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(54) **Title**: ALDEHYDE-TAGGED IMMUNOGLOBULIN POLYPEPTIDES AND METHOD OF USE THEREOF

IgG1
Light chain conserved region:

Figure 1A

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        140       150       160       170       180       189
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD

        200       210       220       230   236
SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
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Heavy chain conserved region:

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      130       140       150       160       170       180
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

      190       200       210       220       230       240
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG

      250       260       270       280       290       300
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN

      310       320       330       340       350       360
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE

      370       380       390       400       410       420
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW

      430       440       450
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
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(57) **Abstract**: The present disclosure provides aldehyde-tagged immunoglobulin (Ig) polypeptides that can be converted by a formylglycine-generating enzyme to produce a 2-formylglycine (FGly)-modified Ig polypeptide. An FGly-modified Ig polypeptide can be covalently and site-specifically bound to a moiety of interest to provide an Ig conjugate. The disclosure also encompasses methods of production of such aldehyde-tagged Ig polypeptides, FGly-modified Ig polypeptides, and Ig conjugates, as well as methods of use of same.

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ALDEHYDE-TAGGED IMMUNOGLOBULIN POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. provisional application serial no. 61/433,042, filed January 14, 2011, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] Antibodies find use in various diagnostic and therapeutic applications. Antibodies can also be used to deliver drugs. However, conjugation of a drug to an antibody can be difficult to control, resulting in a heterogeneous mixture of conjugates that differ in the number of drug molecules attached. This can make controlling the amount administered to a patient difficult.

Literature

[0003] U.S. Patent Publication No. 2010/0210543; WO 2010/096394; U.S. Patent Publication No. 2008/0187956; WO 2009/120611.

SUMMARY

[0004] The present disclosure provides aldehyde-tagged immunoglobulin (Ig) polypeptides that can be converted by a formylglycine-generating enzyme to produce a formylglycine (FGly)-modified Ig polypeptide. An FGly-modified Ig polypeptide can be covalently and site-specifically bound to a moiety of interest to provide an Ig conjugate. The disclosure also encompasses methods of production of such aldehyde-tagged Ig polypeptides, FGly-modified Ig polypeptides, and Ig conjugates, as well as methods of use of same.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Figure 1A depicts a site map showing possible modification sites for generation of an aldehyde tagged Ig polypeptide. The upper sequence is the amino acid sequence of the conserved region of an IgG1 light chain polypeptide (SEQ ID NO:1) and shows possible modification sites in an Ig light chain; the lower sequence is the amino acid sequence of the conserved region of an Ig heavy chain polypeptide (SEQ ID NO:228; GenBank Accession No. AAG00909) and shows possible modification sites in an Ig heavy chain. The heavy and light chain numbering is based on the full-length heavy and light chains.

[0006] Figure 1B depicts an alignment of immunoglobulin heavy chain constant regions for IgG1 (SEQ ID NO:2), IgG2 (SEQ ID NO:4), IgG3 (SEQ ID NO:3), IgG4 (SEQ

ID NO:5), and IgA (SEQ ID NO:6), showing modification sites at which aldehyde tags can be provided in an immunoglobulin heavy chain. The heavy and light chain numbering is based on the full- heavy and light chains.

[0007] Figure 1C depicts an alignment of immunoglobulin light chain constant regions (SEQ ID NOS:1 and 7-10), showing modification sites at which aldehyde tags can be provided in an immuoglobulin immunoglobulin light chain.

[0008] Figure 2 presents a scheme for expression of aldehyde-tagged antibodies and their subsequent chemical conjugation.

[0009] Figure 3 depicts solvent-accessible loop regions in anti-CD19 light chain (upper sequence (SEQ ID NO:11)) and heavy chain (lower sequence (SEQ ID NO:12)) constant regions, with an LCTPSR sulfatase motif in the heavy chain constant region. The signal peptide is shown in lower-case letters; the variable region is underlined; solvent-accessible loop regions in the constant regions are shown in bold and underlined. The LCTPSR sequence is shown in bold and double underlining.

[0010] Figure 4 depicts protein blot analysis of aldehyde-tagged anti-CD19 and aldehyde-tagged anti-CD22 antibodies. The left panel provides a schematic of an antibody and indicates the relative positions of examples of sites of aldehyde tag modification in an Ig heavy chain CH1 region ("CH1 (A)", "CH1 (B)", "CH1 (C)"), Ig heavy chain CH2 region ("CH2 (A)", "CH2 (B)", "CH2 (C)"), CH2/3 region ("CH2/CH3"), and C-terminal region ("C-terminal").

[0011] Figure 5 depicts Western blot analysis of a) aldehyde-tagged anti-CD22 antibodies chemically conjugated with aminooxy-FLAG (Panel A); and b) Western blot analysis of aldehyde-tagged anti-CD19 antibodies and aldehyde-tagged anti-CD22 antibodies chemically conjugated with aminooxy-FLAG.

[0012] Figures 6A and 6B depict a nucleotide sequence (Figure 6A; (SEQ ID NO:13)) encoding the heavy chain of a CD22-specific IgG1 antibody, and the encoded amino acid sequence (Figure 6B; (SEQ ID NO:14)). The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0013] Figures 7A and 7B depict a nucleotide sequence (Figure 7A; (SEQ ID NO:15)) encoding an aldehyde-tagged anti-CD22 immunoglobulin (Ig) heavy chain ("CH1 (A) LCTPSR"), and the encoded amino acid sequence (Figure 7B; (SEQ ID NO:16)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH1 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0014] Figures 8A and 8B depict a nucleotide sequence (Figure 8A; (SEQ ID NO:18)) encoding an aldehyde-tagged anti-CD22 immunoglobulin (Ig) heavy chain ("CH1 (B) LCTPSR"), and the encoded amino acid sequence (Figure 8B; (SEQ ID NO:19)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH1 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0015] Figures 9A and 9B depict a nucleotide sequence (Figure 9A; (SEQ ID NO:20)) encoding an aldehyde-tagged anti-CD22 immunoglobulin (Ig) heavy chain ("CH1 (C) LCTPSR"), and the encoded amino acid sequence (Figure 9B; (SEQ ID NO:21)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH1 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0016] Figures 10A and 10B depict a nucleotide sequence (Figure 10A; (SEQ ID NO:22)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH1 (C) LATPSR"), and the encoded amino acid sequence (Figure 10B; (SEQ ID NO:23)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence in CH1 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0017] Figures 11A and 11B depict a nucleotide sequence (Figure 11A; (SEQ ID NO:25)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH2 (A) LCTPSR"), and the encoded amino acid sequence (Figure 11B; (SEQ ID NO:26)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH2 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0018] Figures 12A and 12B depict a nucleotide sequence (Figure 12A; (SEQ ID NO:27)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH2 (B) LCTPSR"), and the encoded amino acid sequence (Figure 12B; (SEQ ID NO:28)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH2 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0019] Figures 13A and 13B depict a nucleotide sequence (Figure 13A; (SEQ ID NO:29)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH2 (C) LCTPSR"), and the encoded amino acid sequence (Figure 13B; (SEQ ID NO:30)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH2 is underlined. The end of the signal sequence is

denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0020] Figures 14A and 14B depict a nucleotide sequence (Figure 14A; (SEQ ID NO:31)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH2 (C)"), and the encoded amino acid sequences (Figure 14B; (SEQ ID NO:32)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence in CH2 is underlined.

[0021] Figures 15A and 15B depict a nucleotide sequence (Figure 15A; (SEQ ID NO:33)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH2/CH3 LCTPSR"), and the encoded amino acid sequences (Figure 15B; (SEQ ID NO:34)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in CH2/CH3 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0022] Figures 16A and 16B depict a nucleotide sequence (Figure 16A; (SEQ ID NO:35)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("CH2/CH3 LATPSR"), and the encoded amino acid sequences (Figure 16B; (SEQ ID NO:36)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence in CH2/CH3 is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0023] Figures 17A and 17B depict a nucleotide sequence (Figure 17A; (SEQ ID NO:37)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("C-terminal LCTPSR"), and the encoded amino acid sequences (Figure 17B; (SEQ ID NO:38)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in the C-terminal region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0024] Figures 18A and 18B depict a nucleotide sequence (Figure 18A; (SEQ ID NO:39)) encoding an aldehyde-tagged anti-CD22 Ig heavy chain ("C-terminal LATPSR").., and the encoded amino acid sequences (Figure 18B; (SEQ ID NO:40)) The LATPSR (SEQ ID NO:24) sulfatase motif sequence in the C-terminal region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0025] Figures 19A and 19B depict a nucleotide sequence (Figure 19A; (SEQ ID NO:41)) encoding a CD22-specific human Ig kappa light chain, and the encoded amino acid sequence (Figure 19B; (SEQ ID NO:42)). The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0026] Figures 20A and 20B depict a nucleotide sequence (Figure 20A; (SEQ ID NO:43)) encoding an aldehyde-tagged anti-CD22 Ig kappa light chain, and the encoded amino acid sequences (Figure 20B; (SEQ ID NO:44)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0027] Figures 21A and 21B depict a nucleotide sequence (Figure 21A; (SEQ ID NO:45)) encoding an aldehyde-tagged anti-CD22 Ig kappa light chain, and the encoded amino acid sequences (Figure 21B; (SEQ ID NO:46)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0028] Figures 22A and 22B depict a nucleotide sequence (Figure 22A; (SEQ ID NO:47)) encoding the heavy chain of a CD19-specific IgG1 antibody, and the encoded amino acid sequence (Figure 22B; (SEQ ID NO:48)). The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0029] Figures 23A and 23B depict a nucleotide sequence (Figure 23A; (SEQ ID NO:49)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("CH1 (C) LCTPSR"), and the encoded amino acid sequences (Figure 23B; (SEQ ID NO:50)) (CHI (C)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in the CH1 region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0030] Figures 24A and 24B depict a nucleotide sequence (Figure 24A; (SEQ ID NO:51)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("CH1 (C) LATPSR"), and the encoded amino acid sequences (Figure 24B; (SEQ ID NO:52)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence in the CH1 region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0031] Figures 25A and 25B depict a nucleotide sequence (Figure 25A; (SEQ ID NO:53)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("CH2 (B) LCTPSR"), and the encoded amino acid sequences (Figure 25B; (SEQ ID NO:54)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in the CH2 region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0032] Figures 26A and 26B depict a nucleotide sequence (Figure 26A; (SEQ ID NO:55)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("CH2 (B) LATPSR"), and the encoded amino acid sequences (Figure 26B; (SEQ ID NO:56)). The LATPSR (SEQ ID

NO:24) sulfatase motif sequence in the CH2 region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0033] Figures 27A and 27B depict a nucleotide sequence (Figure 27A; (SEQ ID NO:57)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("CH2/CH3 LCTPSR"), and the encoded amino acid sequences (Figure 27B; (SEQ ID NO:58)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in the CH2/CH3 region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0034] Figures 28A and 28B depict a nucleotide sequence (Figure 28A; (SEQ ID NO:59)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("CH2/CH3 LATPSR"), and the encoded amino acid sequences (Figure 28B; (SEQ ID NO:60)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence in the CH2/CH3 region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0035] Figures 29A and 29B depict a nucleotide sequence (Figure 29A; (SEQ ID NO:61)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("C-terminal LCTPSR"), and the encoded amino acid sequences (Figure 29B; (SEQ ID NO:62)). The LCTPSR (SEQ ID NO:17) sulfatase motif sequence in the C-terminal region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0036] Figures 30A and 30B depict a nucleotide sequence (Figure 30A; (SEQ ID NO:63)) encoding an aldehyde-tagged anti-CD19 Ig heavy chain ("C-terminal LATPSR"), and the encoded amino acid sequences (Figure 30B; (SEQ ID NO:64)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence in the C-terminal region is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0037] Figures 31A and 31B depict a nucleotide sequence (Figure 31A; (SEQ ID NO:65)) encoding a CD19-specific human Ig kappa light chain, and the encoded amino acid sequence (Figure 31B; (SEQ ID NO:66)). The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0038] Figures 32A and 32B depict a nucleotide sequence (Figure 32A; (SEQ ID NO:67)) encoding an aldehyde-tagged anti-CD19 Ig kappa light chain, and the encoded amino acid sequences (Figure 32B; (SEQ ID NO:68)). The LCTPSR (SEQ ID NO:17)

sulfatase motif sequence is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

[0039] Figures 33A and 33B depict a nucleotide sequence (Figure 33A; (SEQ ID NO:69)) encoding an aldehyde-tagged anti-CD19 Ig kappa light chain, and the encoded amino acid sequences (Figure 33B; (SEQ ID NO:70)). The LATPSR (SEQ ID NO:24) sulfatase motif sequence is underlined. The end of the signal sequence is denoted by "/". The end of the variable region and the beginning of the constant region is denoted "//".

DEFINITIONS

[0040] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymeric form of amino acids of any length. Unless specifically indicated otherwise, "polypeptide," "peptide," and "protein" can include genetically coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, proteins which contain at least one N-terminal methionine residue (e.g., to facilitate production in a recombinant bacterial host cell); immunologically tagged proteins; and the like.

[0041] "Native amino acid sequence" or "parent amino acid sequence" are used interchangeably herein in the context of an immunoglobulin to refer to the amino acid sequence of the immunoglobulin prior to modification to include a heterologous aldehyde tag.

[0042] The term "antibody" is used in the broadest sense and includes monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, and multispecific antibodies (e.g., bispecific antibodies), humanized antibodies, single-chain antibodies, chimeric antibodies, antibody fragments (e.g., Fab fragments), and the like. An antibody is capable of binding a target antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) Immuno Biology, 5th Ed., Garland Publishing, New York). A target antigen can have one or more binding sites, also called epitopes, recognized by complementarity determining regions (CDRs) formed by one or more variable regions of an antibody.

[0043] "Immunoglobulin polypeptide" as used herein refers to a polypeptide comprising at least a constant region of a light chain polypeptide or at least a constant region of a heavy chain polypeptide.

[0044] An immunoglobulin polypeptide immunoglobulin light or heavy chain variable region is composed of a framework region (FR) interrupted by three hypervariable regions, also called "complementarity determining regions" or "CDRs". The extent of the framework region and CDRs have been defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, 1991). The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs. The CDRs are primarily responsible for binding to an epitope of an antigen.

[0045] The term "natural antibody" refers to an antibody in which the heavy and light chains of the antibody have been made and paired by the immune system of a multi-cellular organism. Spleen, lymph nodes, bone marrow and serum are examples of tissues that produce natural antibodies. For example, the antibodies produced by the antibody producing cells isolated from a first animal immunized with an antigen are natural antibodies.

[0046] Throughout the present disclosure, the numbering of the residues in an immunoglobulin heavy chain and in an immunoglobulin light chain is that as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), expressly incorporated herein by reference.

[0047] A "parent Ig polypeptide" is a polypeptide comprising an amino acid sequence which lacks an aldehyde-tagged constant region as described herein. The parent polypeptide may comprise a native sequence constant region, or may comprise a constant region with pre-existing amino acid sequence modifications (such as additions, deletions and/or substitutions).

[0048] In the context of an Ig polypeptide, the term "constant region" is well understood in the art, and refers to a C-terminal region of an Ig heavy chain, or an Ig light chain. An Ig heavy chain constant region includes CH1, CH2, and CH3 domains (and CH4 domains, where the heavy chain is a μ or an ε heavy chain). In a native Ig heavy chain, the CH1, CH2, CH3 (and, if present, CH4) domains begin immediately after (C-terminal to) the heavy chain variable (VH) region, and are each from about 100 amino acids to about 130 amino acids in length. In a native Ig light chain, the constant region begins begin immediately after (C-terminal to) the light chain variable (VL) region, and is about 100 amino acids to 120 amino acids in length.

[0049] In some embodiments, a "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity; 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 generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays that are well known in the art.

[0050] Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express FcRs (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII.

[0051] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457 92 (1991); Capel et al., Immunomethods 4:25 34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330 41 (1995).

[0052] The term “humanized antibody” or “humanized immunoglobulin” refers to a non-human (e.g., mouse or rabbit) antibody containing one or more amino acids (in a framework region, a constant region or a CDR, for example) that have been substituted with a correspondingly positioned amino acid from a human antibody. In general, humanized antibodies produce a reduced immune response in a human host, as compared to a non-humanized version of the same antibody. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332). In certain embodiments, framework substitutions are identified by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (see, e.g., U.S. Pat. No. 5,585,089; Riechmann et al., Nature 332:323 (1988)). Additional methods for humanizing antibodies contemplated for use in the present invention are described in U.S. Pat. Nos. 5,750,078; 5,502,167; 5,705,154; 5,770,403; 5,698,417; 5,693,493; 5,558,864; 4,935,496; and 4,816,567, and PCT publications WO 98/45331 and WO 98/45332. In particular embodiments, a subject rabbit antibody may be humanized according to the methods set forth in US20040086979 and US20050033031. Accordingly, the antibodies described above may be humanized using methods that are well known in the art.

[0053] The term “chimeric antibodies” refer to antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable

and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as gamma 1 and gamma 3. An example of a therapeutic chimeric antibody is a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody, although domains from other mammalian species may be used.

[0054] By “aldehyde tag” or “ald-tag” is meant an amino acid sequence that contains an amino acid sequence derived from a sulfatase motif which is capable of being converted, or which has been converted, by action of a formylglycine generating enzyme (FGE) to contain a 2-formylglycine residue (referred to herein as “FGly”). The FGly residue generated by an FGE is often referred to in the literature as a “formylglycine”. Stated differently, the term “aldehyde tag” is used herein to refer to an amino acid sequence comprising an “unconverted” sulfatase motif (i.e., a sulfatase motif in which the cysteine or serine residues has not been converted to FGly by an FGE, but is capable of being converted) as well as to an amino acid sequence comprising a “converted” sulfatase motif (i.e., a sulfatase motif in which the cysteine or the serine residue has been converted to FGly by action of an FGE).

[0055] By “conversion” as used in the context of action of a formylglycine generating enzyme (FGE) on a sulfatase motif refers to biochemical modification of a cysteine or serine residue in a sulfatase motif to a formylglycine (FGly) residue (e.g., Cys to FGly, or Ser to FGly).

[0056] By "genetically-encodable" as used in reference to an amino acid sequence of polypeptide, peptide or protein means that the amino acid sequence is composed of amino acid residues that are capable of production by transcription and translation of a nucleic acid encoding the amino acid sequence, where transcription and/or translation may occur in a cell or in a cell-free in vitro transcription/translation system.

[0057] The term "control sequences" refers to DNA sequences that facilitate expression of an operably linked coding sequence in a particular expression system, e.g. mammalian cell, bacterial cell, cell-free synthesis, etc. The control sequences that are suitable for prokaryote systems, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cell systems may utilize promoters, polyadenylation signals, and enhancers.

[0058] A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked

to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate the initiation of translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. Linking is accomplished by ligation or through amplification reactions. Synthetic oligonucleotide adaptors or linkers may be used for linking sequences in accordance with conventional practice.

[0059] The term “expression cassette” as used herein refers to a segment of nucleic acid, usually DNA, that can be inserted into a nucleic acid (e.g., by use of restriction sites compatible with ligation into a construct of interest or by homologous recombination into a construct of interest or into a host cell genome). In general, the nucleic acid segment comprises a polynucleotide that encodes a polypeptide of interest (e.g., an aldehyde tagged-Ig protein), and the cassette and restriction sites are designed to facilitate insertion of the cassette in the proper reading frame for transcription and translation. Expression cassettes can also comprise elements that facilitate expression of a polynucleotide encoding a polypeptide of interest in a host cell. These elements may include, but are not limited to:a promoter, a minimal promoter, an enhancer, a response element, a terminator sequence, a polyadenylation sequence, and the like.

[0060] As used herein the term "isolated" is meant to describe a compound of interest that is in an environment different from that in which the compound naturally occurs. "Isolated" is meant to include compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified.

[0061] As used herein, the term "substantially purified" refers to a compound that is removed from its natural environment and is at least 60% free, at least 75% free, at least 80% free, at least 85% free, at least 90% free, at least 95% free, at least 98% free, or more than 98% free, from other components with which it is naturally associated.

[0062] The term “physiological conditions” is meant to encompass those conditions compatible with living cells, *e.g.*, predominantly aqueous conditions of a temperature, pH, salinity, *etc.* that are compatible with living cells.

[0063] By "reactive partner" is meant a molecule or molecular moiety that specifically reacts with another reactive partner to produce a reaction product. Exemplary reactive partners include a cysteine or serine of sulfatase motif and an FGE, which react to form a reaction product of a converted aldehyde tag containing an FGly in lieu of cysteine or serine in the motif. Other exemplary reactive partners include an aldehyde of a formylglycine

(FGly) residue of a converted aldehyde tag and an "aldehyde-reactive reactive partner", which comprises an aldehyde-reactive group and a moiety of interest, and which reacts to form a reaction product of a modified aldehyde tagged polypeptide having the moiety of interest conjugated to the modified polypeptide through a modified FGly residue.

[0064] "N-terminus" refers to the terminal amino acid residue of a polypeptide having a free amine group, which amine group in non-N-terminus amino acid residues normally forms part of the covalent backbone of the polypeptide.

[0065] "C-terminus" refers to the terminal amino acid residue of a polypeptide having a free carboxyl group, which carboxyl group in non-C-terminus amino acid residues normally forms part of the covalent backbone of the polypeptide.

[0066] By "internal site" as used in referenced to a polypeptide or an amino acid sequence of a polypeptide means a region of the polypeptide that is not at the N-terminus or at the C-terminus.

[0067] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0068] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0069] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace subject matter that are, for example, compounds that are stable

compounds (i.e., compounds that can be made, isolated, characterized, and tested for biological activity). In addition, all sub-combinations of the various embodiments and elements thereof (e.g., elements of the chemical groups listed in the embodiments describing such variables) are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0070] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0071] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an aldehyde-tagged Ig polypeptide" includes a plurality of such polypeptides and reference to "the drug-conjugated Ig polypeptide" includes reference to one or more drug-conjugated Ig polypeptide and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0072] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination.

[0073] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0074] The present disclosure provides aldehyde-tagged immunoglobulin (Ig) polypeptides that can be converted by a formylglycine-generating enzyme (FGE) to produce a formylglycine (FGly)-modified Ig polypeptide. An FGly-modified Ig polypeptide can be covalently and site-specifically bound to a moiety of interest via reaction with an aldehyde-reactive reactive partner to provide an Ig conjugate. The disclosure also encompasses methods of production of such aldehyde-tagged Ig polypeptides, FGly-modified Ig polypeptides, and Ig conjugates, as well as methods of use of same.

[0075] Aldehyde-tagged Ig polypeptides may also be referred to herein as "ald-tagged Ig polypeptides", "ald-tagged Ig heavy chains" or "ald-tagged Ig light chains". Such Ald-tagged Ig polypeptides can be site-specifically decorated with a covalently bound molecule of interest, such as a drug (e.g., a peptide drug, a small molecule drug, and the like), a water-soluble polymer, a detectable label, a synthetic peptide, etc.

[0076] The compositions and methods of the present disclosure exploit a naturally-occurring, genetically-encodable sulfatase motif for use as a tag, referred to herein as an "aldehyde tag" or "ald tag", to direct site-specific modification of the Ig polypeptide. The sulfatase motif of the aldehyde tag, which is based on a motif found in active sites of sulfatases, contains a serine or cysteine residue that is capable of being converted (oxidized) to a 2-formylglycine (FGly) residue by action of a formylglycine generating enzyme (FGE) either *in vivo* (e.g., at the time of translation of an ald tag-containing protein in a cell) or *in vitro* (e.g., by contacting an ald tag-containing protein with an FGE in a cell-free system). The aldehyde moiety of the resulting FGly residue can be used as a "chemical handle" to facilitate site-specific chemical modification of the Ig polypeptide, and thus site-specific attachment of a moiety of interest. For example, a peptide modified to contain an α-nucleophile-containing moiety (e.g., an aminooxy or hydrazide moiety) can be reacted with the FGly-containing Ig polypeptide to yield a conjugate in which the Ig polypeptide and the peptide are linked by a hydrazone or oxime bond, respectively, or via alternative aldehyde-specific chemistries such as reductive amination. The reactivity of the aldehyde thus allows for bioorthogonal and chemoselective modification of the Ig polypeptide, and thus provides a site-specific means for chemical modification that in turn can be exploited to provide for site-specific attachment of a moiety of interest in the final conjugate.

ALDEHYDE TAGGED IMMUNOGLOBULIN POLYPEPTIDES

[0077] The present disclosure provides isolated aldehyde-tagged Ig polypeptides, including aldehyde-tagged Ig heavy chains and aldehyde-tagged Ig light chains, where the

aldehyde-tagged Ig polypeptides, where the aldehyde tag is within or adjacent a solvent-accessible loop region of the Ig constant region, and where the aldehyde tag is not at the C-terminus of the Ig polypeptide.

[0078] In general, an aldehyde tag can be based on any amino acid sequence derived from a sulfatase motif (also referred to as a "sulfatase domain") which is capable of being converted by action of a formylglycine generating enzyme (FGE) to contain a formylglycine (FGly). Such sulfatase motifs may also be referred to herein as an FGE-modification site. Action of FGE is directed in a sequence-specific manner in that the FGE acts at a sulfatase motif positioned within the immunoglobulin polypeptide.

[0079] The present disclosure also provides a library of aldehyde-tagged Ig polypeptides, where the library comprises a plurality (a population) of members, and where each member Ig polypeptide comprises an aldehyde tag at a different location(s) from the other members.

[0080] The present disclosure provides an aldehyde-tagged antibody, where an aldehyde-tagged antibody can include: 1) an aldehyde-tagged Ig heavy chain constant region; and an aldehyde-tagged Ig light chain constant region; 2) an aldehyde-tagged Ig heavy chain constant region; and an Ig light chain constant region that is not aldehyde tagged; or 3) an Ig heavy chain constant region that is not aldehyde tagged; and an aldehyde-tagged Ig light chain constant region. A subject aldehyde-tagged antibody also includes VH and/or VL domains and can bind antigen.

EXEMPLARY ALDEHYDE TAGS

[0081] A minimal sulfatase motif of an aldehyde tag is usually 5 or 6 amino acid residues in length, usually no more than 6 amino acid residues in length. Sulfatase motifs provided in an Ig polypeptide are at least 5 or 6 amino acid residues, and can be, for example, from 5 to 16, 6-16, 5-15, 6-15, 5-14, 6-14, 5-13, 6-13, 5-12, 6-12, 5-11, 6-11, 5-10, 6-10, 5-9, 6-9, 5-8, or 6-8 amino acid residues in length, so as to define a sulfatase motif of less than 16,15, 14, 13, 12, 11, 10, 9, 8 or 7 amino acid residues in length.

[0082] In general, it is normally desirable to minimize the extent of modification of the native amino acid sequence of the target Ig polypeptide, so as to minimize the number of amino acid residues that are inserted, deleted, substituted (replaced), or added (e.g., to the N- or C-terminus). Minimizing the extent of amino acid sequence modification of the target Ig polypeptide is usually desirable so as to minimize the impact such modifications may have upon Ig function and/or structure. Thus, aldehyde tags of particular interest include those that require modification (insertion, addition, deletion, substitution/replacement) of less than 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 amino acid residues of the amino acid sequence

of the target polypeptide. Where an amino acid sequence native to the Ig polypeptide contains one or more residues of the desired sulfatase motif, the total number of modifications of residues can be reduced, e.g., by site-specification modification of amino acid residues flanking native amino acid residues to provides a sequence of a sulfatase motif.

[0083] It should be noted that while aldehyde tags of particular interest are those comprising at least a minimal sulfatase motif (also referred to a "consensus sulfatase motif"), it will be readily appreciated that longer aldehyde tags are both contemplated and encompassed by the present disclosure and can find use in the compositions and methods of the invention. Aldehyde tags can thus comprise a minimal sulfatase motif of 5 or 6 residues, or can be longer and comprise a minimal sulfatase motif which can be flanked at the N- and/or C-terminal sides of the motif by additional amino acid residues. Aldehyde tags of, for example, 5 or 6 amino acid residues are contemplated, as well as longer amino acid sequences of more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid residues.

[0084] In certain embodiments, the sulfatase motif used may be described by the formula:

[0085] $X_1Z_1X_2Z_2X_3Z_3$ (I)

[0086] where

[0087] Z_1 is cysteine or serine (which can also be represented by (C/S));

[0088] Z_2 is either a proline or alanine residue (which can also be represented by (P/A));

[0089] Z_3 is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), usually A, G, L, V, or I;

[0090] X_1 is present or absent and, when present, can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than a aromatic amino acid or a charged amino acid), usually L, M, V, S or T, more usually L, M, S or V, with the proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X_1 is present; and

[0091] X_2 and X_3 independently can be any amino acid, though usually an aliphatic amino acid, a polar, uncharged amino acid, or a sulfur containing amino acid (i.e., other than a aromatic amino acid or a charged amino acid), usually S, T, A, V, G or C, more usually S, T, A, V or G.

[0092] Thus, the present disclosure provides isolated aldehyde-tagged Ig polypeptides, including aldehyde-tagged Ig heavy chains and aldehyde-tagged Ig light chains,

where the aldehyde-tagged Ig polypeptides comprise an Ig constant region amino acid sequence modified to provide a sequence of at least 5 amino acids of the formula $X_1Z_1X_2Z_2X_3Z_3$, where

[0093] Z_1 is cysteine or serine;

[0094] Z_2 is a proline or alanine residue;

[0095] Z_3 is an aliphatic amino acid or a basic amino acid;

[0096] X_1 is present or absent and, when present, is any amino acid, with the proviso that when the heterologous sulfatase motif is at an N-terminus of the polypeptide, X_1 is present;

[0097] X_2 and X_3 are each independently any amino acid,

[0098] where the sequence is within or adjacent a solvent-accessible loop region of the Ig constant region, and wherein the sequence is not at the C-terminus of the Ig heavy chain.

[0099] It should be noted that, following action of an FGE on the sulfatase motif, Z_1 is oxidized to generate a formylglycine (FGly) residue. Furthermore, following both FGE-mediated conversion and reaction with a reactive partner comprising a moiety of interest, FGly position at Z_1 in the formula above is covalently bound to the moiety of interest (e.g., detectable label, water soluble polymer, polypeptide, drug, etc).

[00100] Where the aldehyde tag is present at a location other than the N-terminus of the target polypeptide, X_1 of the formula above can be provided by an amino acid residue of the native amino acid sequence of the target polypeptide. Therefore, in some embodiments, and when present at a location other than the N-terminus of a target polypeptide, sulfatase motifs are of the formula:

[00101] $(C/S)X_1(P/A)X_2Z_3$ (II)

[00102] where X_1 and X_2 independently can be any amino acid, though usually an aliphatic amino acid, a polar, uncharged amino acid, or a sulfur-containing amino acid (i.e., other than an aromatic amino acid or a charged amino acid), usually S, T, A, V, or C, more usually S, T, A, or V; and Z_3 is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), usually A, G, L, V, or I.

[00103] As noted above, the sulfatase motif can contain additional residues at one or both of the N- and C-terminus of the sequence, e.g., such that the aldehyde tag includes both a sulfatase motif and an "auxiliary motif". In one embodiment, the sulfatase motif includes an auxiliary motif at the C-terminus (i.e., following the arginine residue in the formula above) 1, 2, 3, 4, 5, 6, or all 7 of the contiguous residues of an amino acid sequence of AALLTGR

(SEQ ID NO:92), SQLLTGR (SEQ ID NO:93), AAFMTGR (SEQ ID NO:94), AAFLTGR (SEQ ID NO:95), SAFLTGR (SEQ ID NO:96), ASILTGK (SEQ ID NO:97), VSFLTGR (SEQ ID NO:98), ASLLTGL (SEQ ID NO:99), ASILITG (SEQ ID NO:100), VSFLTGR (SEQ ID NO:101), SAIMTGR (SEQ ID NO:102), SAIVTGR (SEQ ID NO:103), TNLWRG (SEQ ID NO:104), TNLWRGQ (SEQ ID NO:105), TNLCAAS (SEQ ID NO:106), VSLWTGK (SEQ ID NO:107), SMLLTG (SEQ ID NO:108), SMLLTGN (SEQ ID NO:109), SMLLTGT (SEQ ID NO:110), ASFMAGQ (SEQ ID NO:111), or ASLLTGL (SEQ ID NO:112), (see, e.g., Dierks et al. (1999) EMBO J 18(8): 2084–2091), or of GSLFTGR (SEQ ID NO:113). However, such additional C-terminal amino acid residues are not required for FGE-mediated conversion of the sulfatase motif of the aldehyde tag, and thus are only optional and may be specifically excluded from the aldehyde tags described herein. In some embodiments the aldehyde tag does not contain an amino acid sequence CGPSR(M/A)S (SEQ ID NO:114) or CGPSR(M/A) (SEQ ID NO:115), which may be present as a native amino acid sequence in phosphonate monoester hydrolases.

[00104] The sulfatase motif of the aldehyde tag is generally selected so as to be capable of conversion by a selected FGE, e.g., an FGE present in a host cell in which the aldehyde tagged polypeptide is expressed or an FGE which is to be contacted with the aldehyde tagged polypeptide in a cell-free in vitro method.

[00105] Selection of aldehyde tags and an FGE that provide for suitable reactive partners to provide for generation of an FGly in the aldehyde tagged target Ig polypeptide can be readily accomplished in light of information available in the art. In general, sulfatase motifs susceptible to conversion by a eukaryotic FGE contain a cysteine and a proline (i.e., a cysteine and proline at Z_1 and Z_2, respectively, in Formula I above (e.g., $X_1CX_2PX_3Z_3$); $CX_1PX_2Z_3$ in Formula II above) and are modified by the "SUMF1-type" FGE (Cosma et al. Cell 2003, 113, (4), 445-56; Dierks et al. Cell 2003, 113, (4), 435-44). Sulfatase motifs susceptible to conversion by a prokaryotic FGE contain either a cysteine or a serine, and a proline in the sulfatase motif (i.e., a cysteine or serine at Z_1, and a proline at Z_2, respectively, in Formula I above (e.g., $X_1(C/S)X_2PX_3Z_3$); $(C/S)X_1PX_2Z_3$ in Formula II above) are modified either by the "SUMF1-type" FGE or the "AtsB-type" FGE, respectively (Szameit et al. J Biol Chem 1999, 274, (22), 15375-81). Other sulfatase motifs susceptible to conversion by a prokaryotic FGE contain either a cysteine or a serine, and either a proline or an alanine in the sulfatase motif (i.e., a cysteine or serine at Z_1, and a proline or alanine at Z_2, respectively, in Formula I or II above (e.g., $X_1CX_2PX_3R$; $X_1SX_2PX_2R$; $X_1CX_2AX_3R$; $X_1SX_2AX_3R$; CX_1PX_2R; SX_1PX_2R; CX_1AX_2R; SX_1AX_2R, $X_1CX_2PX_3Z_3$; $X_1SX_2PX_2\ Z_3$; $X_1CX_2AX_3Z_3$; $X_1SX_2AX_3Z_3$; $CX_1PX_2Z_3$; $SX_1PX_2Z_3$; $CX_1AX_2Z_3$; $SX_1AX_2Z_3$), and are susceptible to

modification by, for example, can be modified by an FGE of a Firmicutes (e.g., *Clostridium perfringens*) (see Berteau et al. *J. Biol. Chem.*.2006; 281:22464-22470) or an FGE of *Mycobacterium tuberculosis*.

[00106] Therefore, for example, where the FGE is a eukaryotic FGE (e.g., a mammalian FGE, including a human FGE), the sulfatase motif is usually of the formula:

[00107] $X_1CX_2PX_3Z_3$

[00108] where

[00109] X_1 may be present or absent and, when present, can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than a aromatic amino acid or a charged amino acid), usually L, M, S or V, with the proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X_1 is present;

[00110] X_2 and X_3 independently can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than a aromatic amino acid or a charged amino acid), usually S, T, A, V, G, or C, more usually S, T, A, V or G; and

[00111] Z_3 is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), usually A, G, L, V, or I;.

[00112] Specific examples of sulfatase motifs include LCTPSR (SEQ ID NO:17), MCTPSR (SEQ ID NO:116), VCTPSR (SEQ ID NO:117), LCSPSR (SEQ ID NO:118), LCAPSR (SEQ ID NO:119), LCVPSR (SEQ ID NO:120), LCGPSR (SEQ ID NO:121), ICTPAR (SEQ ID NO:122), LCTPSK (SEQ ID NO:123), MCTPSK (SEQ ID NO:124), VCTPSK (SEQ ID NO:125), LCSPSK (SEQ ID NO:126), LCAPSK (SEQ ID NO:127), LCVPSK (SEQ ID NO:128), LCGPSK (SEQ ID NO:129), LCTPSA (SEQ ID NO:130), ICTPAA (SEQ ID NO:131), MCTPSA (SEQ ID NO:132), VCTPSA (SEQ ID NO:133), LCSPSA (SEQ ID NO:134), LCAPSA (SEQ ID NO:135), LCVPSA (SEQ ID NO:136), and LCGPSA (SEQ ID NO:137). Other specific sulfatase motifs are readily apparent from the disclosure provided herein.

FORMYLGLYCINE-MODIFIED IG POLYPEPTIDES

[00113] As described in more detail below, a converted aldehyde tagged Ig polypeptide is reacted with a reactive partner containing a moiety of interest to provide for conjugation of the moiety of interest to the FGly residue of the converted aldehyde tagged Ig polypeptide, and production of a modified polypeptide. Modified Ig polypeptides having a

modified aldehyde tag are generally described by comprising a modified sulfatase motif of the formula:

[00114] $X_1(FGly')X_2Z_2X_3Z_3$ (I')

[00115] where

[00116] FGly' is the formylglycine residue having a covalently attached moiety;

[00117] Z_2 is either a proline or alanine residue (which can also be represented by (P/A)); Z_3 is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), usually A, G, L, V, or I;

[00118] X_1 may be present or absent and, when present, can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than a aromatic amino acid or a charged amino acid), usually L, M, V, S or T, more usually L, M or V, with the proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X_1 is present; and

[00119] X_2 and X_3 independently can be any amino acid, though usually an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than a aromatic amino acid or a charged amino acid), usually S, T, A, V, G or C, more usually S, T, A, V or G.

[00120] Thus, the present disclosure provides an Ig polypeptide modified to comprise formylglycine moiety, wherein the Ig polypeptide comprises an FGly-converted sulfatase motif of the formula:

[00121] $X_1(FGly)X_2Z_2X_3Z_3$

[00122] wherein:

[00123] X_1 is present or absent and, when present, is any amino acid, with the proviso that when the sulfatase motif is at an N-terminus of the polypeptide, X_1 is present;

[00124] X_2 and X_3 are each independently any amino acid; and

[00125] Z^3 is a basic amino acid; and

[00126] where the FGly-modified Ig polypeptide presents the FGly group on a solvent-accessible surface when in a folded state.

[00127] The present disclosure also provides a library of FGly-modified Ig polypeptides, where the library comprises a plurality (a population) of members, where each member FGly-modified Ig polypeptide comprises an FGly-modified aldehyde tag, and where each member FGly-modified Ig polypeptide comprises an aldehyde tag at a different location(s) from the other members. Figure 2 depicts an example of a scheme for generating a library of FGly-modified Ig polypeptides, in which each member Ig polypeptide comprises an

aldehyde tag at a different location from the other members. Figure 2 depicts attachment of drug to the FGly-modified polypeptides.

[00128] The present disclosure provides an FGly-modified antibody, where an FGly-modified antibody can include: 1) an FGly-modified Ig heavy chain constant region; and an FGly-modified Ig light chain constant region; 2) an FGly-modified Ig heavy chain constant region; and an Ig light chain constant region that is not FGly-modified; or 3) an Ig heavy chain constant region that is not FGly-modified; and an FGly-modified Ig light chain constant region. A subject FGly-modified antibody also includes VH and/or VL domains and can bind antigen.

[00129] Specific examples of converted sulfatase motifs include L(FGly)TPSR (SEQ ID NO:138), M(FGly)TPSR (SEQ ID NO:139), V(FGly)TPSR (SEQ ID NO:140), L(FGly)SPSR (SEQ ID NO:141), L(FGly)APSR (SEQ ID NO:142), L(FGly)VPSR (SEQ ID NO:143), and L(FGly)GPSR (SEQ ID NO:144), I(FGly)TPAR (SEQ ID NO:145), L(FGly)TPSK (SEQ ID NO:146), M(FGly)TPSK (SEQ ID NO:147), V(FGly)TPSK (SEQ ID NO:148), L(FGly)SPSK (SEQ ID NO:149), L(FGly)APSK (SEQ ID NO:150), L(FGly)VPSK (SEQ ID NO:151), L(FGly)GPSK (SEQ ID NO:152), L(FGly)TPSA (SEQ ID NO:152), M(FGly)TPSA (SEQ ID NO:153), V(FGly)TPSA (SEQ ID NO:154), L(FGly)SPSA (SEQ ID NO:155), L(FGly)APSA (SEQ ID NO:156), L(FGly)VPSA (SEQ ID NO:157), and L(FGly)GPSA (SEQ ID NO:158).

[00130] As described in more detail below, the moiety of interest can be any of a variety of moieties such as a water-soluble polymer, a detectable label, a drug, or a moiety for immobilization of the Ig polypeptide in a membrane or on a surface. As is evident from the above discussion of aldehyde tagged Ig polypeptides, the modified sulfatase motif of the modified polypeptide can be positioned at any desired site of the polypeptide. Thus, the present disclosure provides, for example, a modified polypeptide having a modified sulfatase motif positioned at a site of post-translational modification of a parent of the modified polypeptide (i.e., if the target polypeptide is modified to provide an aldehyde tag at a site of post-translational modification, the later-produced modified polypeptide will contain a moiety at a position corresponding to this site of post-translational modification in the parent polypeptide). For example, then, a modified polypeptide can be produced so as to have a covalently bound, water-soluble polymer at a site corresponding to a site at which glycosylation would normally occur in the parent target polypeptide. Thus, for example, a PEGylated polypeptide can be produced having the PEG moiety positioned at the same or nearly the same location as sugar residues would be positioned in the naturally-occurring parent polypeptide. Similarly, where the parent target polypeptide is engineered to include

one or more non-native sites of post-translational modification, the modified polypeptide can contain covalently attached water-soluble polymers at one or more sites of the modified polypeptide corresponding to these non-native sites of post-translational modification in the parent polypeptide.

MODIFICATION OF A TARGET IG POLYPEPTIDE TO INCLUDE AN ALDEHYDE TAG

[00131] Modification of a target Ig polypeptide to include one or more aldehyde tags can be accomplished using recombinant molecular genetic techniques, so as produce nucleic acid encoding the desired aldehyde tagged Ig polypeptide. Such methods are well known in the art, and include cloning methods, site-specific mutation methods, and the like (see, e.g., Sambrook et al., In "Molecular Cloning: A Laboratory Manual" (Cold Spring Harbor Laboratory Press 1989); "Current Protocols in Molecular Biology" (eds., Ausubel et al.; Greene Publishing Associates, Inc., and John Wiley & Sons, Inc. 1990 and supplements).

Target Immunoglobulin Heavy and Light Chains

[00132] As discussed above, the present disclosure provides aldehyde-tagged Ig polypeptides, FGly-modified aldehyde-tagged Ig polypeptides, and Ig conjugates. The Ig polypeptides used to generate an aldehyde-tagged Ig polypeptide, an FGly-modified aldehyde-tagged Ig polypeptide, or an Ig conjugate, of the present disclosure, include at least an Ig constant region, e.g., an Ig heavy chain constant region (e.g., at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain), or an Ig light chain constant region. Such Ig polypeptides are referred to herein as "target Ig polypeptides."

[00133] A target Ig polypeptide can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 330 amino acids of an amino acid sequence of a heavy chain constant region depicted in Figure 1B. For example, a target Ig polypeptide can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 330 amino acids of the amino acid sequence set forth in SEQ ID NO:2.

[00134] A target Ig polypeptide can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to about 233 amino acids, or from about 200 amino acids to about 236 amino acids, of an amino acid sequence of a light chain constant region depicted in Figure 1C. For example, a target Ig polypeptide can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to about 236 amino acids of the amino acid sequence set forth in SEQ ID NO:1.

[00135] As noted above, a target Ig polypeptide generally includes at least an Ig heavy chain constant region or an Ig light chain constant region, and can further include an Ig variable region (e.g., a V_L region and/or a V_H region). Ig heavy chain constant regions include Ig constant regions of any heavy chain isotype, non-naturally occurring Ig heavy chain constant regions (including consensus Ig heavy chain constant regions). An Ig constant region can be modified to include an aldehyde tag, where the aldehyde tag is present in or adjacent a solvent-accessible loop region of the Ig constant region.

[00136] An Ig constant region can be modified by insertion and/or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids, or more than 16 amino acids, to provide an amino acid sequence of a sulfatase motif as described above.

[00137] In some cases, an aldehyde-tagged Ig polypeptide of the present disclosure comprises an aldehyde-tagged Ig heavy chain constant region (e.g., at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain). The aldehyde-tagged Ig heavy chain constant region can include heavy chain constant region sequences of an IgA, IgM, IgD, IgE, IgG1, IgG2, IgG3, or IgG4 isotype heavy chain or any allotypic variant of same, e.g., human heavy chain constant region sequences or mouse heavy chain constant region sequences, a hybrid heavy chain constant region, a synthetic heavy chain constant region, or a consensus heavy chain constant region sequence, etc., modified to include at least one sulfatase motif that can be modified by an FGE to generate an FGly-modified Ig polypeptide. Allotypic variants of Ig heavy chains are known in the art. See, e.g., Jefferis and Lefranc (2009) *MAbs* 1:4.

[00138] In some cases, an aldehyde-tagged Ig polypeptide of the present disclosure comprises an aldehyde-tagged Ig light chain constant region. The aldehyde-tagged Ig light chain constant region can include constant region sequences of a kappa light chain, a lambda light chain, e.g., human kappa or lambda light chain constant regions, a hybrid light chain constant region, a synthetic light chain constant region, or a consensus light chain constant region sequence, etc., modified to include at least one sulfatase motif that can be modified by an FGE to generate an FGly-modified Ig polypeptide. Exemplary constant regions include human gamma 1 and gamma 3 regions. With the exception of the sufatase motif, a modified constant region may have a wild-type amino acid sequence, or it may have an amino acid sequence that is at least 70% identical (e.g., at least 80%, at least 90% or at least 95% identical) to a wild type amino acid sequence.

[00139] As noted above, an isolated aldehyde-tagged Ig polypeptide of the present disclosure comprises an Ig constant region amino acid sequence modified to provide a sulfatase motif sequence of at least 5 amino acids of the formula described above, where the sequence is within or adjacent a solvent-accessible loop region of the Ig polypeptide constant region. In some embodiments the sulfatase motif is at a position other than, or in addition to, the C-terminus of the Ig polypeptide heavy chain.

[00140] Solvent accessible loop of an antibody can be identified by molecular modeling, or by comparison to a known antibody structure. The relative accessibility of amino acid residues can also be calculated using a method of DSSP (Dictionary of Secondary Structure in Proteins; Kabsch and Sander 1983 Biopolymers 22: 2577-637) and solvent accessible surface area of an amino acid may be calculated based on a 3-dimensional model of an antibody, using algorithms known in the art (e.g., Connolly, J. Appl. Cryst. 16, 548 (1983) and Lee and Richards, J. Mol. Biol. 55, 379 (1971), both of which are incorporated herein by reference).

[00141] As noted above, an isolated aldehyde-tagged Ig polypeptide can comprise a heavy chain constant region modified to include a sulfatase motif as described above, where the sulfatase motif is in or adjacent a surface-accessible loop region of the Ig polypeptide heavy chain constant region. Illustrative examples of surface-accessible loop regions of a heavy chain constant region are presented in Figures 1A and 1B.

[00142] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of an IgG1 heavy chain constant region corresponding to one or more of: 1) amino acids 122-127; 2) amino acids 137-143; 3) amino acids 155-158; 4) amino acids 163-170; 5) amino acids 163-183; 6) amino acids 179-183; 7) amino acids 190-192; 8) amino acids 200-202; 9) amino acids 199-202; 10) amino acids 208-212; 11) amino acids 220-241; 12) amino acids 247-251; 13) amino acids 257-261; 14) amino acid 269-277; 15) amino acids 271-277; 16) amino acids 284-285; 17) amino acids 284-292; 18) amino acids 289-291; 19) amino acids 299-303; 20) amino acids 309-313; 21) amino acids 320-322; 22) amino acids 329-335; 23) amino acids 341-349; 24) amino acids 342-348; 25) amino acids 356-365; 26) amino acids 377-381; 27) amino acids 388-394; 28) amino acids 398-407; 29) amino acids 433-451; and 30) amino acids 446-451; wherein the amino acid numbering is based on the amino acid numbering of human IgG1 as depicted in Figure 1B.

[00143] Exemplary surface-accessible loop regions of an IgG1 heavy chain include: 1) ASTKGP (SEQ ID NO:71); 2) KSTSGGT (SEQ ID NO:72); 3) PEPV (SEQ ID NO:73); 4) NSGALTSG (SEQ ID NO:202); 5) NSGALTSGVHTFPAVLQSSGL (SEQ ID NO:74); 6)

QSSGL (SEQ ID NO:227); 7) VTV; 8) QTY; 9) TQTY (SEQ ID NO:75); 10) HKPSN (SEQ ID NO:76); 11) EPKSCDKTHTCPPCPAPELLGG (SEQ ID NO:77); 12) FPPKP (SEQ ID NO:78); 13) ISRTP (SEQ ID NO:79); 14) DVSHEDPEV (SEQ ID NO:80); 15) SHEDPEV (SEQ ID NO:223; 16) DG; 17) DGVEVHNAK (SEQ ID NO:81); 18) HNA; 19) QYNST (SEQ ID NO:82); 20) VLTVL (SEQ ID NO:83); 21) GKE; 22) NKALPAP (SEQ ID NO:84); 23) SKAKGQPRE (SEQ ID NO:85); 24) KAKGQPR (SEQ ID NO:206); 25) PPSRKELTKN (SEQ ID NO:86); 26) YPSDI (SEQ ID NO:87); 27) NGQPENN (SEQ ID NO:88); 28) TPPVLDSDGS (SEQ ID NO:89); 29) HEALHNHYTQKSLSLSPGK (SEQ ID NO:90); and 30) SLSPGK (SEQ ID NO:175), as shown in Figures 1A and 1B.

[00144] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of an IgG2 heavy chain constant region corresponding to one or more of: 1) amino acids 1-6; 2) amino acids 13-24; 3) amino acids 33-37; 4) amino acids 43-54; 5) amino acids 58-63; 6) amino acids 69-71; 7) amino acids 78-80; 8) 87-89; 9) amino acids 95-96; 10) 114-118; 11) 122-126; 12) 134-136; 13) 144-152; 14) 159-167; 15) 175-176; 16) 184-188; 17) 195-197; 18) 204-210; 19) 216-224; 20) 231-233; 21) 237-241; 22) 252-256; 23) 263-269; 24) 273-282; 25) amino acids 299-302; where the amino acid numbering is based on the numbering of the amino acid sequence set forth in SEQ ID NO:4 (human IgG2; also depicted in Figure 1B).

[00145] Exemplary surface-accessible loop regions of an IgG2 heavy chain include 1) ASTKGP (SEQ ID NO:71); 2) PCSRSTSESTAA (SEQ ID NO:91); 3) FPEPV (SEQ ID NO:168); 4) SGALTSGVHTFP (SEQ ID NO:159); 5) QSSGLY (SEQ ID NO:160); 6) VTV; 7) TQT; 8) HKP; 9) DK; 10) VAGPS (SEQ ID NO:161); 11) FPPKP (SEQ ID NO:78); 12) RTP; 13) DVSHEDPEV (SEQ ID NO:80); 14) DGVEVHNAK (SEQ ID NO:81); 15) FN; 16) VLTVV (SEQ ID NO:162); 17) GKE; 18) NKGLPAP (SEQ ID NO:163); 19) SKTKGQPRE (SEQ ID NO:164); 20) PPS; 21) MTKNQ (SEQ ID NO:165); 22) YPSDI (SEQ ID NO:87); 23) NGQPENN (SEQ ID NO:88); 24) TPPMLDSDGS (SEQ ID NO:166); 25) GNVF (SEQ ID NO:182); and 26) HEALHNHYTQKSLSLSPGK (SEQ ID NO:90), as shown in Figure 1B.

[00146] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of an IgG3 heavy chain constant region corresponding to one or more of: 1) amino acids 1-6; 2) amino acids 13-22; 3) amino acids 33-37; 4) amino acids 43-61; 5) amino acid 71; 6) amino acids 78-80; 7) 87-91; 8) amino acids 97-106; 9) 111-115; 10) 147-167; 11) 173-177; 16) 185-187; 13) 195-203; 14) 210-218; 15) 226-227; 16) 238-239; 17) 246-248; 18) 255-261; 19) 267-275; 20) 282-291; 21) amino acids 303-307; 22) amino acids 313-320; 23) amino

acids 324-333; 24) amino acids 350-352; 25) amino acids 359-365; and 26) amino acids 372-377; where the amino acid numbering is based on the numbering of the amino acid sequence set forth in SEQ ID NO:3 (human IgG3; also depicted in Figure 1B).

[00147] Exemplary surface-accessible loop regions of an IgG3 heavy chain include 1) ASTKGP (SEQ ID NO:71); 2) PCSRSTSGGT (SEQ ID NO:167); 3) FPEPV (SEQ ID NO:168); 4) SGALTSGVHTFPAVLQSSG (SEQ ID NO:169); 5) V; 6) TQT; 7) HKPSN (SEQ ID NO:76); 8) RVELKTPLGD (SEQ ID NO:170); 9) CPRCPKP (SEQ ID NO:171); 10) PKSCDTPPPCPRCPAPELLGG (SEQ ID NO:229); 11) FPPKP (SEQ ID NO:78); 12) RTP; 13) DVSHEDPEV (SEQ ID NO:80); 14) DGVEVHNAK (SEQ ID NO:81); 15) YN; 16) VL; 17) GKE; 18) NKALPAP (SEQ ID NO:84); 19) SKTKGQPRE (SEQ ID NO:164); 20) PPSREEMTKN (SEQ ID NO:172); 21) YPSDI (SEQ ID NO:87); 22) SSGQPENN (SEQ ID NO:173); 23) TPPMLDSDGS (SEQ ID NO:166); 24) GNI; 25) HEALHNR (SEQ ID NO:174); and 26) SLSPGK (SEQ ID NO:175), as shown in Figure 1B.

[00148] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of an IgG4 heavy chain constant region corresponding to one or more of: 1) amino acids 1-5; 2) amino acids 12-23; 3) amino acids 32-36; 4) amino acids 42-53; 5) amino acids 57-62; 6) amino acids 68-70; 7) amino acids 77-79; 8) amino acids 86-88; 9) amino acids 94-95; 10) amino acids 101-102; 11) amino acids 108-118; 12) amino acids 122-126; 13) amino acids 134-136; 14) amino acids 144-152; 15) amino acids 159-167; 16) amino acids 175-176; 17) amino acids 185-186; 18) amino acids 196-198; 19) amino acids 205-211; 20) amino acids 217-226; 21) amino acids 232-241; 22) amino acids 253-257; 23) amino acids 264-265; 24) 269-270; 25) amino acids 274-283; 26) amino acids 300-303; 27) amino acids 399-417; where the amino acid numbering is based on the numbering of the amino acid sequence set forth in SEQ ID NO:5 (human IgG4; also depicted in Figure 1B).

[00149] Exemplary surface-accessible loop regions of an IgG4 heavy chain include 1) STKGP (SEQ ID NO:176); 2) PCSRSTSESTAA (SEQ ID NO:91); 3) FPEPV (SEQ ID NO:168); 4) SGALTSGVHTFP (SEQ ID NO:159); 5) QSSGLY (SEQ ID NO:160); 6) VTV; 7) TKT; 8) HKP; 9) DK; 10) YG; 11) CPAPEFLGGPS (SEQ ID NO:177); 12) FPPKP (SEQ ID NO:78); 13) RTP; 14) DVSQEDPEV (SEQ ID NO:178); 15) DGVEVHNAK (SEQ ID NO:81); 16) FN; 17) VL; 18) GKE; 19) NKGLPSS (SEQ ID NO:179); 20) SKAKGQPREP (SEQ ID NO:180); 21) PPSQEEMTKN (SEQ ID NO:181); 22) YPSDI (SEQ ID NO:87); 23) NG; 24) NN; 25) TPPVLDSDGS (SEQ ID NO:89); 26) GNVF (SEQ ID NO:182); and 27) HEALHNHYTQKSLSLSLGK (SEQ ID NO:183), as shown in Figure 1B.

[00150] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of an IgA heavy chain constant region corresponding to one or more of: 1) amino acids 1-13; 2) amino acids 17-21; 3) amino acids 28-32; 4) amino acids 44-54; 5) amino acids 60-66; 6) amino acids 73-76; 7) amino acids 80-82; 8) amino acids 90-91; 9) amino acids 123-125; 10) amino acids 130-133; 11) amino acids 138-142; 12) amino acids 151-158; 13) amino acids 165-174; 14) amino acids 181-184; 15) amino acids 192-195; 16) amino acid 199; 17) amino acids 209-210; 18) amino acids 222-245; 19) amino acids 252-256; 20) amino acids 266-276; 21) amino acids 293-294; 22) amino acids 301-304; 23) amino acids 317-320; 24) amino acids 329-353; where the amino acid numbering is based on the numbering of the amino acid sequence set forth in SEQ ID NO:6 (human IgA; also depicted in Figure 1B).

[00151] Exemplary surface-accessible loop regions of an IgA heavy chain include 1) ASPTSPKVFPLSL (SEQ ID NO:184); 2) QPDGN (SEQ ID NO:185); 3) VQGFFPQEPL (SEQ ID NO:186); 4) SGQGVTARNFP (SEQ ID NO:187); 5) SGDLYTT (SEQ ID NO:188); 6) PATQ (SEQ ID NO:189); 7) GKS; 8) YT; 9) CHP; 10) HRPA (SEQ ID NO:190); 11) LLGSE (SEQ ID NO:191); 12) GLRDASGV (SEQ ID NO:192); 13) SSGKSAVQGP (SEQ ID NO:193); 14) GCYS (SEQ ID NO:194); 15) CAEP (SEQ ID NO:195); 16) PE; 17) SGNTFRPEVHLLPPPSEELALNEL (SEQ ID NO:196); 18) ARGFS (SEQ ID NO:197); 19) QGSQELPREKY (SEQ ID NO:198); 20) AV; 21) AAED (SEQ ID NO:199); 22) HEAL (SEQ ID NO:200); and 23) IDRLAGKPTHVNVSVVMAEVDGTCY (SEQ ID NO:201), as shown in Figure 1B.

[00152] A sulfatase motif can be provided within or adjacent one or more of these amino acid sequences of such modification sites of an Ig heavy chain. For example, an Ig heavy chain polypeptide can be modified at one or more of these amino acid sequences to provide a sulfatase motif adjacent and N-terminal and/or adjacent and C-terminal to these modification sites. Alternatively or in addition, an Ig heavy chain polypeptide can be modified at one or more of these amino acid sequences to provide a sulfatase motif insertion between any two residues of the Ig heavy chain modifications sites. In some embodiments, an Ig heavy chain polypeptide may be modified to include two motifs, which may be adjacent to one another, or which may be separated by one, two, three, four or more (e.g., from about 1 to about 25, from about 25 to about 50, or from about 50 to about 100, or more, amino acids. Alternatively or in addition, where a native amino acid sequence provides for one or more amino acid residues of a sulfatase motif sequence, selected amino acid residues of the modification sites of an Ig heavy chain polypeptide amino acid sequence can be modified so as to provide a sulfatase motif at the modification site.

[00153] The amino acid sequence of a surface-accessible loop region can thus be modified to provide a sulfatase motif, where the modifications can include substitution and/or insertion. For example, where the modification is in a CH1 domain, the surface-accessible loop region can have the amino acid sequence NSGALTSG (SEQ ID NO:202), and the aldehyde-tagged sequence can be, e.g., NSGALCTPSRG (SEQ ID NO:203), e.g., where the "TS" residues of the NSGALTSG (SEQ ID NO:202) sequence are replaced with "CTPSR," (SEQ ID NO:204) such that the sulfatase motif has the sequence LCTPSR (SEQ ID NO:17). As another example, where the modification is in a CH2 domain, the surface-accessible loop region can have the amino acid sequence NKALPAP (SEQ ID NO:84), and the aldehyde-tagged sequence can be, e.g., NLCTPSRAP (SEQ ID NO:205), e.g., where the "KAL" residues of the NKALPAP (SEQ ID NO:84) sequence are replaced with "LCTPSR," (SEQ ID NO:17) such that the sulfatase motif has the sequence LCTPSR (SEQ ID NO:17). As another example, where the modification is in a CH2/CH3 domain, the surface-accessible loop region can have the amino acid sequence KAKGQPR (SEQ ID NO:206), and the aldehyde-tagged sequence can be, e.g., KAKG**LCTPS**R (SEQ ID NO:207), e.g., where the "GQP" residues of the KAKGQPR (SEQ ID NO:206) sequence are replaced with "LCTPS," (SEQ ID NO:208) such that the sulfatase motif has the sequence LCTPSR (SEQ ID NO:17).

[00154] As noted above, an isolated aldehyde-tagged Ig polypeptide can comprise a light chain constant region modified to include a sulfatase motif as described above, where the sulfatase motif is in or adjacent a surface-accessible loop region of the Ig polypeptide light chain constant region. Illustrative examples of surface-accessible loop regions of a light chain constant region are presented in Figures 1A and 1C.

[00155] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of an Ig light chain constant region corresponding to one or more of: 1) amino acids 130-135; 2) amino acids 141-143; 3) amino acid 150; 4) amino acids 162-166; 5) amino acids 163-166; 6) amino acids 173-180; 7) amino acids 186-194; 8) amino acids 211-212; 9) amino acids 220-225; 10) amino acids 233-236; wherein the amino acid numbering is based on the amino acid numbering of human kappa light chain as depicted in Figure 1C.

[00156] Exemplary surface-accessible loop regions of an Ig light chain (e.g., a human kappa light chain) include: 1) RTVAAP (SEQ ID NO:209); 2) PPS; 3) Gly (see, e.g., Gly at position 150 of the human kappa light chain sequence depicted in Figure 1C); 4) YPREA (SEQ ID NO:210); 5) PREA (SEQ ID NO:226); 6) DNALQSGN (SEQ ID NO:211); 7) TEQDSKDST (SEQ ID NO:212); 8) HK; 9) HQGLSS (SEQ ID NO:213); and 10) RGEC (SEQ ID NO:214), as shown in Figures 1A and 1C.

[00157] Exemplary surface-accessible loop regions of an Ig lambda light chain include QPKAAP (SEQ ID NO:215), PPS, NK, DFYPGAV (SEQ ID NO:216), DSSPVKAG (SEQ ID NO:217), TTP, SN, HKS, EG, and APTECS (SEQ ID NO:218), as shown in Figure 1C.

[00158] In some instances, a target immunoglobulin is modified to include a sulfatase motif as described above, where the sulfatase motif is within, or adjacent to, a region of a rat Ig light chain constant region corresponding to one or more of: 1) amino acids 1-6; 2) amino acids 12-14; 3) amino acids 121-22; 4) amino acids 31-37; 5) amino acids 44-51; 6) amino acids 55-57; 7) amino acids 61-62; 8) amino acids 81-83; 9) amino acids 91-92; 10) amino acids 102-105; wherein the amino acid numbering is based on the amino acid numbering of rat light chain as set forth in SEQ ID NO:10 (and depicted in Figure 1C).

[00159] Non-limiting examples of amino acid sequences of aldehyde-tagged IgG1 heavy chain polypeptides are shown in Figures 7B, 8B, 9B, 11B, 12B, 14,B, 15B, 17B, 23B, 25B, 27B, and 29B, with the LCTPSR (SEQ ID NO:17) sulfatase motif in the CH1 domain (see, e.g., Figure 7B, 8B, 9B, and 23B), CH2 domain (Figures 11B, 12B, 14B, and 25B), CH2/CH3 domain (Figures 15B, and 27B), and near the C-terminus (Figures 17B, and 29B).

[00160] Non-limiting examples of amino acid sequences of aldehyde-tagged kappa light chain polypeptides are shown in Figures 20B and 32B.

[00161] A sulfatase motif can be provided within or adjacent one or more of these amino acid sequences of such modification sites of an Ig light chain. For example, an Ig light chain polypeptide can be modified at one or more of these amino acid sequences to provide a sulfatase motif adjacent and N-terminal and/or adjacent and C-terminal these modification sites. Alternatively or in addition, an Ig light chain polypeptide can be modified at one or more of these amino acid sequences to provide a sulfatase motif insertion between any two residues of the Ig light chain modifications sites. Alternatively or in addition, where a native amino acid sequence provides for one or more amino acid residues of a sulfatase motif sequence, selected amino acid residues of the modification sites of an Ig light chain polypeptide amino acid sequence can be modified so as to provide a sulfatase motif at the modification site.

[00162] The amino acid sequence of a surface-accessible loop region is modified to provide a sulfatase motif, where the modifications can include substitution and/or insertion. For example, where the modification is in a CL region, the surface-accessible loop region can have the amino acid sequence DNALQSGN (SEQ ID NO:211), and the aldehyde-tagged sequence can be, e.g., DNALCTPSRQSGN (SEQ ID NO:219), e.g., where the sequence "CTPSR" (SEQ ID NO:204) is inserted between the "DNAL" (SEQ ID NO:220) and the

"QSGN" (SEQ ID NO:221) sequences of the surface-accessible loop region, such that the sulfatase motif is LCTPSR (SEQ ID NO:17).

[00163] In one embodiment, modification of an Ig constant region does not substantially alter functionality of the heavy chain constant region. For example, the Fc portion (e.g., CH2 and CH3 domains of IgA or IgG antibodies; and CH2, CH3, and CH4 domains of IgM or IgE antibodies) can have various binding and effector functions. Non limiting examples, of Fc binding and effector functions include, e.g., Fc receptor (FcR) binding, C1q binding, and antibody-dependent cell-mediated cytotoxicity (ADCC) activity. Modification of an Ig constant region to provide an aldehyde tag, as described herein, does not substantially increase or decrease one or more of Fc binding, and any effector function of the heavy chain, e.g., the modification does not increase or decrease the FcR binding and/or an effector function by more than about 1%, about 2%, about 5%, or about 10%, compared to a parent Ig polypeptide.

[00164] Modification of an Ig constant region to provide an aldehyde tag, as described herein, does not substantially reduce antigen binding affinity of an antibody comprising the aldehyde-tagged Ig constant region.

[00165] Modification of an Ig constant region to provide an aldehyde tag, as described herein, does not substantially reduce production of the Ig polypeptide, e.g., the aldehyde-tagged Ig polypeptide can be expressed in a host cell and can be folded properly so as to result in a functional polypeptide.

[00166] An aldehyde-tagged Ig heavy chain can include an Ig variable region, or can lack an Ig variable region. Similarly, an aldehyde-tagged Ig light chain can include an Ig variable region, or can lack an Ig variable region. Ig variable regions are well known in the art, and can provide antigen-binding specificity to an Ig polypeptide.

[00167] An aldehyde-tagged Ig heavy chain can include, in addition to an aldehyde tag, one or more additional modifications, e.g., glycosylation, and the like.

[00168] The present disclosure provides an aldehyde-tagged antibody comprising an Ig heavy chain and an Ig light chain, where the Ig heavy chain and/or the Ig light chain comprises an aldehyde tag. An aldehyde-tagged antibody can include an Ig heavy chain with one, two, three, or more aldehyde tags; and an Ig light chain with no aldehyde tags. An aldehyde-tagged antibody can include an Ig heavy chain with no aldehyde tags; and an Ig light chain with one, two, three, or more aldehyde tags. An aldehyde-tagged antibody can include an Ig heavy chain with one, two, three, or more aldehyde tags; and an Ig light chain with one, two, three, or more aldehyde tags.

[00169] An aldehyde-tagged antibody of the present disclosure can have any of a variety of antigen-binding specificities. An aldehyde-tagged antibody can bind any of a variety of antigens, including, e.g., an antigen present on a cancer cell; an antigen present on an autoimmune cell; an antigen present on a pathogenic microorganism; an antigen present on a virus-infected cell (e.g., a human immunodeficiency virus-infected cell), e.g., CD4 or gp120; an antigen present on a diseased cell; and the like. For example, an aldehyde-tagged antibody can bind an antigen, as noted above, where the antigen is present on the surface of the cell.

[00170] For example, an aldehyde-tagged antibody can specifically bind an antigen present on a cancer cell. Non-limiting examples of cancer antigens that can be recognized and bound (e.g., specifically bound) by an aldhehyde-tagged antibody of the present disclosure include antigens present on carcinomas, prostate cancer cells, breast cancer cells, colorectal cancer cells, melanoma cells, T-cell leukemia cells, T-cell lymphoma cells, B-cell lymphoma cells, non-Hodgkin's lymphoma cells, and the like.

[00171] Non-limiting examples of antigens present on particular cancer cells include, e.g., CA125, CA15-3, CA19-9, L6, Lewis Y, Lewis X, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific antigen, prostatic acid phosphatase, epidermal growth factor, MAGE-1, MAGE-2, MAGE-3, MAGE-4, anti-transferrin receptor, p97, MUC1-KLH, HER2, CEA, gp100, MART1, prostate-specific antigen, human chorionic gonadotropin, IL-2 receptor, EphB2, CD19, CD20, CD22, CD52, CD33, CD38, CD40, mucin, P21, MPG, and Neu oncogene product. In some embodiments, the antigen is CD19. In other embodiments, the antigen is CD22.

[00172] Non-limiting examples of antibodies that can be modified to include an aldehyde tag, as described herein, include, but are not limited to, an anti-CD19 antibody, and an anti-CD22 antibody.

FORMYLGLYCINE GENERATING ENZYMES (FGES)

[00173] The enzyme that oxidizes cysteine or serine in a sulfatase motif to FGly is referred to herein as a formylglycine generating enzyme (FGE). As discussed above, "FGE" is used herein to refer to FGly-generating enzymes that mediate conversion of a cysteine (C) of a sulfatase motif to FGly as well as FGly-generating enzymes that mediate conversion of serine (S) of a sulfatase motif to FGly. It should be noted that in general, the literature refers to FGly-generating enzymes that convert a C to FGly in a sulfatase motif as FGEs, and refers to enzymes that convert S to FGly in a sulfatase motif as Ats-B-like. However, for purposes of the present disclosure "FGE" is used generically to refer to both types of FGly-generating enzymes, with the understanding that an appropriate FGE will be selected according to the

target reactive partner containing the appropriate sulfatase motif (i.e., C-containing or S-containing).

[00174] As evidenced by the ubiquitous presence of sulfatases having an FGly at the active site, FGEs are found in a wide variety of cell types, including both eukaryotes and prokaryotes. There are at least two forms of FGEs. Eukaryotic sulfatases contain a cysteine in their sulfatase motif and are modified by the "SUMF1-type" FGE (Cosma et al. Cell 2003, 113, (4), 445-56; Dierks et al. Cell 2003, 113, (4), 435-44). The FGly-generating enzyme (FGE) is encoded by the *SUMF1* gene. Prokaryotic sulfatases can contain either a cysteine or a serine in their sulfatase motif and are modified either by the "SUMF1-type" FGE or the "AtsB-type" FGE, respectively (Szameit et al. J Biol Chem 1999, 274, (22), 15375-81). In eukaryotes, it is believed that this modification happens co-translationally or shortly after translation in the endoplasmic reticulum (ER) (Dierks et al. Proc Natl Acad Sci U S A 1997, 94(22):11963-8). Without being held to theory, in prokaryotes it is thought that SUMF1-type FGE functions in the cytosol and AtsB-type FGE functions near or at the cell membrane. A SUMF2 FGE has also been described in deuterostomia, including vertebrates and echinodermata (see, e.g., Pepe et al. (2003) Cell 113, 445-456, Dierks et al. (2003) Cell 113, 435-444; Cosma et al. (2004) Hum. Mutat. 23, 576-581).

[00175] In general, the FGE used to facilitate conversion of cysteine or serine to FGly in a sulfatase motif of an aldehyde tag of a target polypeptide is selected according to the sulfatase motif present in the aldehyde tag. The FGE can be native to the host cell in which the aldehyde tagged polypeptide is expressed, or the host cell can be genetically modified to express an appropriate FGE. In some embodiments it may be desired to use a sulfatase motif compatible with a human FGE (e.g., the SUMF1-type FGE, see, e.g., Cosma et al. Cell 113, 445-56 (2003); Dierks et al. Cell 113, 435-44 (2003)), and express the aldehyde tagged protein in a human cell that expresses the FGE or in a host cell, usually a mammalian cell, genetically modified to express a human FGE.

[00176] In general, an FGE for use in the methods disclosed herein can be obtained from naturally occurring sources or synthetically produced. For example, an appropriate FGE can be derived from biological sources which naturally produce an FGE or which are genetically modified to express a recombinant gene encoding an FGE. Nucleic acids encoding a number of FGEs are known in the art and readily available (see, e.g., Preusser et al. 2005 J. Biol. Chem. 280(15):14900-10 (Epub 2005 Jan 18); Fang et al. 2004 J Biol Chem. 79(15):14570-8 (Epub 2004 Jan 28); Landgrebe et al. Gene. 2003 Oct 16;316:47-56; Dierks et al. 1998 FEBS Lett. 423(1):61-5; Dierks et al. Cell. 2003 May 16;113(4):435-44; Cosma et al. (2003 May 16) Cell 113(4):445-56; Baenziger (2003 May 16) Cell 113(4):421-2 (review);

Dierks et al. Cell. 2005 May 20;121(4):541-52; Roeser et al. (2006 Jan 3)Proc Natl Acad Sci USA 103(1):81-6; Sardiello et al. (2005 Nov 1) Hum Mol Genet. 14(21):3203-17; WO 2004/072275; WO 2008/036350; U.S. Patent Publication No. 2008/0187956; and GenBank Accession No. NM_182760. Accordingly, the disclosure here provides for recombinant host cells genetically modified to express an FGE that is compatible for use with an aldehyde tag of a tagged target polypeptide. In certain embodiments, the FGE used may be a naturally occurring enzyme (may have a wild type amino acid sequence). In other embodiments, the FGE used may be non-naturally occurring, in which case it may, in certain cases, have an amino acid sequence that is at least 80% identical, at least 90% identical or at least 95% identical to that of a wild type enzyme. Because FGEs have been studied structurally and functionally and the amino acid sequences of several examples of such enzymes are available, variants that retain enzymatic activity should be readily designable.

[00177] Where a cell-free method is used to convert a sulfatase motif-containing polypeptide, an isolated FGE can be used. Any convenient protein purification procedures may be used to isolate an FGE, see, e.g., Guide to Protein Purification, (Deuthser ed.) (Academic Press, 1990). For example, a lysate may be prepared from a cell that produces a desired FGE, and purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, and the like.

EXPRESSION VECTORS AND GENETICALLY MODIFIED HOST CELLS

[00178] The present disclosure provides nucleic acid encoding ald-tagged Ig polypeptides, as well as constructs and host cells containing nucleic acid. Such nucleic acids comprise a sequence of DNA having an open reading frame that encodes an aldehyde tagged Ig polypeptide and, in most embodiments, is capable, under appropriate conditions, of being expressed. "Nucleic acid" encompasses DNA, cDNA, mRNA, and vectors comprising such nucleic acids.

[00179] The present disclosure provides a recombinant nucleic acid comprising a nucleotide sequence encoding an aldehyde-tagged Ig polypeptide, as described above. The recombinant nucleic acid can include:

[00180] 1) a nucleotide sequence encoding an aldehyde-tagged Ig heavy chain constant region (and not an Ig heavy chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VH domain);

[00181] 2) a nucleotide sequence encoding an aldehyde-tagged Ig polypeptide, where the Ig polypeptide comprises an Ig VH domain and an aldehyde-tagged Ig heavy chain constant region;

[00182] 3) a nucleotide sequence encoding an aldehyde-tagged Ig light chain constant region (and not an Ig light chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VL domain);

[00183] 4) a nucleotide sequence encoding an aldehyde-tagged Ig polypeptide, where the Ig polypeptide comprises an Ig VL domain and an aldehyde-tagged Ig light chain constant region;

[00184] 5) a nucleotide sequence encoding an aldehyde-tagged Ig heavy chain constant region (and not an Ig heavy chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VH domain); and a nucleotide sequence encoding an aldehyde-tagged Ig light chain constant region (and not an Ig light chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VL domain);

[00185] 6) a nucleotide sequence encoding an aldehyde-tagged Ig heavy chain constant region (and not an Ig heavy chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VH domain); and a nucleotide sequence encoding an Ig light chain constant region (and not an Ig light chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VL domain), where the Ig light chain constant region is not aldehyde tagged;

[00186] 7) a nucleotide sequence encoding an Ig heavy chain constant region (and not an Ig heavy chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VH domain), where the Ig heavy chain constant region is not aldehyde tagged; and a nucleotide sequence encoding an aldehyde-tagged Ig light chain constant region (and not an Ig light chain variable region, i.e., where the recombinant nucleic acid lacks a nucleotide sequence encoding an Ig VL domain);

[00187] 8) a nucleotide sequence encoding a first aldehyde-tagged Ig polypeptide, where the first aldehyde-tagged Ig polypeptide comprises an Ig VH domain and an aldehyde-tagged Ig heavy chain constant region; and a nucleotide sequence encoding a second aldehyde-tagged Ig polypeptide, where the second aldehyde-tagged Ig polypeptide comprises an Ig VL domain and an aldehyde-tagged Ig light chain constant region;

[00188] 9) a nucleotide sequence encoding a first Ig polypeptide, where the first Ig polypeptide is aldehyde tagged, where the first Ig polypeptide comprises an Ig VH domain and an aldehyde-tagged Ig heavy chain constant region; and a nucleotide sequence encoding a second Ig polypeptide, where the second Ig polypeptide comprises an Ig VL domain and an Ig light chain constant region, where the Ig light chain constant region is not aldehyde tagged; or

[00189] 10) a nucleotide sequence encoding a first Ig polypeptide, where the first Ig polypeptide comprises an Ig VH domain and an Ig heavy chain constant region, where the Ig heavy chain constant region is not aldehyde tagged; and a nucleotide sequence encoding a second Ig polypeptide, where the second Ig polypeptide is aldehyde tagged, where the second Ig polypeptide comprising an Ig VL domain and an aldehyde-tagged Ig light chain constant region.

[00190] The present disclosure provides a recombinant expression vector comprising a nucleic acid as described above, where the nucleotide sequence encoding the Ig polypeptide(s) is operably linked to a promoter. In some embodiments, where a subject recombinant expression vector encodes both Ig heavy and light chains (with or without Ig variable regions), the heavy and light chain-encoding sequences can be operably linked to the same promoter, or to separate promoters.

[00191] Where a recombinant expression vector includes a nucleotide sequence encoding a heavy chain variable (V_H) region and/or a light chain variable (V_L) region, it will be appreciated that a large number of V_H and V_L amino acid sequences, and nucleotide sequences encoding same, are known in the art, and can be used. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

[00192] In those instances in which a recombinant expression vector comprises a nucleotide sequence encoding an Ig heavy or Ig light chain without variable region sequences, the vector can include an insertion site for an Ig variable region 5' of the Ig polypeptide-encoding nucleotide sequence. For example, a recombinant expression vector can comprise, in order from 5' to 3':

[00193] 1) an insertion site for a nucleotide sequence encoding a VH domain; and a nucleotide sequence encoding an aldehyde-tagged Ig heavy chain constant region;

[00194] 2) an insertion site for a nucleotide sequence encoding a VL domain; and a nucleotide sequence encoding an aldehyde-tagged Ig light chain constant region.

[00195] The present disclosure also provides a library of recombinant expression vectors, where the library can include a plurality of member recombinant expression vectors, e.g.:

[00196] 1) a first recombinant expression vector comprising, in order from 5' to 3', an insertion site for a nucleotide sequence encoding a VH domain; and a nucleotide sequence encoding a first aldehyde-tagged Ig heavy chain constant region comprising an aldehyde tag in or adjacent a first surface-accessible loop region;

[00197] 2) a second recombinant expression vector comprising, in order from 5' to 3', an insertion site for a nucleotide sequence encoding a VH domain; and a nucleotide sequence encoding a second aldehyde-tagged Ig heavy chain constant region comprising an aldehyde tag in or adjacent a second surface-accessible loop region;

[00198] 3) a third recombinant expression vector comprising, in order from 5' to 3', an insertion site for a nucleotide sequence encoding a VH domain; and a nucleotide sequence encoding a third aldehyde-tagged Ig heavy chain constant region comprising an aldehyde tag in or adjacent a third surface-accessible loop region;

[00199] and combinations thereof, where each additional member recombinant expression vectors can include nucleotide sequences encoding aldehyde-tagged Ig polypeptides having aldehyde tags in or adjacent a different surface-accessible loop region.

[00200] In some instances, a recombinant expression vector in the library will also include a nucleotide sequence encoding an Ig light chain, which may or may not include a variable region, and which may or may not be aldehyde tagged.

[00201] The present disclosure also provides a library of recombinant expression vectors, where the library can include a plurality of member recombinant expression vectors, e.g.:

[00202] 1) a first recombinant expression vector comprising, in order from 5' to 3', an insertion site for a nucleotide sequence encoding a VL domain; and a nucleotide sequence encoding a first aldehyde-tagged Ig light chain constant region comprising an aldehyde tag in or adjacent a first surface-accessible loop region;

[00203] 2) a second recombinant expression vector comprising, in order from 5' to 3', an insertion site for a nucleotide sequence encoding a VL domain; and a nucleotide sequence encoding a second aldehyde-tagged Ig light chain constant region comprising an aldehyde tag in or adjacent a second surface-accessible loop region;

[00204] 3) a third recombinant expression vector comprising, in order from 5' to 3', an insertion site for a nucleotide sequence encoding a VL domain; and a nucleotide sequence encoding a third aldehyde-tagged Ig light chain constant region comprising an aldehyde tag in or adjacent a third surface-accessible loop region;

[00205] and combinations thereof, where each additional member recombinant expression vectors can include nucleotide sequences encoding aldehyde-tagged Ig polypeptides having aldehyde tags in or adjacent a different surface-accessible loop region.

[00206] In some instances, a recombinant expression vector in the library will also include a nucleotide sequence encoding an Ig heavy chain, which may or may not include a variable region, and which may or may not be aldehyde tagged.

[00207] Figure 2 depicts an example of a scheme for generating a library of aldehyde-tagged Ig polypeptides, in which each member Ig polypeptide comprises an aldehyde tag at a different location from the other members. For example, an Ig heavy chain or an Ig light chain, a "tagged cassette" is modified with aldehyde tags that can be further elaborated chemically. These cassettes can be applied to different Fvs for antibody-drug conjugate production.

[00208] Nucleic acids contemplated herein can be provided as part of a vector (also referred to as a construct), a wide variety of which are known in the art and need not be elaborated upon herein. Exemplary vectors include, but are not limited to, plasmids; cosmids; viral vectors (e.g., retroviral vectors); non-viral vectors; artificial chromosomes (yeast artificial chromosomes (YAC's), BAC's, etc.); mini-chromosomes; and the like. The choice of vector will depend upon a variety of factors such as the type of cell in which propagation is desired and the purpose of propagation.

[00209] Vectors can provide for extrachromosomal maintenance in a host cell or can provide for integration into the host cell genome. Vectors are amply described in numerous publications well known to those in the art, including, e.g., Short Protocols in Molecular Biology, (1999) F. Ausubel, et al., eds., Wiley & Sons. Vectors may provide for expression of the nucleic acids encoding a polypeptide of interest (e.g., an aldehyde tagged polypeptide, an FGE, etc.), may provide for propagating the subject nucleic acids, or both.

[00210] Exemplary vectors that may be used include but are not limited to those derived from recombinant bacteriophage DNA, plasmid DNA or cosmid DNA. For example, plasmid vectors such as pBR322, pUC 19/18, pUC 118, 119 and the M13 mp series of vectors may be used. Bacteriophage vectors may include λgt10, λgt11, λgt18-23, λZAP/R and the EMBL series of bacteriophage vectors. Cosmid vectors that may be utilized include, but are not limited to, pJB8, pCV 103, pCV 107, pCV 108, pTM, pMCS, pNNL, pHSG274, COS202, COS203, pWE15, pWE16 and the charomid 9 series of vectors. Alternatively, recombinant virus vectors may be engineered, including but not limited to those derived from viruses such as herpes virus, retroviruses, vaccinia virus, poxviruses, adenoviruses, adeno-associated viruses, or bovine papilloma virus.

[00211] For expression of a protein of interest (e.g., an aldehyde-tagged Ig polypeptide or an FGE), an expression cassette may be employed. Thus, the present invention provides a recombinant expression vector comprising a subject nucleic acid. The expression vector provides a transcriptional and translational regulatory sequence, and may provide for inducible or constitutive expression, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and

translational termination region. These control regions may be native to the gene encoding the polypeptide (e.g., the Ig polypeptide or the FGE), or may be derived from exogenous sources. In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In addition to constitutive and inducible promoters, strong promoters (e.g., T7, CMV, and the like) find use in the constructs described herein, particularly where high expression levels are desired in an in vivo (cell-based) or in an *in vitro* expression system. Further exemplary promoters include mouse mammary tumor virus (MMTV) promoters, Rous sarcoma virus (RSV) promoters, adenovirus promoters, the promoter from the immediate early gene of human CMV (Boshart et al., *Cell* 41:521-530, 1985), and the promoter from the long terminal repeat (LTR) of RSV (Gorman et al., *Proc. Natl. Acad. Sci. USA* 79:6777-6781, 1982). The promoter can also be provided by, for example, a 5'UTR of a retrovirus.

[00212] Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding proteins of interest. A selectable marker operative in the expression host may be present to facilitate selection of cells containing the vector. In addition, the expression construct may include additional elements. For example, the expression vector may have one or two replication systems, thus allowing it to be maintained in organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. In addition the expression construct may contain a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

[00213] Expression constructs encoding aldehyde tagged Ig polypeptides can also be generated using amplification methods (e.g., a polymerase chain reaction (PCR)), where at least one amplification primer (i.e., at least one of a forward or reverse primer) includes a nucleic acid sequence encoding an aldehyde tag. For example, an amplification primer having an aldehyde tag-encoding sequence is designed to provide for amplification of a nucleic acid encoding an Ig polypeptide. The extension product that results from polymerase-mediated synthesis from the aldehyde tag-containing forward primer produces a nucleic acid amplification product encoding a fusion protein composed of an aldehyde-tagged Ig polypeptide. The amplification product is then inserted into an expression construct of choice to provide an aldehyde tagged polypeptide expression construct.

HOST CELLS

[00214] The present disclosure provides genetically modified host cells comprising a subject nucleic acid, including a genetically modified host cell comprising a recombinant expression vector as described above. Any of a number of suitable host cells can be used in the production of an aldehyde-tagged Ig polypeptide. The host cell used for production of an aldehyde tagged Ig polypeptide can optionally provide for FGE-mediated conversion, so that the Ig polypeptide produced contains an FGly-containing aldehyde tag following expression and modification by FGE. Alternatively the host cell can provide for production of an unconverted aldehyde tagged Ig polypeptide (e.g., due to lack of expression of an FGE that facilitates conversion of the aldehyde tag).

[00215] The aldehyde moiety of a converted aldehyde tag can be used for a variety of applications including, but not limited to, visualization using fluorescence or epitope labeling (e.g., electron microscopy using gold particles equipped with aldehyde reactive groups); protein immobilization (e.g., protein microarray production); protein dynamics and localization studies and applications; and conjugation of proteins with a moiety of interest (e.g., moieties that improve a parent protein's half-life (e.g., poly(ethylene glycol)), targeting moieties (e.g., to enhance delivery to a site of action), and biologically active moieties (e.g., a therapeutic moiety).

[00216] In general, the polypeptides described herein may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. Thus, the present invention further provides a host cell, e.g., a genetically modified host cell that comprises a nucleic acid encoding an aldehyde tagged polypeptide. The host cell can further optionally comprise a recombinant FGE, which may be endogenous or heterologous to the host cell.

[00217] Host cells for production (including large scale production) of an unconverted or (where the host cell expresses a suitable FGE) converted aldehyde tagged Ig polypeptide, or for production of an FGE (e.g., for use in a cell-free method) can be selected from any of a variety of available host cells. Exemplary host cells include those of a prokaryotic or eukaryotic unicellular organism, such as bacteria (e.g., *Escherichia coli* strains, *Bacillus* spp. (e.g., *B. subtilis*), and the like) yeast or fungi (e.g., *S. cerevisiae*, *Pichia* spp., and the like), and other such host cells can be used. Exemplary host cells originally derived from a higher organism such as insects, vertebrates, particularly mammals, (*e.g.* CHO, HEK, and the like), may be used as the expression host cells.

[00218] Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos.

CRL9618 and CRL9096), CHO DG44 cells (Urlaub (1983) *Cell* 33:405), CHO-K1 cells (ATCC CCL-61), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

[00219] Specific expression systems of interest include bacterial, yeast, insect cell and mammalian cell derived expression systems. Representative systems from each of these categories are provided below.

[00220] The product can be recovered by any appropriate means known in the art. Further, any convenient protein purification procedures may be employed, where suitable protein purification methodologies are described in Guide to Protein Purification, (Deuthser ed.) (Academic Press, 1990). For example, a lysate may prepared from a cell comprising the expression vector expressing the ald-tagged Ig polypeptide, and purified using high performance liquid chromatography (HPLC), exclusion chromatography, gel electrophoresis, affinity chromatography, and the like.

METHODS FOR CONVERSION AND MODIFICATION OF AN ALDEHYDE TAG

[00221] Conversion of an aldehyde tag present in an aldehyde tagged Ig polypeptide can be accomplished by cell-based (*in vivo*) or cell-free methods (*in vitro*). Similarly, modification of a converted aldehyde tag of an aldehyde tagged polypeptide can be accomplished by cell-based (in vivo) or cell-free methods (in vitro). These are described in more detail below.

"In vivo" Host Cells Conversion and Modification

[00222] Conversion of an aldehyde tag of an aldehyde tagged polypeptide can be accomplished by expression of the aldehyde tagged polypeptide in a cell that contains a suitable FGE. In this embodiment, conversion of the cysteine or serine of the aldehyde tag occurs during or following translation in the host cell. The FGE of the host cell can be endogenous to the host cell, or the host cell can be recombinant for a suitable FGE that is heterologous to the host cell. FGE expression can be provided by an expression system endogenous to the FGE gene (e.g., expression is provided by a promoter and other control elements present in the native FGE gene of the host cell), or can be provided by from a recombinant expression system in which the FGE coding sequence is operably linked to a heterologous promoter to provide for constitutive or inducible expression.

[00223] Conditions suitable for use to accomplish conjugation of a reactive partner moiety to an aldehyde tagged polypeptide are similar to those described in Mahal et al. (1997 May 16) Science 276(5315):1125-8.

[00224] In some instances, where a method is carried out in a cell, the cell is *in vitro*, e.g., in *in vitro* cell culture, e.g., where the cell is cultured *in vitro* in a single-cell suspension or as an adherent cell.

"In vitro" (Cell-Free) Conversion and Modification

[00225] *In vitro* (cell-free) conversion of an aldehyde tag of an aldehyde tagged Ig polypeptide can be accomplished by contacting an aldehyde tagged polypeptide with an FGE under conditions suitable for conversion of a cysteine or serine of a sulfatase motif of the aldehyde tag to an FGly. For example, nucleic acid encoding an aldehyde tagged Ig polypeptide can be expressed in an in vitro transcription/translation system in the presence of a suitable FGE to provide for production of converted aldehyde tagged Ig polypeptides.

[00226] Alternatively, isolated, unconverted aldehyde tagged Ig polypeptide can be isolated following recombinant production in a host cell lacking a suitable FGE or by synthetic production. The isolated aldehyde tagged Ig polypeptide is then contacted with a suitable FGE under conditions to provide for aldehyde tag conversion. The aldehyde tagged Ig polypeptide can be unfolded by methods known in the art (e.g., using heat, adjustment of pH, chaotropic agents, (e.g., urea, and the like), organic solvents (e.g., hydrocarbons: octane, benzene, chloroform), etc.) and the denatured protein contacted with a suitable FGE. The ald-tagged Ig polypeptide can then be refolded under suitable conditions.

[00227] With respect to modification of converted aldehyde tagged, modification is normally carried out *in vitro*. A converted aldehyde tagged Ig polypeptide is isolated from a production source (e.g., recombinant host cell production, synthetic production), and contacted with a reactive partner-containing drug or other moiety under conditions suitable to provide for conjugation of the drug or other moiety to the FGly of the aldehyde tag.

[00228] In some instances, a combination of cell-based conversion and cell-free conversion is carried out, to generate a converted aldehyde tag; followed by cell-free modification of the converted aldehyde tag. In some embodiments, a combination of cell-free conversion and cell-based conversion is carried out.

MOIETIES FOR MODIFICATION OF IMMUNOGLOBULIN POLYPEPTIDES

[00229] The aldehyde tagged, FGly-containing Ig polypeptides can be subjected to modification to provide for attachment of a wide variety of moieties. Exemplary molecules of interest include, but are not necessarily limited to, a drug, a detectable label, a small molecule, a water-soluble polymer, a synthetic peptide, and the like.

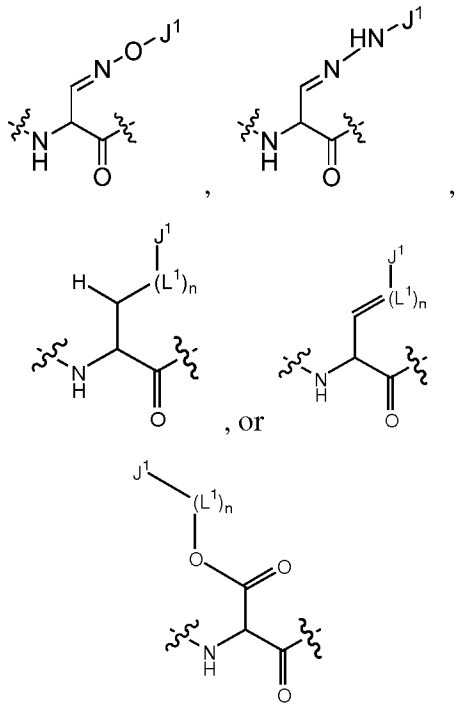
[00230] Thus, the present disclosure provides an Ig polypeptide conjugate (also referred to herein as an "Ig conjugate"), the Ig conjugate comprising:

[00231] an Ig polypeptide (e.g., an Ig heavy chain or an Ig light chain, or an Ig comprising both heavy and light chains) and a covalently conjugated moiety, wherein the Ig polypeptide comprises a modified sulfatase motif of the formula:

[00232] $X_1(FGly')X_2Z_2X_3Z_3$

[00233] where FGly' is of the formula:

[00234]



[00235] wherein J^1 is the covalently bound moiety;

[00236] each L^1 is a divalent moiety independently selected from alkylene, substituted alkylene, alkenylene, substituted alkenylene, alkynylene, alkynylene, arylene, substituted arylene, cycloalkylene, substituted cycloalkylene, heteroarylene, substituted heteroarylene, heterocyclene, substituted heterocyclene, acyl, amido, acyloxy, urethanylene, thioester, sulfonyl, sulfonamide, sulfonyl ester, -O-, -S-, -NH-, and substituted amine;

[00237] n is a number selected from zero to 40;

[00238] Z_2 is a proline or alanine residue;

[00239] X_1 is present or absent and, when present, is any amino acid, with the proviso that when the sulfatase motif is at an N-terminus of the polypeptide, X_1 is present;

[00240] X_2 and X_3 are each independently any amino acid; and

[00241] Z_3 is an aliphatic amino acid or basic amino acid;

[00242] and

[00243] wherein the Ig conjugate presents the covalently bound moiety on a solvent-accessible surface when in a folded state.

[00244] The present disclosure provides an antibody conjugated to a moiety of interest, where an antibody conjugated to a moiety of interest is referred to as an "antibody conjugate." An antibody conjugate of the present disclosure can include: 1) Ig heavy chain constant region conjugated to a moiety of interest; and an Ig light chain constant region conjugated to a moiety of interest; 2) an Ig heavy chain constant region conjugated to a moiety of interest; and an Ig light chain constant region that is not conjugated to a moiety of interest; or 3) an Ig heavy chain constant region that is not conjugated to a moiety of interest; and an Ig light chain constant region conjugated to a moiety of interest. A subject antibody conjugate can also include VH and/or VL domains.

[00245] The moiety of interest is provided as component of a reactive partner for reaction with an aldehyde of the FGly residue of a converted aldehyde tag of the tagged Ig polypeptide. Since the methods of tagged Ig polypeptide modification are compatible with conventional chemical processes, the methods of the present disclosure can exploit a wide range of commercially available reagents to accomplish attachment of a moiety of interest to an FGly residue of an aldehyde tagged Ig polypeptide. For example, aminooxy, hydrazide, or thiosemicarbazide derivatives of a number of moieties of interest are suitable reactive partners, and are readily available or can be generated using standard chemical methods.

[00246] For example, to attach a poly(ethylene glycol) (PEG) moiety to a tagged Ig polypeptide, an aminooxy-PEG can be generated from monoamino-PEGs and aminooxyglycine using standard protocols. The aminooxy-PEG can then be reacted with a converted (e.g., FGly-modified) aldehyde tagged Ig polypeptide to provide for attachment of the PEG moiety. Delivery of a biotin moiety to a converted aldehyde tagged polypeptide can be accomplished using aminooxy biotin, biotin hydrazide or 2,4 dinitrophenylhydrazine.

[00247] Provided the present disclosure, the ordinarily skilled artisan can readily adapt any of a variety of moieties to provide a reactive partner for conjugation to an aldehyde tagged polypeptide as contemplated herein. The ordinarily skilled artisan will appreciate that factors such as pH and steric hindrance (i.e., the accessibility of the aldehyde tag to reaction with a reactive partner of interest) are of importance. Modifying reaction conditions to provide for optimal conjugation conditions is well within the skill of the ordinary artisan, and is routine in the art. In general, it is normally desirable to conduction conjugation reactions at a pH below 7, with a pH of about 5.5, about 6, about 6.5, usually about 5.5 being optimal. Where conjugation is conducted with an aldehyde tagged polypeptide present in or on a living cell, the conditions are selected so as to be physiologically compatible. For example,

the pH can be dropped temporarily for a time sufficient to allow for the reaction to occur but within a period tolerated by the cell having an aldehyde tag (e.g., from about 30 min to 1 hour). Physiological conditions for conducting modification of aldehyde tagged polypeptides on a cell surface can be similar to those used in a ketone-azide reaction in modification of cells bearing cell-surface azides (see, e.g., US 6,570,040).

[00248] In general, the moiety or moieties can provide for one or more of a wide variety of functions or features. Exemplary moieties include detectable labels (e.g., dye labels (e.g., chromophores, fluorophores), biophysical probes (spin labels, nuclear magnetic resonance (NMR) probes), Förster Resonance Energy Transfer (FRET)-type labels (e.g., at least one member of a FRET pair, including at least one member of a fluorophore/quencher pair), Bioluminescence Resonance Energy Transfer (BRET)-type labels (e.g., at least one member of a BRET pair), immunodetectable tags (e.g., FLAG, His(6), and the like), localization tags (e.g., to identify association of a tagged polypeptide at the tissue or molecular cell level (e.g., association with a tissue type, or particular cell membrane)), and the like); light-activated dynamic moieties (e.g., azobenzene mediated pore closing, azobenzene mediated structural changes, photodecaging recognition motifs); water soluble polymers (e.g., PEGylation); purification tags (e.g., to facilitate isolation by affinity chromatography (*e.g.*, attachment of a FLAG epitope, e.g., DYKDDDDK (SEQ ID NO:222)); membrane localization domains (e.g., lipids or glycophosphatidylinositol (GPI)-type anchors); immobilization tags (e.g., to facilitate attachment of the polypeptide to a surface, including selective attachment); drugs (e.g., to facilitate drug targeting, e.g., through attachment of the drug to an antibody); targeted delivery moieties, (e.g., ligands for binding to a target receptor (*e.g.*, to facilitate viral attachment, attachment of a targeting protein present on a liposome, *etc.*)), and the like.

[00249] Specific, non-limiting examples are provided below.

Detectable labels

[00250] The compositions and methods of the present disclosure can be used to deliver a detectable label to an aldehyde tagged Ig, e.g., where J^1 is a detectable label. Exemplary detectable labels include, but are not necessarily limited to, fluorescent molecules (*e.g.*, autofluorescent molecules, molecules that fluoresce upon contact with a reagent, *etc.*), radioactive labels (*e.g.*, ^{111}In, ^{125}I, ^{131}I, ^{212}B, ^{90}Y, ^{186}Rh, and the like); biotin (*e.g.*, to be detected through reaction of biotin and avidin); fluorescent tags; imaging reagents, and the like. Detectable labels also include peptides or polypeptides that can be detected by antibody binding, *e.g.*, by binding of a detectably labeled antibody or by detection of bound antibody through a sandwich-type assay.

Attachment of target molecules to a support

[00251] The methods can provide for conjugation of an aldehyde tagged immunoglobulin to a moiety to facilitate attachment of the immunoglobulin to a solid substratum (*e.g.*, to facilitate assays), or to a moiety to facilitate easy separation (e.g., a hapten recognized by an antibody bound to a magnetic bead). In one embodiment, the methods of the invention are used to provide for attachment of a protein to an array (e.g., chip) in a defined orientation. For example, a polypeptide having an aldehyde tag at a selected site (*e.g.*, at or near the N-terminus) can be generated, and the methods and compositions of the invention used to deliver a moiety to the converted aldehyde tag. The moiety can then be used as the attachment site for affixing the polypeptide to a support (*e.g.*, solid or semi-solid support, particularly a support suitable for use as a microchip in high-throughput assays).

Attachment of molecules for delivery to a target site

[00252] The reactive partner for the aldehyde tagged polypeptide can comprise a small molecule drug, toxin, or other molecule for delivery to the cell and which can provide for a pharmacological activity or can serve as a target for delivery of other molecules.

[00253] Also contemplated is use of a reactive partner that comprises one of a pair of binding partners (e.g., a ligand, a ligand-binding portion of a receptor, a receptor-binding portion of a ligand, etc.). For example, the reactive partner can comprise a polypeptide that serves as a viral receptor and, upon binding with a viral envelope protein or viral capsid protein, facilitates attachment of virus to the cell surface on which the modified aldehyde tagged protein is expressed. Alternatively, the reactive partner comprises an antigen that is specifically bound by an antibody (*e.g.*, monoclonal antibody), to facilitate detection and/or separation of host cells expressing the modified aldehyde tagged polypeptide.

Water-soluble polymers

[00254] In some cases, an Ig conjugate comprises a covalently linked water-soluble polymer, e.g., where J^1 is a water-soluble polymer. A moiety of particular interest is a water-soluble polymer. A "water-soluble polymer" refers to a polymer that is soluble in water and is usually substantially non-immunogenic, and usually has an atomic molecular weight greater than about 1,000 Daltons. The methods and compositions described herein can be used to attach one or more water-soluble polymers to an aldehyde tagged polypeptide. Attachment of a water-soluble polymer (e.g., PEG) of a polypeptide, particularly a pharmaceutically active (therapeutic) polypeptide can be desirable as such modification can increase therapeutic index by increasing serum half-life as a result of increased proteolytic stability and/or

decreased renal clearance. Additionally, attachment of one or more polymers (e.g., PEGylation) can reduce immunogenicity of protein pharmaceuticals.

[00255] In some embodiments, the water-soluble polymer has an effective hydrodynamic molecular weight of greater than about 10,000 Da, greater than about 20,000 to 500,000 Da, greater than about 40,000 Da to 300,000 Da, greater than about 50,000 Da to 70,000 Da, usually greater than about 60,000 Da. In some embodiments, the water-soluble polymer has an effective hydrodynamic molecular weight of from about 10 kDa to about 20 kDa, from about 20 kDa to about 25 kDa, from about 25 kDa to about 30 kDa, from about 30 kDa to about 50 kDa, or from about 50 kDa to about 100 kDa. By "effective hydrodynamic molecular weight" is intended the effective water-solvated size of a polymer chain as determined by aqueous-based size exclusion chromatography (SEC). When the water-soluble polymer contains polymer chains having polyalkylene oxide repeat units, such as ethylene oxide repeat units, each chain can have an atomic molecular weight of between about 200 Da and about 80,000 Da, or between about 1,500 Da and about 42,000 Da, with 2,000 to about 20,000 Da being of particular interest. Unless referred to specifically, molecular weight is intended to refer to atomic molecular weight. Linear, branched, and terminally charged water soluble polymers (e.g., PEG) are of particular interest.

[00256] Polymers useful as moieties to be attached to an aldehyde tagged polypeptide can have a wide range of molecular weights, and polymer subunits. These subunits may include a biological polymer, a synthetic polymer, or a combination thereof. Examples of such water-soluble polymers include: dextran and dextran derivatives, including dextran sulfate, P-amino cross linked dextrin, and carboxymethyl dextrin, cellulose and cellulose derivatives, including methylcellulose and carboxymethyl cellulose, starch and dextrines, and derivatives and hydroylactes of starch, polyalklyene glycol and derivatives thereof, including polyethylene glycol, methoxypolyethylene glycol, polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol, wherein said homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group, heparin and fragments of heparin, polyvinyl alcohol and polyvinyl ethyl ethers, polyvinylpyrrolidone, aspartamide, and polyoxyethylated polyols, with the dextran and dextran derivatives, dextrine and dextrine derivatives. It will be appreciated that various derivatives of the specifically recited water-soluble polymers are also contemplated.

[00257] Water-soluble polymers such as those described above are well known, particularly the polyalkylene oxide based polymers such as polyethylene glycol "PEG" (See. e.g., "Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications", J. M. Harris, Ed., Plenum Press, New York, N.Y. (1992); and "Poly(ethylene glycol) Chemistry

and Biological Applications", J. M. Harris and S. Zalipsky, Eds., ACS (1997); and International Patent Applications: WO 90/13540, WO 92/00748, WO 92/16555, WO 94/04193,WO 94/14758, WO 94/17039, WO 94/18247, WO 94/28937, WO 95/11924, WO 96/00080, WO 96/23794, WO 98/07713, WO 98/41562, WO 98/48837, WO 99/30727, WO 99/32134, WO 99/33483, WO 99/53951, WO 01/26692, WO 95/13312, WO 96/21469, WO 97/03106, WO 99/45964, and U.S. Pat. Nos. 4,179,337; 5,075,046; 5,089,261; 5,100,992; 5,134,192; 5,166,309; 5,171,264; 5,213,891; 5,219,564; 5,275,838; 5,281,698; 5,298,643; 5,312,808; 5,321,095; 5,324,844; 5,349,001; 5,352,756; 5,405,877; 5,455,027; 5,446,090; 5,470,829; 5,478,805; 5,567,422; 5,605,976; 5,612,460; 5,614,549; 5,618,528; 5,672,662; 5,637,749; 5,643,575; 5,650,388; 5,681,567; 5,686,110; 5,730,990; 5,739,208; 5,756,593; 5,808,096; 5,824,778; 5,824,784; 5,840,900; 5,874,500; 5,880,131; 5,900,461; 5,902,588; 5,919,442; 5,919,455; 5,932,462; 5,965,119; 5,965,566; 5,985,263; 5,990,237; 6,011,042; 6,013,283; 6,077,939; 6,113,906; 6,127,355; 6,177,087; 6,180,095; 6,194,580; 6,214,966).

[00258] Exemplary polymers of interest include those containing a polyalkylene oxide, polyamide alkylene oxide, or derivatives thereof, including polyalkylene oxide and polyamide alkylene oxide comprising an ethylene oxide repeat unit of the formula -(CH_2-CH_2-O)-. Further exemplary polymers of interest include a polyamide having a molecular weight greater than about 1,000 Daltons of the formula -[C(O)-X-C(O)-NH-Y-NH]n- or -[NH-Y-NH-C(O)-X-C(O)]$_n$-, where X and Y are divalent radicals that may be the same or different and may be branched or linear, and n is a discrete integer from 2-100, usually from 2 to 50, and where either or both of X and Y comprises a biocompatible, substantially non-antigenic water-soluble repeat unit that may be linear or branched. Further exemplary water-soluble repeat units comprise an ethylene oxide of the formula -(CH_2-CH_2-O)- or -(CH_2-CH_2-O)- . The number of such water-soluble repeat units can vary significantly, with the usual number of such units being from 2 to 500, 2 to 400, 2 to 300, 2 to 200, 2 to 100, and most usually 2 to 50. An exemplary embodiment is one in which one or both of X and Y is selected from: -((CH_2)$_{n1}$-(CH_2-CH_2-O)$_{n2}$-(CH_2)- or -((CH_2)$_{n1}$-(O-CH_2-CH_2)$_{n2}$-(CH_2)$_{n-1}$-), where n1 is 1 to 6, 1 to 5, 1 to 4 and most usually 1 to 3, and where n2 is 2 to 50, 2 to 25, 2 to 15, 2 to 10, 2 to 8, and most usually 2 to 5. A further exemplary embodiment is one in which X is -(CH_2-CH_2)-, and where Y is -(CH_2-(CH_2-CH_2-O)$_3$-CH_2-CH_2-CH_2)- or -(CH_2-CH_2-CH_2-(O-CH_2-CH_2)$_3$-CH_2)-.

[00259] The polymer can include one or more spacers or linkers. Exemplary spacers or linkers include linear or branched moieties comprising one or more repeat units employed in a water-soluble polymer, diamino and or diacid units, natural or unnatural amino acids or

derivatives thereof, as well as aliphatic moieties, including alkyl, aryl, heteroalkyl, heteroaryl, alkoxy, and the like, which can contain, for example, up to 18 carbon atoms or even an additional polymer chain.

[00260] The polymer moiety, or one or more of the spacers or linkers of the polymer moiety when present, may include polymer chains or units that are biostable or biodegradable. For example, Polymers with repeat linkages have varying degrees of stability under physiological conditions depending on bond lability. Polymers with such bonds can be categorized by their relative rates of hydrolysis under physiological conditions based on known hydrolysis rates of low molecular weight analogs, e.g., from less stable to more stable, e.g., polyurethanes (-NH-C(O)-O-) > polyorthoesters (-O-C((OR)(R'))-O-) > polyamides (-C(O)-NH-). Similarly, the linkage systems attaching a water-soluble polymer to a target molecule may be biostable or biodegradable, e.g., from less stable to more stable: carbonate (-O-C(O)-O-)>ester (-C(O)-O-) > urethane (-NH-C(O)-O-) > orthoester (-O-C((OR)(R'))-O-) > amide (-C(O)-NH-). In general, it may be desirable to avoid use of sulfated polysaccharide, depending on the lability of the sulfate group. In addition, it may be less desirable to use polycarbonates and polyesters. These bonds are provided by way of example, and are not intended to limit the types of bonds employable in the polymer chains or linkage systems of the water-soluble polymers useful in the modified aldehyde tagged polypeptides disclosed herein.

Synthetic peptides

[00261] In some cases, an Ig conjugate comprises a covalently linked peptide, e.g., where J^1 is a peptide. Suitable peptides include, but are not limited to, cytotoxic peptides; angiogenic peptides; anti-angiogenic peptides; peptides that activate B cells; peptides that activate T cells; anti-viral peptides; peptides that inhibit viral fusion; peptides that increase production of one or more lymphocyte populations; anti-microbial peptides; growth factors; growth hormone-releasing factors; vasoactive peptides; anti-inflammatory peptides; peptides that regulate glucose metabolism; an anti-thrombotic peptide; an anti-nociceptive peptide; a vasodilator peptide; a platelet aggregation inhibitor; an analgesic; and the like.

[00262] Where J^1 is a peptide, the peptide can be chemically synthesized to include a group reactive with a converted FGly-containing Ig polypeptide. A suitable synthetic peptide has a length of from about 5 amino acids to about 100 amino acids, or longer than 100 amino acids; e.g., a suitable peptide has a length of from about 5 amino acids (aa) to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, from about 40 aa to

about 50 aa, from about 50 aa to about 60 aa, from about 60 aa to about 70 aa, from about 70 aa to about 80 aa, from about 80 aa to about 90 aa, or from about 90 aa to about 100 aa.

[00263] A peptide can be modified to contain an α-nucleophile-containing moiety (e.g., an aminooxy or hydrazide moiety), e.g., can be reacted with the FGly-containing Ig polypeptide to yield a conjugate in which the aldehyde-tagged Ig polypeptide and peptide are linked by a hydrazone or oxime bond, respectively. Exemplary methods of synthesizing a peptide, such that the synthetic peptide comprising a reactive group reactive with a converted aldehyde tag, are described above.

[00264] Suitable peptides include, but are not limited to, hLF-11 (an 11-amino acid N-terminal fragment of lactoferrin), an anti-microbial peptide; granulysin, an anti-microbial peptide; Plectasin (NZ2114; SAR 215500), an anti-microbial peptide; viral fusion inhibitors such as Fuzeon (enfuvirtide), TRI-1249 (T-1249; see, e.g., Matos et al. (2010) *PLoS One* 5:e9830), TRI-2635 (T-2635; see, e.g., Eggink et al. (2009) *J. Biol. Chem.* 284:26941), T651, and TRI-1144; C5a receptor inhibitors such as PMX-53, JPE-1375, and JSM-7717; POT-4, a human complement factor C3 inhibitor; Pancreate (an INGAP derivative sequence, a HIP-human proislet protein); somatostatin; a somatostatin analog such as DEBIO 8609 (Sanvar), octreotide, octreotide (C2L), octreotide QLT, octreotide LAR, Sandostatin LAR, SomaLAR, Somatuline (lanreotide), see, e.g., Deghenghi et al. (2001) *Endocrine* 14:29; TH9507 (Tesamorelin, a growth hormone-releasing factor); POL7080 (a protegrin analog, an anti-microbial peptide); relaxin; a corticotropin releasing factor agonist such as urotensin, sauvagine, and the like; a heat shock protein derivative such as DiaPep277; a human immunodeficiency virus entry inhibitor; a heat shock protein-20 mimic such as AZX100; a thrombin receptor activating peptide such as TP508 (Chrysalin); a urocortin 2 mimic (e.g., a CRF2 agonist) such as urocortin-2; an immune activator such as Zadaxin (thymalfasin; thymosin-α1), see, e.g., Sjogren (2004) *J. Gastroenterol. Hepatol.* 19:S69; a hepatitis C virus (HCV) entry inhibitorE2 peptide such as HCV3; an atrial natriuretic peptide such as HANP (Sun 4936; carperitide); an annexin peptide; a defensin (anti-microbial peptide) such as hBD2-4; a defensin (anti-microbial peptide) such as hBD-3; a defensin (anti-microbial peptide) such as PMX-30063; a histatin (anti-microbial peptide) such as histatin-3, histatin-5, histatin-6, and histatin-9; a histatin (anti-microbial peptide) such as PAC-113; an indolicidin (anti-microbial peptide) such as MX-594AN (Omniganin; CLS001); an indolicidin (anti-microbial peptide) such as Omnigard (MBI-226; CPI-226); an anti-microbial peptide such as an insect cecropin; an anti-microbial peptide such as a lactoferrin (talactoferrin); an LL-37/cathelicidin derivative (an anti-microbial peptide) such as P60.4 (OP-145); a magainin (an anti-microbial peptide) such as Pexiganan (MSI-78; Suponex); a protegrin (an anti-microbial

peptide) such as IB-367 (Iseganan); an agan peptide; a beta-natriuretic peptide such as Natrecor, or Noratak (Nesiritide), or ularitide; bivalarudin (Angiomax), a thrombin inhibitor; a C peptide derivative; a calcitonin such as Miacalcin (Fortical); an enkephalin derivative; an erythropoiesis-stimulating peptide such as Hematide; a gap junction modulator such as Danegaptide (ZP1609); a gastrin-releasing peptide; a ghrelin; a glucagon-like peptide; a glucagon-like peptide-2 analog such as ZP1846 or ZP1848; a glucosaminyl muramyl dipeptide such as GMDP; a glycopeptide antibiotic such as Oritavancin; a teicoplanin derivative such as Dalbavancin; a gonadotropin releasing hormone (GnRH) such as Zoladex (Lupon) or Triptorelin; a histone deacetylase (HDAC) inhibitor depsipeptide such as PM02734 (Irvalec); an integrin such as eptifibatide; an insulin analog such as Humulog; a kahalalide depsipeptide such as PM02734; a kallikrein inhibitor such as Kalbitor (ecallantide); an antibiotic such as Telavancin; a lipopeptide such as Cubicin or MX-2401; a lutenizing hormone releasing hormone (LHRH) such as goserelin; an LHRH synthetic decapeptide agonist analog such as Treistar (triptorelin pamoate); an LHRH such as Eligard; an M2 protein channel peptide inhibitor; metreleptin; a melanocortin receptor agonist peptide such as bremalanotide/PT-141; a melanocortin; a muramyl tripeptide such as Mepact (mifamurtide); a myelin basic protein peptide such as MBP 8298 (dirucotide); an N-type voltage-gated calcium channel blocker such as Ziconotide (Prialt); a parathyroid hormone peptide; a parathyroid analog such as 768974; a peptide hormone analog such as UGP281; a prostaglandin F2-α receptor inhibitor such as PDC31; a protease inhibitor such as PPL-100; surfaxin; a thromobspondin-1 (TSP-1) mimetic such as CVX-045 or ABT 510; a vasoactive intestinal peptide; vasopressin; a Y2R agonist peptide such as RG7089; obinepeptide; and TM30339.

DRUGS FOR CONJUGATION TO AN ALDEHYDE-TAGGED IMMUNOGLOBULIN POLYPEPTIDE

[00265] Any of a number of drugs are suitable for use, or can be modified to be rendered suitable for use, as a reactive partner to conjugate to an ald-tagged-Ig polypeptide. Exemplary drugs include small molecule drugs and peptide drugs. Thus, the present disclosure provides drug-antibody conjugates.

[00266] "Small molecule drug" as used herein refers to a compound, e.g., an organic compound, which exhibits a pharmaceutical activity of interest and which is generally of a molecular weight of no greater than about 800 Da, or no greater than 2000 Da, but can encompass molecules of up to 5kDa and can be as large as about 10 kDa. A small inorganic molecule refers to a molecule containing no carbon atoms, while a small organic molecule refers to a compound containing at least one carbon atom.

[00267] "Peptide drug" as used herein refers to amino-acid containing polymeric compounds, and is meant to encompass naturally-occurring and non-naturally-occurring peptides, oligopeptides, cyclic peptides, polypeptides, and proteins, as well as peptide mimetics. The peptide drugs may be obtained by chemical synthesis or be produced from a genetically encoded source (e.g., recombinant source). Peptide drugs can range in molecular weight, and can be from 200 Da to 10 kDa or greater in molecular weight.

[00268] In some cases, the drug is a cancer chemotherapeutic agent. For example, where an antibody has specificity for a tumor cell, the antibody can be modified as described herein to include an aldehyde tag, can be subsequently converted to an FGly-modified antibody, and can then be conjugated to a cancer chemotherapeutic agent. Cancer chemotherapeutic agents include non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones. Peptidic compounds can also be used.

[00269] Suitable cancer chemotherapeutic agents include dolastatin and active analogs and derivatives thereof; and auristatin and active analogs and derivatives thereof. See, e.g., WO 96/33212, WO 96/14856, and USPN 6,323,315. For example, dolastatin 10 or auristatin PE can be included in an antibody-drug conjugate of the present disclosure. Suitable cancer chemotherapeutic agents also include maytansinoids and active analogs and derivatives thereof (see, e.g., EP 1391213; and Liu et al (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623); and duocarmycins and active analogs and derivatives thereof (e.g., including the synthetic analogues, KW-2189 and CB 1-TM1).

[00270] Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

[00271] Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-

propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

[00272] Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, *etc.*; podophyllotoxins, *e.g.* etoposide, teniposide, *etc.*; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, *etc.*; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.* mitoxantrone; azirinopyrrolo indolediones, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, *etc.*; and the like.

[00273] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

[00274] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

[00275] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, *etc.*; estrogens and pregestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; *etc.*; and adrenocortical suppressants, *e.g.* aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation; therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

[00276] Other suitable chemotherapeutic agents include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, *etc.*; ureas, *e.g.* hydroxyurea; and hydrazines, *e.g.* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc. Other anti-proliferative agents of interest include immunosuppressants, *e.g.* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

[00277] Taxanes are suitable for use. "Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*).

[00278] Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[00279] Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

[00280] Biological response modifiers suitable for use include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN-α; (7) IFN-γ; (8) colony-stimulating factors; and (9) inhibitors of angiogenesis.

Methods for modification of drugs to contain reactive partner for reaction with 2-formylglycine

[00281] Peptide drugs to be conjugated to an ald-tagged Ig polypeptide are modified to incorporate a reactive partner for reaction with an aldehyde of the FGly residue of the ald-tagged Ig polypeptide. Since the methods of ald-tagged polypeptide modification are compatible with conventional chemical processes, any of a wide variety of commercially available reagents can be used to accomplish conjugation. For example, aminooxy, hydrazide, hydrazine, or thiosemicarbazide derivatives of a number of moieties of interest are suitable reactive partners, and are readily available or can be generated using standard chemical methods.

[00282] Where the drug is a peptide drug, the reactive moiety (e.g., aminooxy or hydrazide can be positioned at an N-terminal region, the N-terminus, a C-terminal region, the C-terminus, or at a position internal to the peptide. For example, an exemplary method involves synthesizing a peptide drug having an aminooxy group. In this example, the peptide is synthesized from a Boc-protected precursor. An amino group of a peptide can react with a compound comprising a carboxylic acid group and oxy-N-Boc group. As an example, the amino group of the peptide reacts with 3-(2,5-dioxopyrrolidin-1-yloxy)propanoic acid. Other variations on the compound comprising a carboxylic acid group and oxy-N-protecting group can include different number of carbons in the alkylene linker and substituents on the alkylene linker. The reaction between the amino group of the peptide and the compound comprising a carboxylic acid group and oxy-N-protecting group occurs through standard peptide coupling chemistry. Examples of peptide coupling reagents that can be used include, but not limited to, DCC (dicyclohexylcarbodiimide), DIC (diisopropylcarbodiimide), di-p-toluoylcarbodiimide, BDP (1-benzotriazole diethylphosphate-1-cyclohexyl-3-(2-morpholinylethyl)carbodiimide), EDC (1-(3-dimethylaminopropyl-3-ethyl-carbodiimide hydrochloride), cyanuric fluoride, cyanuric chloride, TFFH (tetramethyl fluoroformamidinium hexafluorophosphosphate), DPPA (diphenylphosphorazidate), BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate), HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate), TBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate), TSTU (O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate), HATU (N-[(dimethylamino)-1-H-1,2,3-triazolo[4,5,6]-pyridin-1-ylmethylene]- -N-methylmethanaminium hexafluorophosphate N-oxide), BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride), PyBOP ((1-H-1,2,3-benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium tetrafluorophopsphate), BrOP

(bromotris(dimethylamino)phosphonium hexafluorophosphate), DEPBT (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one) PyBrOP (bromotris(pyrrolidino)phosphonium hexafluorophosphate). As a non-limiting example, HOBt and DIC can be used as peptide coupling reagents.

[00283] Deprotection to expose the amino-oxy functionality is performed on the peptide comprising an N-protecting group. Deprotection of the N-oxysuccinimide group, for example, occurs according to standard deprotection conditions for a cyclic amide group. Deprotecting conditions can be found in Greene and Wuts, Protective Groups in Organic Chemistry, 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al. Certain deprotection conditions include a hydrazine reagent, amino reagent, or sodium borohydride. Deprotection of a Boc protecting group can occur with TFA. Other reagents for deprotection include, but are not limited to, hydrazine, methylhydrazine, phenylhydrazine, sodium borohydride, and methylamine. The product and intermediates can be purified by conventional means, such as HPLC purification.

[00284] The ordinarily skilled artisan will appreciate that factors such as pH and steric hindrance (i.e., the accessibility of the aldehyde tag to reaction with a reactive partner of interest) are of importance, Modifying reaction conditions to provide for optimal conjugation conditions is well within the skill of the ordinary artisan, and is routine in the art. In general, it is normally desirable to conduction conjugation reactions at a pH below 7, with a pH of about 5.5, about 6, about 6.5, usually about 5.5 being optimal. Where conjugation is conducted with an aldehyde tagged polypeptide present in or on a living cell, the conditions are selected so as to be physiologically compatible. For example, the pH can be dropped temporarily for a time sufficient to allow for the reaction to occur but within a period tolerated by the cell having an aldehyde tag (e.g., from about 30 min to 1 hour). Physiological conditions for conducting modification of aldehyde tagged polypeptides on a cell surface can be similar to those used in a ketone-azide reaction in modification of cells bearing cell-surface azides (see, e.g., US 6,570,040).

[00285] Small molecule compounds containing, or modified to contain, an α-nucleophilic group that serves as a reactive partner with an aldehyde of an FGly of an ald tag are also contemplated for use as drugs in the Ig-drug conjugates of the present disclosure. General methods are known in the art for chemical synthetic schemes and conditions useful for synthesizing a compound of interest (see, e.g., Smith and March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Fifth Edition, Wiley-Interscience, 2001; or Vogel, A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis, Fourth Edition, New York: Longman, 1978).

[00286] Thus small molecules having an aminooxy or hydrazone group for reaction with an aldehyde of an FGly of an ald-tagged Ig polypeptide are available or can be readily synthesized. An aminooxy or hydrazone group can be installed onto a small molecule using standard synthetic chemistry techniques.

IG CONJUGATES

[00287] In some embodiments, a subject Ig-conjugate is an antibody conjugate. For example, the present disclosure provides an antibody conjugate that comprises a subject Ig conjugate, where the antibody conjugate binds an antigen. The antibody conjugate can include a J^1 moiety covalently bound to an Ig heavy chain constant region only, covalently bound to an Ig light chain constant region only, or a J^1 moiety covalently bound to an Ig heavy chain constant region and a J^1 moiety covalently bound to an Ig light chain constant region.

[00288] An antibody conjugate can have any of a variety of antigen-binding specificities, as described above, including, e.g., an antigen present on a cancer cell; an antigen present on an autoimmune cell; an antigen present on a pathogenic microorganism; an antigen present on a virus-infected cell (e.g., a human immunodeficiency virus-infected cell), e.g., CD4 or gp120; an antigen present on a diseased cell; and the like. For example, an antibody conjugate can bind an antigen, as noted above, where the antigen is present on the surface of the cell.

[00289] An antibody conjugate of the present disclosure can include, as the J^1 moiety, any of a variety of compounds, as described above, e.g., a drug (e.g., a peptide drug, a small molecule drug, and the like), a water-soluble polymer, a detectable label, a synthetic peptide, etc.

[00290] An antibody conjugate of the present disclosure can bind antigen with a suitable binding affinity, e.g., from about 5×10^{-6} M to about 10^{-7} M, from about 10^{-7} M to about 5×10^{-7} M, from about 5×10^{-7} M to about 10^{-8} M, from about 10^{-8} M to about 5×10^{-8} M, from about 5×10^{-8} M to about 10^{-9} M, or a binding affinity greater than 10^{-9} M.

[00291] As non-limiting examples, a subject antibody conjugate can bind an antigen present on a cancer cell (e.g., a tumor-specific antigen; an antigen that is over-expressed on a cancer cell; etc.), and the J^1 moiety can be a cytotoxic compound (e.g., a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.). For example, a subject antibody conjugate can be specific for CD19, where the J^1 moiety is a cytotoxic compound (e.g., a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.). As another example, a subject antibody conjugate can be specific for CD22, where the J^1 moiety can be a cytotoxic compound (e.g., a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.).

[00292] As further non-limiting examples, a subject antibody conjugate can bind an antigen present on a cell infected with a virus (e.g., where the antigen is encoded by the virus; where the antigen is expressed on a cell type that is infected by a virus; etc.), and the J^1 moiety can be a viral fusion inhibitor. For example, a subject antibody conjugate can bind CD4, and the J^1 moiety can be a viral fusion inhibitor. As another example, a subject antibody conjugate can bind gp120, and the J^1 moiety can be a viral fusion inhibitor.

FORMULATIONS

[00293] The Ig conjugates (including antibody conjugates) of the present disclosure can be formulated in a variety of different ways. In general, where the Ig conjugate is an Ig-drug conjugate, the Ig conjugate is formulated in a manner compatible with the drug conjugated to the Ig, the condition to be treated, and the route of administration to be used.

[00294] The Ig conjugate (e.g., Ig-drug conjugate) can be provided in any suitable form, e.g., in the form of a pharmaceutically acceptable salt, and can be formulated for any suitable route of administration, e.g., oral, topical or parenteral administration. Where the Ig conjugate is provided as a liquid injectable (such as in those embodiments where they are administered intravenously or directly into a tissue), the Ig conjugate can be provided as a ready-to-use dosage form, or as a reconstitutable storage-stable powder or liquid composed of pharmaceutically acceptable carriers and excipients.

[00295] Methods for formulating Ig conjugates can be adapted from those available in the art. For example, Ig conjugates can be provided in a pharmaceutical composition comprising an effective amount of a Ig conjugate and a pharmaceutically acceptable carrier (e.g., saline). The pharmaceutical composition may optionally include other additives (e.g., buffers, stabilizers, preservatives, and the like). Of particular interest in some embodiments are formulations that are suitable for administration to a mammal, particularly those that are suitable for administration to a human.

METHODS OF TREATMENT

[00296] The Ig-drug conjugates of the present disclosure find use in treatment of a condition or disease in a subject that is amenable to treatment by administration of the parent drug (i.e., the drug prior to conjugation to the Ig). By "treatment" is meant that at least an amelioration of the symptoms associated with the condition afflicting the host is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g. terminated, such that the host no longer suffers from the condition, or at least the symptoms

that characterize the condition. Thus treatment includes: (i) prevention, that is, reducing the risk of development of clinical symptoms, including causing the clinical symptoms not to develop, e.g., preventing disease progression to a harmful state; (ii) inhibition, that is, arresting the development or further development of clinical symptoms, e.g., mitigating or completely inhibiting an active disease; and/or (iii) relief, that is, causing the regression of clinical symptoms.

[00297] In the context of cancer, the term "treating" includes any or all of: reducing growth of a solid tumor, inhibiting replication of cancer cells, reducing overall tumor burden, and ameliorating one or more symptoms associated with a cancer.

[00298] The subject to be treated can be one that is in need of therapy, where the host to be treated is one amenable to treatment using the parent drug. Accordingly, a variety of subjects may be amenable to treatment using an Ig-drug conjugates disclosed herein. Generally such subjects are “mammals”, with humans being of particular interest. Other subjects can include domestic pets (e.g., dogs and cats), livestock (e.g., cows, pigs, goats, horses, and the like), rodents (e.g., mice, guinea pigs, and rats, e.g., as in animal models of disease), as well as non-human primates (e.g., chimpanzees, and monkeys.

[00299] The amount of Ig-drug conjugate administered can be initially determined based on guidance of a dose and/or dosage regimen of the parent drug. In general, the Ig-drug conjugates can provide for targeted delivery and/or enhanced serum half-life of the bound drug, thus providing for at least one of reduced dose or reduced administrations in a dosage regimen. Thus the Ig-drug conjugates can provide for reduced dose and/or reduced administration in a dosage regimen relative to the parent drug prior to being conjugated in an Ig-drug conjugate of the present disclosure.

[00300] Furthermore, as noted above, because the Ig-drug conjugates can provide for controlled stoichiometry of drug delivery, dosages of Ig-drug conjugates can be calculated based on the number of drug molecules provided on a per Ig-drug conjugate basis.

[00301] In some embodiments, multiple doses of an Ig-drug conjugate are administered. The frequency of administration of an Ig-drug conjugate can vary depending on any of a variety of factors, e.g., severity of the symptoms, etc. For example, in some embodiments, an Ig-drug conjugate is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

Methods of treating cancer

[00302] The present disclosure provides methods for delivering a cancer chemotherapeutic agent to an individual having a cancer. The methods are useful for treating a wide variety of cancers, including carcinomas, sarcomas, leukemias, and lymphomas.

[00303] Carcinomas that can be treated using a subject method include, but are not limited to, esophageal carcinoma, hepatocellular carcinoma, basal cell carcinoma (a form of skin cancer), squamous cell carcinoma (various tissues), bladder carcinoma, including transitional cell carcinoma (a malignant neoplasm of the bladder), bronchogenic carcinoma, colon carcinoma, colorectal carcinoma, gastric carcinoma, lung carcinoma, including small cell carcinoma and non-small cell carcinoma of the lung, adrenocortical carcinoma, thyroid carcinoma, pancreatic carcinoma, breast carcinoma, ovarian carcinoma, prostate carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, renal cell carcinoma, ductal carcinoma in situ or bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical carcinoma, uterine carcinoma, testicular carcinoma, osteogenic carcinoma, epithelial carcinoma, and nasopharyngeal carcinoma, etc.

[00304] Sarcomas that can be treated using a subject method include, but are not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, chordoma, osteogenic sarcoma, osteosarcoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, and other soft tissue sarcomas.

[00305] Other solid tumors that can be treated using a subject method include, but are not limited to, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[00306] Leukemias that can be treated using a subject method include, but are not limited to, a) chronic myeloproliferative syndromes (neoplastic disorders of multipotential hematopoietic stem cells); b) acute myelogenous leukemias (neoplastic transformation of a multipotential hematopoietic stem cell or a hematopoietic cell of restricted lineage potential; c) chronic lymphocytic leukemias (CLL; clonal proliferation of immunologically immature and functionally incompetent small lymphocytes), including B-cell CLL, T-cell CLL prolymphocytic leukemia, and hairy cell leukemia; and d) acute lymphoblastic leukemias (characterized by accumulation of lymphoblasts). Lymphomas that can be treated using a subject method include, but are not limited to, B-cell lymphomas (e.g., Burkitt's lymphoma); Hodgkin's lymphoma; non-Hodgkin's B cell lymphoma; and the like.

EXAMPLES

[00307] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

EXAMPLE 1: CLONING OF CD19 AND CD22 SPECIFIC ANTIBODIES

[00308] Genes encoding the CD19 and CD22 specific variable light chain regions were synthesized and cloned into a plasmid containing the human IgG kappa light chain constant region using NcoI and BsiWI restriction sites. The light chain constant region plasmid was either wild-type or contained LCTPSR (SEQ ID NO:17) or LATPSR (SEQ ID NO:24), which were inserted into the plasmid using Quikchange mutagenesis.

[00309] Genes encoding the CD19 and CD22 specific variable heavy chain regions were synthesized and cloned into a plasmid containing the human IgG heavy chain constant region using EcoRI and NheI restriction sites. The heavy chain constant region plasmid was either wild-type or contained LCTPSR (SEQ ID NO:17) or LATPSR (SEQ ID NO:24), which were inserted into the plasmid using Quikchange mutagenesis.

[00310] Figure 3 shows amino acid sequences of anti-CD19 light chain (upper sequence) and heavy chain (lower sequence) constant regions, with an LCTPSR sulfatase motif in the heavy chain constant region. The signal peptide is shown in lower-case letters; the variable region is underlined; solvent-accessible loop regions in the constant regions are shown in bold and underlined. The LCTPSR sequence is shown in bold and double underlining. The initial methionine (M) present in the heavy and light chain amino acid sequences is for purposes of facilitating expression and can be optionally present in these and all heavy and light chains amino acid sequences described herein.

Wild-type anti-CD19 and anti-CD22 sequences

[00311] Amino acid sequences of wild-type (not aldehyde-tagged) anti-CD22 heavy and light chains are shown in Figures 6B and 19B, respectively. Nucleotide sequences encoding wild-type (not aldehyde-tagged) anti-CD22 heavy and light chains are shown in Figures 6A and 15A, respectively.

[00312] Amino acid sequences of wild-type (not aldehyde-tagged) anti-CD19 heavy and light chains are shown in Figures 19B and 31B, respectively. Nucleotide sequences encoding wild-type (not aldehyde-tagged) anti-CD19 heavy and light chains are shown in Figures 19A and 31A, respectively.

Sequences of anti-CD19 and anti-CD22 heavy chains modified to include LCTPSR

[00313] Amino acid sequences of anti-CD22 heavy chain constant regions modified to include the aldehyde tag sequence LCTPSR (which is recognized and converted by FGE) are shown in Figures 7B, 8B, and 9B, where the aldehyde tag is in the CH1 domain; Figure 11B,, 12B and 13B where the aldehyde tag is in the CH2 domain; Figures 15B, where the aldehyde tag is in the CH2/CH3 region; and Figure 17B, where the aldehyde tag is near the C-terminus. Figures 7A, 8A, 9A, 11A, 12A, 13A, and 15A provide nucleotide sequences encoding the amino acid sequences shown in Figures 7A, 8B, 9B, 11B, 12B, 13B and 15B, respectively.

[00314] Amino acid sequences of anti-CD19 heavy chain constant regions modified to include the aldehyde tag sequence LCTPSR (which is recognized and converted by FGE) are shown in Figures 23B, where the aldehyde tag is in the CH1 domain; Figure 25B, where the aldehyde tag is in the CH2 domain; Figure 27B, where the aldehyde tag is in the CH2/CH3 region; and Figure 29B, where the aldehyde tag is near the C-terminus. Figures 19A, 21A, 23A, and 25A provide nucleotide sequences encoding the amino acid sequences shown in Figures 19B, 21B, 23B, and 25B, respectively.

Sequences of anti-CD19 and anti-CD22 heavy chains modified to include LATPSR

[00315] Amino acid sequences of anti-CD22 heavy chain constant regions modified to include the control sequence LATPSR (which is not recognized by FGE) are shown in Figures 10B, where the control sequence is in the CH1 domain; Figure 14B, where the control sequence is in the CH2 domain; Figure 16B, where the control sequence is in the CH2/CH3 region; and Figure 18B, where the control sequence is near the C-terminus. Figures 10A, 14A, 16A, and 18A provide nucleotide sequences encoding the amino acid sequences shown in Figures 10B, 14B, 16B, and 18B, respectively.

[00316] Amino acid sequences of anti-CD19 heavy chain constant regions modified to include the control sequence LATPSR (which is not recognized by FGE) are shown in Figures 24B, where the control sequence is in the CH1 domain; Figure 26B, where the control sequence is in the CH2 domain; Figure 28B where the control sequence is in the CH2/CH3 region; and Figure 30B, where the control sequence is near the C-terminus.

Sequences of anti-CD19 and anti-CD22 light chains modified to include LCTPSR

[00317] An amino acid sequence of an anti-CD22 light chain constant region modified to include the aldehyde tag sequence LCTPSR is shown in Figure 20B. Figure 20A provides a nucleotide sequence encoding the amino acid sequence shown in Figure 20B. Figure 21B provides an amino acid sequence of an anti-CD22 light chain constant region modified to include the control sequence LATPSR; Figure 21A provides a nucleotide sequence encoding the amino acid sequence shown in Figure 21B.

[00318] An amino acid sequence of an anti-CD19 light chain constant region modified to include the aldehyde tag sequence LCTPSR is shown in Figure 32B. Figure 32A provides a nucleotide sequence encoding the amino acid sequence shown in Figure 32B. Figure 33B provides an amino acid sequence of an anti-CD22 light chain constant region modified to include the control sequence LATPSR; Figure 33A provides a nucleotide sequence encoding the amino acid sequence shown in Figure 33B.

EXAMPLE 2: EXPRESSING AND PURIFYING CD19 AND CD22 SPECIFIC ANTIBODIES

[00319] Plasmids containing genes encoding the CD19 or CD22 specific heavy and light chains were transfected into CHO-K1 cells stably expressing human FGE using Lipofectamine 2000 transfection reagent. 12μg of the heavy and light chain plasmids were used for every 10mL of Opti-MEM serum-free medium used. After 5h at 37°C, the Opti-MEM was removed and Ex-Cell 325 protein-free medium was added. After 72 h at 37°C, the media was collected and cleared of debris. Cleared medium was combined with Protein A binding buffer and Protein A resin and incubated with rotation for 1h at room temperature (RT). The mixture was added to a column to let the unbound material flow through. The resin was washed with Protein A binding buffer and then eluted 5 with Protein A elution buffer.

[00320] Anti-CD19 and anti-CD22 heavy chain constant regions were modified to include an aldehyde tag in the CH1 domain, the CH2 domain, or the CH3 domain. Anti-CD19 and anti-CD22 light chains were also modified to include an aldehyde tag. Aldehyde-tagged anti-CD19 and aldehyde-tagged anti-CD22 antibodies were subjected to protein blot analysis. The results are shown in Figure 4.

[00321] As shown in Figure 4, inclusion of an aldehyde tag did not disrupt protein expression, folding, or secretion. "Ald" refers to modification of the constant region to include LCTPSR, a sequence that is recognized by FGE. "C2A" refers to modification of the constant region to include "LATPSR," a sequence that is not recognized by FGE.

[00322] The aldehyde-tagged anti-CD19 and anti-CD22 antibodies include aldehyde tags in both heavy and light chains.

EXAMPLE 3: CONJUGATION OF AMINOOXY FLAG PEPTIDE TO PURIFIED ALDEHYDE-TAGGED ANTIBODY

[00323] Purified antibodies were combined with 1mM aminooxy FLAG peptide and 100mM MES buffer pH 5.5 for 16h at room temperature (RT). Samples were run on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and subjected to Western Blot analysis using an anti-FLAG antibody to detect conjugation of the FLAG peptide to the antibody.

[00324] The results are shown in Figure 5. Figure 5 depicts protein blot analysis of aldehyde-tagged anti-CD19 and aldehyde-tagged anti-CD22 antibodies that were chemically conjugated with aminooxy-FLAG.

[00325] Figure 5A depicts a schematic of protein expression followed by aldehyde specific chemical conjugation. A western blot, probed with goat anti-human IgG or with anti-FLAG antibody, illustrates an example of protein conjugation. No labeling was observed with the C2A (LATPSR)-tagged antibody (lower panel).

[00326] Figure 5B depicts labeling with aminooxy FLAG to the tagged anti-CD19 and Anti-CD22 IgGs. The protein loading and labeling was monitored by Western blot. "CtoA" refers to antibodies modified to include the LATPSR sequence.

[00327] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An isolated aldehyde-tagged immunoglobulin (Ig) polypeptide comprising an Ig constant region amino acid sequence comprising an amino acid sequence of a sulfatase motif,

wherein the sulfatase motif is positioned within or adjacent a solvent-accessible loop region of the Ig polypeptide constant region, and wherein the sulfatase motif is not at the C-terminus of the Ig polypeptide chain.

2. The isolated aldehyde-tagged Ig polypeptide of claim 1, wherein the sulfatase motif comprises an amino acid sequence of the formula $X_1Z_1X_2Z_2X_3Z_3$, wherein

Z_1 is cysteine or serine;

Z_2 is a proline or alanine residue;

Z_3 is an aliphatic amino acid or a basic amino acid;

X_1 is present or absent and, when present, is any amino acid, with the proviso that when the heterologous sulfatase motif is at an N-terminus of the polypeptide, X_1 is present;

X_2 and X_3 are each independently any amino acid,

3. The isolated Ig polypeptide of claim 2, wherein Z_3 is arginine (R).

4. The isolated Ig polypeptide of claim 2, wherein X_1, when present, X_2, and X_3 are each independently an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid.

5. The isolated Ig polypeptide of claim 2, wherein the X_1, when present, is L, M, V, S or T.

6. The isolated Ig polypeptide of claim 2, wherein X_2 and X_3 are each independently S, T, A, V, G, or C.

7. The isolated Ig polypeptide of claim 2, wherein the Ig polypeptide comprises an Ig heavy chain constant region.

8. The isolated Ig polypeptide of claim 7, wherein the Ig heavy chain constant region is an IgG1 heavy chain constant region.

9. The isolated Ig polypeptide of claim 1, wherein the Ig polypeptide comprises an Ig light chain constant region.

10. The isolated Ig polypeptide of claim 9, wherein the Ig light chain constant region is a kappa light chain constant region.

11. The isolated Ig polypeptide of claim 1, wherein the Ig polypeptide comprises two or more sulfatase motifs.

12. An isolated immunoglobulin (Ig) polypeptide modified to comprise 2-formylglycine (FGly) moiety, wherein the FGly-modified sulfatase motif is within or adjacent a solvent-accessible loop region of the Ig polypeptide, wherein the FGly-modified Ig presents the FGly group on a solvent-accessible surface when in a folded state, and wherein the sulfatase motif is not at the C-terminus of the Ig polypeptide chain.

13. The isolated Ig polypeptide of claim 12, wherein the FGly-converted sulfatase motif comprises the formula:

$$X_1(FGly)X_2Z_2X_3Z_3$$

wherein:

X_1 is present or absent and, when present, is any amino acid, with the proviso that when the sulfatase motif is at an N-terminus of the polypeptide, X_1 is present;

X_2 and X_3 are each independently any amino acid; and

Z_3 is a basic amino acid.

14. The FGly-modified Ig polypeptide of claim 12, wherein the FGly-modified Ig polypeptide comprises two or more FGly-converted sulfatase motifs.

15. The FGly-modified Ig polypeptide of claim 12, wherein the FGly-modified Ig polypeptide comprises an Ig heavy chain constant region.

16. The FGly-modified Ig polypeptide of claim 15, wherein the Ig heavy chain constant region is an IgG1 heavy chain constant region.

17. The FGly-modified Ig polypeptide of claim 12, wherein the FGly-modified Ig polypeptide comprises an Ig light chain constant region.

18. The FGly-modified Ig polypeptide of claim 17, wherein the Ig light chain constant region is a Ig kappa light chain constant region.

19. The FGly-modified Ig polypeptide of claim 13, wherein Z_3 is arginine (R).

20. The FGly-modified Ig polypeptide of claim 13, wherein X_1, when present, X_2, and X_3 are each independently an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid.

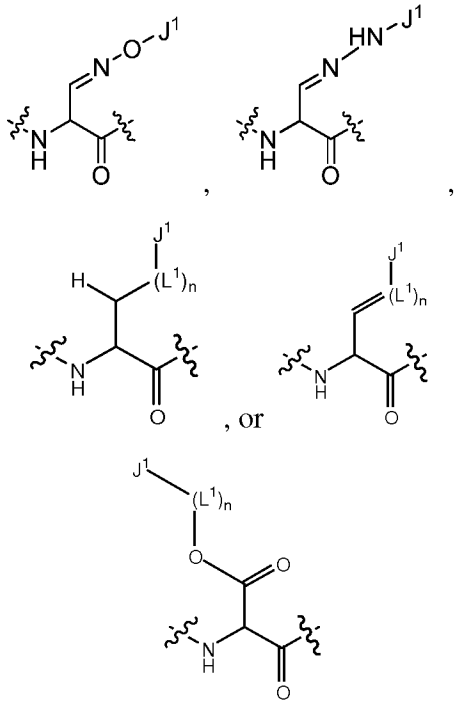
21. The FGly-modified Ig polypeptide of claim 13, wherein the X_1, when present, is L, M, V, S or T.

22. The FGly-modified Ig polypeptide of claim 13, wherein X_2 and X_3 are each independently S, T, A, V, G, or C.

23. An immunoglobulin (Ig) conjugate comprising:

an Ig polypeptide and a covalently conjugated moiety, wherein the Ig polypeptide comprises a modified sulfatase motif comprising an FGly'

where FGly' is of the formula:



wherein J^1 is the covalently bound moiety;

each L^1 is a divalent moiety independently selected from alkylene, substituted alkylene, alkenylene, substituted alkenylene, alkynylene, alkynylene, arylene, substituted arylene, cycloalkylene, substituted cycloalkylene, heteroarylene, substituted heteroarylene, heterocyclene, substituted heterocyclene, acyl, amido, acyloxy, urethanylene, thioester, sulfonyl, sulfonamide, sulfonyl ester, -O-, -S-, -NH-, and substituted amine; and

n is a number selected from zero to 40;

wherein the sulfatase motif is within or adjacent a solvent-accessible loop region of the Ig polypeptide, wherein the sulfatase motif is not at the C-terminus of the Ig polypeptide, and wherein the Ig conjugate presents the covalently bound moiety on a solvent-accessible surface when in a folded state.

24. The Ig conjugate of claim 23, wherein the sulfatase motif comprises the formula:

$$X_1(FGly')X_2Z_2X_3Z_3$$

wherein

Z_2 is a proline or alanine residue;

X_1 is present or absent and, when present, is any amino acid, with the proviso that when the sulfatase motif is at an N-terminus of the polypeptide, X_1 is present;

X_2 and X_3 are each independently any amino acid; and

Z_3 is an aliphatic amino acid or basic amino acid.

25. The Ig conjugate of claim 23, wherein J^1 is selected from a drug, a detectable label, a water-soluble polymer, and a synthetic peptide.

26. The Ig conjugate of claim 23, wherein the Ig conjugate comprises two or more modified sulfatase motifs.

27. The Ig conjugate of claim 25, wherein J^1 is a small molecule drug.

28. The Ig conjugate of claim 27, wherein the small molecule drug is a cancer chemotherapeutic agent.

29. The Ig conjugate of claim 28, wherein the cancer chemotherapeutic agent is an alkylating agent, a nitrosourea, an antimetabolite, an antitumor antibiotic, a vinca alkaloid, or a steroid hormone.

30. The Ig conjugate of claim 25, wherein J^1 is a water-soluble polymer.

31. The Ig conjugate of claim 30, wherein the water-soluble polymer is poly(ethylene glycol).

32. The Ig conjugate of claim 25, wherein J^1 is a detectable label.

33. The Ig conjugate of claim 32, wherein the detectable label is an imaging agent.

34. The Ig conjugate of claim 23, wherein the Ig polypeptide comprises an Ig heavy chain constant region.

35. The Ig conjugate of claim 34, wherein the Ig heavy chain constant region is an IgG1 heavy chain constant region.

36. The Ig conjugate of claim 23, wherein the FGly-modified Ig polypeptide comprises an Ig light chain constant region.

37. The Ig conjugate of claim 36, wherein the Ig light chain constant region is a Ig kappa light chain constant region.

38. The Ig conjugate of claim 24, wherein Z_3 is arginine (R).

39. The Ig conjugate of claim 24, wherein X_1, when present, X_2, and X_3 are each independently an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid.

40. The Ig conjugate of claim 24, wherein the X_1, when present, is L, M, V, S or T.

41. The Ig conjugate of claim 24, wherein X_2 and X_3 are each independently S, T, A, V, G, or C.

42. A recombinant nucleic acid comprising a nucleotide sequence encoding the aldehyde-tagged immunoglobulin (Ig) polypeptide of claim 1.

43. The nucleic acid of claim 42, wherein the Ig polypeptide comprises an Ig heavy chain constant region.

44. The nucleic acid of claim 43, wherein the Ig polypeptide further comprises an Ig variable region.

45. The nucleic acid of claim 42, wherein the Ig polypeptide comprises an Ig light chain constant region.

46. The nucleic acid of claim 45, wherein the Ig polypeptide further comprises an Ig variable region.

47. A recombinant expression vector comprising the nucleic acid of claim 42, wherein the aldehyde-tagged Ig polypeptide-encoding nucleotide sequence is operably linked to a promoter.

48. The recombinant expression vector of claim 47, wherein the Ig polypeptide comprises an Ig heavy chain constant region.

49. The recombinant expression vector of claim 48, wherein the vector comprises an insertion site for an Ig variable region 5' of the aldehyde-tagged Ig polypeptide-encoding nucleotide sequence.

50. The recombinant expression vector of claim 47, wherein the Ig polypeptide comprises an Ig light chain constant region.

51. The recombinant expression vector of claim 50, wherein the vector comprises an insertion site for an Ig variable region 5' of the aldehyde-tagged Ig polypeptide-encoding nucleotide sequence.

52. A library of recombinant expression vectors, the library comprising:

a population of the recombinant expression vectors of claim 47, wherein each member of the population comprises a nucleotide sequence encoding a differently aldehyde-tagged immunoglobulin polypeptide.

53. A library of aldehyde-tagged immunoglobulin (Ig) polypeptides, the library comprising:

a population of aldehyde-tagged Ig polypeptides according to claim 1, or FGly-modified immunoglobulin polypeptides according to claim 12, wherein the population comprises members having differently aldehyde-tagged Ig polypeptides.

54. A formulation comprising:

a) an Ig conjugate of claim 23; and

b) a pharmaceutically acceptable excipient.

55. A method of producing an immunoglobulin-drug conjugate, the method comprising

combining in a reaction mixture

an FGly-modified, aldehyde-tagged immunoglobulin (Ig) polypeptide of claim 12, wherein Z_1 is a 2-formyl-glycine (FGly) residue and

a drug for conjugation to the FGly-modified Ig polypeptide, wherein the drug comprises a aminooxy or hydrazide reactive group,

wherein the drug is provided in the reaction mixture in an amount sufficient to provide for a desired ratio of drug to Ig polypeptide, said combining being under conditions

suitable to promote reaction between an aldehyde of the Ig polypeptide and reactive group of the drug to generate an Ig polypeptide-drug conjugate; and

isolating the Ig polypeptide-drug conjugate from the reaction mixture.

56. A method of delivering a drug to an individual in need thereof, the method comprising administering to the individual an effective amount of an Ig conjugate of claim 23.

57. A method of treating cancer in a subject, the method comprising:

administering to a subject having cancer an effective amount of an immunoglobulin-drug conjugate of claim 23, wherein said administering is effective to treat the cancer in the subject.

58. An aldehyde-tagged antibody comprising an aldehyde-tagged immunoglobulin (Ig) polypeptide of claim 1.

59. The aldehyde-tagged antibody of claim 58, wherein the antibody comprises an aldehyde-tagged Ig heavy chain constant region.

60. The aldehyde-tagged antibody of claim 58, wherein the antibody comprises an aldehyde-tagged Ig light chain constant region.

61. The aldehyde-tagged antibody of claim 58, wherein the antibody comprises an aldehyde-tagged Ig heavy chain constant region and an aldehyde-tagged Ig light chain constant region

62. An antibody comprising a formylglycine (FGly) moiety, wherein the antibody comprises an FGly-modified Ig polypeptide of claim 12.

63. The FGly-modified antibody of claim 62, wherein the antibody comprises an FGly-modified heavy chain constant region.

64. The FGly-modified antibody of claim 62, wherein the antibody comprises an FGly-modified light chain constant region.

65. The FGly-modified antibody of claim 62, wherein the antibody comprises an FGly-modified heavy chain constant region and an FGly-modified light chain constant region.

66. An antibody conjugate comprising an immunoglobulin (Ig) conjugate of claim 21.

67. The antibody conjugate of claim 66, wherein the antibody conjugate comprises a J^1 moiety covalently bound to an Ig heavy chain constant region.

68. The antibody conjugate of claim 66, wherein the antibody conjugate comprises a J^1 moiety covalently bound to an Ig light chain constant region.

69. The antibody conjugate of claim 66, wherein the antibody conjugate comprises a J^1 moiety covalently bound to an Ig heavy chain constant region and a J^1 moiety covalently bound to an Ig light chain constant region.

70. The antibody conjugate of claim 66, wherein the antibody specifically binds a tumor antigen on a cancer cell.

71. The antibody conjugate of claim 70, wherein the J^1 moiety is a cytotoxic agent.

72. The antibody conjugate of claim 66, wherein the antibody specifically binds an antigen on a cell infected by a virus.

73 The antibody conjugate of claim 72, wherein antigen is encoded by the virus.

74. The antibody conjugate of claim 72, wherein the J^1 moiety is a viral fusion inhibitor.

Figure 1A

IgG1
Light chain conserved region:

```
          140         150         160         170         180       189
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD

          200         210         220         230   236
SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
```

Heavy chain conserved region:

```
       130         140         150         160         170         180
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS

       190         200         210         220         230         240
GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG

       250         260         270         280         290         300
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN

       310         320         330         340         350         360
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE

       370         380         390         400         410         420
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW

       430         440         450
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
```

Figure 1B

Seq 1 = *Homo sapiens* IgG1 heavy chain constant region; GenBank P01857.1
Seq 2 = *Homo sapiens* IgG2 heavy chain constant region; GenBank P01859.2
Seq 3 = *Homo sapiens* IgG3 heavy chain constant region; GenBank P01860.2
Seq 4 = *Homo sapiens* IgG4 heavy chain constant region; GenBank AAB59394.1
Seq 5 = *Homo sapiens* IgA heavy chain constant region; GenBank AAAT74070

```
seq1          --ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP-EPVTVSWNSGALTSGVHTFPAVL 178
seq3          --ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFP-EPVTVSWNSGALTSGVHTFPAVL
seq2          --ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFP-EPVTVSWNSGALTSGVHTFPAVL
seq4            STKGPSVFPLAPCSRSTSESTAALGCLVKDYFP-EPVTVSWNSGALTSGVHTFPAVL
seq5          --ASPTSPKVFPLSLCS-TQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQ
                ** ...*.****: .* : . ....:.***:.:** **::*:*....    .:.**.

seq1          QSSG-LYSLSSVVTVPS-SSLGTQTYICNVNHKPSNTKVDKKVE---------------- 220
seq3          QSSG-LYSLSSVVTVPS-SSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCP
seq2          QSSG-LYSLSSVVTVPS-SNFGTQTYTCNVDHKPSNTKVDKTVER---------------
seq4          QSSG-LYSLSSVVTVPS-SSLGTKTYTCNVDHKPSNTKVDKRVES---------------
seq5          DASGDLYTTSSQLTLPATQCLAGKSVTCHVKHY-TNPSQDVTVPCP--------------
              : ** **: ** :*:*: . :. ::  *:*.*  :*.. *  *

seq1          --------------------------------PKSCDKTHTCPPCPAPELLGGPSVFLFPP 249
seq3          EPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPP
seq2          --------------------------------KCCVE---CPPCPAPPVAG-PSVFLFPP
seq4          ---------------------------------KYGPPCPSCPAPEFLGGPSVFLFPP
seq5          --------------------------------VPSTPPTPSPSTPPTPSPSCCHPRLSLHR
                                                .  *:*     . : :

seq1          KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV 309
seq3          KPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSV
seq2          KPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSV
seq4          KPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
seq5          PALEDLLLGSEANLTCTLTGLR-DASGVTFTWTPS--SGKSAVQGPPDRDLCGCYSVSSV
               . : *::.  .::**.:..:  : . * *.*  .  . :.*   * :.:  . : * **
```

Figure 1B, cont'd.

```
seq1  LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS- 368
seq3  LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS-
seq2  LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS-
seq4  LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS-
seq5  LSGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS-GNTFRPEVHLLPPPSEELALNELVT
      *:   : *  ** :.*...:      :.:  *:* : *:. .*:*: ***. :*:: *::

seq1  LTCLVKGFYPSDIAVEWESNGQ--PENNYKTTPPVLDSDG---SFFLYSKLTVDKSRWQQ 423
seq3  LTCLVKGFYPSDIAVEWESSGQ--PENNYNTTPPMLDSDG---SFFLYSKLTVDKSRWQQ
seq2  LTCLVKGFYPSDISVEWESNGQ--PENNYKTTPPMLDSDG---SFFLYSKLTVDKSRWQQ
seq4  LTCLVKGFYPSDIAVEWESNGQ--PENNYKTTPPVLDSDG---SFFLYSRLTVDKSRWQE
seq5  LTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKK
      ****.:** *.*: *.* ...* *.::* * ..  :..   :* : * * *  . *::

seq1  GNVFSCSVMHEALHNHYTQKSLSLSPGK---------- 451 (SEQ ID NO:2)
seq3  GNIFSCSVMHEALHNRFTQKSLSLSPGK---------- (SEQ ID NO:3)
seq2  GNVFSCSVMHEALHNHYTQKSLSLSPGK---------- (SEQ ID NO:4)
seq4  GNVFSCSVMHEALHNHYTQKSLSLSLGK---------- (SEQ ID NO:5)
seq5  GDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEVDGTCY (SEQ ID NO:6)
      *: *** * ****   :****::.   **
```

Figure 1C

Seq1 = *Homo sapiens* kappa light chain constant region; GenBank CAA75031.1
Seq2 = *Homo sapiens* kappa light chain constant region; GenBank BAC0168.1
Seq3 = *Homo sapiens* lambda light chain constant region; GenBank CAA75033
Seq4 = *Mus musculus* light chain constant region; GenBank AAB09710.1
Seq5 = *Rattus norvegicus* light chain constant region; GenBank AAD10133

```
seq1            RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD 189
seq2            RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
seq4            RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQD
seq5            RADAAPTVSIFPPSMEQLTSGGATVVCFVNNFYPRDISVKWKIDGSEQRDGVLDSVTDQD
seq3            QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQ
                :. ***:* :**** *:* :. *::**::.:***   .* ** *.:    .   : ..::

seq1            SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236 (SEQ ID NO:1)
seq2            SKDSTYSLSSTLTLSKADYEKHKLYACEVTHQGLSSPVTKSFNRGEC     (SEQ ID NO:7)
seq4            SKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRGEC     (SEQ ID NO:8)
seq5            SKDSTYSMSSTLSLTKVEYERHNLYTCEVVHKTSSSPVVKSFNRNEC     (SEQ ID NO:9)
seq3            S-NNKYAASSYLSLTPEQWKSHKSYSCQVTHEG--STVEKTVAPTECS     (SEQ ID NO:10)
                * :..*: ** *:*:  ::: *: *:*:..*:   *.: *:.   **
```

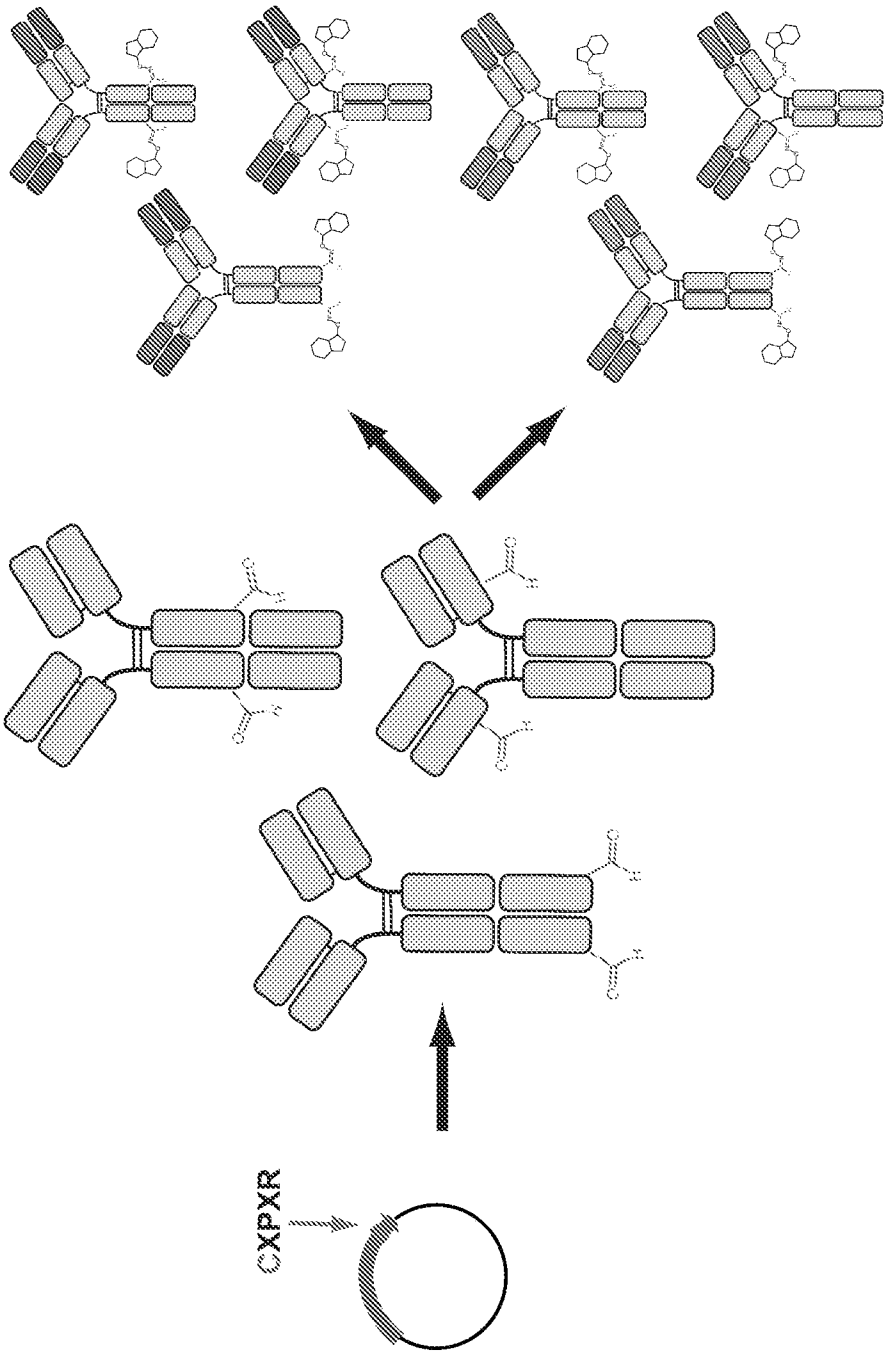


Figure 2

Figure 3

Light chain:

mmssaqflgllllcfqgtrc/DILLTQTPASLAVSLGQPATISCKASQSVDYDGDSYLNWYQQIPGQPPKL
LIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTEDPWTFGGGTKLEIKR**RTVAAP**SVF
IF**PPS**DEQLKS**G**TASVVCLLNNFY**PREA**KVQWKV**DNALQSGN**SQESV**TEQDSKDST**YSLSSTLTLSKADYE
K**HK**VYACEVT**HQGLSS**PVTKSFN**RGEC**

Heavy chain:

mnfglsliflvlvlkgvqc/QVQLQQSGAELVPPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
WPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARPETTTVGPYYYAMDYWGQGTSVTV
SS**ASTKGP**SVFPLAPSS**KSTSGGT**AALGCLVKDYF**PEPV**TVSW**NSGALTSGVHTFPAVLQSSGL**YSLSSV**V
TV**PSSSLG**TQTY**ICNVN**HKPSN**TKVDKKV**EPKSCDKTHTCPPCPAPELLGG**PSVFL**FPPKP**KDTLM**ISRTP
EVTCVVV**DVSHEDPEV**KFNWYV**DGVEVHNAK**TKPREE**QYNST**YRVVS**VLTVL**HQDWLN**GKE**YKCKVS**NKAL
PAP**IEKTI**SKAKGQPR**EPQVYTL**PPSREEMTKN**QVSLTCLVKGF**YPSDI**AVEWES**NGQPENN**YKT**TPPVLD
SDGS**FFLYSKLTVDKSRWQQGNVFSCSVM**HEALHNHYTQKSLSLSPGSLCTPSRGS**

Figure 4

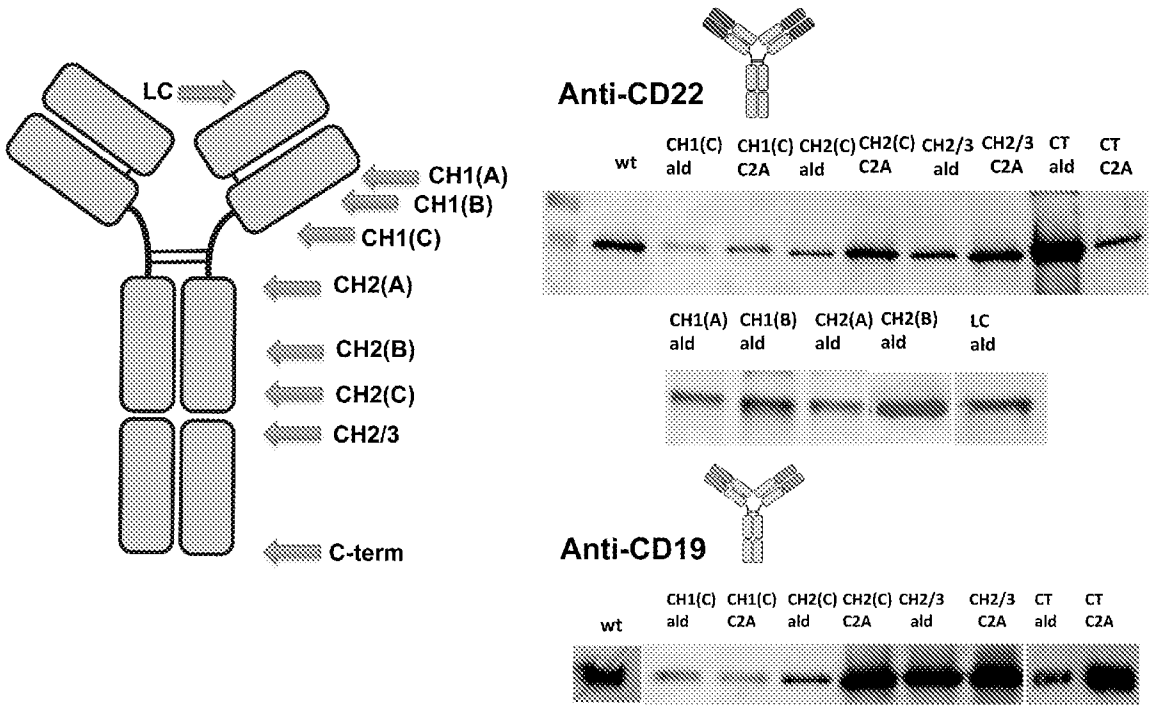
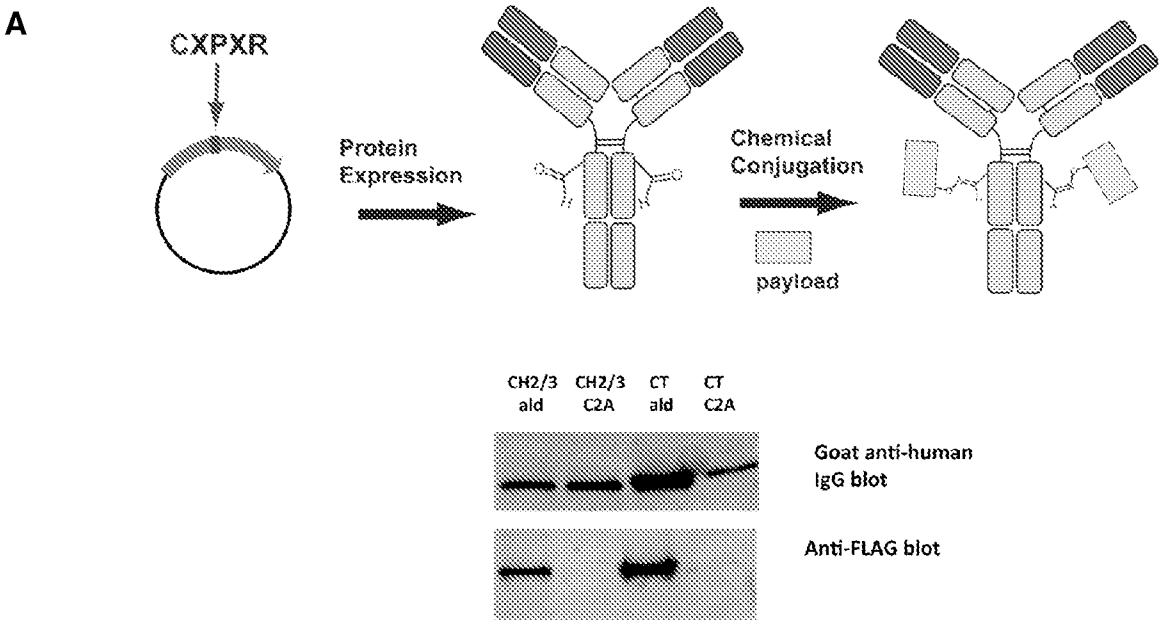

LC
CH1(A)
CH1(B)
CH1(C)
CH2(A)
CH2(B)
CH2(C)
CH2/3
C-term
Anti-CD22
wt
CH1(C) ald
CH1(C) C2A
CH2(C) ald
CH2(C) C2A
CH2/3 ald
CH2/3 C2A
CT ald
CT C2A
CH1(A) ald
CH1(B) ald
CH2(A) ald
CH2(B) ald
LC ald
Anti-CD19
wt
CH1(C) ald
CH1(C) C2A
CH2(C) ald
CH2(C) C2A
CH2/3 ald
CH2/3 C2A
CT ald
CT C2A

Figure 5


A
CXPXR
Protein Expression
Chemical Conjugation
payload
CH2/3 ald
CH2/3 C2A
CT ald
CT C2A
Goat anti-human IgG blot
Anti-FLAG blot

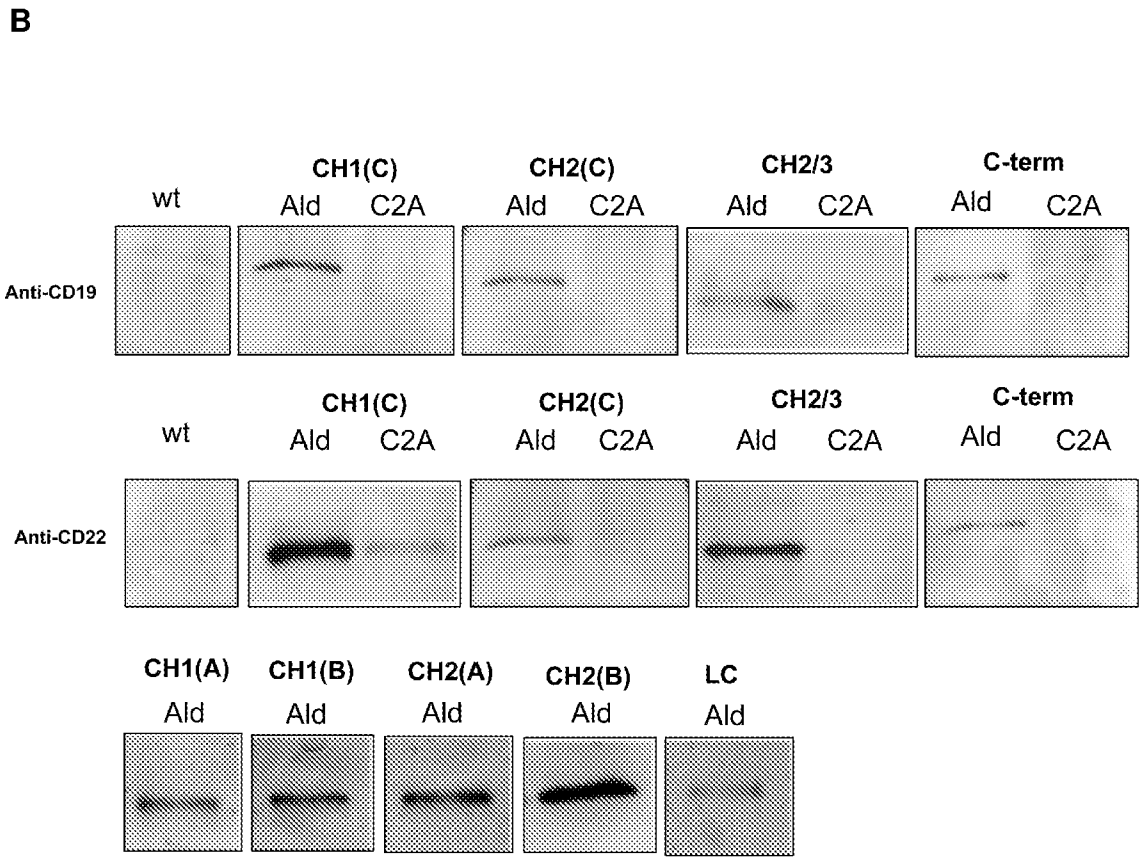

B
wt
CH1(C)
CH2(C)
CH2/3
C-term
Ald
C2A
Anti-CD19
Anti-CD22
CH1(A)
CH1(B)
CH2(A)
CH2(B)
LC

Figure 6A

CD22 specific anti-human IgG1 heavy chain - no aldehyde tag, wild-type

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
TGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 6B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

Figure 7A

CD22 specific anti-human IgG1 heavy chain - CH1(A) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCctgtgtacccctctagaGTCTTCCCCCTGGCACC
CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG
AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC
CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCAC
CCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGC
CCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCG
TCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC
ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCAC
AGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTG
GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCG
TGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC
AACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 7B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGLCTPSRVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

Figure 8A

CD22 specific anti-human IgG1 heavy chain - CH1(B) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGTGTACCCCTTCTAGATC
CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
TGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGAC
CTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT
CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTC
TTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTC
CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
ACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAA
GACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 8B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLCTPSRSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK

Figure 9A

CD22 specific anti-human IgG1 heavy chain - CH1(C) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGtgtacccctctagaGGCGTGCACACCTTCCCGGCTGTCCTA
CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA
GACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCA
AATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCA
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATG
CGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGG
TGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA
CAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 9B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALCTPSRGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

Figure 10A

CD22 specific anti-human IgG1 heavy chain - CH1(C) LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGgctacccccttctagaGGCGTGCACACCTTCCCGGCTGTCCTA
CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA
GACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCA
AATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCA
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATG
CGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGG
TGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA
CAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 10B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALATPSRGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

Figure 11A
CD22 specific anti-human IgG1 heavy chain - CH2(A) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCACTGTGTACCCCTTCTAGAGAACTCCT
GGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA
AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCT
GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
CGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA
GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 11B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPLCTPSRELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

Figure 12A

CD22 specific anti-human IgG1 heavy chain - CH2(B) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCTGTGTACCCCTTCTAGAGAGGTCAAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTG
GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC
CAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC
CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA
CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 12B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDLCTPSREVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

Figure 13A
CD22 specific anti-human IgG1 heavy chain - CH2(C) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACttatgtacccc
ttctagaGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
ACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAA
GACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 13B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNLCTPSRAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK

Figure 14A

CD22 specific anti-human IgG1 heavy chain - CH2(C) LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACttagctacccc
ttctagaGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
ACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAA
GACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 14B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNLATPSRAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK

Figure 15A
CD22 specific anti-human IgG1 heavy chain - CH2/CH3 LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGttatgtacccctctCGAGAACCACAGG
TGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA
CAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 15B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGLCTPSREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

Figure 16A
CD22 specific anti-human IgG1 heavy chain - CH2/CH3 LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGttagctacccccttctCGAGAACCACAGG
TGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA
CAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 16B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGLATPSREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

Figure 17A

CD22 specific anti-human IgG1 heavy chain - C-terminal LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
TGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGATCCTTATGTACCCCTTCTAGAGGATCCTGA

Figure 17B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGSLCTPSRGS

Figure 18A

CD22 specific anti-human IgG1 heavy chain - C-terminal LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTCCA
GCTGCAGGAGTCAGGGGCTGAACTGTCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTT
CTGGCTACACCTTTACTAGCTACTGGCTGCACTGGATAAAACAGAGGCCTGGACAGGGTCTGGAA
TGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTACAATCAGAACTTCAAGGACAAGGC
CACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGG
ACTCTGCAGTCTATTACTGTGCAAGAAGGGATATTACTACGTTCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
TGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGATCCTTAGCTACCCCTTCTAGAGGATCCTGA

Figure 18B

MNFGLSLIFLVLVLKGVQC/QVQLQESGAELSKPGASVKMSCKASGYTFTSYWLHWIKQRPGQGL
EWIGYINPRNDYTEYNQNFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRDITTFYWGQGT
TLTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGSLATPSRGS

Figure 19A
CD22 specific anti-human Ig kappa light chain - no aldehyde tag, wild-type

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGACAT
TCAGCTGACCCAGTCTCCATCATCTCTGGCTGTGTCTGCAGGAGAAAACGTCACTATGAGCTGTA
AGTCCAGTCAAAGTGTTTTATACAGTGCAAATCACAAGAACTACTTGGCCTGGTACCAGCAGAAA
CCAGGGCAGTCTCCTAAACTGCTGATCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCG
CTTCACAGGCAGCGGATCTGGGACAGATTTTACTCTTACCATCAGCAGAGTACAAGTTGAAGACC
TGGCAATTTATTATTGTCACCAATACCTCTCCTCGTGGACGTTCGGTGGAGGGACCAAGCTGGAG
ATCAAACGTCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGT
CTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAG
AGTGTTAG

Figure 19B

MMSSAQFLGLLLLCFQGTRC/DIQLTQSPSSLAVSAGENVTMSCKSSQSVLYSANHKNYLA
WYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYCHQYLSS
WTFGGGTKLEIKR//RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 20A
CD22 specific anti-human Ig kappa light chain -LCTPSR

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGACAT
TCAGCTGACCCAGTCTCCATCATCTCTGGCTGTGTCTGCAGGAGAAAACGTCACTATGAGCTGTA
AGTCCAGTCAAAGTGTTTTATACAGTGCAAATCACAAGAACTACTTGGCCTGGTACCAGCAGAAA
CCAGGGCAGTCTCCTAAACTGCTGATCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCG
CTTCACAGGCAGCGGATCTGGGACAGATTTTACTCTTACCATCAGCAGAGTACAAGTTGAAGACC
TGGCAATTTATTATTGTCACCAATACCTCTCCTCGTGGACGTTCGGTGGAGGGACCAAGCTGGAG
ATCAAACGTCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
GGAAGGTGGATAACGCCCTCTGCACCCCCAGCCGGCAATCGGGTAACTCCCAGGAGAGTGTCACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTA
CGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGA
GCTTCAACAGGGGAGAGTGTTAG

Figure 20B

MMSSAQFLGLLLLCFQGTRC/DIQLTQSPSSLAVSAGENVTMSCKSSQSVLYSANHKNYLAWYQQ
KPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYCHQYLSSWTFGGGTKL
EIKR//RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALCTPSRQSGNSQE
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 21A
CD22 specific anti-human Ig kappa light chain -LATPSR

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGACAT
TCAGCTGACCCAGTCTCCATCATCTCTGGCTGTGTCTGCAGGAGAAAACGTCACTATGAGCTGTA
AGTCCAGTCAAAGTGTTTTATACAGTGCAAATCACAAGAACTACTTGGCCTGGTACCAGCAGAAA
CCAGGGCAGTCTCCTAAACTGCTGATCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCG
CTTCACAGGCAGCGGATCTGGGACAGATTTTACTCTTACCATCAGCAGAGTACAAGTTGAAGACC
TGGCAATTTATTATTGTCACCAATACCTCTCCTCGTGGACGTTCGGTGGAGGGACCAAGCTGGAG
ATCAAACGTCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
GGAAGGTGGATAACGCCCTCGCCACCCCCAGCCGGCAATCGGGTAACTCCCAGGAGAGTGTCACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTA
CGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGA
GCTTCAACAGGGGAGAGTGTTAG

Figure 21B

MMSSAQFLGLLLLCFQGTRC/DIQLTQSPSSLAVSAGENVTMSCKSSQSVLYSANHKNYLAWYQQ
KPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVQVEDLAIYYCHQYLSSWTFGGGTKL
EIKR//RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALATPSRQSGNSQE
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 22A
CD19 specific anti-human IgG1 heavy chain - no aldehyde tag, wild-type

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGC
CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACA
GCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 22B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

Figure 23A
CD19 specific anti-human IgG1 heavy chain - CH1 (C) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGtgtacccctctagaGGC
GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGT
GCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCA
AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCA
AAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT
TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC
GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
A

Figure 23B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALCTP
SRGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

Figure 24A

CD19 specific anti-human IgG1 heavy chain - CH1 (C) LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGgctacccccttctagaGGC
GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGT
GCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCA
AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCA
AAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT
TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC
GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
A

Figure 24B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALATP
SRGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

Figure 25A
CD19 specific anti-human IgG1 heavy chain - CH2 (B) LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACTTATGTACCCCTTCTAGAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAG
GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 25B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNLCTPSRAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

Figure 26A
CD19 specific anti-human IgG1 heavy chain - CH2 (B) LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACttagctacccctctagaGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAG
GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Figure 26B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNLATPSRAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

Figure 27A
CD19 specific anti-human IgG1 heavy chain - CH2/CH3 LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGttat
gtacccttctCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT
TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC
GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
A

Figure 27B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGLCTPSREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

Figure 28A

CD19 specific anti-human IgG1 heavy chain - CH2/CH3 LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGTTAG
CTACCCCTTCTCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT
TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC
GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
A

Figure 28B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGLATPSREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

Figure 29A
CD19 specific anti-human IgG1 heavy chain - C-terminal LCTPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGC
CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACA
GCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGATCCTTATGTACCCC
TTCTAGAGGATCCTGA

Figure 29B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGSLCTPSRGS

Figure 30A

CD19 specific anti-human IgG1 heavy chain - C-terminal LATPSR

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTCAGGTGCA
GCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTT
CTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAGGGTCTTGAG
TGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGC
CACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGG
ACTCTGCGGTCTATTTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTATGCTATG
GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA
AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGC
CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACA
GCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGATCCTTAGCTACCCC
TTCTAGAGGATCCTGA

Figure 30B

MNFGLSLIFLVLVLKGVQC/QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGL
EWIGQIWPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYA
MDYWGQGTSVTVSS//ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGSLATPSRGS

Figure 31A
CD19 specific anti-human Ig kappa light chain - no aldehyde tag, wild-type

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATAT
CTTGCTCACCCAAACTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATCTCCTGCA
AGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATTTGAACTGGTACCAACAGATTCCAGGA
CAGCCACCCAAACTCCTCATCTATGATGCATCCAATCTAGTTTCTGGGATCCCACCCAGGTTTAG
TGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGAAGGTGGATGCTGCAA
CCTATCACTGTCAGCAAAGTACTGAGGATCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATC
AAACGGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGA
AGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC
AGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA
CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT
GTTAG

Figure 31B

MMSSAQFLGLLLLCFQGTRC/DILLTQTPASLAVSLGQRATISCKASQSVDYDGDSYLNWY
QQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTEDPW
TFGGGTKLEIKR//RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 32A
CD19 specific anti-human Ig kappa light chain - LCTPSR

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATAT
CTTGCTCACCCAAACTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATCTCCTGCA
AGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATTTGAACTGGTACCAACAGATTCCAGGA
CAGCCACCCAAACTCCTCATCTATGATGCATCCAATCTAGTTTCTGGGATCCCACCCAGGTTTAG
TGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGAAGGTGGATGCTGCAA
CCTATCACTGTCAGCAAAGTACTGAGGATCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATC
AAACGGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGA
AGGTGGATAACGCCCTCTGCACCCCCAGCCGGCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGA
GAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG

Figure 32B

MMSSAQFLGLLLLCFQGTRC/DILLTQTPASLAVSLGQRATISCKASQSVDYDGDSYLNWYQQIP
GQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTEDPWTFGGGTKLE
IKR//RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALCTPSRQSGNSQES
VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 33A
CD19 specific anti-human Ig kappa light chain -LATPSR

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATAT
CTTGCTCACCCAAACTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATCTCCTGCA
AGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATTTGAACTGGTACCAACAGATTCCAGGA
CAGCCACCCAAACTCCTCATCTATGATGCATCCAATCTAGTTTCTGGGATCCCACCCAGGTTTAG
TGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGAAGGTGGATGCTGCAA
CCTATCACTGTCAGCAAAGTACTGAGGATCCGTGGACGTTCGGTGGAGGCACCAAGCTGGAAATC
AAACGGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGA
AGGTGGATAACGCCCTCGCCACCCCCAGCCGGCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGA
GAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG

Figure 33B

MMSSAQFLGLLLLCFQGTRC/DILLTQTPASLAVSLGQRATISCKASQSVDYDGDSYLNWYQQIP
GQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTEDPWTFGGGTKLE
IKR//RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALATPSRQSGNSQES
VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC