further comprise a half-life extension moiety, such as any of those described herein. For example, a TNFR2 AFFIMER® agent may comprise at least one TNFR2 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., CD3ε chain or CD16) further linked to a half-life extension moiety, such as a fragment crystallizable (Fc) domain (e.g., an FcγR null-binding Fc), 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.

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.

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 half-life 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®-Fc fusion can produce an avidity effect if the AFFIMER® targets are spaced closely enough together or are themselves dimers. This may be desirable or not depending on the target.

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 some embodiments, the Fc region is a FcγR null-binding Fc region. In this context, an Fc fusion ("Fc-fusion"), such as a TNFR2 AFFIMER® agent created as an AFFIMER®-Fc fusion protein, is a polypeptide comprising at least one TNFR2 AFFIMER® 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 TNFR2 AFFIMER® sequence as part of the same polypeptide. An immunoglobulin Fc region may also be linked indirectly to at least one TNFR2 AFFIMER® polypeptide. Various linkers are known in the art and can optionally be used to link an Fc to a polypeptide including a TNFR2 AFFIMER® 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 TNFR2 AFFIMER® agent that comprises a TNFR2 AFFIMER® polypeptide linked to an Fc domain linked to another TNFR2 AFFIMER® polypeptide (TNFR2 AFFIMER® polypeptide-Fc domain-TNFR2 AFFIMER® polypeptide).

There are several reasons for choosing the Fc region of human antibodies for use in generating TNFR2 AFFIMER® agents as TNFR2 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:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV
MHEALHNHYTQKSLSLSPGK (SEQ ID NO: 442)

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: 536)) of the exemplary human IgG1 immunoglobulin Fc domain sequence provided above. In some embodiments, the AFFIMER®-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: 443) or EPKSCDKTHTCPPCPAPELLG (SEQ ID NO: 444).

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: 445);
 EPKSSDKTHTCPPCPAPELLGGPS (SEQ ID NO: 446);
 EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO: 447);
 EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 448);
 DKTHTCPPCPAPELLGGPS (SEQ ID NO: 449); and
 DKTHTCPPCPAPELLGGSS (SEQ ID NO: 450).

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: 451),
 EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 452), and DKTHTCPPCPAPELLGGSS (SEQ ID NO: 453).

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: 448). 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: 446), EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO: 451), and EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 452).

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 NOs: 454-467 or an Fc sequence from the examples provided by SEQ ID NOs: 454-467. 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: 454-467, except that the C-terminal lysine thereof is omitted. In some embodiments, the Fc domain comprises the amino acid sequence selected from SEQ ID NOs: 454-467. In some embodiments, the Fc domain comprises the amino acid sequence selected from SEQ ID NOs: 454-467 except the C-terminal lysine thereof is omitted.

Table 5. Fc domain sequences.

Name	Sequence	SEQ ID NO:
hIgG1a_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVHLHQLDNLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	454
hIgG1a_189 [hIgG1a_191 sans "GK" on C term; A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVHLHQLDNLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSP	455
hIgG1a_191b [A/F subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVHLHQLDNLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	456

hIgG1f_1.1_191 [Contains five point-mutations to alter ADCC function, F subtype]	DKTHTCPPCPAPEAEAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	457
hIgG1f_1.1_186 [Contains five point-mutations to alter ADCC function and C225S (Edlemen numbering); F subtype]	EPKSSDKTHTCPPCPAPEAEAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	458
hIgG1a_(N297G)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	459
hIgG1a_190 [hIgG1a_190 sans "K" on C term; A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG	460
hIgG1a_(N297Q)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	461
hIgG1a_(N297S)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYSS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	462
hIgG1a_(N297A)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	463
hIgG1a_(N297H)_191 [A subtype]	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYHS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	464

hIgG4	DKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK	465
hIgG4_(S241P)	DKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK	466
hIgG1 (Contain two point-mutations to alter ADCC function L20A, L21A)	SEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK	467

“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.

Albumin Fusions

In some embodiments, the AFFIMER® agent is a fusion protein comprising, in addition to at least one AFFIMER® 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: 468) 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.

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: 483) followed by a pro-domain of 6 amino acids (RGVFRR) (SEQ ID NO: 469); this 24 amino acid residue peptide may be referred to as the pre-pro domain. The AFFIMER®-HSA fusion proteins can be expressed and secreted using the HSA pre-pro-domain in the recombinant proteins coding sequence. Alternatively, the

AFFIMER®-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®-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®-containing polypeptide.

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).

AFFIMER® XT

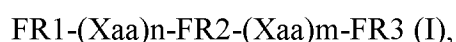
In some embodiments, the molecule that binds a serum protein such as HSA comprises an HSA AFFIMER® polypeptide. Examples of such HSA AFFIMER® polypeptides can be found in WO2022/023540. An HSA AFFIMER® polypeptide provided herein, in some embodiments, is linked to another molecule and extend the half-life of that molecule (e.g., a

therapeutic polypeptide). 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™ polypeptides can also be used to extend the half-life of other peptide or protein therapeutics, such as the TNFR2 AFFIMER® of the present invention.

In some embodiments, an HSA AFFIMER® polypeptide extends the serum half-life of the TNFR2 AFFIMER® polypeptide in vivo. For example, an HSA AFFIMER® polypeptide may extend the half-life of the TNFR2 AFFIMER® polypeptide 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 the TNFR2 AFFIMER® polypeptide 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 TNFR2 AFFIMER® polypeptide not linked to an HSA AFFIMER® polypeptide. In some embodiments, an HSA AFFIMER® polypeptide extends the half-life of the TNFR2 AFFIMER® polypeptide 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 TNFR2 AFFIMER® polypeptide not linked to an HSA AFFIMER® polypeptide. In some embodiments, an HSA AFFIMER® polypeptide extends the half-life of the TNFR2 AFFIMER® polypeptide 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.

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 10⁻⁶M or less.

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% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) identity to MIPGGLSEAK PATPEIQEIV DKVKPQLEEK TNETYGKLEA VQYKTQVLA (SEQ ID NO: 470); FR2 is an amino acid sequence having at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) identity to GTNYYIKVRA GDNKYMHLKV FKSL (SEQ ID NO: 2); FR3 is an amino acid sequence having at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) identity to EDL VLTGYQV DKNKDDELTF (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. Additional variations of this general structure can be found in WO2022/023540.

In all embodiments, each amino acid of (Xaa)_n may be the same or selected from any amino acid. The same applies for (Xaa)_m.

In some embodiments, the TNFR2 AFFIMER® polypeptide comprises an HSA AFFIMER® polypeptide comprising an amino acid sequence selected from any one of SEQ ID NOs: 471-477 (Table 6). In some embodiments, the TNFR2 AFFIMER® polypeptide has an extended serum half-life and comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95% or even 98% identical with a sequence selected from SEQ ID NOs: 471-477 (Table 6). Additional HSA AFFIMER® polypeptides sequences for use with the present invention can be found in WO2022/023540.

Table 6. Exemplary HSA AFFIMER® Polypeptide Sequences

Sequence	SEQ ID NO:
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLADWWQAKWP HSTNYYIKVRAGDNKYMHLKVFNGPYKVHQSSGGADRVL TGYQVDKNKDDELTF	471
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGYWAAKWP GSTNYYIKVRAGDNKYMHLKVFNGPFPNTSYDLQADRVL TGYQVDKNKDDELTF	472
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANWYQQRWP GSTNYYIKVRAGDNKYMHLKVFNGPWHNYGESSGADRVL TGYQVDKNKDDELTF	473
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAVWGPEYQHQ STNYYIKVRAGDNKYMHLKVFNGPNAGWPLVPEADRVL TGYQVDKNKDDELTF	474
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGHYARYWPG STNYYIKVRAGDNKYMHLKVFNGPWAQSKSVHQADRVL TGYQVDKNKDDELTF	475
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLAGFWASKWPG STNYYIKVRAGDNKYMHLKVFNGPFTAVSKKDAADRVL TGYQVDKNKDDELTF	476
MIPRGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVLANFFQRRWPG STNYYIKVRAGDNKYMHLKVFNGPWKFRNTDRGADRVL TGYQVDKNKDDELTF	477

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 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 (TNFR2 in the case of the TNFR2 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.

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: ⁴³K, ⁴⁷Sc, ⁵¹Cr, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, ⁶⁴Cu, ⁶⁷Ga, ⁶⁷Cu, ⁶⁸Ga, ⁷¹Ge, ⁷⁵Br, ⁷⁶Br, ⁷⁷Br, ⁷⁷As, ⁸¹Rb, ⁹⁰Y, ⁹⁷Ru, ^{99m}Tc, ¹⁰⁰Pd, ¹⁰¹Rh, ¹⁰³Pb, ¹⁰⁵Rh, ¹⁰⁹Pd, ¹¹¹Ag, ¹¹¹In, ¹¹³In, ¹¹⁹Sb, ¹²¹Sn, ¹²³I, ¹²⁵I, ¹²⁷Cs, ¹²⁸Ba, ¹²⁹Cs, ¹³¹I, ¹³¹Cs, ¹⁴³Pr, ¹⁵³Sm, ¹⁶¹Tb, ¹⁶⁶Ho, ¹⁶⁹Eu, ¹⁷⁷Lu, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁸⁹Re, ¹⁹¹Os, ¹⁹³Pt, ¹⁹⁴Ir, ¹⁹⁷Hg, ¹⁹⁹Au, ²⁰³Pb, ²¹¹At, ²¹²Pb, ²¹²Bi and ²¹³Bi. 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.

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®-containing 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.

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®-containing polypeptide or can be included as part of a fusion protein with the AFFIMER®-containing polypeptide. An illustrative example would be a diphtheria toxin-AFFIMER® fusion protein. In the case of non-peptide entities, the addition to the AFFIMER®-containing polypeptide will generally be by way of chemical conjugation to the AFFIMER®-containing 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 is sensitive to an environmental condition (such as pH) that permits the conjugated moiety to be released from the AFFIMER®-containing polypeptide, such as in a diseased tissue (or tissue to be protected if the conjugated moiety functions to protect healthy tissue).

Spacers

In some embodiments, an AFFIMER® polypeptide-drug conjugate comprises a spacer or bond (L1) between the half-life extension moiety and the substrate recognition sequence (SRS) cleavable by the enzyme, e.g., present in an inflammatory 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 responses are contemplated herein. In some embodiments, linkers are non-protein-coding.

In some embodiments, L1 is a hydrocarbon (straight chain or cyclic) such as 6-maleimidocaproyl, maleimidopropanoyl and maleimido ethyl cyclohexane-1-carboxylate, or L1 is N-Succinimidyl 4-(2-pyridylthio) pentanoate, N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate, N-Succinimidyl (4-iodo-acetyl) aminobenzoate.

In some embodiments, L1 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), L1 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.

Encoded AFFIMER® Polynucleotide for In vivo Delivery

An alternative approach to the delivery of therapeutic AFFIMER® agents, such as a TNF2 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® polynucleotide, 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® polynucleotide 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® polynucleotide can be adapted, including viral vectors, naked DNA and RNA.

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 TNFR2 AFFIMER® polynucleotide of the present disclosure.

An effective encoded AFFIMER® gene transfer approach results in 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. Likewise, polyadenylation of a transcribed encoded AFFIMER® transcript can also be important for nuclear export, translation, and mRNA stability. Therefore, in some embodiments, the encoded AFFIMER® polynucleotide will include a promoter and/or a polyadenylation signal sequence, as well as other regulatory elements well known in the art, such as enhancers, intronic sequences, post transcriptional regulatory agents and the like.

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® polynucleotides 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. TNFR2 AFFIMER® polypeptides may be encoded and delivered *in vivo* using retroviral vectors, such as gammaretroviruses or lentiviruses. In some embodiments the viral vector is pseudotyped with an envelope is chosen from one of amphotropic, polytropic, xenotropic, 10A1, GALV, VSV-G, Baboon endogenous virus, RD114, rhabdovirus, alphavirus, measles or influenza virus envelopes.

Non-viral Vectors

Exemplary nucleic acids or polynucleotides for the encoded TNFR2 AFFIMER® agents 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, α -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. Therefore, the encoded TNFR2 AFFIMER® polynucleotide may be delivered by plasmid DNA, minicircle DNA, mRNA, RNA replicons and the like. Means for delivery include transfection using electroporation, the use of lipofection and other transfection reagents, naked DNA delivery, gold particle delivery and other means known in the art.

Expression Methods and Systems for Protein Production

TNFR2 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 AFFIMER® polypeptide may be secreted or associated

with the expressing cell's membrane, for example through anchoring or tethering, such as by inclusion of a transmembrane domain.

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.

5 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
10 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
15 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
20 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
25 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
30 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 metallothioneine 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

5 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);

10 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);

15 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 (Mogam et al., Nature, 315 338-340, 1985; Kollias et al. (1986), Cell 46: 89-94); the myelin basic protein gene control

20 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

25 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.

30 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.),
 5 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
 10 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
 15 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
 20 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
 25 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
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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 (CaMV) 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, lymphotic 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, VERO, 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 Cell 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 aprt- 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 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® 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®/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.

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, for systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis and/or cancer. The methods of use may be in vitro, ex vivo, or in vivo methods.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE), commonly referred to simply as lupus, is a chronic autoimmune disease that can cause swelling (inflammation) and pain throughout the body. There are several different types of lupus. Systemic lupus erythematosus is the most common.

5 Other types of lupus include:

Cutaneous lupus erythematosus: This type of lupus affects the skin — cutaneous is a term meaning skin. Individuals with cutaneous lupus erythematosus may experience skin issues like a sensitivity to the sun and rashes. Hair loss can also be a symptom of this condition.

10 Drug-induced lupus: These cases of lupus are caused by certain medications. People with drug-induced lupus may have many of the same symptoms of systemic lupus erythematosus, but it is usually temporary.

15 Neonatal lupus: A rare type of lupus, neonatal lupus is a condition found in infants at birth. Children born with neonatal lupus have antibodies that were passed to them from their mother — who either had lupus at the time of the pregnancy or may have the condition later in life. Not every baby born to a mother with lupus will have the disease.

20 Therapies that may be used in combination with the TNFR2 AFFIMER® polypeptides provided herein include, for example: Steroids (corticosteroids, including prednisone); Hydroxychloroquine (Plaquenil®); Azathioprine (Imuran®); Methotrexate (Rheumatrex®); Cyclophosphamide (Cytoxan®) and mycophenolate mofetil (CellCept®); Belimumab (Benlysta®); and/or Rituximab (Rituxan®).

Lupus Nephritis

25 Lupus nephritis is a frequent complication in people who have systemic lupus erythematosus — more commonly known as lupus. Lupus nephritis occurs when lupus autoantibodies affect structures in your kidneys that filter out waste. This causes kidney inflammation and may lead to blood in the urine, protein in the urine, high blood pressure, impaired kidney function or even kidney failure. As many as half of adults with systemic lupus develop lupus nephritis. Systemic lupus causes immune system proteins to damage the kidneys, harming their ability to filter out waste.

Rheumatoid Arthritis

Rheumatoid arthritis is a type of chronic (ongoing) arthritis that occurs in joints on both sides of the body, such as hands, wrists and knees. The short-term goals of rheumatoid arthritis medications are to reduce joint pain and swelling and/or to improve joint function. The long-term goal is to slow or stop the disease process, particularly joint damage.

Arthritis is a general term that describes inflammation in joints. Rheumatoid arthritis is a type of chronic (ongoing) arthritis (resulting in pain and swelling) that occurs generally in joints symmetrically (on both sides of the body, such as hands, wrists and knees). This involvement of several joints helps distinguish rheumatoid arthritis from other types of arthritis.

In addition to affecting the joints, rheumatoid arthritis may occasionally affect the skin, eyes, lungs, heart, blood, nerves or kidneys.

Therapies that may be used in combination with the TNFR2 AFFIMER® agents provided herein to treat rheumatoid arthritis can include, for example:

Drugs that decrease pain and inflammation. These products include non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen (MOTRIN®), naproxen (ALEVE®), and other similar products. Another type of drug – the COX-2 inhibitor – also falls into this drug category, providing relief of the signs and symptoms of rheumatoid arthritis. Celecoxib (CELEBREX®), one COX-2 inhibitor, is available and used in the United States. The COX-2 inhibitors were designed to have fewer bleeding side effects on the stomach.

Disease-modifying antirheumatic drugs (DMARDs). Unlike other NSAIDs, DMARDs can actually slow the disease process by modifying the immune system. Older DMARDs include methotrexate (TREXALL®), gold salts, penicillamine (CUPRIMINE®), hydroxychloroquine (PLAQUENIL®), sulfasalazine (AZULFIDINE®), cyclosporine (SANDIMMUNE®), cyclophosphamide (CYTOXAN®) and leflunomide (ARAVA®). Currently, methotrexate, leflunomide, hydroxychloroquine, and sulfasalazine are the most commonly used. (Cyclosporine, cyclophosphamide, gold salts, and penicillamine are not typically used anymore.)

Biologics. Beyond these more "traditional" DMARDs, newer medications have been approved. Currently, there are seven different classes of medications and, in some cases, there are different kinds in each class. (Some of them, such as the class anti-TNFs, have been used since 2000.) Collectively, these DMARDs are known by another name – biologic agents (or

biologic response agents). Compared with the traditional DMARDs, these products target the molecules that cause inflammation in rheumatoid arthritis. Inflammatory cells in the joints are involved in the development of rheumatoid arthritis itself. The biologic agents cut down the inflammatory process that ultimately causes the joint damage seen in rheumatoid arthritis. The older DMARDs work one step further out than the biologics; they work by modifying the body's own immune response to the inflammation. By attacking the cells at a more specific level of the inflammation itself, biologics are considered to be more effective and more specifically targeted. The biologic agents include etanercept (ENBREL®), infliximab (REMICADE®), adalimumab (HUMIRA®), anakinra (KINARET®), abatacept (ORENCIA®), rituximab (RITUXAN®), certolizumab pegol (CIMZIA®), golimumab (SYMPONI®), tocilizumab (ACTEMRA®) and tofacitinib (XELJANJ®). Some of the biologics are used in combination with the traditional DMARDs, especially with methotrexate.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease. With these conditions, the immune system mistakenly attacks healthy cells. In people with MS, the immune system attacks cells in the myelin, the protective sheath that surrounds nerves in the brain and spinal cord. Damage to the myelin sheath interrupts nerve signals from the brain to other parts of the body. The damage can lead to symptoms affecting the brain, spinal cord and eyes.

There are four types of multiple sclerosis:

Clinically isolated syndrome (CIS): When someone has a first episode of MS symptoms, healthcare providers often categorize it as CIS. Not everyone who has CIS goes on to develop multiple sclerosis.

Relapsing-remitting MS (RRMS): This is the most common form of multiple sclerosis. People with RRMS have flare-ups -- also called relapse or exacerbation -- of new or worsening symptoms. Periods of remission follow (when symptoms stabilize or go away).

Primary progressive MS (PPMS): People diagnosed with PPMS have symptoms that slowly and gradually worsen without any periods of relapse or remission.

Secondary progressive MS (SPMS): In many cases, people originally diagnosed with RRMS eventually progress to SPMS. With secondary-progressive multiple sclerosis, one continues to accumulate nerve damage. Symptoms progressively worsen. While one may still

experience some relapses or flares (when symptoms increase), one no longer has periods of remission afterward (when symptoms stabilize or go away).

Therapies that may be used in combination with the TNFR2 AFFIMER® polypeptides provided herein include, for example:

- 5 Disease-modifying therapies (DMTs): Several medications have FDA approval for long-term MS treatment. These drugs help reduce relapses (also called flare-ups or attacks). They slow down the disease's progression. And they can prevent new lesions from forming on the brain and spinal cord.

10 Relapse management medications: If there is a severe attack, a neurologist may recommend a high dose of corticosteroids. The medication can quickly reduce inflammation. They slow damage to the myelin sheath surrounding nerve cells.

Physical rehabilitation: Multiple sclerosis can affect physical function. Staying physically fit and strong will help maintain mobility.

15 Mental health counseling: Coping with a chronic condition can be emotionally challenging. MS can sometimes affect mood and memory. Working with a neuropsychologist or getting other emotional support is an essential part of managing the disease.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a group of disorders that cause chronic inflammation (pain and swelling) in the intestines.

20 Crohn's disease and ulcerative colitis are the main types of IBD. Types include:

Crohn's disease causes pain and swelling in the digestive tract. It can affect any part from the mouth to the anus. It most commonly affects the small intestine and upper part of the large intestine.

25 Ulcerative colitis causes swelling and sores (ulcers) in the large intestine (colon and rectum).

Microscopic colitis causes intestinal inflammation that's only detectable with a microscope.

Therapies that may be used in combination with the TNFR2 AFFIMER® polypeptides provided herein include, for example: aminosalicylates (an anti-inflammatory medicine like

sulfasalazine, mesalamine or balsalazide) minimize irritation to the intestines; antibiotics treat infections and abscesses; biologics interrupt signals from the immune system that cause inflammation; corticosteroids, such as prednisone, keep the immune system in check and manage flares; immunomodulators calm an overactive immune system; antidiarrheal medication; nonsteroidal anti-inflammatory drugs (NSAIDs); vitamins and supplements like probiotics.

Graft versus Host Disease

Graft versus host disease (GvHD) is a condition that might occur after an allogeneic transplant. In GvHD, the donated bone marrow or peripheral blood stem cells view the recipient's body as foreign, and the donated cells/bone marrow attack the body.

There are two forms of GvHD: Acute graft versus host disease (aGvHD); and Chronic graft versus host disease (cGvHD).

Psoriasis

Psoriasis is a chronic skin disorder, which means a skin condition that doesn't go away. People with psoriasis have thick, pink or red patches of skin covered with white or silvery scales. The thick, scaly patches are called plaques. Psoriasis usually starts in early adulthood, though it can begin later in life. In addition to red, scaly patches, symptoms of psoriasis include: itchiness, cracked, dry skin, scaly scalp, skin pain, nails that are pitted, cracked or crumbly, and joint pain.

Therapies that may be used in combination with the TNFR2 AFFIMER® polypeptides provided herein include, for example: Steroid creams, Moisturizers for dry skin, anthralin (a medication to slow skin cell production), Medicated lotions, shampoos and bath solutions to improve scalp psoriasis, Vitamin D3 ointment, Vitamin A or retinoid creams, light therapy, PUVA (a treatment combines a medication called psoralen with exposure to a special form of UV light), methotrexate, retinoids, cyclosporine, and/or immune therapies.

Sjogren's Syndrome

Sjögren's syndrome is a lifelong autoimmune disorder that reduces the amount of moisture produced by glands in the eyes and mouth. It is named for Henrik Sjögren, a Swedish eye doctor who first described the condition. While dry mouth and dry eyes are the primary

symptoms, most people who have these problems don't have Sjögren's syndrome. Dry mouth is also called xerostomia.

There are two forms of Sjögren's syndrome: primary Sjögren's syndrome, which develops on its own, not because of any other health condition, and secondary Sjögren's syndrome, which develops in addition to other autoimmune diseases like rheumatoid arthritis, lupus and psoriatic arthritis.

Therapies that may be used in combination with the TNFR2 AFFIMER® polypeptides provided herein include, for example, treatments for dry eyes (e.g., artificial tears, prescription eye drops, punctal plugs, surgery, autologous serum drops), treatments for dry mouth (e.g., saliva producers), treatments for joint or organ problems (e.g., pain relievers, anti-rheumatics, immunosuppressants, steroids, antifungals, and treatments for vaginal dryness.

Myasthenia gravis

Myasthenia gravis (MG) is an autoimmune disease, meaning the body's immune system mistakenly attacks its own parts. MG affects the communication between nerves and muscles (the neuromuscular junction).

People with MG lose the ability to control muscles voluntarily. They experience muscle weakness and fatigue of various severity. They may not be able to move muscles in the eyes, face, neck and limbs. MG is a lifelong neuromuscular disease.

MG affects about 20 out of every 100,000 people. Experts estimate that 36,000 to 60,000 Americans have this neuromuscular disease. The actual number of people affected may be higher, as some people with mild cases may not know they have the disease. MG mostly affects women aged 20 to 40 and men aged 50 to 80. About one in 10 cases of MG occur in teenagers (juvenile MG). The illness can affect people of all ages but is rare in children.

Autoimmune MG is the most common form of this neuromuscular disease. Autoimmune MG may be:

Ocular: The muscles that move the eyes and eyelids weaken. The eyelids may droop, or you may not be able to keep your eyes open. Some people have double vision. Eye weakness is often the first sign of MG. Nearly half of people with ocular MG evolve into the generalized form within two years of the first symptom.

Generalized: Muscle weakness affects the eye and other body parts such as the face, neck, arms, legs and throat. It may be difficult to speak or swallow, lift the arms over the head, stand up from a seated position, walk long distances and climb stairs.

Therapies that may be used in combination with the TNFR2 AFFIMER® polypeptides provided herein include, for example, Medications, Monoclonal antibodies, IV immunoglobulin (IVIG), Plasma exchange (plasmapheresis), and/or Surgery.

Cancer

The binding of TNF to TNFR2 has been shown promote activation in tumour cells and tumour-associated cell types, as well as being affecting the immune response, such as tumour infiltrating lymphocytes, to create an environment in which the tumour can progress. Indeed, TNFR2 expression in tumours is associated with disease progression and poor clinical outcomes (Sheng Y, et al.). Therefore, the TNFR2 AFFIMER® polypeptides of the present invention may be useful in the treatment of cancer, either as monotherapy or in conjunction with other cancer treatments such as immunotherapies, e.g. CAR-T therapies, checkpoint inhibitors, and conventional cancer treatments.

Pharmaceutical preparations

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, 22nd 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 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.

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).

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. 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 ug to 100 mg/kg of body weight, from 0.1 ug to 100 mg/kg of body weight, from 1ug 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. In some embodiments, a method for treating inflammatory or autoimmune disease 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 another therapeutic 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.

Further embodiments of the invention are set out in the clauses below.

1. An engineered polypeptide that binds specifically to TNFR2 with a K_d of $1 \times 10^{-6} \text{M}$ or less, wherein the engineered polypeptide is a variant of a Stefin A protein.
2. The engineered polypeptide of clause 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 MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVV-(Xaa)n-Xaa2-TNYYIKVRAGDNKYMHLKVF-Xaa3-Xaa4-Xaa5-(Xaa)m-Xaa6-D-Xaa7-VLTGYQVDKNKDDELDTGF (SEQ ID NO: 4),
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 clause 2 wherein the amino acid sequence has 100% identity to an amino acid sequence of MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVV-(Xaa)n-Xaa2-TNYYIKVRAGDNKYMHLKVF-Xaa3-Xaa4-Xaa5-(Xaa)m-Xaa6-D-Xaa7-VLTGYQVDKNKDDELDTGF (SEQ ID NO: 4),
wherein Xaa, individually for each occurrence, is an amino acid residue; and n and m are each, independently, an integer from 3 to 20.
4. The engineered polypeptide of clause 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 MIPGGLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVD-(Xaa)n-GTNYYIKVRAGDNKYMHLKVF-KSL-(Xaa)m-EDLVLTGYQVDKNKDDELDTGF (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.

5. The engineered polypeptide of clause 4 wherein the amino acid sequence has 100% identity to an amino acid sequence of

5 MIPGGLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVD-(Xaa)_n-GTNYYIKVRAGDNKYMHLKVFKSL-(Xaa)_m-EDLVLTYGYQVDKNKDELDTGF (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.

10 6. The engineered polypeptide of any one of clauses 1 to 5 wherein n is an integer from 5 to 18.

7. The engineered polypeptide of clause 6 wherein n is an integer from 7 to 15.

8. The engineered polypeptide of clause 7 wherein n is an integer from 8 to 12.

9. The engineered polypeptide of any one of clauses 1 to 8 wherein m is an
15 integer from 5 to 18.

10. The engineered polypeptide of clause 9 wherein m is an integer from 7 to 15.

11. The engineered polypeptide of clause 10 wherein m is an integer from 8 to 12.

12. The engineered polypeptide of any one of clauses 1 to 11 that binds to TNFR2 with a K_d of 1×10^{-7} M.

20 13. The engineered polypeptide of clause 12 that binds to TNFR2 with a K_d of 1×10^{-8} M.

14. The engineered polypeptide of any one of clauses 1 to 13 wherein (Xaa)_n comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6-102.

15. The engineered polypeptide of clause 14 wherein (Xaa)_n consists of an amino
25 acid sequence selected from the group consisting of SEQ ID NO: 6-102.

16. The engineered polypeptide of any one of clauses 1 to 15 wherein (Xaa)_m comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 103-199.

17. The engineered polypeptide of clause 16 wherein (Xaa)_m consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 103-199.

30 18. The engineered polypeptide of clause 14, wherein (Xaa)_n comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 11, 13, 16, 17, 24, 25, 29, 33, 41, 54, 65, 67, 73, 75, 82, 89, 90, 98 and 99.

19. The engineered polypeptide of clause 18, wherein (Xaa)_n consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 11, 13, 16, 17, 24, 25, 29, 33, 41, 54, 65, 67, 73, 75, 82, 89, 90, 98 and 99.

20. The engineered polypeptide of clause 16, wherein (Xaa)_m comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 108, 110, 113, 114, 121, 122, 126, 130, 138, 151, 162, 164, 170, 172, 179, 186, 187, 195 and 196.

21. The engineered polypeptide of clause 20, wherein (Xaa)_m consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 108, 110, 113, 114, 121, 122, 126, 130, 138, 151, 162, 164, 170, 172, 179, 186, 187, 195 and 196.

22. The engineered polypeptide of clause 1 comprising an amino acid sequence at least 75%, at least 85%, or at least 95% identity to the amino acid sequence of SEQ ID Nos: 200, 205, 207, 210, 211, 218, 219, 223, 227, 235, 248, 259, 261, 267, 269, 276, 283, 284, 292 or 293, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues.

23. The engineered polypeptide of clause 22 comprising an amino acid sequence having 100% identity to the amino acid sequence of SEQ ID Nos: 200, 205, 207, 210, 211, 218, 219, 223, 227, 235, 248, 259, 261, 267, 269, 276, 283, 284, 292 or 293, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues. .

24. The engineered polypeptide of any one of clauses 1 to 23 wherein the Stefin A polypeptide is a mammalian Stefin A polypeptide.

25. The engineered polypeptide of any one of clauses 1 to 24 wherein the Stefin A polypeptide is a human Stefin A polypeptide.

26. The engineered polypeptide of any one of clauses 1 to 25, further comprising a signal peptide.

27. A fusion protein comprising a dimer, a trimer or a tetramer of the engineered polypeptide of any one of clauses 1 to 26, optionally comprising one or more linkers.

28. The fusion protein of clause 27, wherein the linker is a rigid linker.

29. The fusion protein of clause 28, wherein the rigid linker is a peptide linker comprising the sequence (EAAAK)_n, wherein n is 1-6.

30. A fusion protein comprising one or more of the engineered polypeptide of any one of clauses 1 to 29 linked to a therapeutic or diagnostic moiety.

31. The fusion protein of clause 30, wherein the therapeutic moiety is a therapeutic protein or peptide.

32. The fusion protein of clause 31, wherein the therapeutic moiety is an antibody.

33. The fusion protein of clause 30, wherein the diagnostic moiety is a fluorescent protein.

34. The engineered polypeptide of any one of clauses 1 to 26 or the fusion protein of any one of clauses 27 to 33 further comprising a half-life extension moiety.

5 35. The engineered polypeptide or fusion protein of clause 34, wherein the half-life extension moiety is selected from antibody Fc domains, human serum albumin, serum binding proteins.

36. The engineered polypeptide or fusion protein of clause 34, wherein the half-life extension moiety is a variant of a Stefin A protein that binds specifically to human serum
10 albumin with a K_d of $1 \times 10^{-6}M$ or less.

37. The engineered polypeptide or fusion protein of any one of clauses 1 to 36, wherein the K_d is measured by Biacore kinetic analysis.

38. The engineered polypeptide or fusion protein of clause 37, wherein the K_d is measured as set out in EXAMPLE 3, 'Biacore assay'.

15 39. The engineered polypeptide or fusion protein of any one of clauses 1 to 38, wherein the engineered polypeptide binds TNFR2 as a monomer with an IC_{50} in a competitive binding assay with TNFR2 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.

40. The engineered polypeptide or fusion protein of any one of clauses 1 to 39,
20 wherein the TNFR2 is mammalian TNFR2.

41. The engineered polypeptide or fusion protein of clause 40, wherein the TNFR2 is human TNFR2.

42. The engineered polypeptide or fusion protein of clause 40, wherein the TNFR2 is mouse TNFR2.

25 43. The engineered polypeptide or fusion protein of clause 34, wherein the half-life extension moiety is selected from the group consisting of SEQ ID Nos: 471-477.

44. A polynucleotide or set of polynucleotides encoding the engineered polypeptide or the fusion protein of any one of clauses 1 to 43.

45. The polynucleotide or set of polynucleotides of clause 44, comprising a nucleic
30 acid sequence having at least 80%, at least 90%, at least 95, or at least 98% identity to a sequence selected from the group consisting of SEQ ID Nos: 297, 302, 304, 307, 308, 315, 316, 320, 324, 332, 345, 356, 358, 364, 366, 373, 380, 381, 389 and 290, wherein the sequence optionally excludes the nucleotides encoding one or more of the twenty one carboxy terminal residues.

46. A delivery vehicle comprising the polynucleotide of either clause 44 or 45.

47. The delivery vehicle of clause 46, which is a viral delivery vehicle.

48. The delivery vehicle of clause 47, wherein the viral delivery vehicle is an adenoviral vector, a retroviral vector, a lentiviral vector, or an adeno-associated viral (AAV) vector.

49. The delivery vehicle of clause 46, which is a non-viral delivery vehicle.

50. The delivery vehicle of clause 49, wherein the non-viral delivery vehicle is a liposome or a lipid nanoparticle.

51. A plasmid or minicircle comprising the polynucleotide of any one of clauses 44 to 50.

52. A messenger RNA (mRNA) comprising an open reading frame encoding the engineered polypeptide of any one of clauses 1 to 43.

53. The mRNA of clause 52 further comprising an open reading frame encoding an immune cell binding.

54. A lipid nanoparticle, optionally a cationic lipid nanoparticle, comprising the mRNA of either clause 52 or 53.

55. The lipid nanoparticle of clause 54, wherein the lipid nanoparticle is a cationic lipid nanoparticle.

56. A pharmaceutical composition comprising the engineered polypeptide, the fusion protein, or the delivery vehicle of any one of clauses 1 to 50, and optionally a pharmaceutically acceptable excipient.

57. A conjugate comprising the engineered polypeptide or the fusion protein of any one of clauses 1 to 43 linked to a pharmacologically active moiety.

58. A method comprising administering to a subject the pharmaceutical composition of clause 56.

59. The method of clause 58, wherein the subject has systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis or cancer.

60. An engineered polypeptide, fusion protein, or delivery vehicle of any one of clauses 1 to 50 for use as a medicament.

61. The engineered polypeptide, the fusion protein, or the delivery vehicle of clause 60 for use in the treatment of systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis or cancer.

62. Use of an engineered polypeptide, the fusion protein, or the delivery vehicle of any one of clauses 1 to 50 in the manufacture of a medicament for the treatment of systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis or cancer.

63. An engineered polypeptide that competes for binding to TNFR2 with an engineered polypeptide according to any one of clauses 1 to 43.

64. An engineered polypeptide which binds the same epitope on TNFR2 as that of an engineered polypeptide according to any one of clauses 1 to 43.

65. The engineered polypeptide or fusion protein of any one of clauses 1 to 64 which comprises a signal sequence and/or a transmembrane domain.

Further embodiments of the invention are set out in the clauses below.

1B. An engineered polypeptide that binds specifically to TNFR2 with a K_d of $1 \times 10^{-6} \text{M}$ or less, wherein the engineered polypeptide is a variant of a Stefin A protein.

2B. The engineered polypeptide of clause 1B 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 MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKKTGETYGGKLEAVQYKTQV (SEQ ID NO: 484) Xaa2-(Xaa) n -Xaa3-TNYYIKVRAGDNKYMHLKVF (SEQ ID NO: 485)-Xaa4-Xaa5-Xaa6-(Xaa) m -Xaa7-D-Xaa8-VLTGYQVDKNKDDDELTF (SEQ ID NO: 486),

wherein Xaa, individually for each occurrence, is any number of amino acid residues;

5 and n and m are each, independently, an integer from 3 to 20.

3B. The engineered polypeptide of clause 2B wherein the amino acid sequence has 100% identity to an amino acid sequence of MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKKTGETYGGKLEAVQYKTQV (SEQ ID NO: 484) Xaa2-(Xaa) n -Xaa3-TNYYIKVRAGDNKYMHLKVF (SEQ ID NO: 485)-Xaa4-Xaa5-Xaa6-
10 (Xaa) m -Xaa7-D-Xaa8-VLTGYQVDKNKDDDELTF (SEQ ID NO: 486),

wherein Xaa, individually for each occurrence, is any number of amino acid residues; and n and m are each, independently, an integer from 3 to 20.

4B. The engineered polypeptide of clause 1B comprising an amino acid sequence having at least 80%, at least 90%, at least 95, or at least 98% identity to an amino acid
15 sequence of

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKKTGETYGGKLEAVQYKTQV (SEQ ID NO: 484) Xaa2-(Xaa) n -Xaa3-TNYYIKVRAGDNKYMHLKVF (SEQ ID NO: 485)-Xaa4-Xaa5-Xaa6-(Xaa) m -Xaa7-D-Xaa8-VLTGYQVDKNKDDDELTF (SEQ ID NO: 486),

or MIPGGLSEAKPATPEIQEIVDKVKPQLEEKKTGETYGGKLEAVQYKTQVD-
20 (Xaa) n -GTNYYIKVRAGDNKYMHLKVFKSL-(Xaa) m -EDLVLTGYQVDKNKDDDELTF (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.

5B. The engineered polypeptide of clause 4B wherein the amino acid sequence has
25 100% identity to an amino acid sequence of

MIPGGLSEAKPATPEIQEIVDKVKPQLEEKKTGETYGGKLEAVQYKTQVD-(Xaa) n -GTNYYIKVRAGDNKYMHLKVFKSL-(Xaa) m -EDLVLTGYQVDKNKDDDELTF (SEQ ID NO: 5),

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

6B. The engineered polypeptide of any one of clauses 1B to 5B wherein n is an integer from 5 to 18.

7B. The engineered polypeptide of clause 6B wherein n is an integer from 7 to 15.

8B. The engineered polypeptide of clause 7B wherein n is an integer from 8 to 12.

9B. The engineered polypeptide of any one of clauses 1B to 8B wherein m is an integer from 5 to 18.

10B. The engineered polypeptide of clause 9B wherein m is an integer from 7 to 15.

5 11B. The engineered polypeptide of clause 10B wherein m is an integer from 8 to 12.

12B. The engineered polypeptide of any one of clauses 1B to 11B that binds to TNFR2 with a K_d of $1 \times 10^{-7}M$.

10 13B. The engineered polypeptide of clause 12B that binds to TNFR2 with a K_d of $1 \times 10^{-8}M$.

14B. The engineered polypeptide of any one of clauses 1B to 13B wherein (Xaa)_n comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6-102.

15B. The engineered polypeptide of clause 14B wherein (Xaa)_n consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 6-102.

15 16B. The engineered polypeptide of any one of clauses 1B to 15B wherein (Xaa)_m comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 103-199.

17B. The engineered polypeptide of clause 16B wherein (Xaa)_m consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 103-199.

18B. The engineered polypeptide of clause 14B, wherein (Xaa)_n comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 11, 13, 16, 17, 24, 25, 29, 33, 41, 54, 65, 67, 73, 75, 82, 89, 90, 98 and 99.

19B. The engineered polypeptide of clause 18B, wherein (Xaa)_n consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 11, 13, 16, 17, 24, 25, 29, 33, 41, 54, 65, 67, 73, 75, 82, 89, 90, 98 and 99.

20B. The engineered polypeptide of clause 16B, wherein (Xaa)_m comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 108, 110, 113, 114, 121, 122, 126, 130, 138, 151, 162, 164, 170, 172, 179, 186, 187, 195 and 196.

21B. The engineered polypeptide of clause 20B, wherein (Xaa)_m consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 108, 110, 113, 114, 121, 122, 126, 130, 138, 151, 162, 164, 170, 172, 179, 186, 187, 195 and 196.

22B. The engineered polypeptide of clause 1B comprising an amino acid sequence at least 75%, at least 85%, or at least 95% identity to the amino acid sequence of SEQ ID Nos: 200, 205, 207, 210, 211, 218, 219, 223, 227, 235, 248, 259, 261, 267, 269, 276, 283, 284, 292

or 293, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues.

23B. The engineered polypeptide of clause 22B comprising an amino acid sequence having 100% identity to the amino acid sequence of SEQ ID Nos: 200, 205, 207, 210, 211, 218, 219, 223, 227, 235, 248, 259, 261, 267, 269, 276, 283, 284, 292 or 293, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues. .

24B. The engineered polypeptide of any one of clauses 1B to 23B wherein the Stefin A polypeptide is a mammalian Stefin A polypeptide.

25B. The engineered polypeptide of any one of clauses 1B to 24B wherein the Stefin A polypeptide is a human Stefin A polypeptide.

26B. The engineered polypeptide of any one of clauses 1B to 25B, further comprising a signal peptide.

27B. A fusion protein comprising a dimer, a trimer or a tetramer of the engineered polypeptide of any one of clauses 1B to 26B, optionally comprising one or more linkers.

28B. The fusion protein of clause 27B, wherein the linker is a rigid linker.

29B. The fusion protein of clause 28B, wherein the rigid linker is a peptide linker comprising the sequence (EAAAK)_n, wherein n is 1-6.

30B. A fusion protein comprising one or more of the engineered polypeptide of any one of clauses 1B to 29B linked to a therapeutic or diagnostic moiety.

31B. The fusion protein of clause 30B, wherein the therapeutic moiety is a therapeutic protein or peptide.

32B. The fusion protein of clause 31B, wherein the therapeutic moiety is an antibody.

33B. The fusion protein of clause 30B, wherein the diagnostic moiety is a fluorescent protein.

34B. The engineered polypeptide of any one of clauses 1B to 26B or the fusion protein of any one of clauses 27B to 33B further comprising a half-life extension moiety.

35B. The engineered polypeptide or fusion protein of clause 34B, wherein the half-life extension moiety is selected from antibody Fc domains, human serum albumin, serum binding proteins.

36B. The engineered polypeptide or fusion protein of clause 34B, wherein the half-life extension moiety is a variant of a Stefin A protein that binds specifically to human serum albumin with a K_d of $1 \times 10^{-6}M$ or less.

37B. The engineered polypeptide or fusion protein of any one of clauses 1B to 36B, wherein the K_d is measured by Biacore kinetic analysis.

38B. The engineered polypeptide or fusion protein of clause 37B, wherein the K_d is measured as set out in EXAMPLE 3, 'Biacore assay'.

5 39B. The engineered polypeptide or fusion protein of any one of clauses 1B to 38B, wherein the engineered polypeptide binds TNFR2 as a monomer with an IC₅₀ in a competitive binding assay with TNFR2 of 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.

10 40B. The engineered polypeptide or fusion protein of any one of clauses 1B to 39B, wherein the TNFR2 is mammalian TNFR2.

41B. The engineered polypeptide or fusion protein of clause 40B, wherein the TNFR2 is human TNFR2.

42B. The engineered polypeptide or fusion protein of clause 40B, wherein the TNFR2 is mouse TNFR2.

15 43B. The engineered polypeptide or fusion protein of clause 34B, wherein the half-life extension moiety is selected from the group consisting of SEQ ID Nos: 471-477.

44B. A polynucleotide or set of polynucleotides encoding the engineered polypeptide or the fusion protein of any one of clauses 1B to 43B.

20 45B. The polynucleotide or set of polynucleotides of clause 44B, comprising a nucleic acid sequence having at least 80%, at least 90%, at least 95, or at least 98% identity to a sequence selected from the group consisting of SEQ ID Nos: 297, 302, 304, 307, 308, 315, 316, 320, 324, 332, 345, 356, 358, 364, 366, 373, 380, 381, 389 and 290, wherein the sequence optionally excludes the nucleotides encoding one or more of the twenty one carboxy terminal residues.

25 46B. A delivery vehicle comprising the polynucleotide of either clause 44B or 45B.

47B. The delivery vehicle of clause 46B, which is a viral delivery vehicle.

48B. The delivery vehicle of clause 47B, wherein the viral delivery vehicle is an adenoviral vector, a retroviral vector, a lentiviral vector, or an adeno-associated viral (AAV) vector.

30 49B. The delivery vehicle of clause 46B, which is a non-viral delivery vehicle.

50B. The delivery vehicle of clause 49B, wherein the non-viral delivery vehicle is a liposome or a lipid nanoparticle.

51B. A plasmid or minicircle comprising the polynucleotide of any one of clauses 44B to 50B.

52B. A messenger RNA (mRNA) comprising an open reading frame encoding the engineered polypeptide of any one of clauses 1B to 43B.

53B. The mRNA of clause 52B further comprising an open reading frame encoding an immune cell binding.

5 54B. A lipid nanoparticle, optionally a cationic lipid nanoparticle, comprising the mRNA of either clause 52B or 53B.

55B. The lipid nanoparticle of clause 54B, wherein the lipid nanoparticle is a cationic lipid nanoparticle.

56B. A pharmaceutical composition comprising the engineered polypeptide, the fusion protein, or the delivery vehicle of any one of clauses 1B to 50B, and optionally a pharmaceutically acceptable excipient.

57B. A conjugate comprising the engineered polypeptide or the fusion protein of any one of clauses 1B to 43B linked to a pharmacologically active moiety.

58B. A method comprising administering to a subject the pharmaceutical composition of clause 56B.

59B. The method of clause 58B, wherein the subject has systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis or cancer.

60B. An engineered polypeptide, fusion protein, or delivery vehicle of any one of clauses 1B to 50B for use as a medicament.

61B. The engineered polypeptide, the fusion protein, or the delivery vehicle of clause 60B for use in the treatment of systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental

allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis or cancer.

62B. Use of an engineered polypeptide, the fusion protein, or the delivery vehicle of any one of clauses 1B to 50B in the manufacture of a medicament for the treatment of systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis or cancer.

63B. An engineered polypeptide that competes for binding to TNFR2 with an engineered polypeptide according to any one of clauses 1B to 43B.

64B. An engineered polypeptide which binds the same epitope on TNFR2 as that of an engineered polypeptide according to any one of clauses 1B to 43B.

65B. The engineered polypeptide or fusion protein of any one of clauses 1B to 64B which comprises a signal sequence and/or a transmembrane domain.

EXAMPLES

The aim of these experiments was to identify human and mouse TNFR2 specific agonistic AFFIMER® polypeptides.

Example 1. Selection of TNFR2 Binding AFFIMER® Polypeptides from Phage Display Library

Identification of candidate clones

TNFR2 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

expressing AFFIMER® proteins were incubated with human (or mouse in later experiments) TNFR2 as required, the sequences of which are shown in Table 7.

Table 7. TNFR protein sequences used during selection (from Sino Biologicals).

Protein	Target sequence	Plasmid Catalog number	SEQ ID NO:
Human TNFR2	MAPVAVWAALAVGLELWAAHALPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWNWVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFVARPGTETSDVVCCKPCAPGTFSTNTSSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPPPAEGSTGDFALPVGLIVGVTALGLLIIGVVNCVIMTQVKKKPLCLQREAKVPHLPADKARGTQGPQQHLLITAPSSSSSSLESSASALDRRAPTRNQPPAPGVASGAGEARASTGSSDSSPGGHGTQVNVTCIVNVCSSSDHSSQCSSQASSTMGDTDSPPSESPKDEQVPFSKEECAFRSQLETPETL LGSTEEKPLPLGVDPDAGMKPS	Cat: HG10417-CY	478
Human TNFR1	MGLSTVPDLLLPLVLELLLVGIYPSGVIGLVPHLGDREKRDSVCPQGYIHPQNNISICCTKCHKGTLYNDPCPGPGQDTCRECESGSFTASENHLRHCLSCSKCRKEMQVEISSCTVDRDTCVCGCRKNQYRHYWSENLFQCFNCSLCLNGTVHLSCQEKQNTVCTCHAGFFLRENECVSCSNCKKSELECTKLCLPQIENVKGTEDSGTTVLLPLVIFFGCLCLLSLLFIGLMYRQYRWKSKLYSIVCGKSTPEKEGELEGTTTKPLAPNPSFSPPTPGFTPTLGFSPVPSSTFTSSSTYTPGDCPNFAAPRREVAPPYQADPILATALASDPIPNPLQKWEDSAHKPQSLDTPATLYAVVENVPPLRWKEFVRRGLSDHEIDRLELQNGRCLREAQYMLATWRRRTPRREATLELLGRVLRDMDLLGCLEDIEEALCGPAALPPAPSLLR	HG10872-CY	479
Cynomolgus TNFR2	MAPAAVWAALAVGLELWAAHALPAQVAFTPYAPEPGGTCRLREYYDQTAQMCCSKCPCPPGQHAKVFCTKTSDTVCDSCEDSTYTQLWNWVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFVARPGTETSDVVCCKPCAPGTFSTNTSSSTDICRPHQICHVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPAPSTAPGTSFLLPVGPSPPAEGSTGDIVLPVGLIVGVTALGLLIIGVVNCVIMTQVKKKPLCLQRETKVPHLPADKARGAQGPQQHLLITVPSSSSSSLESSASALDRRAPTRNQPPAPGAEKASGAGEARASTGSSDSSPGGHGTQVNVTCIVNVCSSSDHSSQCSSQASSTTGDTDASPSPKDEQVPFSKEECAFRSQLETPETL LGSTGEKPLPLGVDPDAGMKPS	CG90102-ACR	480
Mouse TNFR2	MAPAALWVALVFELQLWATGHTVPAQVVLTPYKPEPGYECQISQ EYYDRKAQMCCAKCPGQYVKHFCNKTSDTVCDCEASMYTQVWNQFRTCLSCSSSCTTDQVEIRACTKQQRNVCACEAGRYCALKTHSGSCRQCMRLSKCGPGFVASSRAPNGNVLCACAPGTFSDTTSSTDVCRPHRICSIILAI PGNASTDAVCAPESPTLSAIPRTLYVSQPEPTRSQPLDQEPGPSQTPSILTSLGSTPIIEQSTKGGISLPIGLIVGVTSLGLMLGLVNCIILVQRKKKPSCLQRDAKVPHVPDEK SQDAVGLEQQHLLTTAPSSSSSSLESSASAGDRRAPPGGHPQARVMAEAQGFQEARASSRISDSSHGSHGTHVNVTCIVNVCSSSDHSSQCSSQASATVGDPAKPSASPKDEQVPFSQEECPSPQPCETTE TLQSHEKPLPLGVDPDMGMKPSQAGWFDQIAVKVA	MG50128-CY	481
Mouse TNFR1	MGLPTVPGLLLSLVLLALLMGIHPSGVTGLVPSLGDREKRDSLCPQGYVHSHKNNISICCTKCHKGTLYVSDCPSPGRDTCRECEKGTFTASQNYLRQCLSCKTCRKEMQVEISPCQADKDTVCGCKENQFQRYLSETHFQCVDSPCFNGTVTIPCKETQNTVCNCHAGFFLRESECVPCSHCKKNEECMKLCLPPPLANVTNPQDSGTAVLLPLVIL LGLCLLSFIFISLMCRYPRWRPEVYSIIICRDPVPVKEEKAGKPLTPAPSPAFSPTSGFNPTLGFSTPGFSSPVSSSTPISPIFGPSNWHFMPVSEVVPTQGADPLLYESLCSVPAPTSVQKWEDSAHPQRPD	MG50496-CY	482

	NADLAIFYAVVDGVPPARWKEFMRFMGLSEHEIERLEMQNGRCL REAQYSMLEAWRRRTPRHEDTLEVGLVLSKMNLAGCLENILEA LRNPAPSSTTRLPR		
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Table 8. Commercial antigens used during selection. TNFR2 antigens having the sequences shown in Table 7 as well as other proteins shown below were tested in different formats for biotinylation and use in TNFR2 AFFIMER® selection and other assays.

Antigen	Supplier	Catalogue number	Tag	Source	Predicted MW (kDa)
hTNFR2-hFc-his-Avi (biotin-conjugated)	BPS	100205	Human IgG1, 6x His, Avi, Biotin	HEK293	75
hTNFR2-hFc-his-Avi	BPS	79363	Human IgG1, 6x His, Avi	HEK293	75
hTNFR2-his	Acro Biosystems	AMS.TN2-H5227	6X His Tag	HEK293	26
hTNFR2-his-avi (biotin conjugated)	Acro Biosystems	AMS.TN2-H82E3	6X His and Avi Tag, Biotin	HEK293	26
hTNFR2-his	Sino Biologicals	10417-H08H	6x His	HEK293	45
hTNFR2	Peprtech	310-12	N/A	<i>E. coli</i>	20
mTNFR2-mFc	R&D Biosystems	9707-R2-050	Mouse IgG2a	NS0	52
mTNFR2-his	Sino Biologicals	50128-M08H	6x His	HEK293	27
mTNFR2-hFc	Sino Biologicals	50128-M02H	Human IgG1 Fc	HEK293	52
hTNFa	Peprtech	300-01-A	N/A	<i>E. coli</i>	17.4
mTNFa	Peprtech	315-01A	N/A	<i>E. coli</i>	17.3
hFc (IgG1)	AcroBiosystems	FCC- H5214	N/A	HEK293	26
hFc-Avi (IgG1) (biotin conjugated)	AcroBiosystems	IG1-H8213	Avi Tag, Biotin	HEK293	28.4
mFc (IgG2a)	AcroBiosystems	IGA-M5207	N/A	HEK293	26.4
mFc (IgG2a) biotinylated	AcroBiosystems	IGA-M8210	N/A	HEK293	28.2

hTNFR1-hFc	AcroBiosystems	TN1-H5251	Human IgG1 Fc	HEK293	47.9
mTNFR1-hFc-His	R&D Systems	430-RI/CF	Human IgG1 Fc	NS0	48.6

In some cases the TNFR2 was biotinylated and captured on alternating streptavidin and neutravidin beads, alternatively the TNFR2 was passively absorbed to a surface. Unbound phage particles were then washed away and, following washing, bound phage were eluted. Elution of bound phage was accomplished by 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, murine and cynomolgus TNFR2 was immobilized on a plate, lysates were added, and the amount of TNFR2-binding AFFIMER® polypeptide in each plate was measured using a detector antibody specific to the Myc tag expressed on the candidate AFFIMER® polypeptides. The phagemid vectors encoding AFFIMER® polypeptides with the best human TNFR2-binding activity were sequenced to identify DNA sequences of candidate clones for further development. TNFR1 polypeptides were also used to distinguish specificity. The loop 2 and loop 4 amino acid sequences of each of these candidate clones are shown in Table 1.

EXAMPLE 2: AFFIMER® production in mammalian cells as soluble protein and screening

Cloning into vector of interest

To assess the ability of the selected AFFIMER® proteins to bind TNFR2 when expressed at the surface of a mammalian cell, transiently transfected cell pools were generated for 30 ‘fast tracked’ clones based on being the most repeated sequences during passive selection. These clones were used for initial characterisation and assay optimisation. TNFR2 AFFIMER® sequences and TNFR2 targets were cloned from the phagemid of Example 1 into the mammalian expression vector pD609kanR (ATUM custom vector) for expression in Expi293F™ cells (ThermoFisher). The average concentration obtained from expression was 2.8 mg/mL, with 19 clones selected based on purity and assessed by for degradation.

These 19 clones were screened on Expi293F™hTNFR2 expressing cells by flow cytometry at 1 µM and 0.1 µM while being compared to the SQT-GLY and 3T0-GLY negative controls which contain glycines only in Loop 2 and Loop 4 and thus are not specific binders. The signal detected on transfected cells was subtracted by the signal obtained on the untransfected Expi293F™cells. Of these, 16 clones demonstrate binding to hTNFR2-Expi293F™cells at 1 µM >10% after binding to parental cell line was subtracted. Clones 15, 22 and 23 did not show such binding (Figure 2).

The hTNFR2 mammalian purified AFFIMER® proteins were screened in the HEK Blue™ TNFα SEAP reporter assay (Invivogen) according to manufacturer’s to determine if they could trigger the NFκB pathway and the SEAP production via binding to TNFR2 when they are clustered by an anti-his antibody coated on the plate. In this experiment, 5 of the fast tracked clones (01, 09, 12, 21 and 26) showed the ability to trigger the release of SEAP, with clone 12 showing the most consistent agonism.

A further 90 AFFIMER® polypeptides were cloned from phagemid and similarly characterised. 55 clones were taken forward and binding confirmed by Mirrorball analysis. Further characterisation was done by competition ELSA to test their ability to block the interaction between hTNFR2-hFc-his-avi and recombinant TNF-alpha. A series of experiments were carried out to determine the EC80 of hTNFR2-hFc-his-Avi when binding to coated hTNF alpha (593 pM). AFFIMER® proteins were tested at three different concentrations (10, 1 and 0.1 µM) when possible, in presence of hTNFR2 antigen at its determined EC80. Detection of hTNFR2-hFc-his-Avi bound was done by anti-hFc-HRP antibody. Among the 75 clones tested, 32 clones, that showed a % inhibition greater than 65% at 10 µM, were selected to be

characterized further to determine their IC₅₀ (Figure 3). clones 06, 21, 26, 27, 44, 108 and 115 showed full inhibition of the interaction between TNF α and hTNFR2 at the tested concentrations.

At this point, these 55 clones as well as the 14 clones from the fast track group (13 agonist, one negative, clone 06) were cloned into the pDisplay vector (Thermo Fisher). That vector contains a PDGFR protein transmembrane domain in C-term to allow Affimer expression at the surface of the expressing cells and not as soluble proteins. Additional tags such as HA in N-term are also encoded by this vector allowing an easy verification for Affimer expression. Once cloned these Affimer were transiently transfected into Expi293F™ cells for SEAP analysis as previously described. Taking all the data together, 43 clones were selected for additional characterisation.

EXAMPLE 3: Characterisation of 43 selected clones

Biacore assay

The selected 43 clones were tested in a Biacore kinetic analysis as soluble proteins. Briefly Multicycle kinetics were performed using a CM5 chip on which the dimeric antigen hTNFR2-hFc-his-avi was immobilized at 500RU. AFFIMER® proteins were titrated down in HBS-EP+ buffer from 1 μ M in 1:2 dilution series. Association time and Dissociation used were 200 sec and 400 sec respectively at a 20 μ l/min flow rate with a regeneration with 10 mM Glycine pH 1.5 for 30 sec at 30 μ l/min. Twelve clones showed very low response and no KD value could be estimated for them. Clone 09 was fitted using the 1:1 binding model however, the reported KD value was too high (>E-05) to be trusted with the range of concentration used. For 16 clones, the reached signal was adequate and they could be fitted successfully using the 1:1 Binding model, the curve and their fit are compiled in. Two clones (21 and 69) that are dimeric were fitted using both 1:1 binding model and steady state in order to estimate there KD, while in 12 clones, low Rmax made it difficult to fit them using the 1:1 binding model and instead the Steady State model was used. In that case, the obtained KD value was not reported, an order of magnitude was instead. Results are shown in Figure 4.

Binding ELISA crossreactivity

TNFR2 AFFIMER® clones' specificity for hTNFR2 was assessed by ELISA when the clones binding to TNFR2 was compared to their binding to TNFR1. None of the clones showed binding to TNFR1 as a recombinant antigen. hTNFR1 was also expressed in Expi293F™ cells, and no binding was observed to this cellularly expressed hTNFR1, confirming no crossreactivity between the hTNFR2 AFFIMER® polypeptide binders with hTNFR1.

In addition to their binding to human TNFR2, the clones were tested as well for binding to the mouse TNFR2 to verify if any of them was cross reactive. There were very little chances for this to happen as the homology between the two proteins is ~68% for the extra cellular domain, which was confirmed as no clone demonstrated binding to mTNFR2 expressing Expi293F™ cells.

HEK293 – HEK Blue assay characterization

The 43 lead clones were transiently transfected in HEK 293 cells, along with 4 controls, SQT-Gly, 3t0-Gly, Empty vector, and clone 06 which has shown to be negative. Approximately 29h post transfection the cells viability was determined using a cell counter and the expression of the AFFIMER® was determined by flow cytometry using anti-HA antibody. 48 hours post transfection the AFFIMER® expressing HEK293 cells were co-cultured with HEK Blue reporter cells at 4 different cell densities, 40000, 20000, 10000 and 5000 with a fixed number of HEK Blue cells of 50000 cells/well. In parallel, the positive control MR2-1 was also incubated with HEK Blue cells for 24h at a range of concentrations. After 24h, the release of SEAP in the supernatant was quantified using Quanti-Blue and measurement of Absorbance 640nm. Staining of the AFFIMER®s with monoclonal anti-HA antibody showed expression levels >90% in both experiments. In the co-culture reporter assay, the effect of the TNFR2 AFFIMER® proteins displayed on HEK293 on the HEK-Blue cells was visible, as well as the MR2-1 clone effect, allowing the selection of 16 clones which were consistently ranked within the best 20 clones in both experiments. These results are compiled in Figure 4, with the following clones identified: 001, 007, 009, 012, 013, 026, 027, 037, 044, 059, 069, 079, 100, 103, 112 and 115.

Cell binding to Human and Cynomolgus TNFR2

The 16 best agonists were characterized in soluble format for its binding to human TNFR2 overexpressing Expi293F™ and cynomolgus TNFR2 over-expressing Expi293F. The

experiment was repeated 3 times and 2 different batches of transiently transfected cells were used. All clones showed consistently specific binding to hTNFR2-Expi293F™ cells. Clones 26 and 69 showed the best EC50s on both human and cynomolgus TNFR2 expressing cells. 13 out of 16 tested clones show cross reactivity with cynomolgus TNFR2. The 3 non-crossreactive clones were clones 001, 007 and 009. Without necessarily being the best binders, clones DAW02-013, DAW02-044 and DAW02-112 are the clones with the least difference between the binding to human TNFR2 and cynomolgus TNFR2 over-expressing cells when all 3 experiments are compiled (Figure 5).

EXAMPLE 4. In-line fusion homodimer constructs

Clones 001, 009 and 012 were chosen to be formatted as in-line fusion (ILF) homodimers, to investigate if that format would be an advantage to trigger agonism compared to the monomeric form of the clones, as soluble protein and when displayed on cells. Two constructs were used for the generation of the ILF homodimers with a flexible linker, one codon optimised for mammalian expression and one not codon optimised. Both constructs were cloned into mammalian secretion vector (pD609-KanR) to purify recombinant protein and assess their expression levels, protein quality, binding kinetics to hTNFR2-hFc-his-avi by Biacore and agonistic activity in reporter assay with HEK reporter cells. In addition, both constructs were cloned into pDisplay vector for expression on Expi293F™ cell surface and assessment of their expression levels, binding to hTNFR2-hFc-his-avi and agonistic activity in co culture reporter assay with HEK reporter cells. Codon optimisation did not improve yields or purity of the soluble ILF homodimers, which were confirmed by HPLC.

When analysed by Biacore, clones 001 and 009 showed increased affinity in their soluble ILF format as compared to the soluble monomer format. Clone 12 showed no response. No difference was observed between the codon optimisation constructs. In SEAP assays only Clone 12 showed improved profile as a soluble ILF format, however when displayed on cells, in SEAP assays as well as flow cytometry presentation assays, no difference could be determined between the ILF and monomer formats.

Summary of hTNFR2 AFFIMER® polypeptide selection and characterisation.

More than 2500 monoclonal phage were screened by phage ELISA with a hit rate greater than 60% with good diversity (20-40%). In the screening in ELISA as cell crude extract, more

than 400 clones showed binding to the hTNFR2 antigen. At this stage, it was decided to introduce another step in the selection screening campaign to reduce the number of clones to cloned into the mammalian expression vector. In parallel, it was also decided to fast-track 30 clones from the passive selection directly to expression as soluble AFFIMER® proteins to facilitate assay development and screening cascade building for the rest of the clones. 20 of these clones passed the protein production quality criteria of more than 80% purity by SEC-HPLC and were used to set up various assays as well as being characterised. 5 out of 20 clones demonstrated agonist activity in a preliminary agonist assay where monomeric soluble AFFIMER® protein were clustered with an anti-his antibody via their histidine tag and incubated with the HEK reporter cell line.

Alongside assay development with fast-tracked clones, the phage screening campaign was finalised and a total of 364 human TNFR2-binding AFFIMER® proteins were purified from the phagemid and tested for binding to a TNFR2 over-expressing cell line. Following on from this assay, 90 human TNFR2-binding AFFIMER® proteins were re-formatted into the mammalian expression vector. 55 of these clones passed the protein production quality criteria of more than 80% purity by SEC-HPLC and were carried forward for the rest of the assays.

The total number of clones (fast-tracked and non-fast tracked) was narrowed down from 75 to 43 following a series of experiment that looked at AFFIMER® soluble proteins binding to hTNFR2 overexpressing cells, agonist assay with HEK reporter cells and preliminary data from agonist assay using HEK reporter cells in co-culture with AFFIMER® proteins expressed at the surface of Expi293F™ cells. These 43 best clones were characterised as soluble proteins for their binding to human TNFR2 by Biacore where 16 out of 43 had their KD successfully calculated using a 1:1 binding model. None of the clones showed binding to human TNFR1 by ELISA or mouse TNFR2 by cell binding. As AFFIMER® proteins displayed at the surface of HEK293, these 43 clones were tested for their ability to trigger TNFR2 agonism in a HEK reporter cell line when in co-culture. A large majority of the tested clones demonstrated a dose dependent effect on the HEK reporter cells (80%). 16 AFFIMER® proteins were selected as best agonists and taken through additional characterisation to assess cynomolgus TNFR2 binding and human TNFR1 binding on cells. The selected 16 hTNFR2 AFFIMER® polypeptides are clones 001, 007, 009, 012, 013, 026, 027, 037, 044, 059, 069, 079, 100, 103, 112 and 115.

The use of in-line fusion homodimers as soluble proteins vs monomers showed advantages for binding to the recombinant TNFR2 by Biacore and possible advantage in the anti-his clustering agonism assay with HEK reporter cells. However, no advantage was demonstrated compared to the monomers when the in-line fusion AFFIMER® proteins were displayed on cells to trigger the reporter cell line agonism pathway via TNFR2. The reason for the in-line fusion homodimers not performing better than the monomers in this last assay could have been that the in-line fusion format was not well displayed on the cell surface and only one of the two AFFIMER® proteins was available for binding or that the limitation of the reporter assay had been reached and it was not possible to discriminate between the two formats.

10 EXAMPLE 5. Mouse TNFR2 AFFIMER® polypeptides

Phage selection and screening campaign

Murine TNFR2 antigens having the sequence as shown in Table 7 but in different formats were tested for suitability for use in assays to select for mTNFR2 AFFIMER® polypeptides. Of the three formats (mFc, hFc and His tagged), mTNFR2-mFc was chosen for biotinylation and use in the AFFIMER® selections due to its better performance in the assays.

Selections

Passive and solution selections were performed on the two AFFIMER® phage libraries as described for the hTNFR2 AFFIMER® selection. Enrichment was observed when the mTNFR2 concentration was maintained at 10 nM at round 3. More than 2000 phage-displayed AFFIMER® proteins were screened by monoclonal phage ELISA from round 2 and round 3 outputs. Of these clones, over 900 demonstrated mTNFR2 binding, with no detectable non-specific binding to mouse Fc, plastic, streptavidin or neutravidin. These were defined as “hits” based on the criteria of absorbance 450nm-630nm above 0.5 on mTNFR2-coated plates and below 0.05 on plates coated with control antigens (i.e. mouse Fc, plastic, neutravidin). Sequencing of phage ELISA hits identified 281 unique clone sequences, of which 109 demonstrated mTNFR2 binding after ELISA on the cell crude extracts. Further analysis of binding using mTNFR2-Expi293F™ cells narrowed this field to 58 clones, identified as clones 122-179 in Table 2.

Soluble protein characterisation

As described for the hTNFR2 AFFIMER® polypeptide characterisation above, the identified mTNFR2 AFFIMER® polypeptides were cloned from phagemid to mammalian vectors for expression in Expi293F™ cells. The expressed proteins were then assessed by SDS-PAGE and HPLC for purity and Biacore for affinity, as well as cell based binding of mTNFR2 in mTNFR2-Expi293F™ cells. Specificity for mTNFR2 was tested using ELISA and cell based assays using mTNFR1 and mTNFR2 as the targets, confirming the identified clones' specificity. To complete the soluble protein characterisation, the AFFIMER® polypeptides were tested for their ability to block mTNFR2-mFC binding to mTNFα in ELISA, with four clones (157, 160, 171 and 172) showing clear competitive ability at the range tested.

T cell stimulation assays

A T cell stimulation assay was used as a screening tool since activated T cells express high levels of TNFR2, when stimulated with HM102 (TNFR2 agonist) the CD25 expression increases.

The mTNFR2 AFFIMER® polypeptides were first expressed on the cell surface of HEK293s. Then, these cells were co-cultured for 48 hours with BALB/c splenocytes and finally the CD25 expression increase was evaluated on CD4 and CD8 positive T cells. Expression of the AFFIMER® proteins on the HEK293 cell surface was assessed by flow cytometry staining of the HA tag present at the beginning of the open reading frame prior to the AFFIMER® proteins and cell anchoring protein. In order to assess the effect of the AFFIMER® proteins-HEK on the CD4+ and CD8+ cells, after 48h incubation the cells were stained with the following panel of markers, Live/Dead stain, CD90.2, CD4, CD8 and CD25.

A dose response was seen in CD25 expression in CD4+ and CD8+ T cells in response to HEK293 cells expressing mTNFR2 AFFIMER® proteins, similar to the response observed for HM102 TNFR2 agonist. The response obtained in CD8+ T cells is stronger than in CD4+ T cells. However, when focusing on the two highest cell numbers used, a similar ranking of the clones was obtained between the CD8+ and CD4+ T cell subsets.

In two rounds of this assay experiment the six top-ranked AFFIMER® proteins were consistent 174, 160, 175, 125, 157 and 179.

Summary of mTNFR2 AFFIMER® polypeptide selection and characterisation.

More than 2000 monoclonal phage were screened by phage ELISA with a moderate 35-60% hit rate, with a good diversity (25-35%). In the screening in ELISA as cell crude extract, 109 clones showed binding to mTNFR2 antigen. Using the same screening cascade than for the human programme, it was decided to purify the identified binders from the phagemid and to test them for binding to a TNFR2 over-expressing cell line. Following on from this assay, 60 mouse TNFR2-binding AFFIMER® proteins were reformatted into the mammalian expression vector, produced and tested for their biophysical properties as soluble proteins. 22 Affimer proteins passed the protein production quality criteria of purity by SEC-HPLC greater than 80%. The 22 clones were characterised in various assays as soluble proteins, then were expressed at the surface of HEK293 for a co-culture assay with splenocytes.

Although only 10 out of the 22 Affimer proteins showed triple digit nanomolar binding affinities (KD) when tested on Biacore, it was considered that even an Affimer protein with a higher KD value could be a good agonist once displayed on the cell surface. Additionally, the performance of the clones as soluble proteins was also dependent on whether they were a natural dimer or trimer. For these reasons, Biacore data were considered in the context of mTNFR2 agonism activity and were not used to discriminate clones. When tested on cells expressing mouse TNFR2, 20 out of 22 clones demonstrated specific binding with a wide range of EC50 values. As for Biacore data, cell binding results were only considered in the context of mouse TNFR2 agonism activity and were not used to discriminate clones. None of the clones bound to TNFR1 either as recombinant proteins or expressed on a cell's surface. 5 out of 22 clones demonstrated full inhibition of the interaction between TNF-alpha and TNFR2 in a competition ELISA.

The 22 mouse clones were also expressed at the surface of HEK293 cells for a co-culture assay with splenocytes. The main read out for the assay was the CD25 expression increase on CD4+ T cells and CD8+ T cells. A large majority of the tested clones demonstrated a dose dependent effect on the CD4+ T cells and CD8+ T cells (over 70%). Clones were ranked looking at the CD25 expression on both CD4+ and CD8+ T cells. Based on the results of this key assay, six mTNFR2 AFFIMER® proteins were identified: 174, 160, 175, 125, 157 and 179.

WHAT IS CLAIMED IS:

1. An engineered polypeptide that binds specifically to TNFR2 with a K_d of $1 \times 10^{-6}M$ 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

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVV-(Xaa)n-Xaa2-TNYYIKVRAGDNKYMHLKVF-Xaa3-Xaa4-Xaa5-(Xaa)m-Xaa6-D-Xaa7-VLTGYQVDKNKDDELTGF (SEQ ID NO: 4),

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 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

MIPGGLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQVD-(Xaa)n-GTNYYIKVRAGDNKYMHLKVFKSL-(Xaa)m-EDLVLTGYQVDKNKDDELTGF (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.

4. 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

MIP-Xaa1-GLSEAKPATPEIQEIVDKVKPQLEEKGTGETYGKLEAVQYKTQV (SEQ ID NO: 484) Xaa2-(Xaa)n-Xaa3-TNYYIKVRAGDNKYMHLKVF (SEQ ID NO: 485)-Xaa4-Xaa5-Xaa6-(Xaa)m-Xaa7-D-Xaa8-VLTGYQVDKNKDDELTGF (SEQ ID NO: 486),

wherein Xaa, individually for each occurrence, is any number of amino acid residues; and n and m are each, independently, an integer from 3 to 20.

5. The engineered polypeptide of any one of the preceding claims that binds to TNFR2 with a K_d of $1 \times 10^{-7}M$, preferably a K_d of $1 \times 10^{-8}M$.

6. The engineered polypeptide of claim 2 or claim 3 wherein (Xaa)_n comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6-102 and/or (Xaa)_m comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 103-199.

5

7. The engineered polypeptide of claim 6, wherein (Xaa)_n comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 11, 13, 16, 17, 24, 25, 29, 33, 41, 54, 65, 67, 73, 75, 82, 89, 90, 98 and 99, and/or (Xaa)_m comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 108, 110, 113, 114, 121, 122, 126, 130, 138, 151, 162, 164, 170, 172, 179, 186, 187, 195 and 196.

10

8. The engineered polypeptide of claim 1 comprising an amino acid sequence at least 75%, at least 85%, or at least 95% identity to the amino acid sequence of SEQ ID Nos: 200, 205, 207, 210, 211, 218, 219, 223, 227, 235, 248, 259, 261, 267, 269, 276, 283, 284, 292 and 293, wherein the sequence optionally excludes one or more of the twenty one carboxy terminal residues.

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9. The engineered polypeptide of any one of the preceding claims, further comprising a signal peptide.

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10. The engineered polypeptide of any one of the preceding claims, further comprising a transmembrane domain.

11. A fusion protein comprising a dimer, a trimer or a tetramer of the engineered polypeptide of any one of the preceding claims, optionally comprising a linker.

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12. The fusion protein of claim 11 wherein the linker is a rigid linker.

13. The fusion protein of claim 12, wherein the rigid linker is a peptide linker comprising the sequence (EAAAK)_n, optionally wherein n is 1-6.

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14. A fusion protein comprising one or more of the engineered polypeptide of any one of the preceding claims linked to a therapeutic or diagnostic moiety.

15. The fusion protein of claim 14, wherein the therapeutic moiety is a therapeutic protein or peptide, optionally an antibody.

16. The fusion protein of claim 15, wherein the diagnostic moiety is a fluorescent protein.

17. The engineered polypeptide of any one of claims 1-10 or the fusion protein of any one of claim 11-16 further comprising a half-life extension moiety.

18. The engineered polypeptide or fusion protein of claim 17, wherein the half-life extension moiety is selected from antibody Fc domains, human serum albumin, serum binding proteins.

19. The engineered polypeptide or fusion protein of claim 18, 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 1×10^{-6} M or less.

20. The engineered polypeptide or fusion protein of claim 19, wherein the half-life extension moiety is selected from the group consisting of SEQ ID Nos: 471-477.

21. A polynucleotide or set of polynucleotides encoding the engineered polypeptide of any one of the preceding claims or the fusion protein of any one of the preceding claims.

22. The polynucleotide or set of polynucleotides comprising a nucleic acid sequence having at least 80%, at least 90%, at least 95, or at least 98% identity to a sequence selected from the group consisting of SEQ ID Nos: 297, 302, 304, 307, 308, 315, 316, 320, 324, 332, 345, 356, 358, 364, 366, 373, 380, 381, 389 and 290, wherein the sequence optionally excludes the nucleotides encoding twenty carboxy terminal residues. .

23. A delivery vehicle comprising the polynucleotide of any one of the preceding claims.

24. The delivery vehicle of claim 23, which is a viral delivery vehicle.

25. The delivery vehicle of claim 24, wherein the viral delivery vehicle is an adenoviral vector, a retroviral vector, a lentiviral vector, or an adeno-associated viral (AAV) vector.

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26. The delivery vehicle of claim 24, which is a non-viral delivery vehicle.

27. The delivery vehicle of claim 26, wherein the non-viral delivery vehicle is a liposome or a lipid nanoparticle.

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28. A plasmid or minicircle comprising the polynucleotide of any one of the preceding claims.

29. A messenger RNA (mRNA) comprising an open reading frame encoding the engineered polypeptide of any one of the preceding claims.

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30. The mRNA of claims 29 further comprising an open reading frame encoding an immune cell binding.

31. A lipid nanoparticle, optionally a cationic lipid nanoparticle, comprising the mRNA of claim 29 or 30.

20

32. A pharmaceutical composition comprising the engineered polypeptide, the fusion protein, or the delivery vehicle of any one of preceding claims, and optionally a pharmaceutically acceptable excipient.

25

33. A conjugate comprising the engineered polypeptide or the fusion protein of any one of claims linked to a pharmacologically active moiety.

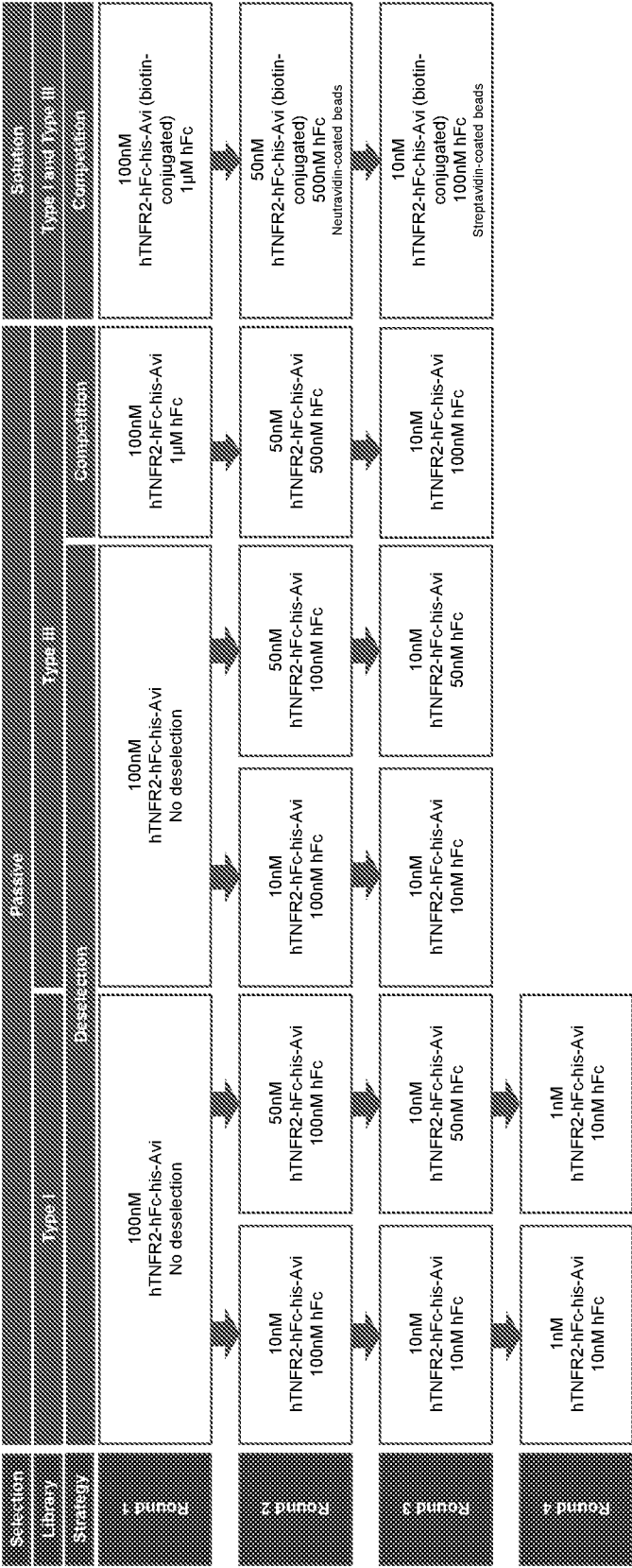
34. A method comprising administering to a subject the pharmaceutical composition of claim 32.

30

35. The method of claim 34, wherein the subject has systemic lupus erythematosus (SLE), lupus nephritis (e.g., drug-induced lupus nephritis), immune

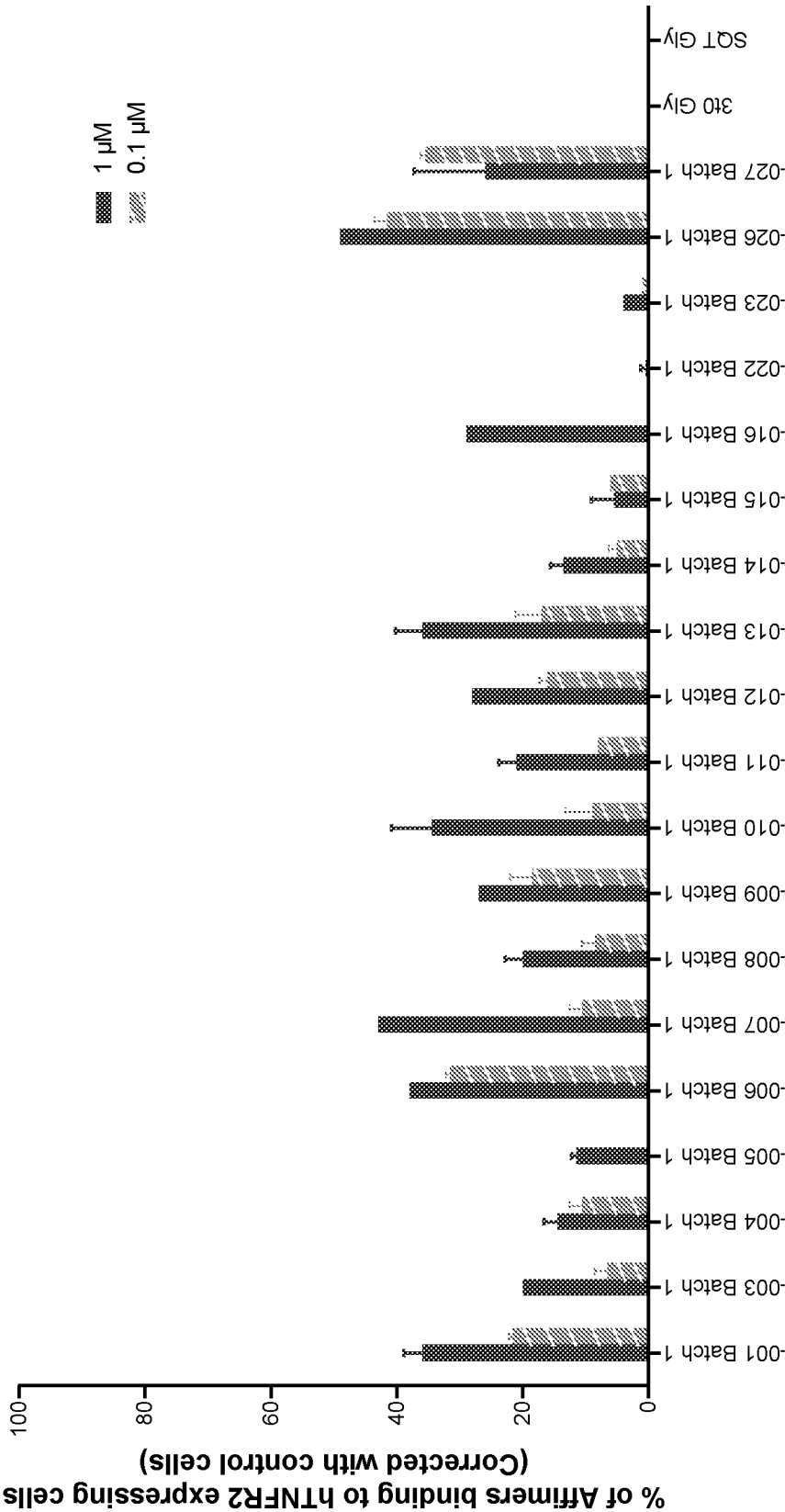
thrombocytopenia (ITP), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD) (e.g., Crohn's disease and colitis/ulcerative colitis), Graft versus Host Disease (GvHD) (relating to stem cell transplants) also called allograft rejection, transplant/Solid Organ Transplantation (SOT), primary biliary cholangitis (PBC), psoriasis, 5 psoriatic arthritis, collagen-induced arthritis, experimental allergic encephalomyelitis (EAE), oophoritis, allergic rhinitis, asthma, Sjogren's syndrome, atopic eczema, Myasthenia gravis, Grave's disease, glomerulosclerosis and/or cancer

Figure 1



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Figure 2



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Figure 3

Clone Name	cELISA % inhibition at 3µM		cELISA IC50 (nM)		Blocker
LG experiment number	Expt 1	Expt 2	Expt 1	Expt 2	
DAW02-001	68.8	73.6	NA	NA	Yes
DAW02-003	37.6	43.2	NA	NA	Maybe
DAW02-004	47.7	48	NA	NA	Maybe
DAW02-006	98.4	98.5	53.8	51.55	Yes
DAW02-007	73.9	81.1	NA	NA	Yes
DAW02-008	37.4	35	NA	NA	Maybe
DAW02-009	75.9	64.5	NA	NA	Yes
DAW02-010	70.8	77.7	NA	NA	Yes
DAW02-011	31.2	40.4	NA	NA	Maybe
DAW02-012	83.3	88.8	NA	NA	Yes
DAW02-013	42.3	51.1	NA	NA	Maybe
DAW02-014	71.7	82.7	NA	NA	Yes
DAW02-015	27	29.8	NA	NA	Maybe
DAW02-021	93.4	96	63.54	18.39	Yes
DAW02-026	97.7	100.2	198.5	62.68	Yes
DAW02-027	85.5	90.2	147.8	79.87	Yes
DAW02-036	34.4	50.4	NA	NA	Maybe
DAW02-037	33.8	48.2	NA	NA	Maybe
DAW02-038	24.8	37	NA	NA	Maybe
DAW02-044	97.8	99.1	183.8	128.4	Yes
DAW02-045	41	56.1	NA	NA	Maybe
DAW02-058	33.1	44.3	NA	NA	Maybe
DAW02-059	74.4	81.2	NA	NA	Yes
DAW02-060	56.7	66.1	NA	NA	Yes
DAW02-069	77.6	88.5	230	76.23	Yes
DAW02-072	37.2	54.6	NA	NA	Maybe
DAW02-080	48.3	58	NA	NA	Maybe
DAW02-100	74.1	84.5	NA	NA	Yes
DAW02-108	83	88.9	200	103.5	Yes
DAW02-115	92.2	95.8	50.59	25.96	Yes
DAW02-117	31.5	44.9	NA	NA	Maybe
DAW02-120	34.4	50.4	NA	NA	Maybe

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Figure 4

clone	40000 cells - Absorbance 640 nm	Ranking	40000 cells - Absorbance 640 nm	Ranking
001	1.946	1	1.375	2
007	1.525	7	1.356	4
009	1.692	3	1.172	5
012	1.6835	4	1.359	3
013	1.0985	11	0.9945	7
026	1.537	6	1.031	6
027	0.9695	15	0.6555	17
037	1.079	12	0.8675	11
044	1.6825	5	0.978	8
059	1.281	9	0.6525	18
069	1.124	10	0.7195	15
079	1.3055	8	0.8445	12
100	1.808	2	1.462	1
103	1.0065	13	0.965	10
112	0.8235	18	0.5885	19
115	0.973	14	0.77	13

Figure 5

clone	Expts. triplicate			Cynomolgus EC ₅₀ Estimation
-001	Low binding	No binding	No binding	No binding
-007	Low binding	No binding	No binding	No binding
-009	No binding	Low binding	No binding	No binding
-012	361.03	98.54	123.1	~70 nM
-013	15.37	114.6	24.40	~35 nM
-026	4.36	23.24	2.490	~6 nM
-027	12.72	37.1	20.03	~21 nM
-037	28.9	No full curve	81.75	~42 nM
-044	7.00	88.3	41.93	~27 nM
-059	12.11	76.98	24.43	~28 nM
-069	5.174	8.68	0.99	~4 nM
-079	82.17	818.6	182.2	~222 nM
-100	No full curve	59.13	No full curve	~59 nM
-103	Low binding	320.8	374.2	~351 nM
-112	18.08	27.26	30.55	~21 nM
-115	24.18	70.53	58.52	~48 nM

clone	Expts. triplicate			Human EC ₅₀ Estimation
-001	19.84	8.22	17.22	~12 nM
-007	51.98	16.46	111.6	~45 nM
-009	36.21	44.77	38.74	~40 nM
-012	48.76	26.81	21.89	~30 nM
-013	48.76	24.82	15.91	~27 nM
-026	5.60	2.81	0.29	~2 nM
-027	9.18	0.98	3.07	~6 nM
-037	169.6	123.7	82.86	~120 nM
-044	14.48	39.14	11.46	~18 nM
-059	40.67	10.34	7.81	~15 nM
-069	5.176	No full curve	0.083	~1 nM
-079	97.01	154.8	177.2	~139 nM
-100	48.08	37.81	9.48	~28 nM
-103	114.1	215.7	98.61	~124 nM
-112	54.48	19.37	7.97	~28 nM
-115	7.39	13.94	2.97	~7 nM

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2023/052513

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K14/715 C07K14/81 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, WPI Data, Sequence Search		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/227018 A1 (SILVERBACK THERAPEUTICS INC [US]) 13 December 2018 (2018-12-13) abstract pages 1-2, paragraph 4 pages 3-4, paragraph 7 page 53, paragraph 126 page 54, paragraph 132 - page 55, paragraph 133 page 103, paragraph 173 page 104, paragraph 176 page 105, paragraph 179 ----- -/--	1-35
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 23 October 2023		Date of mailing of the international search report 08/11/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Grötzinger, Thilo

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2023/052513

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/097385 A1 (GLAXO GROUP LTD [GB]; HAMBLIN PAUL ANDREW [GB] ET AL.) 2 September 2010 (2010-09-02) abstract page 3, line 7 - line 14 page 7, line 9 - line 11 page 8, line 18 - line 22 page 11, line 39 - page 12, line 2 page 13, line 21 - line 24 page 16, line 11 - line 17 -----	1-35
A	WO 2021/249786 A1 (AVACTA LIFE SCIENCES LTD [GB]) 16 December 2021 (2021-12-16) abstract page 35, line 19 - page 36, line 18 -----	1-35
A	WO 2019/008335 A1 (AVACTA LIFE SCIENCES LTD [GB]) 10 January 2019 (2019-01-10) abstract page 29, line 26 - line 35; sequence 1 -----	1-35

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2023/052513

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2023/052513

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018227018 A1	13-12-2018	AU 2018279105 A1 CA 3065852 A1 CN 110891605 A EP 3634485 A1 JP 2020523319 A US 2020199247 A1 WO 2018227018 A1	19-12-2019 13-12-2018 17-03-2020 15-04-2020 06-08-2020 25-06-2020 13-12-2018
WO 2010097385 A1	02-09-2010	CA 2753287 A1 EP 2401298 A1 JP 2012518398 A US 2011305692 A1 WO 2010097385 A1	02-09-2010 04-01-2012 16-08-2012 15-12-2011 02-09-2010
WO 2021249786 A1	16-12-2021	NONE	
WO 2019008335 A1	10-01-2019	AU 2018295447 A1 AU 2023208125 A1 CA 3067682 A1 CN 111132999 A EP 3649151 A1 IL 271340 A JP 2020532949 A KR 20200024869 A SG 11201912298Q A TW 201920256 A US 2020181239 A1 WO 2019008335 A1	16-01-2020 12-10-2023 10-01-2019 08-05-2020 13-05-2020 30-01-2020 19-11-2020 09-03-2020 30-01-2020 01-06-2019 11-06-2020 10-01-2019