(19) DANMARK

(10) DK/EP 3677591 T5

(12) Rettet oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 07 K 14/56 (2006.01)* *A 61 K 38/21 (2006.01)* *A 61 P 35/00 (2006.01)*
A 61 P 35/02 (2006.01) *C 07 K 16/28 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2024-08-26**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2022-12-28**

(86) Europæisk ansøgning nr.: **19213030.0**

(86) Europæisk indleveringsdag: **2013-04-29**

(87) Den europæiske ansøgnings publiceringsdag: **2020-07-08**

(62) Stamansøgningsnr: **13721243.7**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **Anti-CD38 antistoffer og fusioner til svækket interferon alpha-2b**

(56) Fremdragne publikationer:
WO-A1-01/97844
WO-A1-2011/154453
WO-A1-2012/092612
WO-A2-2005/103083
WO-A2-2007/042309
WO-A2-2013/059885
US-A1- 2002 164 788

DESCRIPTION

FIELD OF THE DISCLOSURE

[0001] This disclosure relates generally to the field of antibody engineering. More specifically, this disclosure relates to antibodies that bind specifically to CD38, as well as constructs comprising such antibodies and attenuated interferon-alpha ligands, and methods of treatment using these constructs. In these constructs, the antibodies direct the ligands to cells that express both CD38 and receptors for the ligands, and the attenuated interferon-alpha reduces interferon signaling in cells that do not express CD38.

BACKGROUND OF THE DISCLOSURE

[0002] Various publications, including patents, published applications, technical articles, scholarly articles, and gene or protein accession numbers are cited throughout the specification.

[0003] CD38 is a 46kDa type II transmembrane glycoprotein. It has a short N-terminal cytoplasmic tail of 20 amino acids, a single transmembrane helix and a long extracellular domain of 256 amino acids. It is expressed on the surface of many immune cells including CD4 and CD8 positive T cells, B cells, NK cells, monocytes, plasma cells and on a significant proportion of normal bone marrow precursor cells. In some instances, the expression of CD38 in lymphocytes may be dependent on the differentiation and activation state of the cell, for example, resting T and B cells may be negative while immature and activated lymphocytes may be predominantly positive for CD38 expression. CD38 mRNA expression has been detected in non-hemopoeitic organs such as the pancreas, brain, spleen and liver (Koguma, T. (1994) Biochim. Biophys. Acta 1223:160).

[0004] CD38 is a multifunctional ectoenzyme that is involved in transmembrane signaling and cell adhesion. It is also known as cyclic ADP ribose hydrolase because it can transform NAD^+ and $NADP^+$ into cADPR, ADPR and NAADP, depending on extracellular pH. These products induce Ca^{2+} -mobilization inside the cell, which can lead to tyrosine phosphorylation and activation of the cell. CD38 is also a receptor that can interact with a ligand, CD31. Activation of receptor via CD31 leads to intracellular events including Ca^{2+} mobilization, cell activation, proliferation, differentiation and migration.

[0005] CD38 is expressed at high levels on multiple myeloma cells, in most cases of T- and B-lineage acute lymphoblastic leukemias, some acute myelocyticleukemias, follicular center cell lymphomas and T lymphoblastic lymphomas. CD38 is also expressed on B-lineage chronic lymphoblastic leukemia (B-CLL) cells. In some cases, B-CLL patients presenting with a CD38+

clone are characterized by an unfavorable clinical course with a more advanced stage of disease, poor responsiveness to chemotherapy and shorter survival time. The use of antibodies to CD38 has been proposed for the treatment of CD38-expressing cancers and hematological malignancies. It may therefore be advantageous to provide alternative antibodies to CD38 which have desirable manufacturing, stability and immunogenic properties.

[0006] Numerous peptide and polypeptide ligands have been described to function by interacting with a receptor on a cell surface, and thereby stimulating, inhibiting, or otherwise modulating a biological response, usually involving signal transduction pathways inside the cell that bears the said receptor. Examples of such ligands include peptide and polypeptide hormones, cytokines, chemokines, growth factors, and apoptosis-inducing factors.

[0007] Due to the biological activities of such ligands, many have potential uses as therapeutics. Several peptide or polypeptide ligands have been approved by regulatory agencies as therapeutic products including, for example, human growth hormone, insulin, interferon (IFN)-alpha2b, IFN-alpha2a, IFNβ, erythropoietin, G-CSF and GM-CSF.

[0008] While these and other ligands have demonstrated potential in therapeutic applications, they may also exhibit toxicity when administered to human patients. One reason for toxicity is that most of these ligands trigger receptors on a variety of cells, including cells other than those that mediate the desired therapeutic effect. A consequence of such "off target" activity of ligands is that many ligands are currently not suitable for use as therapeutic agents because the ligands cannot be administered at sufficiently high dosages to produce maximal or optimal therapeutic effects on the target cells which mediate the therapeutic effect.

[0009] For example it has been known since the mid-1980's that interferons, in particular IFN-alpha, are able to increase apoptosis and decrease proliferation of certain cancer cells. IFN-alpha has been approved by the FDA for the treatment of several cancers including melanoma, renal cell carcinoma, B cell lymphoma, multiple myeloma, chronic myelogenous leukemia (CML) and hairy cell leukemia. A direct effect of IFN-alpha on the tumor cells is mediated by the IFN-alpha binding directly to the type I IFN receptor on those cells and stimulating apoptosis, terminal differentiation or reduced proliferation. A further indirect effect of IFN-alpha on non-cancer cells is to stimulate the immune system, which may produce an additional anti-cancer effect by causing the immune system to reject the tumor.

[0010] These biological activities are mediated by type I interferon receptors on the surface of the cancer cells which, when stimulated, initiate various signal transduction pathways leading to reduced proliferation and/or the induction of terminal differentiation or apoptosis. The type I interferon receptor is, however, also present on most non-cancerous cells. Activation of this receptor on non-cancerous cells by IFN-alpha causes the expression of numerous pro-inflammatory cytokines and chemokines, leading to toxicity and untoward effects. Such toxicity may cause severe flu-like symptoms, which prevents the dosing of IFN-alpha to a subject at levels that exert the maximum anti-proliferative and pro-apoptotic activity on the cancer cells.

[0011] When IFN-alpha2b is used to treat multiple myeloma, its utility resides, at least in part, in its binding to type I interferon receptors on the myeloma cells, which in turn triggers apoptosis and/or reduced proliferation and hence limits disease progression. Unfortunately, however, this IFN also binds healthy cells within the body, triggering a variety of other cellular responses, some of which are harmful.

[0012] A publication by Ozzello (Breast Cancer Research and Treatment 25:265-76, 1993) describes chemically conjugating human IFN-alpha to a tumor-targeting antibody, thereby localizing the direct inhibitory activity of IFN-alpha to the tumor as a way of reducing tumor growth rates, and demonstrated that such conjugates have anti-tumor activity in a xenograft model of a human cancer. The mechanism of the observed anti-cancer activity was attributed to a direct effect of IFN-alpha on the cancer cells, since the human IFN-alpha used in the experiments did not interact appreciably with the murine type I IFN receptor, which could have led to an indirect anti-cancer effect. Because of this lack of binding of the human IFN-alpha to the murine cells, the toxicity of the antibody-IFN-alpha conjugate relative to free INF-alpha was not assessed.

[0013] Antibodies and IFN-alpha may also be connected together in the form of a fusion protein. For example, WO 01/97844 describes a direct fusion of human IFN-alpha to the C-terminus of the heavy chain of an IgG specific for the tumor antigen CD20.

[0014] In general, IFN may be targeted to cancer cells. While this approach may result in an increase in activity of the IFN against cancer cells, it does not completely address the issue of undesired activity of the IFN on healthy cells. Fusing IFN-alpha to the C-terminus of the heavy chain of an IgG may prolong the half-life of the IFN alpha leading to undesirable adverse events. Accordingly, there exists a need to decrease off-target activity of ligand-based drugs, while retaining the "on-target" therapeutic effect of such ligands.

[0015] In the prior art WO 2011/154453 discloses human anti CD38 antibodies suitable for the treatment of several diseases (e.g. autoimmune diseases), also used in combination with Interferon alpha 2b, WO 2012/092612 discloses monoclonal anti CD38 antibodies, exhibiting an affinity in the nanomolar range and mediating CDC and ADCC, US 2002/164788 discloses a humanised antibody specific for human CD38, WO 2007/042309 discloses murine, chimeric and fully human antibodies against CD38, WO 2005/103083 discloses human antagonistic antibodies to CD38 that mediate ADCC and CDC and reduce the growth of human myeloma xenografts in a murine model.

SUMMARY OF THE DISCLOSURE

[0016] The first aspect of the present invention provides a polynucleotide comprising a nucleic acid sequence encoding an antibody that specifically binds to CD38, the antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 185.

[0017] The nucleic acid sequence may encode an antibody comprising a human IgG1 constant region.

[0018] The nucleic acid sequence may encode an antibody comprising a human IgG4 constant region. The human IgG4 heavy chain constant region may comprise a proline at position 228 according to the EU numbering system. The human IgG4 heavy chain constant region may also comprise a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256 of the constant region according to the EU numbering system.

[0019] The polynucleotide may comprise a nucleic acid sequence comprising SEQ ID NO: 685, which encodes a heavy chain variable region, and a nucleic acid sequence comprising SEQ ID NO: 691, which encodes a light chain variable region.

[0020] The nucleic acid sequence may also encode an interferon alpha-2b fused to the antibody, wherein the interferon alpha-2b comprises an alanine to aspartic acid or alanine to glycine substitution at the position corresponding to position 168 of the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence may encode an interferon alpha-2b comprising the amino acid sequence of SEQ ID NO: 647 or SEQ ID NO: 650.

[0021] The nucleic acid sequence may also encode an interferon alpha-2b that is an N-terminally truncated interferon alpha-2b. The N-terminally truncated interferon alpha-2b may not have the twenty-three N-terminal amino acids.

[0022] The nucleic acid sequence may encode an interferon alpha-2b comprising the amino acid sequence of SEQ ID NO: 649 or SEQ ID NO: 651.

[0023] In a second aspect, the present invention provides a vector comprising the polynucleotide according to the first aspect.

[0024] In a third aspect, the present invention provides an in vitro transformed mammalian cell comprising the vector of the second aspect.

[0025] The disclosure features new anti-CD38 antibodies and constructs comprising an anti-CD38 antibody and attenuated IFN-alpha. The antibodies, which comprise one or a plurality of mutations in their heavy and/or light chain variable regions retain the ability to specifically bind to CD38, including CD38 expressed on the surface of cells. The antibodies may be fused, for example, to an attenuated form of interferon alpha to form an anti-CD38 antibody-attenuated interferon fusion construct.

[0026] Disclosed herein is an isolated antibody that binds specifically to CD38 which comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 559 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 664. Also disclosed is an isolated antibody that binds specifically to CD38 which comprises a heavy chain

variable region comprising the amino acid sequence of SEQ ID NO: 665 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 666. Also disclosed is an isolated antibody that binds specifically to CD38 which comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 739 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 664. The heavy chain variable region amino acid sequence of SEQ ID NO: 559 excludes the amino acid sequence of SEQ ID NO: 13. The light chain variable region amino acid sequence of SEQ ID NO: 664 excludes the amino acid sequence of SEQ ID NO: 14. Also disclosed is an isolated antibody that binds specifically to CD38 which comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 200, SEQ ID NO: 514 or SEQ ID NO: 697, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 202, SEQ ID NO: 516, SEQ ID NO: 544, SEQ ID NO: 698 or SEQ ID NO: 737, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 204, SEQ ID NO: 222, SEQ ID NO: 518, SEQ ID NO: 534, SEQ ID NO: 535, SEQ ID NO: 536, SEQ ID NO: 699 or SEQ ID NO: 738, and may further comprise a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 233, SEQ ID NO: 319, SEQ ID NO: 583, SEQ ID NO: 590 or SEQ ID NO: 696, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 235, SEQ ID NO: 307, SEQ ID NO: 311, SEQ ID NO: 585, SEQ ID NO: 591 or SEQ ID NO: 605, a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 237, SEQ ID NO: 321, SEQ ID NO: 324, SEQ ID NO: 587 or SEQ ID NO: 594.

[0027] The heavy chain variable region disclosed herein may comprise the amino acid sequence of SEQ ID NO: 34, SEQ ID NO: 18, SEQ ID NO: 665, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 179, SEQ ID NO: 180,SEQ ID NO: 156, SEQ ID NO: 197, SEQ ID NO: 152, SEQ ID NO: 720, SEQ ID NO: 721, SEQ ID NO: 722, SEQ ID NO: 723, SEQ ID NO: 739, SEQ ID NO: 740, SEQ ID NO: 741, SEQ ID NO: 742, SEQ ID NO: 728, SEQ ID NO: 730, SEQ ID NO: 731. The light chain variable region disclosed herein may comprise the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 68, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 660, SEQ ID NO: 661, SEQ ID NO: 662, SEQ ID NO: 663. SEQ ID NO: 161, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 188, SEQ ID NO: 198 or SEQ ID NO: 700, SEQ ID NO: 701, SEQ ID NO: 704, SEQ ID NO: 705, SEQ ID NO: 706, SEQ ID NO: 707, SEQ ID NO: 708, SEQ ID NO: 709, SEQ ID NO: 710, SEQ ID NO: 711.

[0028] The antibody preferably is capable of binding to CD38-positive cells. The antibody may bind to a CD38-positive cell with an EC50 value of less than about 100 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 75 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 50 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 30 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 25 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 20 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 15 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 13 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 10 nM.

[0029] The antibody may be a monoclonal antibody, and is preferably a fully human antibody. The antibody may comprise an FAb. The antibody may comprise a human IgG1 constant region or a human IgG4 constant region. The IgG1 or the IgG4 constant region may comprise a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256 according to the EU numbering system. The IgG4 constant region may comprise a proline at position 228 according to the EU numbering system, and the proline at position 228 may be in addition to a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256.

[0030] The antibody may be fused to attenuated interferon alpha-2b. The interferon alpha-2b may comprise a substitution of the alanine at position 145 to glycine or aspartic acid, including an interferon alpha-2b having the amino acid sequence of SEQ ID NO: 649 or SEQ ID NO: 651. The attenuated interferon alpha-2b may be fused directly to the C-terminus of the IgG1 or IgG4 constant region, and the antibody may comprise the amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 652, SEQ ID NO: 653, SEQ ID NO: 654, SEQ ID NO: 655, SEQ ID NO: 656, SEQ ID NO: 657, SEQ ID NO: 658, or SEQ ID NO: 694. The antibody, including the antibody fused to an attenuated interferon alpha-2b may be comprised in a composition comprising a pharmaceutically acceptable carrier.

[0031] Isolated polynucleotides encoding the antibodies disclosed herein and the antibodies fused to an attenuated interferon alpha-2b are disclosed herein. The polynucleotide may comprise the nucleic acid sequence of SEQ ID NO: 667, SEQ ID NO: 670, SEQ ID NO: 671, SEQ ID NO: 672, SEQ ID NO: 673, SEQ ID NO: 674, SEQ ID NO: 668, SEQ ID NO: 669, SEQ ID NO: 675, SEQ ID NO: 676, or SEQ ID NO: 677, SEQ ID NO: 678, SEQ ID NO: 679, SEQ ID NO: 680, SEQ ID NO: 681, SEQ ID NO: 682, SEQ ID NO: 683, SEQ ID NO: 684, SEQ ID NO: 686, SEQ ID NO: 687, SEQ ID NO: 688, SEQ ID NO: 689, SEQ ID NO: 690, SEQ ID NO: 692, SEQ ID NO: 693, SEQ ID NO: 695 SEQ ID NO: 702, SEQ ID NO: 703, SEQ ID NO: 712, SEQ ID NO: 713, SEQ ID NO: 714, SEQ ID NO: 715, SEQ ID NO: 716, SEQ ID NO: 717, SEQ ID NO: 718, SEQ ID NO: 719, SEQ ID NO: 724, SEQ ID NO: 725, SEQ ID NO: 726, SEQ ID NO: 727 SEQ ID NO: 732, SEQ ID NO: 733, SEQ ID NO: 734, SEQ ID NO: 735, SEQ ID NO: 743, SEQ ID NO: 744, SEQ ID NO: 745, SEQ ID NO: 746 . The polynucleotides may comprise a vector. The vector may be used, for example, to transform a cell. A transformed cell comprising such polynucleotides is disclosed herein. The transformed cell may comprise a mammalian cell, a yeast cell, or an insect cell.

[0032] Stable cells that express the antibodies are also disclosed herein. Antibody-expressing cells may be mammalian cells. Preferred cells are Chinese Hamster Ovary (CHO) cells.

[0033] Kits comprising antibodies fused to attenuated interferon alpha-2b are disclosed herein. The kits comprise the anti-CD38-attenuated interferon alpha-2b fusion construct, and instructions for using the construct in a method for inhibiting the proliferation of a tumor cell expressing CD38 and a receptor for interferon alpha-2b on its surface, instructions for using the construct in a method for inducing apoptosis in a tumor cell expressing CD38 and a receptor for interferon alpha-2b on its surface, instructions for using the construct in a method

for treating a tumor comprising cells expressing CD38 and a receptor for interferon alpha-2b on their surface in a subject in need thereof, and optionally, a pharmaceutically acceptable carrier. Kits comprising anti-CD38 antibodies are disclosed herein, and such kits comprise the anti-CD38 antibody and instructions for using the antibody in a method for detecting a CD38-positive tumor cell in a tissue sample isolated from a subject, the antibody may optionally be fused to an attenuated interferon alpha-2b protein. Any reference to a method of treatment practised on the human or animal body is to be interpreted as substances and compositions for use in such treatment.

[0034] The anti-CD38 antibody-attenuated interferon alpha-2b fusion constructs may be used as a threrapy in the treatment of a tumor comprising cells expressing CD38 and a receptor for interferon alpha-2b on their surface. Generally, a treatment method comprises administering to a subject having the tumor an anti-CD38 antibody-attenuated interferon alpha-2b fusion construct in an amount effective to treat the tumor. The construct may comprise any construct described or exemplified herein. The subject is preferably a mammal, more preferably a non-human primate, and most preferably a human being. The tumor may comprise a B-cell lymphoma, multiple myeloma, non-Hodgkin's lymphoma, chronic myelogenous leukemia, chronic lymphocytic leukemia or acute myelogenous leukemia.

[0035] The anti-CD38 antibodies, optionally fused to an attenuated interferon alpha-2b protein, may be used in a method for detecting CD38 or a CD38-positive tumor cell in a tissue sample isolated from a subject. Generally, the method comprises contacting an antibody that binds specifically to CD38 with a tissue sample isolated from a subject and detecting a complex of the antibody and CD38 or a CD38-positive cell in the tissue sample. The tissue sample may be known to have or be suspected of having CD38-positive tumor cells. The tissue may comprise blood or bone marrow. The CD38-positive tumor cell may be a CD38-positive B-cell lymphoma cell, multiple myeloma cell, non-Hodgkin's lymphoma cell, chronic myelogenous leukemia cell, chronic lymphocytic leukemia cell, or acute myelogenous leukemia cell. The subject is preferably a mammal, more preferably a non-human primate, and most preferably a human being. The method may include the step of isolating the tissue sample from the subject. The method may further comprise contacting the antibody with a tissue sample that does not include any CD38-positive cells, for example, to serve as a negative control.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036]

Figure 1 shows an example of an anti-CD38-attenuated interferon fusion construct.

Figures 2A and 2B show sequences of heavy chain variable regions of X02.1, related constructs, and the most homologous germline antibody sequence. CDRs defined by the Kabat numbering system are underlined.

Figures 3A and 3B show sequences of light chain variable regions of X02.1, related constructs,

and the most homologous germline antibody sequence. CDRs defined by the Kabat numbering system are underlined.

Figures 4A-4D show the sequences of light chain variable regions of A02.1 and related constructs. CDRs defined by the Kabat numbering system are underlined

Figure 5 shows the consensus variable heavy chain sequence of A02.1 and related constructs. Boxed regions contain CDRs (as indicated) as defined by the Kabat numbering system and the enhanced Chothia numbering system. CDRs defined by the Kabat numbering system are shown in bold. CDRs defined by the enhanced Chothia numbering system are underlined.

Figure 6 shows the consensus variable light chain sequence of A02.1 and related constructs. Boxed regions contain CDRs (as indicated) as defined by the Kabat numbering system and the enhanced Chothia numbering system. CDRs defined by the Kabat numbering system are shown in bold. CDRs defined by the enhanced Chothia numbering system are underlined

Figures 7A-7C show sequences of heavy chain variable regions of humanized heavy chain variable regions. CDRs defined by the Kabat numbering system are underlined.

Figures 8A-8C show sequences of heavy chain variable regions of humanized light chain variable regions. CDRs defined by the Kabat numbering system are underlined.

Figures 9A and 9B show the variable heavy chain of A10.0 and related constructs. CDRs defined by the Kabat numbering system are underlined.

Figures 10A and 10B show the variable light chain of A10.0 and related constructs. CDRs defined by the Kabat numbering system are underlined.

Figure 11 shows the variable heavy chain consensus sequence of A10.0 and related constructs. Boxed regions contain CDRs (as indicated) as defined by the Kabat numbering system and the enhanced Chothia numbering system. CDRs defined by the Kabat numbering system are shown in bold. CDRs defined by the enhanced Chothia numbering system are underlined.

Figure 12 shows the variable light chain consensus sequence of A10.0 and related constructs. Boxed regions contain CDRs (as indicated) as defined by the Kabat numbering system and the enhanced Chothia numbering system. CDRs defined by the Kabat numbering system are shown in bold. CDRs defined by the enhanced Chothia numbering system are underlined.

Figure 13 shows the binding activity of A02.1 variants to the CD38-expressing multiple myeloma cell line ARP-1 as measured by flow cytometry. The assay details are described in the Examples of this specification.

Figure 14 shows the binding activity of A02.1 variants to the CD38-expressing multiple myeloma cell line NCI-H929 as measured by flow cytometry. The assay details are described in the Examples of this specification.

Figures 15 and 16 show the anti-proliferative activity of A02.1 variants on the multiple myeloma

cell line ARP-1. A-isotype is an irrelevant specificity antibody fused with the attenuated interferon as a control. The assay details are described in the Examples (Cell proliferation assay).

Figure 17 shows the anti-proliferative activity of IFN-alpha2b (Intron A) compared with A02.1 and A10.0 and their corresponding unfused antibodies X02.1 and X10.0 on the multiple myeloma cell line ARP-1. A-isotype is an irrelevant specificity antibody fused with the attenuated interferon as a control. The assay details are described in the Examples (Cell proliferation assay).

Figure 18 shows the relative fold change of Annexin V production in the CD38-expressing multiple myeloma cell line NCI-H929 when treated with A02.1 and A10.0 and their corresponding unfused antibodies X02.1 and X10.0 for 24 hours compared to an untreated control. A-isotype is an irrelevant specificity antibody fused with the attenuated interferon as a control. The assay details are described in the Examples (Annexin V assay).

Figure 19 shows the relative fold change of caspase activation in the CD38-expressing multiple myeloma cell line H929 of IFN-alpha2b (Intron A) vs. A02.1 and related constructs in comparison to untreated cells. Isotype 145D is an irrelevant specificity antibody fused with the attenuated interferon as a control. The assay details are described in the Examples (Caspase assay).

Figure 20 shows the off-target activity of IFN-alpha2b (Intron A) versus A02.6 and A02.6 fused to wild-type IFN-alpha2b (A02.6 (wt. IFN)) on the CD38-negative cells. The assay details are described in the Examples (HEK-BLUE™).

Figure 21 shows the relative fold change of Annexin V production in the CD38-expressing multiple myeloma cell line H929 between IgG1 and IgG4 subtypes of anti-CD38-attenuated IFN-alpha fusion protein constructs. A-isotype is a non-specific IgG4 antibody fused with the attenuated interferon as a control. The antibodies, A02.12 and A10.0 contain IgG4 constant regions fused to attenuated IFN-alpha while A02.112 and A10.59 contain IgG1 constant regions fused to attenuated IFN-alpha. The assay details are described in the Examples (Annexin V/7AAD assay).

Figure 22 shows the binding activity of A10.0 variants to the CD38-expressing multiple myeloma cell line NCI-H929 as measured by flow cytometry. The assay details are described in the Examples of this specification.

Figure 23 shows caspase activation in the CD38-expressing multiple myeloma cell line H929 of A10.0 and A10.38 compared to untreated cells. A-isotype is an irrelevant specificity antibody fused to the attenuated IFN as a control. The assay details are described in the Examples (Caspase assay).

Figure 24 shows the relative fold change of caspase activation in the CD38 expressing multiple myeloma cell line H929 by A10.0 variants compared to untreated cells. The assay details are described in the Examples (Caspase assay).

Figure 25 shows the relative fold change of production of Annexin V in the CD38-expressing multiple myeloma cell line H929 by A10.0 variants. The assay details are described in the Examples (Annexin V/7AAD assay).

Figure 26 shows the anti-proliferative activity of IFN-alpha2b (Intron A) compared with A02.6, A10.0, A10.38 and parental A10A2.0 chimeric antibody constructs on the Burkitt's lymphoma cell line Daudi. A-isotype is an irrelevant specificity antibody fused to the attenuated IFN as a control. The assay details are described in the Examples (Cell proliferation assay).

Figure 27 shows the effects of humanized A10.0 versus the parental A10A2.0 chimeric antibody attenuated interferon construct on the growth of subcutaneous H929 myeloma tumors in SCID mice. The bar labeled "treatment phase" shows the duration of treatment with the compounds.

Figure 28 shows the non-antibody antigen targeted IFN activity of A10.0 variants fused to the same attenuated IFN-alpha2b protein. The assay details are described in the Examples ("Off-target assays"- iLite gene reporter assay).

Figure 29 shows the "Off-target" activity of IFN-alpha2b (Intron A) compared with A10.0 variants and the parental A10A2.0 chimeric antibody fused to wild-type IFN-alpha2b (A10A2.0 chimeric (wt.IFN)). The assay details are described in the Examples ("Off-target assays"- HEK-BLUE™).

Figure 30 shows variable heavy chain consensus sequences of X910/12-HC-L0-Interferon-alpha (A145D) IgG4 and related sequences. Boxed regions contain CDRs (as indicated) as defined by the Kabat numbering system and the enhanced Chothia numbering system. CDRs defined by the Kabat numbering system are shown in bold. CDRs defined by the enhanced Chothia numbering system are underlined.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0037] Various terms relating to aspects of disclosure are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art, unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definition provided herein.

[0038] The terms subject and patient are used interchangeably and include any animal. Mammals are preferred, including companion and farm mammals, as well as rodents, including mice, rabbits, and rats, and other rodents. Non-human primates, such as Cynomolgus monkeys, are more preferred, and human beings are highly preferred.

[0039] A molecule such as an antibody has been "isolated" if it has been altered and/or

removed from its natural environment by the hand of a human being.

[0040] As used herein, the singular forms "a," "an," and "the" include plural referents unless expressly stated otherwise.

[0041] An anti-CD38 antibody-attenuated interferon alpha-2b fusion construct includes, but is not limited to, any antibody described or exemplified herein that binds specifically to CD38 that is fused to an attenuated interferon alpha-2b protein, including an interferon alpha-2b of SEQ ID NO: 647, SEQ ID NO: 648, SEQ ID NO: 649, SEQ ID NO: 650, or SEQ ID NO: 651. Fusing an unmutated interferon alpha-2b protein, such as SEQ ID NO: 7, to an anti-CD38 antibody may attenuate the biologic activities of the interferon molecule. In this disclosure, attenuated interferon, attenuated interferon alpha-2b, IFN-alpha2b A145D, and IFN-alpha2b A145G are used interchangeably.

[0042] Specificity is not necessarily an absolute designation but may constitute a relative term signifying the degree of selectivity of an antibody IFN-alpha fusion protein construct for an antigen-positive cell compared to an antigen-negative cell. Specificity of an antibody IFN-alpha fusion protein construct for an antigen-positive cell is mediated by the variable regions of the antibody, and usually by the complementarity determining regions (CDRs) of the antibody. A construct may have 100-fold specificity for antigen-positive cells compared to antigen-negative cells.

[0043] Human CD38 comprises the amino acid sequence of SEQ ID NO: 1, and cynomolgus monkey CD38 comprises the amino acid sequence of SEQ ID NO: 2.

[0044] It has been further observed that interferon-alpha2b can be attenuated in terms of its biologic activity which is mediated through the interferon binding to an interferon receptor on a cell surface by introducing certain amino acid changes into the protein sequence. An attenuated interferon molecule can be fused to antibodies that specifically bind to CD38, such that the antibody may serve as a delivery vehicle for the attenuated interferon to CD38-positive cells with a resulting diminution of off target interferon activity caused by the attenuated interferon molecule. It has been further observed that fusing the attenuated interferon to the CD38 antibodies does not significantly affect the capacity of the antibody to specifically bind to CD38 on cells expressing CD38, including cells in the body of animals. It has been further observed that variants of the CD38 antibodies can be engineered and expressed such that the antibodies have reduced immunogenicity and enhanced stability and half life without a significant loss of specificity or affinity of the antibody to the CD38 antigen. These variant antibodies can be fused to an attenuated interferon.

[0045] Accordingly, antibodies that specifically bind to CD38 are featured. It has also been observed that such anti-CD38 antibodies may be employed as delivery vehicles for attenuated ligands such as interferon alpha. Without intending to be limited to any particular theory or mechanism of action, it is believed that the antibodies direct the interferon alpha to which they are attached to CD38-positive cells, where the interferon may interact with its receptor. It is

believed that the antibody, as a delivery vehicle, compensates for the diminished capacity of the interferon molecule to bind to its receptor. In this sense, the attenuated interferon has reduced capacity to interact with its receptor on healthy cells, and particularly cells that do not express CD38. It is believed that by bringing the attenuated interferon into proximity with its receptor on CD38-positive cells, the antibodies may enhance the capacity of the attenuated interferon to bind to its relevant receptor and induce a therapeutic effect, while exhibiting a diminished capacity to induce undesirable effects on healthy cells that do not express CD38.

[0046] The antibodies may be polyclonal, but in some aspects, are not polyclonal. The antibodies preferably are monoclonal. The antibodies are preferably full length antibodies. Full length antibodies generally comprise a variable region heavy chain and a variable region light chain. The antibodies may comprise derivatives or fragments or portions of antibodies that retain the antigen-binding specificity, and also preferably retain most or all of the affinity, of the parent antibody molecule (e.g., for CD38). For example, derivatives may comprise at least one variable region (either a heavy chain or light chain variable region). Other examples of suitable antibody derivatives and fragments include, without limitation, antibodies with polyepitopic specificity, bispecific antibodies, multi-specific antibodies, diabodies, single-chain molecules, as well as FAb, F(Ab')2, Fd, Fabc, and Fv molecules, single chain (Sc) antibodies, single chain Fv antibodies (scFv), individual antibody light chains, individual antibody heavy chains, fusions between antibody chains and other molecules, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and other multimers. Single chain Fv antibodies may be multivalent. All antibody isotypes may be used to produce antibody derivatives, fragments, and portions. Antibody derivatives, fragments, and/or portions may be recombinantly produced and expressed by any cell type, prokaryotic or eukaryotic.

[0047] Disclosed herein an isolated antibody may refer to a monoclonal antibody to which IFN-alpha, or an attenuated IFN-alpha, has been fused to the C-terminus of the heavy chain IgG constant region. When the monoclonal antibody has a binding specificity to CD38 and the IFN-alpha is attenuated IFN-alpha 2b, the isolated antibody is also referred to as an Anti-CD38 attenuated IFN-alpha fusion protein, or an Anti-CD38 attenuated IFN-alpha fusion construct herein.

[0048] In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FWR or FR). Each VH and VL is composed of three CDRs and four FWRs, arranged from amino-terminus to carboxy-terminus in the following order: FWR1, CDR1, FWR2, CDR2, FWR3, CDR3, FWR4. Typically, the antigen binding properties of an antibody are less likely to be disturbed by changes to FWR sequences than by changes to the CDR sequences. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and

IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass.

[0049] The antibodies may be derived from any species. For example, the antibodies may be mouse, rat, goat, horse, swine, bovine, camel, chicken, rabbit, donkey, llama, dromedary, shark, or human antibodies, as well as antibodies from any other animal species. For use in the treatment of humans, non-human derived antibodies may be structurally altered to be less antigenic upon administration to a human patient, including by chimerization or humanization or superhumanization.

[0050] The antibodies disclosed herein may be humanized antibodies. Humanized antibodies are those wherein the amino acids directly involved in antigen binding, e.g., the complementarity determining regions (CDR), and in some cases the framework regions (FWR), or portions thereof, of the heavy and/or light chains are not of human origin, while the rest of the amino acids in the antibody are human or otherwise of human origin, e.g., a human antibody scaffold. Humanized antibodies also include antibodies in which one or more residues of the human protein are modified by one or more amino acid substitutions and/or one or more FWR residues of the human protein are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found in neither the human antibody or in the non-human antibody. A humanized antibody may be a super-humanized antibody, e.g., as described in U.S. Pat. No. 7,732,578. The antibodies may be humanized chimeric antibodies.

[0051] The antibodies disclosed herein may be fully human. Fully human antibodies are those where the whole molecule is human or otherwise of human origin, or includes an amino acid sequence identical to a human form of the antibody. Fully human antibodies include those obtained from a human V gene library, for example, where human genes encoding variable regions of antibodies are recombinantly expressed. Fully human antibodies may be expressed in other organisms (e.g., mice and xenomouse technology) or cells from other organisms transformed with genes encoding human antibodies. Fully human antibodies may nevertheless include amino acid residues not encoded by human sequences, e.g., mutations introduced by random or site directed mutations.

[0052] The antibodies may be full length antibodies of any class, for example, IgG1, IgG2 or IgG4. The constant domains of such antibodies are preferably human. The variable regions of such antibodies may be of non-human origin, or preferably are human in origin or are humanized. Antibody fragments may also be used in place of the full length antibodies.

[0053] The antibodies may be minibodies. Minibodies comprise small versions of whole antibodies, which encode in a single chain the essential elements of a whole antibody. For example, the minibody may be comprised of the VH and VL domains of a native antibody fused to the hinge region and CH3 domain of an immunoglobulin molecule.

[0054] The antibody disclosed herein may comprise non-immunoglobulin derived protein frameworks. For example, reference may be made to (Ku & Schutz, Proc. Natl. Acad. Sci. USA

92: 6552-6556, 1995) which describes a four-helix bundle protein cytochrome b562 having two loops randomized to create CDRs, which have been selected for antigen binding.

[0055] Natural sequence variations may exist among heavy and light chains and the genes encoding them, and therefore, persons having ordinary skill in the art would expect to find some level of variation within the amino acid sequences, or the genes encoding them, of the antibodies described and exemplified herein. These variants preferably maintain the unique binding properties (e.g., specificity and affinity) of the parent antibody. Such an expectation is due in part to the degeneracy of the genetic code, as well as to the known evolutionary success of conservative amino acid sequence variations, which do not appreciably alter the nature of the encoded protein. Accordingly, such variants and homologs are considered substantially the same as one another and are included within the scope of the disclosure. The antibodies thus include variants having single or multiple amino acid substitutions, deletions, additions, or replacements that retain the biological properties (e.g., binding specificity and binding affinity) of the parent antibodies. The variants are preferably conservative, but may be non-conservative.

[0056] Amino acid positions assigned to CDRs and FWRs may be defined according to Kabat Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., 1987 and 1991 (also referred to herein as the Kabat numbering system). In addition, the amino acid positions assigned to CDRs and FWRs may be defined according to the Enhanced Chothia Numbering Scheme (http://www.bioinfo.org.uk/mdex.html). The heavy chain constant region of an antibody can be defined by the EU numbering system (Edelman, GM et al. (1969)., Proc. Natl. Acad. USA, 63, 78-85).

[0057] According to the numbering system of Kabat, VH FWRs and CDRs may be positioned as follows: residues 1-30 (FWR1), 31-35 (CDR1), 36-49 (FWR2), 50-65 (CDR2), 66-94 (FWR3), 95-102 (CDR3) and 103-113 (FWR4), and VL FWRs and CDRs are positioned as follows: residues 1-23 (FWR1), 24-34 (CDR1), 35-49 (FWR2), 50-56 (CDR2), 57-88 (FWR3), 89-97 (CDR3) and 98-107 (FWR4). In some instances, variable regions may increase in length and according to the Kabat numbering system some amino acids may be designated by a number followed by a letter. This specification is not limited to FWRs and CDRs as defined by the Kabat numbering system, but includes all numbering systems, including the canonical numbering system or of Chothia et al. (1987) J. Mol. Biol. 196:901-17; Chothiaet al. (1989) Nature 342:877-83; and/or Al-Lazikani et al. (1997) J. Mol. Biol. 273:927-48; the numbering system of Honnegher et al. (2001) J. Mol. Biol., 309:657-70; or the IMGT system discussed in Giudicelli et al., (1997) Nucleic Acids Res. 25:206-11. The CDRs may be defined according to the Kabat numbering system.

[0058] For any of the heavy chain CDR2 subdomains described herein, according to the Kabat numbering system, the five C-terminal amino acids may not participate directly in antigen binding, and accordingly, it will be understood that any one or more of these five C-terminal amino acids may be substituted with another naturally-occurring amino acid without substantially adversely affecting antigen binding. For any of the light chain CDR1 subdomains

described herein, according to the Kabat numbering system, the four N-terminal amino acids may not participate directly in antigen binding, and accordingly, it will be understood that any one or more of these four amino acids may be substituted with another naturally-occurring amino acid without substantially adversely affecting antigen binding. For example, as described by Padlan et al. (1995) FASEB J. 9:133-139, the five C terminal amino acids of heavy chain CDR2 and/or the four N-terminal amino acids of light chain CDR1 may not participate in antigen binding. Both the heavy chain CDR2 and the light chain CDR1 may not directly participate in antigen binding.

[0059] Chemical analogues of amino acids may be used in the antibodies described and/or exemplified herein. The use of chemical analogues of amino acids is useful, for example, for stabilizing the molecules such as if required to be administered to a subject. The analogues of the amino acids contemplated herein include, but are not limited to, modifications of side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

[0060] The antibodies may comprise post-translational modifications or moieties, which may impact antibody activity or stability. These modifications or moieties include, but are not limited to, methylated, acetylated, glycosylated, sulfated, phosphorylated, carboxylated, and amidated moieties and other moieties that are well known in the art. Moieties include any chemical group or combinations of groups commonly found on immunoglobulin molecules in nature or otherwise added to antibodies by recombinant expression systems, including prokaryotic and eukaryotic expression systems.

[0061] Examples of side chain modifications contemplated by the disclosure include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

[0062] The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

[0063] The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivation, for example, to a corresponding amide.

[0064] Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-

chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

[0065] Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

[0066] Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

[0067] Crosslinkers may be used, for example, to stabilize 3D conformations of the antibodies and constructs, using homo-bifunctional crosslinkers such as the bifunctional imido esters having (CH_2)n spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH).

[0068] The antibodies may be affinity matured, or may comprise amino acid changes that decrease immunogenicity, for example, by removing predicted MHC class II-binding motifs. The therapeutic utility of the antibodies described herein may be further enhanced by modulating their functional characteristics, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), serum half-life, biodistribution and binding to Fc receptors or the combination of any of these. This modulation can be achieved by protein-engineering, glyco-engineering or chemical methods. Depending on the therapeutic application required, it could be advantageous to either increase or decrease any of these activities. An example of glyco-engineering used the Potelligent® method as described in Shinkawa T. et al. (2003) J. Biol. Chem. 278: 3466-73.

[0069] The antibodies may include modifications that modulate its serum half-life and biodistribution, including modifications that modulate the antibody's interaction with the neonatal Fc receptor (FcRn), a receptor with a key role in protecting IgG from catabolism, and maintaining high serum antibody concentration. Serum half-life modulating modifications may occur in the Fc region of IgG1 or IgG4, including the triple substitution of M252Y/S254T/T256E (Numbering according to the EU numbering system (Edelman, G.M. et al. (1969) Proc. Natl. Acad. USA 63, 78-85)), (*e.g.,* SEQ ID NO: 656, SEQ ID NO: 657, SEQ ID NO: 658, SEQ ID NO: 694), as described in U.S. Pat. No. 7,083,784. Other substitutions may occur at positions 250 and 428, see e.g., U.S. Pat. No 7,217,797, as well as at positions 307, 380 and 434, see, e.g., WO 00/42072. Examples of constant domain amino acid substitutions which modulate binding to Fc receptors and subsequent function mediated by these receptors, including FcRn binding and serum half-life, are described in U.S. Publ. Nos. 2009/0142340, 2009/0068175, and 2009/0092599. Naked antibodies may have the heavy chain C-terminal lysine omitted or removed to reduce heterogeneity. The substitution of S228P (EU numbering) in the human IgG4 can stabilize antibody Fab-arm exchange in vivo (Labrin *et al.* (2009) Nature

Biotechnology 27:8; 767-773).

[0070] The glycans linked to antibody molecules are known to influence interactions of antibody with Fc receptors and glycan receptors and thereby influence antibody activity, including serum half-life. Hence, certain glycoforms that modulate desired antibody activities can confer therapeutic advantage. Methods for generating engineered glycoforms include but are not limited to those described in U.S. Pat. Nos. 6,602,684, 7,326,681, and 7,388,081 and PCT Publ. No. WO 08/006554. Alternatively, the antibody sequences may be modified to remove relevant glycoform attachment sites.

[0071] The antibodies may be labeled or conjugated to any chemical or biomolecule moieties. Labeled antibodies may find use in therapeutic, diagnostic, or basic research applications. Such labels/conjugates can be detectable, such as fluorochromes, radiolabels, enzymes, fluorescent proteins, and biotin. The labels/conjugates may be chemotherapeutic agents, toxins, isotopes, and other agents used for treating conditions such as the killing of cancer cells. Chemotherapeutic agents may be any which is suitable for the purpose to which the antibody is being used.

[0072] The antibodies may be derivatized by known protecting/blocking groups to prevent proteolytic cleavage or enhance activity or stability.

[0073] The antibodies preferably have a binding affinity for an epitope on CD38 that includes a dissociation constant (Kd) of less than about 1×10^{-2} M. The Kd may be less than about 1×10^{-3} M. The Kd may be less than about 1×10^{-4} M. The Kd may be less than about 1×10^{-5} M. The Kd may be less than about 1×10^{-6} M. The Kd may be less than about 1×10^{-7} M. The Kd may be less than about 1×10^{-8} M. The Kd may be less than about 1×10^{-9} M. The Kd may be less than about 1×10^{-10} M. The Kd may be less than about 1×10^{-11} M. The Kd may be less than about 1×10^{-12} M. The Kd may be less than about 1×10^{-13} M. The Kd may be less than about 1×10^{-14} M. The Kd may be less than about 1×10^{-15} M. Affinity values refer to those obtained by standard methodologies, including surface plasmon resonance such as Biacore™ analyses or analysis using an Octet® Red 96 (Forte Bio) Dip-and-Read system.

[0074] The antibodies may comprise a single chain Fv molecule (scFv), Fab, or full IgG. Any such antibodies may comprise a heavy chain having an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% sequence identity with the amino acid sequence of SEQ ID NO: 659 or SEQ ID NO: 665 or SEQ ID NO: 736, provided that a heavy chain comprising the amino acid sequence of SEQ ID NO: 659 or variant thereof excludes the amino acid sequence of SEQ ID NO: 13. It will be understood that antibodies comprising amino acid changes in their heavy chain retain the capability to specifically bind to CD38. The retained CD38 specific binding activity (including affinity) is preferably about the same as the binding activity (including affinity) of an antibody without any amino acid changes

in the heavy chain, although the binding activity (including affinity) may be lesser or greater than an antibody without any amino acid changes in the heavy chain. The antibody may comprise a light chain having an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% sequence identity with the amino acid sequence of SEQ ID NO: 664 or SEQ ID NO: 666, provided that a light chain comprising the amino acid sequence of SEQ ID NO: 664 or variant thereof excludes the amino acid sequence of SEQ ID NO: 14. It will be understood that antibodies comprising amino acid changes in their light chain retain the capability to specifically bind to CD38. The retained CD38 specific binding activity (including affinity) is preferably about the same as the binding activity (including affinity) of an antibody without any amino acid changes in the light chain, although the binding activity (including affinity) may be lesser or greater than an antibody without any amino acid changes in the light chain.

[0075] Disclosed herein the heavy chain FWR1 may comprise the amino acid sequence of SEQ ID NO: 199, SEQ ID NO: 206, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 389, SEQ ID NO: 396, SEQ ID NO: 400, SEQ ID NO: 404, SEQ ID NO: 408, SEQ ID NO: 412, SEQ ID NO: 416, SEQ ID NO: 420, SEQ ID NO: 424, SEQ ID NO: 428, SEQ ID NO: 432, SEQ ID NO: 466, SEQ ID NO: 470, SEQ ID NO: 472, SEQ ID NO: 474, SEQ ID NO: 476, SEQ ID NO: 478, SEQ ID NO: 480, SEQ ID NO: 482, SEQ ID NO: 486, SEQ ID NO: 488, SEQ ID NO: 513, SEQ ID NO: 537, SEQ ID NO: 542, SEQ ID NO: 547, SEQ ID NO: 552, SEQ ID NO: 557, SEQ ID NO: 562, SEQ ID NO: 567, SEQ ID NO: 572, SEQ ID NO: 577 or SEQ ID NO: 748, and the heavy chain FWR1 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 199, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 389, SEQ ID NO: 396, SEQ ID NO: 400, SEQ ID NO: 404, SEQ ID NO: 408, SEQ ID NO: 412, SEQ ID NO: 416, SEQ ID NO: 420, SEQ ID NO: 424, SEQ ID NO: 428, SEQ ID NO: 432, SEQ ID NO: 466, SEQ ID NO: 470, SEQ ID NO: 472, SEQ ID NO: 474, SEQ ID NO: 476, SEQ ID NO: 478, SEQ ID NO: 480, SEQ ID NO: 482, SEQ ID NO: 486, SEQ ID NO: 488, SEQ ID NO: 513, SEQ ID NO: 537, SEQ ID NO: 542, SEQ ID NO: 547, SEQ ID NO: 552, SEQ ID NO: 557, SEQ ID NO: 562, SEQ ID NO: 567, SEQ ID NO: 572, SEQ ID NO: 577 or SEQ ID NO: 748. The heavy chain FWR2 may comprise the amino acid sequence of SEQ ID NO: 201, SEQ ID NO: 211, SEQ ID NO: 229, SEQ ID NO: 391, SEQ ID NO: 397, SEQ ID NO: 401, SEQ ID NO: 405, SEQ ID NO: 409, SEQ ID NO: 413, SEQ ID NO: 417, SEQ ID NO: 421, SEQ ID NO: 425, SEQ ID NO: 429, SEQ ID NO: 433, SEQ ID NO: 515 , SEQ ID NO: 520, SEQ ID NO: 521, SEQ ID NO: 522, SEQ ID NO: 523, SEQ ID NO: 524, SEQ ID NO: 525, SEQ ID NO: 538, SEQ ID NO: 543, SEQ ID NO: 548, SEQ ID NO: 553, SEQ ID NO: 558, SEQ ID NO: 563, SEQ ID NO: 568, SEQ ID NO: 573, SEQ ID NO: 578, SEQ ID NO: 749 or SEQ ID NO: 750, and the heavy chain FWR2 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 201, SEQ ID NO: 211, SEQ ID NO: 229, SEQ ID NO: 391, SEQ

ID NO: 397, SEQ ID NO: 401, SEQ ID NO: 405, SEQ ID NO: 409, SEQ ID NO: 413, SEQ ID NO: 417, SEQ ID NO: 421, SEQ ID NO: 425, SEQ ID NO: 429, SEQ ID NO: 433, SEQ ID NO: 515 , SEQ ID NO: 520, SEQ ID NO: 521, SEQ ID NO: 522, SEQ ID NO: 523, SEQ ID NO: 524, SEQ ID NO: 525, SEQ ID NO: 538, SEQ ID NO: 543, SEQ ID NO: 548, SEQ ID NO: 553, SEQ ID NO: 558, SEQ ID NO: 563, SEQ ID NO: 568, SEQ ID NO: 573, SEQ ID NO: 578, SEQ ID NO: 749 or SEQ ID NO: 750. The heavy chain FWR3 may comprise the amino acid sequence of SEQ ID NO: 203, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 221, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 230, SEQ ID NO: 393, SEQ ID NO: 399, SEQ ID NO: 403, SEQ ID NO: 407, SEQ ID NO: 411, SEQ ID NO: 415, SEQ ID NO: 419, SEQ ID NO: 423, SEQ ID NO: 427, SEQ ID NO: 431, SEQ ID NO: 435, SEQ ID NO: 468, SEQ ID NO: 517, SEQ ID NO: 530, SEQ ID NO: 531, SEQ ID NO: 532, SEQ ID NO: 533, SEQ ID NO: 540, SEQ ID NO: 545, SEQ ID NO: 550, SEQ ID NO: 555, SEQ ID NO: 560, SEQ ID NO: 565, SEQ ID NO: 570, SEQ ID NO: 575, SEQ ID NO: 580, SEQ ID NO: 751 or SEQ ID NO: 752 and the heavy chain FWR3 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 203, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 221, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 230, SEQ ID NO: 393, SEQ ID NO: 399, SEQ ID NO: 403, SEQ ID NO: 407, SEQ ID NO: 411, SEQ ID NO: 415, SEQ ID NO: 419, SEQ ID NO: 423, SEQ ID NO: 427, SEQ ID NO: 431, SEQ ID NO: 435, SEQ ID NO: 468, SEQ ID NO: 517, SEQ ID NO: 530, SEQ ID NO: 531, SEQ ID NO: 532, SEQ ID NO: 533, SEQ ID NO: 540, SEQ ID NO: 545, SEQ ID NO: 550, SEQ ID NO: 555, SEQ ID NO: 560, SEQ ID NO: 565, SEQ ID NO: 570, SEQ ID NO: 575, SEQ ID NO: 580 ,SEQ ID NO: 751 or SEQ ID NO: 752. The heavy chain FWR4 may comprise the amino acid sequence of SEQ ID NO: 205, SEQ ID NO: 395, SEQ ID NO: 519, SEQ ID NO: 541, SEQ ID NO: 546, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 561, SEQ ID NO: 566, SEQ ID NO: 571, SEQ ID NO: 576, SEQ ID NO: 581 or SEQ ID NO: 753, and the heavy chain FWR4 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 205, SEQ ID NO: 395, SEQ ID NO: 519, SEQ ID NO: 541, SEQ ID NO: 546, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 561, SEQ ID NO: 566, SEQ ID NO: 571, SEQ ID NO: 576, SEQ ID NO: 581 or SEQ ID NO: 753. It will be understood that antibodies comprising amino acid changes in the heavy chain framework region(s) (FWR1, FWR2, FWR3, and/or FWR4) retain the capability to specifically bind to CD38. The retained CD38 specific binding activity (including affinity) is preferably about the same as the binding activity (including affinity) of an antibody without any amino acid changes in any heavy chain framework region(s), although the binding activity (including affinity) may be lesser or greater than an antibody without any amino acid changes in any heavy chain framework region(s).

[0076] The heavy chain CDR1 may comprise the amino acid sequence of SEQ ID NO: 200, SEQ ID NO: 224, SEQ ID NO: 390, SEQ ID NO: 514, SEQ ID NO: 526, SEQ ID NO: 527, SEQ ID NO: 528, SEQ ID NO: 529, or SEQ ID NO: 697 and the heavy chain CDR1 may comprise an

amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 200, SEQ ID NO: 224, SEQ ID NO: 390, SEQ ID NO: 514, SEQ ID NO: 526, SEQ ID NO: 527, SEQ ID NO: 528, SEQ ID NO: 529, or SEQ ID NO: 697. The heavy chain CDR2 may comprise the amino acid sequence of SEQ ID NO: 202, SEQ ID NO: 392, SEQ ID NO: 398, SEQ ID NO: 402, SEQ ID NO: 406, SEQ ID NO: 410, SEQ ID NO: 414, SEQ ID NO: 418, SEQ ID NO: 422, SEQ ID NO: 426, SEQ ID NO: 430, SEQ ID NO: 434, SEQ ID NO: 467, SEQ ID NO: 471, SEQ ID NO: 473, SEQ ID NO: 475, SEQ ID NO: 477, SEQ ID NO: 479, SEQ ID NO: 481, SEQ ID NO: 483, SEQ ID NO: 485, SEQ ID NO: 487, SEQ ID NO: 489, SEQ ID NO: 516, SEQ ID NO: 539, SEQ ID NO: 544, SEQ ID NO: 549, SEQ ID NO: 554, SEQ ID NO: 559, SEQ ID NO: 564, SEQ ID NO: 569, SEQ ID NO: 574, SEQ ID NO: 579, SEQ ID NO: 698 or SEQ ID NO: 737 and the heavy chain CDR2 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 202, SEQ ID NO: 392, SEQ ID NO: 398, SEQ ID NO: 402, SEQ ID NO: 406, SEQ ID NO: 410, SEQ ID NO: 414, SEQ ID NO: 418, SEQ ID NO: 422, SEQ ID NO: 426, SEQ ID NO: 430, SEQ ID NO: 434, SEQ ID NO: 467, SEQ ID NO: 471, SEQ ID NO: 473, SEQ ID NO: 475, SEQ ID NO: 477, SEQ ID NO: 479, SEQ ID NO: 481, SEQ ID NO: 483, SEQ ID NO: 485, SEQ ID NO: 487, SEQ ID NO: 489, SEQ ID NO: 516, SEQ ID NO: 539, SEQ ID NO: 544, SEQ ID NO: 549, SEQ ID NO: 554, SEQ ID NO: 559, SEQ ID NO: 564, SEQ ID NO: 569, SEQ ID NO: 574, SEQ ID NO: 579, SEQ ID NO: 698 or SEQ ID NO: 737. The heavy chain CDR3 may comprise the amino acid sequence of SEQ ID NO: 204, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 228, SEQ ID NO: 231, SEQ ID NO: 394, SEQ ID NO: 469, SEQ ID NO: 518, SEQ ID NO: 534, SEQ ID NO: 535, SEQ ID NO: 536, SEQ ID NO: 699 or SEQ ID NO: 738 and the heavy chain CDR3 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 204, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 228, SEQ ID NO: 231, SEQ ID NO: 394, SEQ ID NO: 469, SEQ ID NO: 518, SEQ ID NO: 534, SEQ ID NO: 535, SEQ ID NO: 536, SEQ ID NO: 699 or SEQ ID NO: 738. It will be understood that antibodies comprising amino acid changes in the heavy chain complementarity determining region(s) (CDR1, CDR2, and/or CDR3) retain the capability to specifically bind to CD38. The retained CD38 specific binding activity (including affinity) is preferably about the same as the binding activity (including affinity) of an antibody without any amino acid changes in any heavy chain complementarity determining region(s), although the binding activity (including affinity) may be lesser or greater than an antibody without any amino acid changes in any heavy chain complementarity determining region(s).

[0077] The light chain FWR1 may comprise the amino acid sequence of SEQ ID NO: 232, SEQ ID NO: 247, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 436, SEQ ID NO: 443, SEQ ID NO: 447, SEQ ID NO: 451, SEQ ID NO: 455, SEQ ID NO: 459, SEQ ID NO: 463, SEQ ID NO: 490, SEQ ID NO: 497, SEQ ID NO: 501, SEQ ID NO: 509, SEQ ID NO: 582,

SEQ ID NO: 607, SEQ ID NO: 614, SEQ ID NO: 618, SEQ ID NO: 622, SEQ ID NO: 626, SEQ ID NO: 630, SEQ ID NO: 634 or SEQ ID NO: 638 and the light chain FWR1 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 232, SEQ ID NO: 247, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 436, SEQ ID NO: 443, SEQ ID NO: 447, SEQ ID NO: 451, SEQ ID NO: 455, SEQ ID NO: 459, SEQ ID NO: 463, SEQ ID NO: 490, SEQ ID NO: 497, SEQ ID NO: 501, SEQ ID NO: 509, SEQ ID NO: 582, SEQ ID NO: 607, SEQ ID NO: 614, SEQ ID NO: 618, SEQ ID NO: 622, SEQ ID NO: 626, SEQ ID NO: 630, SEQ ID NO: 634 or SEQ ID NO: 638. The light chain FWR2 may comprise the amino acid sequence of SEQ ID NO: 234, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 438, SEQ ID NO: 444, SEQ ID NO: 448, SEQ ID NO: 452, SEQ ID NO: 456, SEQ ID NO: 460, SEQ ID NO: 464, SEQ ID NO: 492, SEQ ID NO: 498, SEQ ID NO: 502, SEQ ID NO: 506, SEQ ID NO: 510, SEQ ID NO: 584, SEQ ID NO: 592, SEQ ID NO: 593, SEQ ID NO: 609, SEQ ID NO: 615, SEQ ID NO: 619, SEQ ID NO: 623, SEQ ID NO: 627, SEQ ID NO: 631, SEQ ID NO: 635 or SEQ ID NO: 639 and the light chain FWR2 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 234, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 438, SEQ ID NO: 444, SEQ ID NO: 448, SEQ ID NO: 452, SEQ ID NO: 456, SEQ ID NO: 460, SEQ ID NO: 464, SEQ ID NO: 492, SEQ ID NO: 498, SEQ ID NO: 502, SEQ ID NO: 506, SEQ ID NO: 510, SEQ ID NO: 584, SEQ ID NO: 592, SEQ ID NO: 593, SEQ ID NO: 609, SEQ ID NO: 615, SEQ ID NO: 619, SEQ ID NO: 623, SEQ ID NO: 627, SEQ ID NO: 631, SEQ ID NO: 635 or SEQ ID NO: 639. The light chain FWR3 may comprise the amino acid sequence of SEQ ID NO: 236, SEQ ID NO: 245, SEQ ID NO: 265, SEQ ID NO: 266, SEQ ID NO: 267, SEQ ID NO: 271, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 316, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 323, SEQ ID NO: 327, SEQ ID NO: 331, SEQ ID NO: 335, SEQ ID NO: 339, SEQ ID NO: 343, SEQ ID NO: 347, SEQ ID NO: 351, SEQ ID NO: 355, SEQ ID NO: 359, SEQ ID NO: 363, SEQ ID NO: 367, SEQ ID NO: 371, SEQ ID NO: 375, SEQ ID NO: 379, SEQ ID NO: 383, SEQ ID NO: 387, SEQ ID NO: 440, SEQ ID NO: 445, SEQ ID NO: 449, SEQ ID NO: 453, SEQ ID NO: 457, SEQ ID NO: 461, SEQ ID NO: 465, SEQ ID NO: 494, SEQ ID NO: 499, SEQ ID NO: 503, SEQ ID NO: 507, SEQ ID NO: 511, SEQ ID NO: 586, SEQ ID NO: 611, SEQ ID NO: 616, SEQ ID NO: 620, SEQ ID NO: 624, SEQ ID NO: 628, SEQ ID NO: 632, SEQ ID NO: 636 or SEQ ID NO: 640, and the light chain FWR3 comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at

least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 236, SEQ ID NO: 245, SEQ ID NO: 265, SEQ ID NO: 266, SEQ ID NO: 267, SEQ ID NO: 271, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 316, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 323, SEQ ID NO: 327, SEQ ID NO: 331, SEQ ID NO: 335, SEQ ID NO: 339, SEQ ID NO: 343, SEQ ID NO: 347, SEQ ID NO: 351, SEQ ID NO: 355, SEQ ID NO: 359, SEQ ID NO: 363, SEQ ID NO: 367, SEQ ID NO: 371, SEQ ID NO: 375, SEQ ID NO: 379, SEQ ID NO: 383, SEQ ID NO: 387, SEQ ID NO: 440, SEQ ID NO: 445, SEQ ID NO: 449, SEQ ID NO: 453, SEQ ID NO: 457, SEQ ID NO: 461, SEQ ID NO: 465, SEQ ID NO: 494, SEQ ID NO: 499, SEQ ID NO: 503, SEQ ID NO: 507, SEQ ID NO: 511, SEQ ID NO: 586, SEQ ID NO: 611, SEQ ID NO: 616, SEQ ID NO: 620, SEQ ID NO: 624, SEQ ID NO: 628, SEQ ID NO: 632, SEQ ID NO: 636 or SEQ ID NO: 640. The light chain FWR4 may comprise the amino acid sequence of SEQ ID NO: 238, SEQ ID NO: 442, SEQ ID NO: 446, SEQ ID NO: 450, SEQ ID NO: 454, SEQ ID NO: 458, SEQ ID NO: 462, SEQ ID NO: 496, SEQ ID NO: 500, SEQ ID NO: 504, SEQ ID NO: 508, SEQ ID NO: 512, SEQ ID NO: 588, SEQ ID NO: 613, SEQ ID NO: 617, SEQ ID NO: 621, SEQ ID NO: 625, SEQ ID NO: 629, SEQ ID NO: 633, SEQ ID NO: 637 or SEQ ID NO: 641 and the light chain FWR4 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 238, SEQ ID NO: 442, SEQ ID NO: 446, SEQ ID NO: 450, SEQ ID NO: 454, SEQ ID NO: 458, SEQ ID NO: 462, SEQ ID NO: 496, SEQ ID NO: 500, SEQ ID NO: 504, SEQ ID NO: 508, SEQ ID NO: 512, SEQ ID NO: 588, SEQ ID NO: 613, SEQ ID NO: 617, SEQ ID NO: 621, SEQ ID NO: 625, SEQ ID NO: 629, SEQ ID NO: 633, SEQ ID NO: 637 or SEQ ID NO: 641. It will be understood that antibodies comprising amino acid changes in the light chain framework region(s) (FWR1, FWR2, FWR3, and/or FWR4) retain the capability to specifically bind to CD38. The retained CD38 specific binding activity (including affinity) is preferably about the same as the binding activity (including affinity) of an antibody without any amino acid changes in any light chain framework region(s), although the binding activity (including affinity) may be lesser or greater than an antibody without any amino acid changes in any light chain framework region(s).

[0078] The light chain CDR1 may comprise the amino acid sequence of SEQ ID NO: 233, SEQ ID NO: 250, SEQ ID NO: 525, SEQ ID NO: 255, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 319, SEQ ID NO: 322, SEQ ID NO: 325, SEQ ID NO: 329, SEQ ID NO: 333, SEQ ID NO: 337, SEQ ID NO: 341, SEQ ID NO: 345, SEQ ID NO: 349, SEQ ID NO: 353, SEQ ID NO: 357, SEQ ID NO: 361, SEQ ID NO: 365, SEQ ID NO: 369, SEQ ID NO: 373, SEQ ID NO: 377, SEQ ID NO: 381, SEQ ID NO: 385, SEQ ID NO: 437, SEQ ID NO: 491, SEQ ID NO: 583, SEQ ID NO: 589, SEQ ID NO: 590, SEQ ID NO: 608, or SEQ ID NO: 696, and the light chain CDR1 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at

least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 233, SEQ ID NO: 250, SEQ ID NO: 525, SEQ ID NO: 255, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 319, SEQ ID NO: 322, SEQ ID NO: 325, SEQ ID NO: 329, SEQ ID NO: 333, SEQ ID NO: 337, SEQ ID NO: 341, SEQ ID NO: 345, SEQ ID NO: 349, SEQ ID NO: 353, SEQ ID NO: 357, SEQ ID NO: 361, SEQ ID NO: 365, SEQ ID NO: 369, SEQ ID NO: 373, SEQ ID NO: 377, SEQ ID NO: 381, SEQ ID NO: 385, SEQ ID NO: 437, SEQ ID NO: 491, SEQ ID NO: 583, SEQ ID NO: 589, SEQ ID NO: 590, SEQ ID NO: 608, or SEQ ID NO: 696. The light chain CDR2 may comprise the amino acid sequence of SEQ ID NO: 235, SEQ ID NO: 249, SEQ ID NO: 253, SEQ ID NO: 264, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 317, SEQ ID NO: 326, SEQ ID NO: 330, SEQ ID NO: 334, SEQ ID NO: 338, SEQ ID NO: 342, SEQ ID NO: 346, SEQ ID NO: 350, SEQ ID NO: 354, SEQ ID NO: 358, SEQ ID NO: 362, SEQ ID NO: 366, SEQ ID NO: 370, SEQ ID NO: 374, SEQ ID NO: 378, SEQ ID NO: 382, SEQ ID NO: 386, SEQ ID NO: 439, SEQ ID NO: 493, SEQ ID NO: 585, SEQ ID NO: 591, SEQ ID NO: 605, SEQ ID NO: 610 or SEQ ID NO: 747, and the light chain CDR2 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 235, SEQ ID NO: 249, SEQ ID NO: 253, SEQ ID NO: 264, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 317, SEQ ID NO: 326, SEQ ID NO: 330, SEQ ID NO: 334, SEQ ID NO: 338, SEQ ID NO: 342, SEQ ID NO: 346, SEQ ID NO: 350, SEQ ID NO: 354, SEQ ID NO: 358, SEQ ID NO: 362, SEQ ID NO: 366, SEQ ID NO: 370, SEQ ID NO: 374, SEQ ID NO: 378, SEQ ID NO: 382, SEQ ID NO: 386, SEQ ID NO: 439, SEQ ID NO: 493, SEQ ID NO: 585, SEQ ID NO: 591, SEQ ID NO: 605, SEQ ID NO: 610 or SEQ ID NO: 747. The light chain CDR3 may comprise the amino acid sequence of SEQ ID NO: 237, SEQ ID NO: 244, SEQ ID NO: 251, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 268, SEQ ID NO: 269, SEQ ID NO: 270, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 321, SEQ ID NO: 324, SEQ ID NO: 328, SEQ ID NO: 332, SEQ ID NO: 336, SEQ ID NO: 340, SEQ ID NO: 344, SEQ ID NO: 348, SEQ ID NO: 352, SEQ ID NO: 356, SEQ ID NO: 360, SEQ ID NO: 364, SEQ ID NO: 368, SEQ ID NO: 372, SEQ ID NO: 376, SEQ ID NO: 380, SEQ ID NO: 384, SEQ ID NO: 388, SEQ ID NO: 441, SEQ ID NO: 495, SEQ ID NO: 587, SEQ ID NO: 594, SEQ ID NO: 595, SEQ ID NO: 596, SEQ ID NO: 597, SEQ ID NO: 598, SEQ ID NO: 599, SEQ ID NO: 600, SEQ ID NO: 601, SEQ ID NO: 602, SEQ ID NO: 603, SEQ ID NO: 604, SEQ ID NO: 606 or SEQ ID NO: 612, and the light chain CDR3 may comprise an amino acid sequence having at least about 85%, at least about 90%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the amino acid sequence of SEQ ID NO: 237, SEQ ID NO: 244, SEQ ID NO: 251, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 268, SEQ ID NO: 269, SEQ ID NO: 270, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 321, SEQ ID NO: 324, SEQ ID NO: 328, SEQ ID NO: 332, SEQ ID NO: 336, SEQ ID NO: 340, SEQ ID NO: 344, SEQ ID NO: 348, SEQ ID NO: 352, SEQ ID NO: 356, SEQ ID NO: 360, SEQ ID NO: 364, SEQ ID NO: 368, SEQ ID NO: 372, SEQ ID NO: 376, SEQ ID NO: 380, SEQ ID NO: 384, SEQ

ID NO: 388, SEQ ID NO: 441, SEQ ID NO: 495, SEQ ID NO: 587, SEQ ID NO: 594, SEQ ID NO: 595, SEQ ID NO: 596, SEQ ID NO: 597, SEQ ID NO: 598, SEQ ID NO: 599, SEQ ID NO: 600, SEQ ID NO: 601, SEQ ID NO: 602, SEQ ID NO: 603, SEQ ID NO: 604, SEQ ID NO: 606 or SEQ ID NO: 612. It will be understood that antibodies comprising amino acid changes in the light chain complementarity determining region(s) (CDR1, CDR2, and/or CDR3) retain the capability to specifically bind to CD38. The retained CD38 specific binding activity (including affinity) is preferably about the same as the binding activity (including affinity) of an antibody without any amino acid changes in any light chain complementarity determining region(s), although the binding activity (including affinity) may be lesser or greater than an antibody without any amino acid changes in any light chain complementarity determining region(s).

[0079] The antibody may comprise particular heavy and light chain pairs. The heavy chains having the amino acid sequences of SEQ ID NO: 659 may be paired with any light chains having the amino acid sequences of SEQ ID NO: 664, or the heavy chains having the amino acid sequences of SEQ ID NO: 665 may be paired with any light chains having the amino acid sequences of SEQ ID NO: 666, or the heavy chain having the amino acid sequences of SEQ ID NO: 736 may be paired with any light chains having the amino acid sequences of SEQ ID NO: 664.

[0080] Variable heavy and variable light chain pairs disclosed herein may comprise pairs from the following table:

Antibody Name	**Variable Heavy SEQ ID NO: (amino acid)**	**Variable Light SEQ ID NO: (amino acid)**
A02.10	19	14
A02.11	20	14
A02.112	34	65
A02.12	34	65
A02.13	35	65
A02.16	34	92
A02.17	34	93
A02.18	34	73
A02.19	34	74
A02.2	13	65
A02.20	34	75
A02.21	34	76
A02.22	34	77
A02.23	34	78
A02.24	34	79
A02.25	34	80
A02.26	34	81

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A02.27	34	82
A02.28	34	83
A02.29	34	84
A02.3	17	65
A02.30	34	85
A02.31	34	86
A02.32	34	87
A02.33	34	88
A02.34	34	89
A02.35	34	90
A02.36	34	91
A02.37	34	66
A02.38	34	113
A02.39	34	112
A02.4	18	65
A02.40	111	65
A02.41	110	65
A02.43	110	113
A02.44	111	112
A02.46	34	67
A02.47	34	68
A02.48	34	69
A02.49	34	70
A02.5	19	65
A02.50	34	71
A02.51	34	72
A02.52	34	94
A02.53	34	95
A02.54	34	96
A02.55	34	97
A02.56	34	98
A02.57	34	99
A02.58	34	100
A02.59	34	101

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A02.6	20	65
A02.60	34	102
A02.61	34	103
A02.62	34	104
A02.63	34	105
A02.64	34	106
A02.65	34	107
A02.66	34	108
A02.67	34	109
A02.8	17	14
A02.9	18	14
A10.1	165	161
A10.10	174	161
A10.11	175	161
A10.12	176	161
A10.13	177	161
A10.14	178	161
A10.15	179	161
A10.16	180	161
A10.17	156	181
A10.18	156	182
A10.19	156	183
A10.2	166	161
A10.20	156	184
A10.21	156	185
A10.22	156	186
A10.23	156	187
A10.24	156	188
A10.25	156	189
A10.26	156	190
A10.27	156	191
A10.28	156	192
A10.29	156	193
A10.3	167	161

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A10.30	156	194
A10.31	156	195
A10.32	156	196
A10.35	197	161
A10.36	156	198
A10.38	152	161
A10.39	152	181
A10.4	168	161
A10.40	152	182
A10.41	152	183
A10.42	152	184
A10.43	152	185
A10.44	152	186
A10.45	152	187
A10.46	152	188
A10.47	152	189
A10.48	152	190
A10.49	152	191
A10.5	169	161
A10.50	152	192
A10.51	152	193
A10.52	152	194
A10.53	152	195
A10.54	152	196
A10.57	152	198
A10.59	156	161
A10.6	170	161
A10.7	171	161
A10.8	172	161
A10.9	173	161
A10A2.0 (chimeric)	148	157
A10A2.1	149	158
A10A2.10	150	160
A10A2.11	150	161

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A10A2.12	150	162
A10A2.13	150	163
A10A2.14	150	164
A10A2.15	151	158
A10A2.16	151	159
A10A2.17	151	160
A10A2.18	151	161
A10A2.19	151	162
A10A2.2	149	159
A10A2.20	151	163
A10A2.21	151	164
A10A2.22	152	158
A10A2.23	152	159
A10A2.24	152	160
A10A2.25	152	161
A10A2.26	152	162
A10A2.27	152	163
A10A2.28	152	164
A10A2.29	153	158
A10A2.3	149	160
A10A2.30	153	159
A10A2.31	153	160
A10A2.32	153	161
A10A2.33	153	162
A10A2.34	153	163
A10A2.35	153	164
A10A2.36	154	158
A10A2.37	154	159
A10A2.38	154	160
A10A2.39	154	161
A10A2.4	149	161
A10A2.40	154	162
A10A2.41	154	163
A10A2.42	154	164

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A10A2.43	154	158
A10A2.44	155	159
A10A2.45	155	160
A10A2.46	155	161
A10A2.47	155	162
A10A2.48	155	163
A10A2.49	155	164
A10A2.5	149	162
A10A2.50	156	158
A10A2.51	156	159
A10A2.52	156	160
A10A2.53	156	161
A10A2.54	156	162
A10A2.55	156	163
A10A2.56	156	164
A10A2.6	149	163
A10A2.7	149	164
A10A2.8	150	158
A10A2.9	150	159
A5D1.0 (chimeric)	114	125
A5D1.1	115	126
A5D1.10	116	129
A5D1.11	116	130
A5D1.12	116	131
A5D1.13	117	126
A5D1.14	117	127
A5D1.15	117	128
A5D1.16	117	129
A5D1.17	117	130
A5D1.18	117	131
A5D1.19	118	126
A5D1.2	115	127
A5D1.20	118	127
A5D1.21	118	128

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A5D1.22	118	129
A5D1.23	118	130
A5D1.24	118	131
A5D1.25	119	126
A5D1.26	119	127
A5D1.27	119	128
A5D1.28	119	129
A5D1.29	119	130
A5D1.3	115	128
A5D1.30	119	131
A5D1.31	120	126
A5D1.32	120	127
A5D1.33	120	128
A5D1.34	120	129
A5D1.35	120	130
A5D1.36	120	131
A5D1.37	121	126
A5D1.38	121	127
A5D1.39	121	128
A5D1.4	115	129
A5D1.40	121	129
A5D1.41	121	130
A5D1.42	121	131
A5D1.43	122	126
A5D1.44	122	127
A5D1.45	122	128
A5D1.46	122	129
A5D1.47	122	130
A5D1.48	122	131
A5D1.49	123	126
A5D1.5	115	130
A5D1.50	123	127
A5D1.51	123	128
A5D1.52	123	129

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A5D1.53	123	130
A5D1.54	123	131
A5D1.55	124	126
A5D1.56	124	127
A5D1.57	124	128
A5D1.58	124	129
A5D1.59	124	130
A5D1.6	115	131
A5D1.60	124	131
A5D1.7	116	126
A5D1.8	116	127
A5D1.9	116	128
A5E8.0 (chimeric)	132	143
A5E8.1	133	144
A5E8.10	135	145
A5E8.11	135	146
A5E8.12	135	147
A5E8.13	136	144
A5E8.14	136	145
A5E8.15	136	146
A5E8.16	136	147
A5E8.17	137	144
A5E8.18	137	145
A5E8.19	137	146
A5E8.2	133	145
A5E8.20	137	147
A5E8.21	138	144
A5E8.22	138	145
A5E8.23	138	146
A5E8.24	138	147
A5E8.25	139	144
A5E8.26	139	145
A5E8.27	139	146
A5E8.28	139	147

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
A5E8.29	140	144
A5E8.3	133	146
A5E8.30	140	145
A5E8.31	140	146
A5E8.32	140	147
A5E8.33	141	144
A5E8.34	141	145
A5E8.35	141	146
A5E8.36	141	147
A5E8.37	142	144
A5E8.38	142	145
A5E8.39	142	146
A5E8.4	133	147
A5E8.40	142	147
A5E8.5	134	144
A5E8.6	134	145
A5 E8.7	134	146
A5E8.8	134	147
A5E8.9	135	144
X02.10	19	14
X02.100	13	58
X02.101	13	59
X02.102	13	60
X02.103	13	61
X02.104	13	62
X02.105	13	63
X02.106	13	64
X02.107	13	65
X02.108	32	14
X02.11	20	14
X02.110	33	14
X02.114	13	660
X02.115	13	661
X02.116	13	662

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
X02.117	13	663
X02.118	34	700
X02.119	34	701
X02.120	728	700
X02.121	729	700
X02.122	730	700
X02.123	731	700
X02.124	728	701
X02.125	729	701
X02.126	730	701
X02.127	731	701
X02.68	21	14
X02.69	22	14
X02.70	23	14
X02.71	24	14
X02.72	25	14
X02.73	26	14
X02.74	27	14
X02.75	28	14
X02.76	29	14
X02.77	30	14
X02.78	31	14
X02.8	17	14
X02.80	13	38
X02.81	13	39
X02.82	13	40
X02.83	13	41
X02.84	13	42
X02.85	13	43
X02.86	13	44
X02.87	13	45
X02.88	13	46
X02.89	13	47
X02.9	18	14

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
X02.90	13	48
X02.91	13	49
X02.92	13	50
X02.93	13	51
X02.94	13	52
X02.95	13	53
X02.96	13	54
X02.97	13	55
X02.98	13	56
X02.99	13	57
X10.100	720	706
X10.101	721	706
X10.102	722	706
X10.103	723	706
X10.104	739	706
X10.105	740	706
X10.106	741	706
X10.107	742	706
X10.108	720	707
X10.109	721	707
X10.110	722	707
X10.111	723	707
X10.112	739	707
X10.113	740	707
X10.114	741	707
X10.115	742	707
X10.116	720	708
X10.117	721	708
X10.118	722	708
X10.119	723	708
X10.120	739	708
X10.121	740	708
X10.122	741	708
X10.123	742	708

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
X10.124	720	709
X10.125	721	709
X10.126	722	709
X10.127	723	709
X10.128	739	709
X10.129	740	709
X10.130	741	709
X10.131	742	709
X10.132	720	710
X10.133	721	710
X10.134	722	710
X10.135	723	710
X10.136	739	710
X10.137	740	710
X10.138	741	710
X10.139	742	710
X10.140	720	711
X10.141	721	711
X10.142	722	711
X10.143	723	711
X10.144	739	711
X10.145	740	711
X10.146	741	711
X10.147	742	711
X10.60	156	704
X10.61	156	705
X10.62	156	706
X10.63	156	707
X10.64	156	708
X10.65	156	709
X10.66	156	710
X10.67	156	711
X10.68	720	161
X10.69	721	161

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)
X10.70	722	161
X10.71	723	161
X10.72	739	161
X10.73	740	161
X10.74	741	161
X10.75	742	161
X10.76	152	704
X10.77	152	705
X10.78	152	706
X10.79	152	707
X10.80	152	708
X10.81	152	709
X10.82	152	710
X10.83	152	711
X10.84	720	704
X10.85	721	704
X10.86	722	704
X10.87	723	704
X10.88	739	704
X10.89	740	704
X10.90	741	704
X10.91	742	704
X10.92	720	705
X10.93	721	705
X10.94	722	705
X10.95	723	705
X10.96	739	705
X10.97	740	705
X10.98	741	705
X10.99	742	705
X910/12-HC-L0-IFN-alpha (A145D) IgG4	110	112
X913/15-HC-L0-IFN-alpha (A145D) IgG4	111	113

[0081] The antibodies may be fused to attenuated ligands, for example, to form antibody-attenuated ligand constructs, which show an elevated antigen-specificity index with respect to activating signaling pathways due to the action of the attenuated ligand *on* a cell surface receptor. These constructs are based on the observation that, in the context of an antibody-ligand construct, the ligand portion can be mutated in *such* a way that the ligand activity on antigen-negative cells is dramatically attenuated, while the ligand activity on antigen-positive cells is only modestly, if at all, attenuated. Such constructs display one, two, three, four or five orders of magnitude greater potency on antigen-positive cells compared to antigen negative cells than does the free ligand. The antibody-attenuated ligand construct may retain at least 1%, at least 10%, at least 20%, at least 30%, at least 40% or at least 50% of the potency on antigen-positive cells as the non-attenuated free (i.e., not attached to an antibody) ligand. The antibody-attenuated ligand construct may retain at least 30%, at least 50%, at least 75% or at least 90% of the maximal activity of the non-attenuated free (i.e. not attached to an antibody) ligand. Maximal activity includes the amount of signaling activity (or downstream effect thereof) at the high, plateau portion of a dose-response curve, where further increases in the *age*nt does not further increase the amount of response.

[0082] The antibody fusion to and inclusion of an attenuating mutation(s) in the interferon ligand may increase the antigen-specificity index (ASI) by greater than 10-fold, preferably greater than 50-fold, preferably greater than 100-fold, preferably greater than 1000-fold, or preferably greater than 10,000 fold, relative to an antibody without a fusion. The ASI comprises the fold-increased potency in signaling activity of the antibody-IFN ligand construct relative to the free non-mutated polypeptide ligand on target antigen-positive cells, multiplied by the fold decreased potency in signaling activity relative to the free non-mutated polypeptide ligand on target antigen-negative cells. Potency may be quantitatively represented by the EC_{50} value, which is the mathematical midpoint of a dose-response curve, in which the dose refers to the concentration of ligand or antibody-ligand construct in an assay, and response refers to the quantitative response of the cells to the signaling activity of the ligand at a particular dose. Thus, for example, when a first compound is shown to possess an EC_{50} (expressed for example in Molar units) that is 10-fold lower than a second compound's EC_{50} on the same cells, typically when measured by the same method, the first compound is said to have a 10-fold higher potency. Conversely, when a first compound is shown to possess an EC_{50} that is 10-fold higher than a second compound's EC_{50} on the same cells, typically when measured by the same method, the first compound is said to have a 10-fold lower potency.

[0083] The antibodies are preferably capable of binding to CD38-positive cells. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 100 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 75 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 50 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 30 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 25 nM. The antibody

may bind to a CD38-positive cell with an EC_{50} value of less than about 20 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 18 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 15 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 13 nM. The antibody may bind to a CD38-positive cell with an EC_{50} value of less than about 10 nM.

[0084] The interferon joined to the antibody preferably comprises alterations in its amino acid sequence, including point mutations and/or deletions that render the interferon less active in stimulating its respective receptors on cells that lack cell surface expression of the CD38 antigen to which the antibody binds. A highly preferred variant of interferon alpha comprises an amino acid change at position 168 of the interferon alpha 2b molecule of SEQ ID NO: 7. For example, the amino acid at position 168, which is an alanine in the parent IFN-alpha2b molecule, is preferably changed to a glycine (Gly/G) (SEQ ID NO: 650) or aspartic acid (Asp/D) (SEQ ID NO: 647). The IFN-alpha2b may be truncated at its N-terminus when the IFN-alpha2b is fused to an IgG heavy chain constant domain such as the human IgG1 or human IgG4 heavy chain constant domain. The truncated IFN-alpha2b does not have the twenty three N-terminal amino acids of SEQ ID NO: 7 (Met 1 through Gly 23 are deleted), and the truncated IFN-alpha2b comprises the amino acid sequence of SEQ ID NO: 648. The truncated IFN-alpha2b may also comprise the amino acid change at what was formerly position 168, but which becomes position 145 in the truncated protein (e.g., alanine 168 becomes alanine 145). In the truncated IFN-alpha2b, the alanine is preferably changed to a glycine (Gly/G) (SEQ ID NO: 651) or aspartic acid (Asp/D) (SEQ ID NO: 649). Interferon with A145D alteration (SEQ ID NO: 647 or SEQ ID NO: 649) is particularly preferred as the attenuated ligand fused to the antibodies of the disclosure. Any of these point-mutated, attenuated versions of IFN-alpha may be joined to any antibody described herein, for example, as an antibody-attenuated interferon construct.

[0085] The linkage between the antibody and the interferon preferably comprises a fusion, for example, a peptide bond between the N- or the C-terminus of the interferon and the Nor C-terminus of the heavy or the light chain of the antibody. No linker may be present between the antibody and the interferon, and the antibody and interferon may thus be directly fused. It is believed that direct fusion, without an intervening linker peptide, provides at least a measurable degree of attenuation of the interferon protein, and it is also believed that this attenuation is additive with the attenuation of the interferon protein that stems from the mutations introduced into the interferon protein, including those described or exemplified herein.

[0086] Polynucleotide sequences that encode antibodies and their subdomains (e.g., FWRs and CDRs) are featured in the disclosure. Polynucleotides include, but are not limited to, RNA, DNA, cDNA, hybrids of RNA and DNA, and single, double, or triple stranded strands of RNA, DNA, or hybrids thereof.

[0087] The polynucleotide of the invention comprises a nucleic acid sequence encoding an antibody that specifically binds to CD38, wherein the antibody comprises a heavy chain

variable region comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 185.

[0088] The polynucleotides may encode the heavy chain of an antibody that specifically binds to an epitope on CD38. The polynucleotides disclosed herein may encode a heavy chain comprising the amino acid sequence of any of SEQ ID NO: 667, SEQ ID NO: 668, SEQ ID NO: 679, SEQ ID NO: 680, SEQ ID NO: 681, SEQ ID NO: 682, SEQ ID NO: 683, SEQ ID NO: 684, SEQ ID NO: 686, SEQ ID NO: 695, SEQ ID NO: 724, SEQ ID NO: 725, SEQ ID NO: 726, SEQ ID NO: 727, SEQ ID NO: 732, SEQ ID NO: 733, SEQ ID NO: 734, SEQ ID NO: 735, SEQ ID NO: 743, SEQ ID NO: 744, SEQ ID NO: 745 or SEQ ID NO: 746. The polynucleotides disclosed herein may encode a light chain comprising the amino acid sequence of any of SEQ ID NO: 669, SEQ ID NO: 670, SEQ ID NO: 671, SEQ ID NO: 672, SEQ ID NO: 673, SEQ ID NO: 674, SEQ ID NO: 675, SEQ ID NO: 676, SEQ ID NO: 677, SEQ ID NO: 678, SEQ ID NO: 688, SEQ ID NO: 689, SEQ ID NO: 690, SEQ ID NO: 692, SEQ ID NO: 693, SEQ ID NO: 702, SEQ ID NO: 703, SEQ ID NO: 712, SEQ ID NO: 713, SEQ ID NO: 714, SEQ ID NO: 715, SEQ ID NO: 716, SEQ ID NO: 717, SEQ ID NO: 718 or SEQ ID NO: 719. The polynucleotides disclosed herein may comprise the nucleic acid sequence of any of SEQ ID NO: 667, SEQ ID NO: 668, SEQ ID NO: 679, SEQ ID NO: 680, SEQ ID NO: 681, SEQ ID NO: 682, SEQ ID NO: 683, SEQ ID NO: 684, SEQ ID NO: 686, SEQ ID NO: 695, SEQ ID NO: 724, SEQ ID NO: 725, SEQ ID NO: 726, SEQ ID NO: 727, SEQ ID NO: 732, SEQ ID NO: 733, SEQ ID NO: 734, SEQ ID NO: 735, SEQ ID NO: 743, SEQ ID NO: 744, SEQ ID NO: 745, SEQ ID NO: 746, SEQ ID NO: 669, SEQ ID NO: 670, SEQ ID NO: 671, SEQ ID NO: 672, SEQ ID NO: 673, SEQ ID NO: 674, SEQ ID NO: 675, SEQ ID NO: 676, SEQ ID NO: 677, SEQ ID NO: 678, SEQ ID NO: 688, SEQ ID NO: 689, SEQ ID NO: 690, SEQ ID NO: 692, SEQ ID NO: 693, SEQ ID NO: 702, SEQ ID NO: 703, SEQ ID NO: 712, SEQ ID NO: 713, SEQ ID NO: 714, SEQ ID NO: 715, SEQ ID NO: 716, SEQ ID NO: 717, SEQ ID NO: 718 or SEQ ID NO: 719. The polynucleotides disclosed herein may comprise a nucleic acid sequence having at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with any of SEQ ID NO: 667, SEQ ID NO: 668, SEQ ID NO: 679, SEQ ID NO: 680, SEQ ID NO: 681, SEQ ID NO: 682, SEQ ID NO: 683, SEQ ID NO: 684, SEQ ID NO: 686, SEQ ID NO: 695, SEQ ID NO: 724, SEQ ID NO: 725, SEQ ID NO: 726, SEQ ID NO: 727, SEQ ID NO: 732, SEQ ID NO: 733, SEQ ID NO: 734, SEQ ID NO: 735, SEQ ID NO: 743, SEQ ID NO: 744, SEQ ID NO: 745, SEQ ID NO: 746, SEQ ID NO: 669, SEQ ID NO: 670, SEQ ID NO: 671, SEQ ID NO: 672, SEQ ID NO: 673, SEQ ID NO: 674, SEQ ID NO: 675, SEQ ID NO: 676, SEQ ID NO: 677, SEQ ID NO: 678, SEQ ID NO: 688, SEQ ID NO: 689, SEQ ID NO: 690, SEQ ID NO: 692, SEQ ID NO: 693, SEQ ID NO: 702, SEQ ID NO: 703, SEQ ID NO: 712, SEQ ID NO: 713, SEQ ID NO: 714, SEQ ID NO: 715, SEQ ID NO: 716, SEQ ID NO: 717, SEQ ID NO: 718 or SEQ ID NO: 719, and such variants may preferably encode the same amino acids encoded by the polynucleotide sequence of SEQ ID NO: 667, SEQ ID NO: 668, SEQ ID NO: 679, SEQ ID NO: 680, SEQ ID NO: 681, SEQ ID NO: 682, SEQ ID NO: 683, SEQ ID NO: 684, SEQ ID NO: 686, SEQ ID NO: 695, SEQ ID NO: 724, SEQ ID NO: 725, SEQ ID NO: 726, SEQ ID NO: 727, SEQ ID NO: 732, SEQ ID NO: 733, SEQ ID NO: 734, SEQ ID NO: 735, SEQ ID

NO: 743, SEQ ID NO: 744, SEQ ID NO: 745, SEQ ID NO: 746, SEQ ID NO: 669, SEQ ID NO: 670, SEQ ID NO: 671, SEQ ID NO: 672, SEQ ID NO: 673, SEQ ID NO: 674, SEQ ID NO: 675, SEQ ID NO: 676, SEQ ID NO: 677, SEQ ID NO: 678, SEQ ID NO: 688, SEQ ID NO: 689, SEQ ID NO: 690, SEQ ID NO: 692, SEQ ID NO: 693, SEQ ID NO: 702, SEQ ID NO: 703, SEQ ID NO: 712, SEQ ID NO: 713, SEQ ID NO: 714, SEQ ID NO: 715, SEQ ID NO: 716, SEQ ID NO: 717, SEQ ID NO: 718 or SEQ ID NO: 719. Preferably, the antibodies encoded by the polynucleotide variants will specifically bind to CD38 with an affinity about equal to the affinity of the antibody encoded by the parent (non-variant) polynucleotide sequence. Affinity may be measured, for example, according to any technique described or exemplified herein, including techniques described in the Examples. Complements of the polynucleotide sequences and the variant polynucleotide sequences are also within the scope of the disclosure.

[0089] Also encompassed within the invention are vectors comprising the polynucleotides of the invention. The vectors may be expression vectors. Recombinant expression vectors containing a sequence encoding a polypeptide of interest are thus provided. The expression vector may contain one or more additional sequences, such as but not limited to regulatory sequences, a selection marker, a purification tag, or a polyadenylation signal. Such regulatory elements may include a transcriptional promoter, enhancers, mRNA ribosomal binding sites, or sequences that control the termination of transcription and translation.

[0090] Expression vectors, especially mammalian expression vectors, may include one or more nontranscribed elements, such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, other 5' or 3' flanking nontranscribed sequences, 5' or 3' nontranslated sequences (such as necessary ribosome binding sites), a polyadenylation site, splice donor and acceptor sites, or transcriptional termination sequences. An origin of replication that confers the ability to replicate in a specific host may also be incorporated.

[0091] The vectors may be used to transform any of a wide array of host cells well known to those of skill in the art, and preferably host cells capable of expressing antibodies. Vectors include without limitation, plasmids, phagemids, cosmids, baculoviruses, bacmids, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), and baculovirus, as well as other bacterial, eukaryotic, yeast, and viral vectors. Suitable host cells include without limitation CHO cells, HEK293 cells, or any eukaryotic stable cell line known or produced, and also include bacteria, yeast, and insect cells.

[0092] The antibodies may also be produced by hybridoma cells; methods to produce hybridomas being well known and established in the art.

[0093] It has been observed in accordance with the disclosure that when interferon alpha ligand, having one or more mutations that substantially decrease the affinity of the ligand for an interferon receptor, is linked to an anti-CD38 antibody that targets the mutated interferon alpha ligand to target cells which display the antibody's corresponding antigen, the ligand's activity on target antigen-positive cells is maintained while the ligand's activity on non-target antigen-

negative cells is substantially reduced. The net result is a ligand signaling molecule that has a much greater potency in activation of its receptors on antigen-positive target cells compared to antigen-negative non-target cells, which provides a means for reducing toxicity arising from off-target ligand activity.

[0094] A polypeptide construct may comprise an IFN-alpha variant linked to an anti-CD38 antibody or antigen binding portion thereof. Such a polypeptide will be capable of exerting with high potency the IFN's anti-proliferative activity on CD38-positive tumor cells while exerting a much lower potency on CD38-negative, non-tumor cells within the body.

[0095] The disclosure also provides compositions comprising the antibodies and antibody-attenuated interferon constructs of the disclosure. These compositions can further comprise at least one of any suitable auxiliary, such as, but not limited to one or more, diluents, binders, stabilizers, buffers, salts, lipophilic solvents, preservatives, adjuvants, or other suitable carrier and/or excipient. Pharmaceutically acceptable auxiliaries are preferred. The compositions may comprise any of the antibodies and antibody-attenuated interferon constructs described and/or exemplified herein and an acceptable carrier such as a pharmaceutically acceptable carrier. Suitable carriers include any media that does not interfere with the biological activity of the antibody and/or the interferon and preferably is not toxic to a host to which it is administered. The carrier may be an aqueous solution, such as water, saline, or alcohol, or a physiologically compatible buffer, such as Hanks's solution, Ringer's solution, or physiological saline buffer. The carrier may contain formulatory agents, such as suspending, stabilizing and/or dispersing agents

[0096] Pharmaceutical excipients and additives useful in the composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and other known sugars; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination any suitable weight or volume. Exemplary protein excipients include serum albumin, such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and other known proteins. Representative amino acids which can also function in a buffering capacity include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, and aspartame. One preferred amino acid is histidine. A second preferred amino acid is arginine.

[0097] Carbohydrate excipients suitable for use in the composition include, for example, monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, and sorbose; disaccharides, such as lactose, sucrose, trehalose, and cellobiose; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, and starches; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), and myoinositol. Preferred carbohydrate excipients for use in the disclosure are mannitol, trehalose, and raffinose.

[0098] Antibody compositions can also include a buffer or a pH adjusting agent; typically, the

buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts, such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts, such as citrate.

[0099] Additionally, the compositions of the disclosure can include polymeric excipients/additives, such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (*e.g.*, cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, antimicrobial agents, antioxidants, antistatic agents, surfactants (*e.g.*, polysorbates such as "TWEEN® 20" and "TWEEN® 80"), lipids (*e.g.*, phospholipids, fatty acids), steroids (*e.g.*, cholesterol), and chelating agents (*e.g.*, EDTA).

[0100] The compositions may also be formulated in sustained release vehicles or depot preparations. For example, the compositions may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Liposomes and emulsions are well-known examples of delivery vehicles suitable for use as carriers for hydrophobic drugs.

[0101] The compositions may be formulated for administration to a subject in any suitable dosage form. The compositions may be formulated for oral, buccal, nasal, transdermal, parenteral, injectable, intravenous, subcutaneous, intramuscular, rectal, or vaginal administrations. The compositions may be formulated in a suitable controlled-release vehicle, with an adjuvant, or as a depot formulation.

[0102] Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions.

[0103] An anti-CD38-attenuated interferon alpha-2b fusion construct may be used, for example, to inhibit, reduce, decrease, block, or prevent proliferation of a cell that expressed CD38 on its surface. Methods for inhibiting or reducing proliferation of a cell that expresses CD38 on its surface generally comprise contacting a cell expressing CD38 with an anti-CD38-attenuated interferon alpha-2b fusion construct in an amount effective to inhibit or reduce proliferation of the cell. The antibody that specifically binds to CD38 may be any antibody described or exemplified herein. The attenuated interferon alpha 2b may comprise IFN-alpha2b A145D or IFN-alpha2b A145G. The cell may be a lymphocyte, an autoimmune lymphocyte, or a tumor cell such as a leukemia cell, a multiple myeloma cell, or a lymphoma cell. The anti-CD38-attenuated interferon alpha-2b fusion construct may be comprised in a composition, for example, with a pharmaceutically acceptable carrier and optionally one or more auxiliaries or excipients, including any such carrier, auxiliary, or excipient described or exemplified herein. The methods may be carried out *in vitro, ex vivo, in vivo,* or *in situ.*

[0104] An anti-CD38-attenuated interferon alpha-2b fusion construct may also be used, for example, to induce, facilitate, or enhance apoptosis of a cell that expressed CD38 on its surface. Methods for inducing apoptosis in a cell that expresses CD38 on its surface generally comprise contacting a cell expressing CD38 with an anti-CD38-attenuated interferon alpha-2b fusion construct in an amount effective to induce apoptosis in the cell. The antibody that specifically binds to CD38 may be any antibody described or exemplified herein. The attenuated interferon alpha 2b may comprise IFN-alpha2b A145D or IFN-alpha2b A145G. The cell may be a lymphocyte, an autoimmune lymphocyte, or a tumor cell such as a leukemia cell, a multiple myeloma cell, or a lymphoma cell. The anti-CD38-attenuated interferon alpha-2b fusion construct may be comprised in a composition, for example, with a pharmaceutically acceptable carrier and optionally one or more auxiliaries or excipients, including any such carrier, auxiliary, or excipient described or exemplified herein. The methods may be carried out *in vitro, ex vivo, in vivo,* or *in situ.*

[0105] An anti-CD38-attenuated interferon alpha-2b fusion construct may also be used to treat a subject having a tumor that comprises and/or is mediated, at least in part, by cells that express CD38 on their surface. Methods for treating a tumor comprising cells expressing CD38 on their surface generally comprise administering to a subject in need thereof an anti-CD38-attenuated interferon alpha-2b fusion construct in an amount effective to treat the tumor in the subject. Effective treatment may include, for example, inhibiting or reducing proliferation of CD38-positive cells in the tumor and/or inducing apoptosis of CD38-positive cells in the tumor. The antibody that specifically binds to CD38 may be any antibody described or exemplified herein. The attenuated interferon alpha 2b may comprise IFN-alpha2b A145D or IFN-alpha2b A145G. The anti-CD38-attenuated interferon alpha-2b fusion construct may be comprised in a composition, for example, with a pharmaceutically acceptable carrier and optionally one or more auxiliaries or excipients, including any such carrier, auxiliary, or excipient described or exemplified herein.

[0106] The anti-CD38-attenuated interferon alpha-2b fusion constructs or composition comprising such constructs may be administered to the tumor by administering the constructs of composition to the blood. The anti-CD38-attenuated interferon alpha-2b fusion constructs or composition comprising such constructs may be administered such that the construct diffuses via blood flow to and/or into the tumor cells. The construct may be internalized by a tumor cell.

[0107] Use of an anti-CD38 antibody or anti-CD38 antibody-attenuated interferon alpha-2b fusion construct in the treatment of tumors are disclosed herein. Methods for treating tumors with an anti-CD38 antibody or anti-CD38 antibody-attenuated interferon alpha-2b fusion construct are disclosed herein. Any anti-CD38 antibody or anti-CD38 antibody-attenuated interferon alpha-2b fusion construct described or exemplified herein may be used. Tumors that may be treated include, but are not limited to AIDS related cancers, acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin),

bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-Cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynecological cancers, hematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, Li-Fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, multiple myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(NSCLC), ocular cancers, esophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (SCLC), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia and Wilms' tumor. In an embodiment the tumor is selected from a group of multiple myeloma or non-Hodgkin's lymphoma.

[0108] The methods may be used for treatment of multiple myeloma, leukemia, or lymphoma in a subject in need thereof. Such methods may further comprise treating the subject with a retinoid, such as all-trans retinoic acid. When the cell surface associated antigen is CD38, the tumor or cancer may be selected from multiple myeloma, non-Hodgkin's lymphoma, chronic myelogenous leukemia, chronic lymphocytic leukemia or acute myelogenous leukemia.

[0109] An anti-CD38-attenuated interferon alpha-2b fusion construct may be combined with other drugs and/or used in addition to other cancer treatment regimens or modalities such as radiation therapy or surgery. When anti-CD38-attenuated interferon alpha-2b fusion constructs are used in combination with known therapeutic agents the combination may be administered

either in sequence (either continuously or broken up by periods of no treatment) or concurrently or as a mixture. In the case of cancer, there are numerous known anticancer agents that may be used in this context. Treatment in combination is also contemplated to encompass the treatment with either the anti-CD38-attenuated interferon alpha-2b fusion construct followed by a known treatment, or treatment with a known agent followed by treatment with the anti-CD38-attenuated interferon alpha-2b fusion construct, for example, as maintenance therapy. For example, in the treatment of cancer it is contemplated that the anti-CD38-attenuated interferon alpha-2b fusion construct may be administered in combination with an alkylating agent (such as mechlorethamine, cyclophosphamide, chlorambucil, ifosfamidecysplatin, or platinum-containing alkylating-like agents such as cisplatin, carboplatin and oxaliplatin), an antimetabolite (such as a purine or pyrimidine analogue or an antifolate agent, such as azathioprine and mercaptopurine), an anthracycline (such as Daunorubicin, Doxorubicin, EpirubicinIdarubicin, Valrubicin, Mitoxantrone, or anthracycline analog), a plant alkaloid (such as a vinca alkaloid or a taxane, such as Vincristine, Vinblastine, Vinorelbine, Vindesine, paclitaxel or Dosetaxel), a topoisomerase inhibitor (such as a type I or type II topoisomerase inhibitor), a Podophyllotoxin (such as etoposide or teniposide), or a tyrosine kinase inhibitor (such as imatinibmesylate, Nilotinib, or Dasatinib).

[0110] In the case of the treatment of multiple myeloma, an anti-CD38-attenuated interferon alpha-2b fusion construct may be administered in combination with other suitable therapies, such as treatment of the subject with the administration of steroids such as dexamethasone, proteasome inhibitors (such as bortezomib or carfilzomib), immunomodulatory drugs (such as thalidomide, lenalidomide or pomalidomide), or induction chemotherapy followed by autologous hematopoietic stem cell transplantation, with or without other chemotherapeutic agents such as Melphalan hydrochloride or the chemotherapeutic agents listed above.

[0111] In the case of the treatment of Hodgkin's lymphoma, an anti-CD38-attenuated interferon alpha-2b fusion construct may be administered in combination with current therapeutic approaches, such as ABVD (Adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine), or Stanford V (doxorubicin, bleomycin, vinblastine, vincristine, mechlorethamine, etoposide, prednisone), or BEACOPP (doxorubicin, bleomycin, vincristine, cyclophosphamide, procarbazine, etoposide, prednisone).

[0112] In the case of non-Hodgkin's lymphoma or other lymphomas, an anti-CD38-attenuated interferon alpha-2b fusion construct may be administered in combination current therapeutic approaches. Examples of drugs approved for non-Hodgkin lymphoma include Abitrexate (Methotrexate), Adriamycin PFS (Doxorubicin Hydrochloride), Adriamycin RDF (Doxorubicin Hydrochloride), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Arranon (Nelarabine), Bendamustine Hydrochloride, Bexxar (Tositumomab and Iodine I 131 Tositumomab), Blenoxane (Bleomycin), Bleomycin, Bortezomib, Chlorambucil, Clafen (Cyclophosphamide), Cyclophosphamide, Cytoxan (Cyclophosphamide), DenileukinDiftitox, DepoCyt (Liposomal Cytarabine), Doxorubicin Hydrochloride, DTIC-Dome (Dacarbazine), Folex (Methotrexate), Folex PFS (Methotrexate), Folotyn (Pralatrexate), Ibritumomab Tiuxetan, Istodax (Romidepsin), Leukeran (Chlorambucil), Linfolizin (Chlorambucil), Liposomal Cytarabine,

Matulane (Procarbazine Hydrochloride), Methotrexate, Methotrexate LPF (Methotrexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Mozobil (Plerixafor), Nelarabine, Neosar (Cyclophosphamide), Ontak (DenileukinDiftitox), Plerixafor, Pralatrexate, Rituxan (Rituximab), Rituximab, Romidepsin, Tositumomab and Iodine I 131 Tositumomab, Treanda (Bendamustine Hydrochloride), Velban (Vinblastine Sulfate), Velcade (Bortezomib), and Velsar (Vinblastine Sulfate), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vorinostat, Zevalin (IbritumomabTiuxetan), Zolinza (Vorinostat). Examples of drug combinations used in treating non-Hodgkin lymphoma include CHOP (C = Cyclophosphamide, H = Doxorubicin Hydrochloride (Hydroxydaunomycin), O = Vincristine Sulfate (Oncovin), P = Prednisone); COPP (C = Cyclophosphamide, O = Vincristine Sulfate (Oncovin), P = Procarbazine Hydrochloride, P = Prednisone); CVP (C = Cyclophosphamide, V = Vincristine Sulfate, P = Prednisone); EPOCH (E = Etoposide, P = Prednisone, O = Vincristine Sulfate (Oncovin), C = Cyclophosphamide, H = Doxorubicin Hydrochloride (Hydroxydaunomycin)); ICE (I = Ifosfamide, C = Carboplatin, E = Etoposide) and R-CHOP (R = Rituximab, C = Cyclophosphamide, H = Doxorubicin Hydrochloride (Hydroxydaunomycin), O = Vincristine Sulfate (Oncovin), P = Prednisone.

[0113] An anti-CD38 antibody, or an anti-CD38-attenuated interferon alpha-2b fusion construct may be used to detect CD38-positive cells, including CD38-positive tumor cells. They may be used in methods for detecting a CD38-positive tumor cell in a tissue sample isolated from a subject, which methods may generally comprise contacting an anti-CD38 antibody, or an anti-CD38-attenuated interferon alpha-2b fusion construct, with a tissue sample isolated from a subject and detecting a complex of the antibody or construct and a CD38-positive cell in the tissue sample. The tissue sample preferably is blood. The cell may be a CD38-positive B-cell lymphoma cell, multiple myeloma cell, non-Hodgkin's lymphoma cell, chronic myelogenous leukemia cell, chronic lymphocytic leukemia cell, or acute myelogenous leukemia cell. The method may further comprise isolating the tissue sample from the subject.

[0114] The disclosure also features kits comprising any of the antibodies and anti-CD38-attenuated interferon alpha-2b fusion constructs described and exemplified herein. The kits may be used to supply antibodies and other agents for use in diagnostic, basic research, or therapeutic methods, among others.

[0115] A kit may comprise an anti-CD38-attenuated interferon alpha-2b fusion construct, the construct optionally comprised in a composition comprising a pharmaceutically acceptable carrier, and instructions for using the kit in one or more of a method for inhibiting or reducing proliferation of a tumor cell expressing CD38 on its surface, a method for inducing apoptosis in a tumor cell expressing CD38 on its surface, and/or a method for treating a tumor that comprises and/or is mediated by cells expressing CD38 on their surface. Such methods may be any method described or exemplified herein. The kits may comprise a pharmaceutically acceptable carrier. The kits may comprise one or more pharmaceutically acceptable auxiliaries and/or one or more pharmaceutically acceptable excipients. In the kits, the anti-CD38 antibody may be any antibody described or exemplified herein, and the attenuated interferon alpha-2b may comprise any attenuated interferon alpha-2b described or exemplified herein. The

constructs may be comprised in sterile solutions ready for injection or intravenous administration, or may comprise a sterile, lyophilized form ready to be combined with a carrier just prior to use.

[0116] A kit may comprise an anti-CD38 antibody and instructions for using the kit in a method for detecting CD38-positive cells in a sample, including a tissue sample isolated from a subject. The anti-CD38 antibody may be any antibody described or exemplified herein. The antibody may optionally be fused to an attenuated interferon alpha-2b protein.

[0117] The following examples are provided to describe the disclosure in greater detail. They are intended to illustrate, not to limit, the disclosure.

Reference Example 1

Optimization of X355/02-HC-L0-IFN-alpha (A145D) IgG4

[0118] Other anti-CD38-attenuated IFN fusion proteins are described in PCT Application No. PCT/AU2012/001323. These include the antibody construct designated in the PCT application as X355/02-HC-L0- IFN-alpha (A145D) IgG4. In this specification, X355/02-HC-L0-IFN-alpha (A145D) IgG4 has been renamed as A02.1. The heavy chain sequence of the antibody comprises the amino acid sequence of SEQ ID NO: 11, and the light chain sequence comprises the amino acid sequence of SEQ ID NO: 12. The variable light chain of A02.1 (SEQ ID NO: 14) was co-expressed with its variable heavy chain A02.1 (SEQ ID NO: 13) formatted on a human IgG4 constant region containing the substitution S228P (EU Numbering) (SEQ ID NO: 3). This antibody is referred to herein as X02.1. A02.1 includes a fusion to IFN-alpha2b whilst X02.1 does not, despite both antibodies sharing identical heavy chain and light chains sequences.

[0119] A BLAST search (Altschul SF (1997) Nucleic Acids Res. 25:3389-3402) against a database of human germline immunoglobulin genes was performed using the amino acid sequence of the variable heavy chain of X02.1. The closest human germline variable heavy chain gene was IGHV4-61*01 (SEQ ID NO: 16). An alignment of the X02.1 VH and IGHV4-61*01 is shown in Figure 2. The X02.1 variable heavy region differs by eight amino acids from its closest germline amino acid sequence. In order to reduce the immunogenicity of the X02.1 heavy chain variable region, germline amino acid residue substitutions could be produced at residues where it differs from the germline sequence and the resulting antibody variants tested for anti-CD38 binding activity.

[0120] Several heavy chain variants of the X02.1 parental sequence are detailed in Figure 2. These heavy chain variable regions were formatted onto the IgG4 S228P constant region, and co-expressed with the A02.1 light chain. Tables 1a and 1b detail the sequences of the variants

tested along with their ability to bind human CD38 as assessed using flow cytometry and surface plasmon resonance (SPR). Briefly, antibody chains were transiently co-expressed in CHO cells and purified via Protein A chromatography as described in Example 5. Flow binding assays as described in Example 5 were used to assess the variants. The EC_{50} of the dose response curve obtained for each antibody is also given in Tables 1a and 1b.

Table 1a

Antibody Designation	**Variable Heavy Chain Amino Acid Substitution (Relative to X02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**	**CD38 binding by SPR**	**ARP-1 flow binding assay (EC_{50} in µg/mL)**
X02.8	L74S	17	14	2.30×10^{-8}	18.3
X02.9	H40P	18	14	2.63×10^{-8}	N/T
X02.10	T(82A)S	19	14	2.07×10^{-8}	N/T
X02.11	L74S, 178F R81K, T(82A)S	20	14	2.39×10^{-8}	18.1
X02.108	178F	32	14	2.63×10^{-8}	N/T
X02.110	R81K	33	14	2.07×10^{-8}	N/T
N/T- Protein was not able to be purified and was not tested.					

Table 1b

Antibody Designation	**Variable Heavy Chain Amino Acid Substitution (Relative to X02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO: [Verify]**	**CD38 binding by SPR**	**ARP-1 flow binding assay (EC_{50} in µg/mL)**
X02.69	Q2V	22	14	3.68×10^{-11}	16.8
X02.71	I29V	24	14	1.29×10^{-10}	3.5
X02.78	S32G	31	14	2.04×10^{-11}	N/T
N/T - Protein not purified or tested.					

[0121] SPR binding of the variants detailed in Table 1a was evaluated separately to those of Table 1b. The K_D (M)of the parental antibody X02.1 ranged from 2.7×10^{-8} to 3.78×10^{-10} in the SPR binding experiments. Flow cytometry binding experiments showed antibodies X02.8,

X02.11, X02.69 and X02.71 bound strongly to the CD38 positive cell line ARP-1.

[0122] Antibodies with the above amino acid substitutions were subsequently explored in the context of a fusion protein through conjugation to attenuated IFN-alpha2b (termed A02 when linked to IFN, with the number following the decimal representing the same variant having the X02 designation). These heavy chain variable regions were formatted onto an IgG4 constant region comprising the substitution S228P fused to A145D attenuated IFN-alpha2b and co-expressed in CHO or HEK cells with the A02.1 light chain as described in Example 5. Proteins that were successfully purified from cell supernatant were then tested in a flow binding assay to the cell line ARP-1. The EC_{50} value of the dose response curve for each antibody is given in Table 2. All antibody-attenuated IFN fusion constructs tested bound to the CD38 positive cell line ARP-1. It was observed that heavy chain variant X02.9 (not fused to IFN) could not easily be purified whereas an identical variant fused to IFN (A02.9) was purified. In some cases, attenuated IFN fusion proteins could be expressed and purified, when the equivalent monoclonal antibody appeared more difficult to be expressed and/or purify.

Table 2

Anti-CD38-attenuated IFN fusion protein	**Variable Heavy Chain Amino Acid Substitution (Relative to A02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**	**Protein A capture by SPR (RU)***	**CD38 binding by SPR (RU) at 350 sec***	**ARP-1 flow binding assay (EC_{50} in µg/mL)**
A02.8	L74S	17	14	4697	833	1.9
A02.9	H40P	18	14	4718	841	1.0
A02.10	T(82A)S	19	14	4647	804	1.5
A02.11	L74S, I78F, R81K, T(82A)S	20	14	4483	827	3.5

*The amount of Anti-CD38 attenuated IFN fusion protein in the cell culture supernatant is indicated by the Protein A capture by SPR. The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase.

[0123] BLAST searches using the amino acid sequence of the A02.1 variable light chain were performed against the database of human germline immunoglobulin genes. The closest human germline variable light chain gene was IGLV5-37*01. An amino acid sequence alignment of A02.1VL and IGLV5-37*01 is given in Figure 3. This alignment illustrates a 12 amino acid difference between these sequences.

[0124] Several amino acid substitutions were made in the X02.1 variable light chain. These substitutions are shown in Figure 3. Co-expression of these light chain variable regions with the X02.1 variable heavy chain formatted onto an IgG4 constant region containing the substitution S228P was performed in CHO cells as described in Example 5.

[0125] Antibodies purified from CHO cell supernatants were subsequently tested in flow cytometry-based binding assays to the CD38 positive cell line ARP-1. Table 3 details EC_{50} values of the dose response curve obtained for each antibody.

Table 3

Antibody Designation	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	CD38 binding by SPR (KD)	ARP-1 flow binding assay (EC_{50} in µg/mL)	Protein A HPLC mg/L
X02.95	A2P	13	53	4.40×10^{-12}	N/T	11.1
X02.96	A8P	13	54	2.50×10^{-10}	30.0	19.9
X02.97	L11S	13	55	7.51×10^{-12}	Low Binding	15.0
X02.98	R29S	13	56	5.84×10^{-12}	N/T	8.7
X02.99	Y30S	13	57	3.21×10^{-12}	4.4	11.5
X02.100	H(54A)D	13	58	1.60×10^{-7}	Low Binding	18.9
X02.101	V(66B)A	13	59	2.89×10^{-11}	13.7	21.0
X02.102	T68A	13	60	1.20×10^{-10}	20.4	18.0
X02.103	S70T	13	61	1.28×10^{-9}	9.4	27.9
X02.104	T90I	13	62	1.0×10^{-8}	9.7	23.1
X02.105	S92P	13	63	3.31×10^{-8}	12.7*	19.3
X02.106	G95A	13	64	4.47×10^{-8}	N/T	11.6

N/T- Protein not purified or not tested.
Low Binding - Minimal binding observed, not sufficient for an EC_{50} value.

*Antibody was tested in a flow binding assay against H929 cell line. Reported value is the EC_{50} in µg/mL.

[0126] Antibodies X02.96, X02.99, X02.101, X02.102, X02.103 and X02.104 bound strongly to the CD38 positive ARP-1 cell line. X02.105 was able to bind strongly to the CD38 positive, H929 cell line.

[0127] Amino acid sequence analysis of the variable heavy chain sequence of X02.1 and A02.1 identified amino acids that could potentially undergo oxidation or isomerization. These include a potential isomerization site at D101 and a potential oxidation site at M(100C). To remove the potential isomerization and oxidation sites, amino acid substitutions were made as follows: D(101)E (SEQ ID NO: 30), M(100C)L (SEQ ID NO: 29) and the combination of both D(101)E and M(100C)L (SEQ ID NO: 27) (Figure 2). Antibodies were made with combinations of these amino acid substitutions in the variable heavy chain as shown in Table 4. Antibody heavy chain variable regions were formatted with an IgG4 constant region containing the substitution S228P and co-expressed with the A02.1 light chain in CHO cells. Antibodies were then purified by Protein A chromatography and screened for binding to ARP-1 cells by flow cytometry. The binding data obtained is shown in Table 4.

Table 4

Antibody Designation	**Variable Heavy Chain Amino Acid Substitution (Relative to X02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**	**CD38 binding by SPR (KD)**	**ARP-1 flow binding assay (EC_{50} in µg/mL)**	**Protein A HPLC mg/L**
X02.76	M(100C)L	29	14	1.58×10^{-13}	4.1	24.8
X02.77	D101E	30	14	9.11×10^{-12}	3.7	15.6
X02.74	M(100C)L, D101E	27	14	6.85×10^{-11}	N/T	21.8
N/T - Protein was not purified or not tested.						

[0128] Antibodies X02.76 and X02.77 maintained their strong binding to the ARP-1 cell line indicating that the amino acids substitutions to remove the potential oxidation and isomerization sites in the X02.1 and A02.1 heavy chain had little impact on their CD38 binding activity. Combining these substitutions to form antibody X02.74 resulted in an antibody that did not purify using the protocol in Example 5.

[0129] Amino acid analysis of the variable light chain sequence of X02.1 and A02.1 identified amino acids that could potentially undergo oxidation or deamidation. These included a potential deamidation site at N69 and potential oxidation site at M89. Additionally, a putative N-linked glycosylation site was predicted to exist within CDR3 of the light chain at position N94. The presence of N-linked glycans can cause heterogeneity in therapeutic proteins, complicating development. To remove these potential issues the following point variants were

synthesized: N69A (SEQ ID NO: 39), M89L (SEQ ID NO: 52) and M89I (SEQ ID NO: 51), N94T (SEQ ID NO: 48), N94Q (SEQ ID NO: 38), G95P (SEQ ID NO: 50) and S96A (SEQ ID NO: 45) (see Figure 3). Antibodies were generated by co-expression of the heavy- and light chains in CHO cells as detailed in Table 5. Antibodies were purified by Protein A chromatography and screened for binding to ARP-1 cells by flow cytometry. The binding data obtained is presented in Table 5.

Table 5

Antibody Designation	**Variable Light Chain Amino Acid Substitution (Relative to A02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**	**CD38 binding by SPR**	**ARP-1 flow binding assay (EC_{50} in µg/mL)**	**Protein A HPLC mg/L**
X02.81	N69A	13	39	2.67×10^{-10}	N/T	12.6
X02.93	M89I	13	51	2.56×10^{-11}	18.7	20.0
X02.94	M89L	13	52	3.48×10^{-12}	8.7	18.9
X02.90	N94T	13	48	5.52×10^{-10}	26.2	23.0
X02.80	N94Q	13	38	1.44×10^{-9}	13.2	30.3
X02.92	G95P	13	50	Low Binding	Low Binding	18.1
X02.87	S96A	13	45	1.99×10^{-9}	37.5	18.9
N/T - Protein was not purified or tested. Low Binding - Minimal binding observed, not sufficient for an EC_{50} or KD value.						

[0130] X02.94 bound the CD38 positive cell line ARP-1 indicating that the substitution M89L had little impact on CD38 binding activity. The substitution N94Q in antibody X02.80 removed the potential N-linked glycosylation motif with minimal impact on CD38 binding activity as measure by flow cytometry (Table 5). Other substitutions that remove this glycosylation motif either resulted in antibodies that could not easily be purified or antibodies that exhibited attenuated binding to the CD38 positive cell line ARP-1. The potential deamidation site at position 69 was removed through substitution to alanine, though this antibody (X02.81) was not easily purified.

[0131] Other antibodies tested that comprised X02.1 variable heavy chain variants are listed in Table 6. These heavy chain variable regions were formatted on an IgG4 constant region

containing an S228P substitution. These heavy chains were co-expressed with the A02.1 light chain in CHO cells. The antibodies were expressed and the resulting antibodies tested in flow cytometry-based assays for binding to the CD38-positive cell ARP-1. All variable heavy chain substitutions with the exception of T23K (SEQ ID NO: 21; X02.68) had minimal impact on binding to the CD38 positive cell line ARP-1 in flow cytometry-based assays.

Table 6

Antibody Designation	**Variable Heavy Chain Amino Acid Substitution (Relative to X02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**	**CD38 binding by SPR**	**ARP-1 flow binding assay (EC_{50} in µg/mL)**	**Protein A HPLC mg/L**
X02.73	S19K	26	14	3.85×10^{-10}	4.3	20.6
X02.68	T23K	21	14	1.06×10^{-11}	N/T	17.0
X02.70	V71R	23	14	3.54×10^{-9}	7.2	32.8
X02.75	T73K	28	14	6.38×10^{-10}	4.9	17.8
X02.72	T83R	25	14	1.63×10^{-9}	3.6	30.6
N/T- Protein not purified or tested.						

[0132] Antibodies comprising other light chain variable region substitutions in the X02.1 sequence were also produced. These variant light chains were combined with the X02.1 heavy chain formatted onto an IgG4 constant region containing the substitution S228P and expressed in CHO cells as described in Example 5. A summary of the heavy- and light chains used to produce these antibody variants is given in Table 7. Antibodies X02.83, X02.85, X02.91, X02.82 bound strongly to the CD38 positive cell line ARP-1.

Table 7

Antibody Designation	**Variable Light Chain Amino Acid Substitution (Relative to A02.1)**	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**	**CD38 binding via SPR**	**ARP-1 flow binding assay (EC_{50} in µg/mL)**	**Protein A HPLC mg/L**
X02.83	E17A	13	41	2.69×10^{-9}	8.2	32.8
X02.86	D(27A)G	13	44	4.28×10^{-9}	120.4	30.0

Antibody Designation	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	CD38 binding via SPR	ARP-1 flow binding assay (EC_{50} in µg/mL)	Protein A HPLC mg/L
X02.85	ΔD(L66A)*** ΔV(L66B)***	13	43	2.70 $\times 10^{-10}$	6.3	17.6
X02.107	E83I, D85T	13	65	2.47 $\times 10^{-8}$##	N/T	10.0*
X02.91	P26R	13	49	7.07 $\times 10^{-10}$	17.6	21.1
X02.88	N32R	13	46	Low binding	Low Binding	33.5
X02.82	Y49R	13	40	N/T	3.8	27.9
X02.89	Y51R	13	47	Low binding	No Binding	25.7
X02.84	Y49R, Y51R	13	42	Low binding	No Binding	35.0

N/T- Protein was not purified or tested.
Low Binding - Minimal binding observed, not sufficient for an EC_{50} value.

***Δ indicates that this amino acid present in A02.1 light chain was removed from this sequence.

*estimated protein value based on protein A capture level by SPR.

##The SPR binding for X02.107 was evaluated in a separate experiment in which the KD of the parental antibody X02.1 was 2.7×10^{-8}. The KD of the parental antibody X02.1 is 3.78×10^{-10} in the SPR binding experiment for all other antibodies tested.

[0133] Substitutions causing little impact on CD38 binding activity and the purification of X02 variant antibodies were subsequently produced as armed antibodies through fusion to A145D attenuated IFN-alpha2b. X02.1 light chain substitutions were combined and the resulting variants co-expressed with point- and combinatorial variants of the X02.1 heavy chain in HEK293E cells, as listed in Table 8. These antibodies were primarily focused on removing the potential X02.1 light chain deamidation site, an oxidation site from CDR3 of the X02.1 heavy chain and a putative strong MHC Class II binding peptide from framework region 3 of the X02.1 heavy chain predicted via *in silico* analyses (Epibase, Lonza, UK), Figure 4.

Table 8

Anti-CD38-attenuated IFN fusion protein	Variable Heavy Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Light SEQ ID NO:
A02.2	None	13	E83I, D85T	65
A02.3	L74S	17	E83I, D85T	65
A02.4	H40P	18	E83I, D85T	65
A02.5	T(82A)S	19	E83I, D85T	65
A02.6	L74S, I78F, R81K, T(82A)S	20	E83I, D85T	65
A02.12	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T	65
A02.13	H40P, L74S, I78F, R81K, T(82A)S	35	E83I, D85T	65
A02.37	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, M89L	66
A02.46	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, N69Q	67
A02.47	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, N69T	68
A02.48	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, N69G	69
A02.49	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, N69H	70
A02.50	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, N69K	71
A02.51	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, N69P	72

[0134] The antibodies listed in Table 8 were analyzed for protein expression and binding to CD38 via surface plasmon resonance (SPR). Potency assays were also performed using cell culture supernatant taken from transfected cells to assess the relative activity of each of these anti-CD38-attenuated IFN fusion proteins as outlined in Example 5. The data obtained is given in Table 9.

Table 9

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)
A02.2	30.2	756	N/T	N/T	N/T
A02.3	21.6	835	N/T	N/T	N/T
A02.4	27.3	809	N/T	N/T	N/T
A02.5	22.0	788	N/T	N/T	N/T
A02.6	33.7	895	N/T	N/T	N/T
A02.12	25.3	N/A	2.7	7.0	236
A02.46	3.1	914	2.2	5.8	1190
A02.47	26.5	1455	3.10	4.44	N/T
A02.48	3.4	921	2.0	5.6	562
A02.49	3.0	946	2.1	5.5	875
A02.50	3.1	809	1.9	6.1	1681
A02.51	1.8	368	2.0	5.8	3741

The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refers to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. N/A - Not Available; N/T - Not Tested.

[0135] Of the proteins tested A02.12 expressed well and demonstrated potency in the Annexin V, Caspase and cell proliferation assays. Substitution of N69T in antibody A02.47 did not affect expression levels or potency in Annexin V or Caspase Assays suggesting that removal of this deamidation site is possible. Substitution N69T could be incorporated into other constructs herein to remove this putative deamidation site with minimal losses in the functional activity of the resulting antibody.

Reference Example 2

***In silico* immunogenicity analysis of the A02.1 light chain amino acid sequence**

[0136] Putative immunogenic epitopes were identified in the light chain variable region amino acid sequence of A02.1 using the Epibase analysis software (Lonza, UK). To remove putative immunogenic epitopes, substitutions were introduced into the A02.1 variable light chain (Figure

4). Light chains with lower predicted immunogenicity were co-expressed in HEK293E cells with the A02.12 heavy chain variable region (SEQ ID NO: 34) formatted onto an IgG4 constant region containing the substitution S228P fused to A145D-attenuated IFN. The antibody variants produced are detailed in Table 10.

Table 10

Anti-CD38-attenuated IFN fusion protein	Variable Heavy Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Light SEQ ID NO:
A02.18	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L47E	73
A02.19	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L47G	74
A02.20	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L47N	75
A02.21	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L47P	76
A02.22	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L47S	77
A02.23	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L48E	78
A02.24	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, L48P	79
A02.25	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, Y49E	80
A02.26	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, Y49Q	81
A02.27	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, Y50P	82
A02.28	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, Y50N	83
A02.29	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, Y50T	84
A02.30	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, Y51D	85
A02.31	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, S52E	86
A02.32	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, S52H	87
A02.33	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, S52Q	88
A02.34	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, H(54A)N	89

Anti-CD38-attenuated IFN fusion protein	Variable Heavy Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Light SEQ ID NO:
A02.35	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, H(54A)P	90
A02.36	L74S, I78F, R81K, T(82A)S, M(100C)L	34	E83I, D85T, K(54B)D	91

[0137] The above antibodies were analyzed for protein expression, binding to CD38 via SPR, and potency using the cell culture supernatant screen as described in Example 5. The results of these assays are detailed in Table 11. These data indicate that substitution of some residues to lower the predicted immunogenicity of the antibody results in Anti-CD38-attenuated IFN fusion proteins that express and have functional potency in the Annexin V, Caspase and Cell Proliferation Assays.

Table 11

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)
A02.18	9.3	745	N/T	N/T	N/T
A02.19	8.0	741	N/T	N/T	N/T
A02.20	9.1	N/T	2.2	5.6	37
A02.21	3.3	DNB	N/T	N/T	N/T
A02.22	10.4	738	N/T	N/T	N/T
A02.23	15.9	192	2.7	6.9	N/T
A02.24	23.5	87	1.4	2.3	N/T
A02.25	25.7	80	3.0	4.3	2477
A02.26	35.0	383	3.2	6.0	66
A02.27	12.3	DNB	1.3	2.5	29910
A02.28	16.1	422	2.7	6.3	N/T
A02.29	19.7	150	2.7	5.8	133
A02.30	25.2	122	1.9	2.5	N/T
A02.31	28.4	359	3.0	7.1	514
A02.32	13.5	663	2.7	7.7	60
A02.33	11.2	107	N/T	N/T	N/T
A02.34	16.6	407	4.8	5.1	503

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)
A02.35	11.2	738	2.4	4.8	3050
A02.36	16.7	192	2.8	8.0	N/T
The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refers to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. DNB - Did not bind; N/T - Not Tested.					

Reference Example 3

Multiple amino acid substitutions yield optimized A02.1 variants

[0138] By combining substitutions that improve the immunogenicity, manufacturability or potency of the anti-CD38 antibodies described above into a single gene construct, highly optimized anti-CD38 antibodies and anti-CD38-attenuated IFN fusion proteins were obtained. Table 12 summarizes such combinatorial substitutions and details heavy- and light chain combinations co-expressed in HEK293E cells and subsequently tested.

Table 12

Anti-CD38-attenuated IFN fusion protein	Variable Heavy Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Light SEQ ID NO:
A02.16	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S E83I, D85T, M89L, N94Q	92
A02.17	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S E83I, D85T, M89L, N94E	93
A02.52	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52Q, E83I, D85T, M89L, N94E	94
A02.53	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52Q, E83I, D85T, M89L, N94E	95
A02.54	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52Q, E83I, D85T, M89L, N94Q	96

Anti-CD38-attenuated IFN fusion protein	Variable Heavy Chain Amino Acid Substitution (Relative to A02.1)	Variable Heavy SEQ ID NO:	Variable Light Chain Amino Acid Substitution (Relative to A02.1)	Variable Light SEQ ID NO:
A02.55	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52Q, E83I, D85T, M89L, N94Q	97
A02.56	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52E, M89L, E83I, D85T, N94E	98
A02.57	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52E, E83I, D85T, M89L, N94E	99
A02.58	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52E, E83I, D85T, M89L, N94Q	100
A02.59	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52E, E83I, D85T, M89L, N94Q	101
A02.60	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52Q, N69Q, E83I, D85T, M89L, N94E	102
A02.61	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S,S52Q, N69Q, E83I, D85T, M89L, N94E	103
A02.62	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52Q, N69Q, E83I, D85T, M89L, N94Q	104
A02.63	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52Q, N69Q, E83I, D85T, M89L, N94Q	105
A02.64	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52E, N69Q, E83I, D85T, M89L, N94E	106
A02.65	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52E, N69Q, E83I, D85T, M89L, N94E	107
A02.66	L74S, I78F, R81K, T(82A)S, M(100C)L	34	R29G, Y30S, S52E, N69Q, E83I, D85T, M89L, N94Q	108
A02.67	L74S, I78F, R81K, T(82A)S, M(100C)L	34	Y30S, S52E, N69Q, E83I, D85T, M89L, N94Q	109

[0139] Each antibody described in Table 12 was analyzed for protein expression, binding to CD38 via SPR, and potency using cell culture supernatant. The resulting data is given in Table

13. These results demonstrate that combining substitutions predicted to be beneficial in silicogave rise to some Anti-CD38-attenuated IFN fusion proteins that expressed and had functional potency in the Annexin V, Caspase and Cell Proliferation Assays.

Table 13

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)
A02.14	8.1	1247	2.18	5.47	398
A02.15	29.6	1409	3.92	5.62	491
A02.16	5.7	1050	2.4	7.9	636
A02.17	10.0	1103	3.7	5.7	467
A02.52	2.8	416	2.0	5.4	2665
A02.53	3.0	545	2.2	4.7	6338
A02.54	1.8	250	1.9	5.9	15350
A02.55	2.1	436	2.2	4.5	12740
A02.56	2.0	178	1.7	4.3	13860
A02.57	2.7	345	2.5	6.6	6363
A02.58	2.3	273	1.9	4.9	9142
A02.59	1.2	388	2.0	4.7	6176
A02.60	1.3	DNB	1.5	3.3	185600
A02.61	1.2	DNB	1.7	4.1	65160
A02.62	1.3	DNB	1.8	5.0	55590
A02.63	1.2	DNB	1.7	3.4	152100
A02.64	1.1	DNB	1.8	3.2	89120
A02.65	1.4	DNB	1.6	4.1	37240
A02.66	1.3	DNB	1.6	4.0	57540
A02.67	1.6	DNB	2.3	3.9	82760

The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM DNB - Did not bind; N/T - Not Tested.

Reference Example 4

Pairing of different heavy and light chain Anti-CD38 antibodies

[0140] In order to determine if functional anti-CD38-attenuated IFN fusion proteins could be obtained, the heavy (SEQ ID NO: 110) and light (SEQ ID NO: 112) chains from the antibody X910/12-HC-L0- IFN-alpha (A145D) IgG4 described in PCT/AU2012/001323, and the heavy (SEQ ID NO: 111) and light (SEQ ID NO: 113) chains from the antibody X913/15-HC-L0- IFN-alpha (A145D) IgG4 described in PCT/AU2012/001323, were paired with each other in various combinations and with heavy and lights chains described in the foregoing examples. A summary of the heavy and light chain pairings is provided listed in Table 14.

Table 14

Anti-CD38-attenuated IFN fusion protein	**Variable Heavy SEQ ID NO:**	**Variable Light SEQ ID NO:**
A02.38	34	113
A02.39	34	112
A02.40	111	65
Anti-CD38-attenuated IFN fusion protein	**Variable Heavy SEQ ID NO:**	**Variable Light Seq ID NO:**
A02.41	110	65
X913/15-HC-L0- IFN-alpha (A145D) IgG4	111	113
A02.43	110	113
A02.44	111	112
X910/12-HC-L0- IFN-alpha (A145D) IgG4	110	112

[0141] Each antibody produced was analyzed for protein expression, binding to CD38 via SPR, and potency using the cell culture supernatant potency assays. The results of these assays are presented in Table 15a. These data show that pairing different heavy and light chains from distinct antibodies gave rise to some Anti-CD38-attenuated IFN fusion proteins that expressed and had functional potency in the Annexin V, Caspase and Cell Proliferation Assays.

Table 15a

Anti-CD38-attenuated IFN fusion protein	**Protein A HPLC (mg/L)**	**CD38 binding by SPR (RU) at 350 sec***	**Annexin V Assay (Fold change relative to untreated cells)**	**Caspase Assay (Fold change relative to untreated cells)**
A02.38	2.9	DNB	0.9	4.3
A02.39	21.3	66	2.5	6.6
A02.40	2.1	DNB	1.0	4.4

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)
A02.41	50.0	165	2.6	6.7
X913/15-HC-L0- IFN-alpha (A145D) IgG4	8.2	96	2.2	5.4
A02.43	2.5	DNB	0.9	4.1
A02.44	2.2	DNB	1.0	4.5
X910/12-HC-L0- IFN-alpha (A145D) IgG4	27.1	125	2.6	5.9
The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. DNB - Did not bind.				

[0142] A selection of the above Anti-CD38-attenuated IFN fusion proteins were purified and analyzed for binding to CD38 positive cells in cell based assays. In addition, potency assays were repeated to give a more accurate determination of the relative activity of each Anti-CD38-attenuated IFN fusion protein. The results of these assays are given in Table 15b.

Table 15b

Anti-CD38-attenuated IFN fusion protein	ARP-1 Flow binding (EC_{50} in µg/mL)	Annexin V Assay(Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC50 (pM)	Figures
A02.1	1.45	1.86	4.2	278.2	13, 14, 15, 16, 17, 18, 19
A02.2	2.46	1.94	4.4	175.7	13, 19
A02.3	0.96	1.86	3.5	254.9	13, 19
A02.4	1.50	1.88	3.8	198.3	13, 19
A02.5	1.45	1.88	4.3	146.3	13, 19
A02.6	1.42	2.03	3.5	102.3	13, 15, 19, 20
A02.8	1.93	1.91	3.8	125.5	13, 19
A02.9	1.03	1.96	3.6	107.1	13, 19

Anti-CD38-attenuated IFN fusion protein	ARP-1 Flow binding (EC_{50} in µg/mL)	Annexin V Assay(Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC50 (pM)	Figures
A02.10	1.48	1.95	3.8	125.6	13, 19
A02.11	3.48	1.98	3.9	374	13, 19
A02.12	3.40*	1.40	3.4	23.66	14,16, 19, 21
A02.14	10.01*	1.82	2.5	398.20	19
A02.15	1.97*	3.09	6.8	491.70	19
A02.16	3.89*	2.66	5.2	636.80	14, 19
A02.17	9.32*	2.23	3.2	467.1	14, 19
A02.18	1.64	1.55	3.7	78.72	19
A02.19	1.07	1.63	3.5	230.3	19
A02.20	15.92*	1.61	2.9	36	19
A02.22	1.58	1.91	3.8	207	19
A02.25	0.37*	1.42	2.4	2477	19
A02.26	37.99*	1.56	2.0	66	19
A02.27	LB*	1.06	1.0	29910	14, 16, 19
A02.29	0.48*	1.55	3.0	133	19
A02.31	0.26*	1.78	2.1	514	14, 16, 19
A02.32	LB*	1.83	3.3	605	19
A02.33	1.54	1.96	3.7	741	16, 19
A02.34	0.89*	3.06	4.7	503	19
A02.35	3.44*	1.71	1.5	3050	19
A02.37	LB*	2.05	4.3	128	19
A02.39	LB*	1.92	4.3	3714	19
A02.41	0.78*	2.01	3.4	554	14, 19
A02.42	LB*	1.52	3.3	310	19
A02.45	0.71*	1.66	1.6	1697	19
A02.47	16.35*	1.53	4.69	144.3	19

The flow binding refers to the concentration of antibody required to achieve 50% of maximal mean fluorescence intensity. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. LB - low binding, not sufficient for an EC_{50} value.

*Antibody was tested in a flow binding assay against H929 cell line. Reported value is the EC_{50} in µg/mL.

[0143] Figure 5 lists the consensus variable heavy chain and Figure 6 lists the consensus variable light chain of A02.1 related constructs with functional activity. It could be further envisioned that combinations of substitutions could be made such as those described for Anti-CD38 antibodies X02.114, X02.115, X02.116, X02.117, X02.118, X02.119 (Figure 6), X02.120, X02.121, X02.122, X02.123, X02.124, X02.125, X02.126 or X02.127 (Figure 30). Further the above Anti-CD38 antibodies could also be constructed as Anti-CD38-attenuated IFN fusion proteins and tested for functional activity as described herein.

H929 multiple myeloma xenograft model

[0144] The *in vivo* potency of A02.1 has been tested previously in the NCI- H929 s.c. multiple myeloma model as described in Example 5. A02.1 was shown to have potent anti-tumor activity. The data is presented in PCT/AU2012/001323.

[0145] The H929 multiple myeloma xenograft model could be used to test the anti-tumor activity of any of the Anti-CD38-attenuated IFN fusion proteins described above.

Attenuated IFN is required for potent apoptotic and caspase activation in tumor cell lines

[0146] Using the Annexin V assay and the Caspase Assay it was demonstrated that the potent apoptotic activity and caspase activation is dependent on the Anti-CD38-attenuated IFN fusion proteins containing an attenuated IFN (Table 16a, Figure 18). In the Annexin V Assay the attenuated IFN containing proteins (A02.1 and A02.6) had 2-fold greater activity than the proteins not containing attenuated IFN (X02.1 and X02.6).

Table 16a

Anti-CD38-attenuated IFN fusion protein	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)
A02.1	3.57	5.60
X02.1	1.50	2.34
A02.6	2.03	3.5

Anti-CD38-attenuated IFN fusion protein	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)
X02.6	1.04	0.40
Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM.		

Example 1

General Methods

[0147] Production of antibodies and antibody-fusion constructs in HEK-293E cells. DNA plasmids encoding protein constructs (antibodies and antibody-IFN-alpha2b related constructs) were prepared using HiSpeed Plasmid Maxi Kit (Qiagen, Valencia, CA) and then transfected into HEK293E cells (CNRC, Montreal, Canada), grown in F17 synthetic medium supplemented with 0.45% (w/v) D-(+)-Glucose (Sigma, Castle Hill, NSW), 25 µg/mL Geneticin (Invitrogen, Carlsbad, CA), and 1 × GlutaMAX (Invitrogen, Carlsbad, CA) using a commercially available transfection reagent and OptiMEM medium (Invitrogen, Carlsbad, CA). After allowing for expression for 6 days in an incubator supplied with 5% CO_2 and 120 rpm shaking, the culture media was isolated and subjected to affinity purification using Protein A Mab Select SuRe™ agarose beads (GE Healthcare, Piscataway, NJ). Purified protein constructs were buffer-exchanged into 0.2M arginine HCl, 25mM citric acid, 71.5mM sodium hydroxide at pH 6.0 using a PD Midi-Trap G-25 column (GE Healthcare, Piscataway, NJ) or a HiPrep 26/10 Desalting column (HiTrap Desalting HiPrep 26/10 Desalting). Purified protein constructs were then concentrated using 50 kDa Amicon Ultra centrifugal filter devices (Millipore, Billerica, MA), followed by protein concentration determination by reading absorbance at 280 nm.

Production of antibodies and antibody-fusion constructs in CHO cells.

[0148] DNA plasmids encoding protein constructs (antibodies and antibody-IFN-alpha2b related constructs) were prepared using HiSpeed Plasmid Maxi Kit (Qiagen, Valencia, CA) and then transfected into CHO cells (Lonza) grown in Freestyle™ CHO Expression Medium (Invitrogen, Carlsbad, CA) using a commercially available transfection reagent and OptiPro SFM™ medium (Invitrogen, Carlsbad, CA). After allowing for expression for 6 days in an incubator supplied with 10% CO_2 and 120 rpm shaking, the culture media was isolated and subjected to affinity purification using Protein A Mab Select SuReagarose beads (GE Healthcare, Piscataway, NJ). Purified protein constructs were buffer-exchanged into 0.2M

arginine.HCl, 25mM citric acid, 71.5mM sodium hydroxide at pH 6.0 using a PD Midi-Trap G-25 column (GE Healthcare, Piscataway, NJ) or a HiPrep 26/10 Desalting column (HiTrap Desalting HiPrep 26/10 Desalting). Purified protein constructs were then concentrated using 50 kDa Amicon Ultra centrifugal filter devices (Millipore, Billerica, MA), followed by protein concentration determination by reading absorbance at 280 nm.

[0149] Anti-CD38-attenuated IFN fusion proteins binding to CD38 as measured by Surface Plasmon Resonance (SPR). The capacity of anti-CD38 antibodies and anti-CD38-attenuated IFN fusion proteins to bind to human CD38 were measured using unpurified transfected cell supernatant prepared 7:1 with Non Specific Binding Reducer (GE Healthcare, Piscataway, NJ). Briefly, using a Biacore™ 3000 or a T200, Protein A was immobilized onto Flow Cell (FC) 1 (FC1) and FC2 (or alternatively FC3 and FC4) of a CM5 research grade sensor chip using amine coupling, giving approximately 1500 RU. FC2 (or FC4) was used as a reference throughout the experiments. The experiments were run at 37° C in HBS-P+ buffer (0.01 M HEPES, 0.15 M NaCl, 0.005% v/v Surfactant P20, pH 7.4). At a flow rate of 20 µl/min, both flow cells were regenerated with 10µL 50mM sodium hydroxide before 40 µL supernatant containing the protein was passed over FC1 (or FC3) only. 30µL of CD38 (10 µg/mL in running buffer) or 30 µL running buffer was injected over FC1 and FC2 with a 5 minute dissociation time. Both surfaces were regenerated twice with sodium hydroxide. Results were generated using the BIAevaluation software provided with the machine. Microsoft Excel was used for calculations. BIAevaluation software automatically subtracted the reference sensorgram giving a trace of FC2-1 (or FC4-3) for each sample. A double reference was performed for each antibody tested by subtracting the sensorgram with a CD38 injection from the sensorgram with a blank running buffer injection. The Protein A capture refers to the response units measured from a sensorgram at a fixed timepoint of 412.5s and this corresponds to the level of protein captured on the Protein A surface. CD38 binding is the response units measured at 507.5s and is an indication of the level of bound CD38 to the protein captured sensor. CD38 dissociation is the response units measured at 865.5s and is an indication of the level of CD38 bound to the protein captured surface after approximately 300s of dissociation phase. BIAevalution was used to fit the sensorgram using a Langmuir 1:1 equation in order to generate an equilibrium association constant (KD)

Protein A HPLC.

[0150] Supernatants were analyzed by Protein A HPLC using a POROS A/20 2.1x30mm Id column (Applied Biosystems) connected to an Agilent 1100 chromatography system. The column was equilibrated with PBS pH7.4, and protein was eluted with PBS adjusted to pH 2.2. A standard curve was generated using known amounts of a monoclonal antibody in PBS. The chromatograms, at the wavelengths of 215 nm or 280 nm, were integrated using the manufacturer's software and the area under the curve (AUC) reported and interpolated against the generated standard curve to estimate concentration.

Flow cytometry binding of antibodies and Anti-CD38-attenuated IFN fusion proteins to

a human CD38 positive cell line, ARP-1 and H929.

[0151] The multiple myeloma cell line ARP-1 was a gift from Bart Barlogie MD, PhD, Director of the Myeloma Institute at the University of Arkansas Medical Center (Little Rock, AK). It is described in Hardin J. et al. (1994) Blood. 84:3063-70). The multiple myeloma cell line NCI-H929 (H929) was purchased from ATCC (CRL-9068, Gazdar, Blood 67: 1542-1549, 1986).

[0152] The ability of the antibodies or antibody-interferon constructs to bind the human CD38-positive myeloma cell lines ARP-1 or H929 in flow cytometry-based assays was tested. ARP-1 cells or H929 cells (5×10^5, as judged by trypan blue exclusion) were incubated with each protein or with a human IgG4 monoclonal antibody with irrelevant specificity protein construct at various concentrations in 50 µL of FACS buffer (PBS plus 1% fetal calf serum, FCS, 0.2M HEPES, 0.5M EDTA) in 96 well plates for 60 minutes on ice in the dark. Cells were washed three times with FACS buffer before incubation for 30 minutes in 50 µL of FACS buffer containing goat anti-human IgG (Fc-specific, conjugated to fluorescein isothiocyanate, FITC; Sigma-Aldrich, St. Louis, MO). After washing three times with FACS buffer, cells were fixed with 50µL of PBS containing 4% formaldehyde v/v and incubated at 4° C in the dark for 16 hours. Incubated cells in suspension were diluted with an additional 150 µL of FACS buffer and analyzed for binding by flow cytometry on a FACS Canto II (BD Biosciences, San Diego, CA) using forward scatter, side scatter and fluorescence intensity in the FITC channel. The value reported is the mean fluorescence intensity (MFI).

Target Assays

[0153] Daudi cell proliferation assay: This assay was used to quantify the anti-proliferative activity of IFNs and antibody-IFN fusion protein constructs on cells that display CD38. Daudi cells express CD38 as a cell surface associated antigen. The viability of cells was measured using the reagent CellTiter-Glo®, Cat #G7570, from Promega (Madison, Wisconsin). This is a luminescence-based assay that determines the viability of cells in culture based on quantitation of ATP. The signal strength is proportional to the number of viable cells in a microtiter plate well. The details of the assay are as follows: Daudi cells (obtained from ATCC, Manassas, VA) were cultured in a T75 flask (TPP, Trasadingen, Switzerland, cat# 90076) to a preferred density of between 0.5×10^5 and 0.8×10^5 viable cells/mL in RPMI 1640 (Mediatech, Inc., Manassas, VA, cat # 10-040- CV) with 10% Fetal Bovine Serum (FBS; Hyclone, Logan, UT cat# SH30070.03). Cells were harvested by centrifuging at 400 × g for five minutes, decanting the supernatant, and resuspending the cell pellet in RPMI 1640 + 10% FBS. Cells were then counted and the density was adjusted to 3.0×10^5 cells/mL in RPMI 1640 + 10% FBS. Then, 50µL of cell suspension was aliquoted into each well of a 96 well round bottom tissue culture plate (hereafter, "experimental plate") (TPP, cat# 92067). On a separate, sterile 96 well plate (hereafter, "dilution plate"; Costar, Corning, NY cat# 3879), test articles were serially diluted in

duplicate in RPMI 1640 + 10% FBS. Then, 50 µL/well was transferred from the dilution plate to the experimental plate. The experimental plate was then incubated for four days at 37° C with 5% CO_2. A mixture of the manufacturer-supplied assay buffer and assay substrate (hereafter, "CellTiter-Glo® reagent", mixed according to the manufacturer's instructions) was added to the experimental plate at 100 µL/well. The plate was shaken for two minutes.

[0154] Then, 100 µL/well was transferred from the experimental plate to a 96 well flat bottom white opaque plate (hereafter, "assay plate"; BD Biosciences, Franklin 5 Lakes, NJ cat# 35 3296). The content of the assay plate was then allowed to stabilize in the dark for 15 minutes at room temperature. The plate was read on a Victor 3V Multilabel Counter (Perkin Elmer, Waltham, MA, model# 1420-041) on the luminometry channel and the luminescence was measured. Results are presented as "relative luminescence units" (RLU).
Data was analyzed using Prism 5 (Graphpad, San Diego, CA) using non-linear regression and three parameter curve fit to determine the midpoint of the curve (EC50).

[0155] ARP-1 Cell proliferation assay: This assay was used to quantify the anti-proliferative activity of IFNs and antibody-IFN fusion protein constructs against CD38 antigen positive cells. ARP-1 cells express CD38 as cell surface associated antigens. The viability of cells was measured using the reagent CellTiter-Glo®, Cat #G7570, from Promega (Madison, Wisconsin). This is a luminescence-based assay that determines the viability of cells in culture by quantitation of ATP. The signal strength is proportional to the number of viable cells in a microtiter plate well.

[0156] The details of the assay are as follows: ARP-1 cells were cultured in a T175 flask (Costar, Corning, NY Lakes, NJ, cat# CLS431080) to a preferred density of between 2.0×10^5 and 2.0×10^6 viable cells/mL in RPMI 1640 (Life Technologies, Mulgrave, VIC, cat # 11875-093) with 10% Fetal Bovine Serum (FBS; AusGeneX, Molendinar, QLD, Australia cat# FBS500S). Cells were harvested by centrifuging at 400 × g for five minutes, decanting the supernatant, and resuspending the cell pellet in RPMI 1640 + 10% FBS. Cells were then counted and the density was adjusted to 2.0×10^5 cells/mL in RPMI 1640 + 10% FBS. Then, 50 µL of the cell suspension was aliquoted into each well of a 96-well flat bottom white opaque plate (hereafter, "experimental plate"; Costar, Corning, NY Lakes, NJ, cat# CLS3917). On a separate, sterile 96-well plate (hereafter, "dilution plate"; Costar, Corning, NY cat# 3799), test articles were serially diluted in duplicate in RPMI 1640 + 10% FBS. Subsequently, 50 µL/well was transferred from the dilution plate to the experimental plate. The experimental plate was then incubated for four days at 37° C with 5% CO_2. Each experimental plate included the parental antibody IFN construct as the relative control.

[0157] A mixture of the manufacturer-supplied assay buffer and assay substrate (CellTiter-Glo® reagent, mixed according to the manufacturer's instructions) was added to the experimental plate at 100 µL/well. The plate was shaken for two minutes. The content of the assay plate was then allowed to stabilize in the dark for 15 minutes at room temperature. The

plate was read on a FLUOstar Galaxy plate reader (BMG Labtech, Durham, NC) on the luminometry channel and the luminescence was measured. Data was analyzed using Prism 5 (Graphpad, San Diego, CA) using non-linear regression and three parameter curve fit to determine the midpoint of the curve (EC_{50}).

[0158] Annexin V assay: H929 cells were harvested by centrifuging at 400 × *g* for five minutes, decanting the supernatant, and resuspending the cell pellet in RPMI 1640 + 10% FBS. Cells were then counted and the density was adjusted to 1.0 × 10^6 cells/mL in RPMI 1640 + 10% FBS. Then, 50 µL of the cell suspension was aliquoted into each well of 96-well round bottom clear plates (hereafter, "experimental plate;" Costar, Corning, NY cat# CL3799). On a separate, sterile 96-well plate (hereafter, "dilution plate"; Costar, Corning, NY cat# CL3799), test articles were diluted to 40 nM in quaduplicate in RPMI 1640 + 10% FBS. Subsequently, 50 µL/well was transferred from the dilution plate to the experimental plate. The experimental plate was then incubated for 24 hours at 37° C with 5% CO_2. The cells were then centrifuged at 400 × *g* for 5 min, supernatant decanted and resuspended in 100 µL of HEPES buffer containing Annexin V-FITC (1/200) and 7-AAD (1/50). The cells were then incubated for 15 min at room temperature and subsequently analyzed for Annexin V and 7-AAD staining by flow cytometry on a FACS Canto II (BD Biosciences, San Diego, CA) using forward scatter, side scatter, FITC and PerCP-Cy5.5 channels. Annexin V positive cells refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM and is expressed as fold change relative to untreated cells.

[0159] Caspase assay: Activated caspases 2, 3, 6, 7, 8, 9, 10 were measured with the reagent Homogeneous Caspases Assay, fluorimetric Cat #12236869001, from Roche (West Sussex, UK) after treatment with test antibodies. The details of the assay follow.

[0160] ARP-1 cells, which express high levels of CD38, were cultured in a T175 flask (Costar, Corning, NY, cat# CLS431080) to a preferred density of between 2.0 × 10^5 and 2.0 × 10^6 viable cells/mL in RPMI 1640 (Life Technologies, Mulgrave, VIC, cat # 11875-093) with 10% FBS (AusGeneX, Molendinar, QLD, Australia cat# FBS500S). Cells were harvested by centrifuging at 400 × *g* for five minutes, decanting the supernatant, and resuspending the cell pellet in RPMI 1640 Phenol red-free (Life Technologies, Mulgrave, VIC, cat # 11835-030) + 10% FBS. Cells were then counted and the density was adjusted to 2.0 × 10^5 cells/mL in RPMI 1640 Phenol red free + 10% FBS. Then, 50 µL of the cell suspension was aliquoted into each well of a 96-well flat bottom black-walled clear bottom plate (hereafter, "experimental plate"; Costar, Corning, NY cat# CLS3603). On a separate, sterile 96-well plate (hereafter, "dilution plate"; Costar, Corning, NY cat# 3799), test articles were diluted to 40 nM in quadruplicate in RPMI 1640 Phenol red free + 10% FBS. Subsequently, 50 µL/well was transferred from the dilution plate to the experimental plate. The experimental plate was then incubated for 24 hours at 37° C with 5% CO_2. The manufacturer-supplied assay buffer was added to the manufacturer-supplied substrate and mixed according to the manufacturer's instructions to create the "substrate solution." Then, 100 µL of the substrate solution was added to each well of the assay plate. The plate was shaken for 2 minutes. The plate was then incubated at room

temperature for 15 minutes in the dark and finally read on FLUOstar Galaxy plate reader (BMG Labtech, Durham, NC) with an excitation filter 470-500 nm and emission filter 500-560 nm and the fluorescence measured and presented as fold change relative to untreated cells. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM.

Off-Target Assays

[0161] iLite gene reporter assay: The "off-target" iLite assay (PBL Interferon Source, Piscataway, NJ, Cat# 51100) was performed largely as described by the manufacturer, with the addition of a human IgG blocking step. The iLite cell line is described by the manufacturer as "a stable transfected cell line derived from a commercially available pro-monocytic human cell line characterized by the expression of MHC Class II antigens, in particular the human lymphocyte antigen (HLADR), on the cell surface." The cell line contains a stably transfected luciferase gene, the expression of which is driven by an interferon-response element (IRE), which allows for interferon activity to be quantified based on luminescence output. The manufacturer supplied iLite plate (hereafter, assay plate) and diluent were removed from the -80° C freezer and allowed to equilibrate to room temperature. Then, 50 µL of the diluent was added per well to the assay plate. The vial of manufacturer-supplied reporter cells was removed from the -80° C freezer and thawed in a 37° C water bath. Then, 25 µL aliquots of cells were dispensed into each well of the assay plate. Next, 12.5 µL of 8 mg/mL human IgG that was diluted into RPMI 1640 + 10% FBS (Sigma Chemicals, St. Louis, MO; cat# 14506) was added per well. The contents were mixed and incubated at 37° C for 15 minutes. On a separate "dilution plate," test articles were serially diluted in duplicate in RPMI 1640 + 10% FBS. Then, 12.5 µL of the test articles were transferred from the dilution plate to the assay plate. The assay plate was then incubated at 37° C with 5% CO_2 for 17 hours. The manufacturer-supplied assay buffer and substrate were removed from the -80° C freezer and allowed to equilibrate to room temperature for two hours. The manufacturer-supplied assay buffer was added to the manufacturer-supplied substrate vial and mixed well according to the manufacturer's instructions to create the "luminescence solution." Then, 100 µL of the luminescence solution was added to each well of the assay plate. The plate was shaken for 2 minutes. The plate was then incubated at room temperature for 5 minutes in the dark and finally read on a Victor 3V Multilabel Counter on a luminometry channel and the luminescence measured and presented as RLU. The data was analyzed with Graphpad Prism 5 as described for the "on-target (Daudi) assay." To test anti-CD38 antibody-IFN fusion protein constructs in the iLite assay, manufacturer-supplied diluent was supplemented with 0.25 mg/mL anti-CD38 antibody (same antibody clone being tested as an antibody-IFN fusion protein construct, to block any binding of the anti-CD38 antibody-IFN fusion protein constructs to the CD38 expressed on the iLite cells).

[0162] HEK-Blue™ Off-target assay: The assay was used to quantify the ability of antibody-IFN fusion constructs to bind interferon-alpha/β receptor (IFNAR) using the HEK-Blue™ IFN-alpha/

β cell line (InvivoGen, San Diego, CA). The "off-target (HB-IFN) assay" was performed largely as described by the manufacturer of the HEK-Blue™ IFN-alpha/β cell line. HEK-Blue™ IFN-alpha/β Cells are specifically designed to monitor the activation of the JAK-STAT pathway, which is induced by type I IFNs. The cells were generated by introducing the human STAT2 and IRF9 genes into HEK293 cells to obtain a fully active type I IFN signalling pathway. The HEK-Blue™ IFN-alpha/β Cells stably express a reporter gene, secreted embryonic alkaline phosphatase (SEAP), under the control of the ISG54 promoter. ISG54 is a well-known ISG activated through an ISRE-dependent mechanism by type I IFNs. Upon IFN-alpha or IFNβ stimulation, HEK-Blue™ IFN-alpha/β cells activate the JAK-STAT pathway and then the expression of the SEAP reporter gene. SEAP is secreted into the media and can be quantitated using the colorimetric reagent QUANTI-Blue™. Briefly, HEK-Blue IFN-alpha/β cells (Invivogen, San Diego CA cat# hkb-ifnab) were thawed and cultured in DMEM media (Mediatech, Manassas VA, cat# 10-013-CV) + 10% FBS (Hyclone, Logan UT, cat# SH30070.03) that had been heat inactivated (HI FBS). When the cells reached 60-80% confluence, they were lifted with Cell Stripper (Mediatech, cat# 25-056-CI). Cells were washed twice in DMEM + HI FBS and counted. Cells were adjusted to 3.3×10^5 viable cells/mL in DMEM + HI FBS and 150 µL was aliquoted per well into a flat bottom 96 well tissue culture plate (hereafter, the "experimental plate"). Then, 50µL of IFN-alpha2b or fusion protein construct, diluted into DMEM + HI FBS, was added per well. The plate was incubated at 37° C 5% CO_2 for 16-24 hours. QUANTI-Blue (Invivogen, cat# rep-qb1) was prepared according to the manufacturer's directions. QUANTI-Blue (150 µL) was aliquoted into each well of a flat bottom plate (hereafter, the "assay plate"). Then, 50 µL supernatant per well from the experimental plate was transferred to assay plate. Assay plate was then incubated at 37° C for 1-3 hours. Assay plate absorbance at 630nm was read on a model 1420-41 Victor 3V Multilabel Counter from Perkin-Elmer. Data was analyzed using Graph Pad Prism.

H929 xenograft model

[0163] The effect of different doses of the A10.38 and A10.0 anti-CD38-attenuated IFN-alpha fusion protein constructs, were compared to the non-CD38-targeted fusion protein construct, on myeloma tumor growth. For these comparisons, the NCI- H929 s.c. multiple myeloma model was used.

[0164] The multiple myeloma cell line, NCI-H929 (ATCC CRL-9068, Gazdar, Blood 67: 1542-1549, 1986) is grown subcutaneously in immunocompromised (SCID) mice.

[0165] Eight to twelve week old CB.17 SCID mice were injected subcutaneously in the flank with 1×10^7 NCI-H929 tumor cells in 50% Matrigel™. When average tumor size reached 170-350 mm^3, mice were grouped into 4 cohorts of 7 mice each and treatment began at time zero (T0). All treatments were given by intraperitoneal injection, (i.p.) twice weekly for 3 weeks (indicated by bar under graph). All compounds were dosed at 100 µg/dose (approximately 4.5

mg/kg) except vehicle group. Tumor volume was measured twice weekly by caliper measurement. Endpoint was tumor volume of 2,000 mm^3.

[0166] The effect of different doses of the A02. 6, A10.0 and A10.38 anti-CD38-attenuated IFN-alpha fusion protein constructs, were compared to vehicle, on myeloma tumor growth. For these comparisons, the NCI- H929 s.c. multiple myeloma model was used.

[0167] The multiple myeloma cell line, NCI-H929 (ATCC CRL-9068, Gazdar, Blood 67: 1542-1549, 1986) is grown subcutaneously in immunocompromised (SCID) mice.

[0168] Eight to twelve week old CB.17 SCID mice were injected subcutaneously in the flank with 1×10^7 NCI-H929 tumor cells in 50% Matrigel. When average tumor size reaches 90mm^3, mice will be grouped into 4 cohorts of 5 mice each and treatment begin at time zero (T0). All treatments will be given by intraperitoneal injection, (i.p.) twice weekly for 3 weeks (indicated by bar under graph). All compounds will be dosed at 100 µg/dose (approximately 4.5 mg/kg) except vehicle group. Tumor volume will be measured twice weekly by caliper measurement.

Reference Example 5

Anti-CD38-attenuated IFN fusion protein with alternative constant region

[0169] A02.12 comprises an anti-CD38-attenuated IFN fusion protein in which the constant region of the protein is HC-L0-IFN-alpha (A145D) IgG4 (SEQ ID NO: 9). The heavy chain variable region of this antibody was reformatted onto an IgG1 constant region fused to A145D attenuated IFN-alpha2b (SEQ ID NO: 10). Co-expression of this heavy chain with the light chain of X02.107 (SEQ ID NO: 65) in HEK293E cells yielded antibody A02.112. Comparison of antibodies A02.12 and A02.112 using flow cytometry-based CD38-binding assays and potency assays demonstrates that other antibody constant regions, such as human IgG1, may also be used resulting in antibody-attenuated IFN fusion proteins with potent biologic activity equivalent to those generated using a human IgG4 constant region (Table 16b).

Table 16b

Anti-CD38-attenuated IFN fusion protein	Flow binding (EC_{50} in µg/mL)	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)	Figures
A02.12	3.40*	1.40	2.70	23.66	Fig 21
A02.112	<0.3*	3.14	3.74	N/T	Fig 21
*Antibody was tested in a flow binding assay against H929 cell line. Reported value					

is the EC_{50} in µg/mL.Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. N/T - Not Tested.

Reference Example 6

Humanization of R5D1, R5E8 and R10A2 variable regions

[0170] Rat-derived anti-CD38 antibodies R5D1, R5E8 and R10A2 are described in PCT/AU2012/001323 and were selected for humanization. The variable regions of these antibodies were superhumanized as described in U.S. Publ. No. 2003/0039649. Briefly, canonical structures were assigned to each rodent heavy and light chain through inspection of their respective amino acid sequences. R10A2 was assigned the canonical structure 2-1-1/1-2 (V_L/V_H), R5E8 was assigned the canonical structure 4-1-1/1-2, and R5D1 was assigned the canonical structure 2-1-1/1-2. Human germline sequences of the same canonical structure were used as acceptor frameworks for the grafting of donor CDRs. Variants of the resulting superhumanized antibody genes containing amino acid substitutions at positions within their sequences deemed likely to be important for maintenance of their binding activity were also designed. The different heavy chain superhumanized variable regions are shown in Figure 7. The different light chain superhumanized variable regions are shown in Figure 8.

[0171] Heavy chain variable region sequences were subcloned into vector pEE6.4 containing a human IgG4 constant region possessing the substitution S228P fused to A145D attenuated IFN-alpha2b (SEQ ID NO: 9). Light chain variable regions were subcloned into vector pEE12.4 containing a human kappa constant region (SEQ ID NO: 5). Antibodies were produced through co-expression of heavy chains in pEE6.4 and light chains in pEE12.4 in CHO cells as described previously. Table 17 summarises the heavy- and light chain pairings used to produce each superhumanized 5D1-based protein. Table 18 details the heavy- and light chain pairings for the superhumanized 5E8-based protein generated, whilst the heavy- and light chain pairings used to generate superhumanzied 10A2-based proteins are given in Table 19. One-shot equilibrium dissociation constant (K_D) ranking of the superhumanized antibodies was performed by BIAcore™ analysis of the resulting CHO transfection supernatants. The method was used to determine if the antibodies expressed (Protein A capture) and had a level of binding activity to human CD38.

Table 17

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
A5D1.0 (chimeric)	114	125	2.28×10^{-9}	N/A
A5D1.1	115	126	2.95×10^{-8}	175
A5D1.2	115	127	2.95×10^{-8}	289
A5D1.3	115	128	2.35×10^{-8}	248
A5D1.4	115	129	2.85×10^{-8}	427
A5D1.5	115	130	1.84×10^{-7}	269
A5D1.6	115	131	2.32×10^{-8}	338
A5D1.7	116	126	1.05×10^{-8}	132
A5D1.8	116	127	6.80×10^{-9}	263
A5D1.9	116	128	9.93×10^{-8}	128
A5D1.10	116	129	5.69×10^{-9}	358
A5D1.11	116	130	1.64×10^{-8}	250
A5D1.12	116	131	5.61×10^{-9}	345
A5D1.13	117	126	1.44	213

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
			$\times 10^{-8}$	
A5D1.14	117	127	1.52×10^{-8}	344
A5D1.15	117	128	1.46×10^{-8}	167
A5D1.16	117	129	1.37×10^{-8}	524
A5D1.17	117	130	3.28×10^{-8}	410
A5D1.18	117	131	1.01×10^{-8}	396
A5D1.19	118	126	1.01×10^{-8}	245
A5D1.20	118	127	1.07×10^{-8}	282
A5D1.21	118	128	7.94×10^{-9}	351
A5D1.22	118	129	8.97×10^{-9}	566
A5D1.23	118	130	2.14×10^{-8}	336
A5D1.24	118	131	8.01×10^{-9}	319
A5D1.25	119	126	DNB	165
A5D1.26	119	127	DNB	286
A5D1.27	119	128	DNB	265
A5D1.28	119	129	DNB	493

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
A5D1.29	119	130	DNB	275
A5D1.30	119	131	DNB	263
A5D1.31	120	126	1.05×10^{-7}	206
A5D1.32	120	127	1.20×10^{-7}	318
A5D1.33	120	128	9.83×10^{-8}	176
A5D1.34	120	129	1.06×10^{-7}	497
A5D1.35	120	130	6.07×10^{-7}	211
A5D1.36	120	131	8.58×10^{-8}	331
A5D1.37	121	126	1.01×10^{-7}	184
A5D1.38	121	127	1.21×10^{-7}	315
A5D1.39	121	128	9.55×10^{-8}	191
A5D1.40	121	129	1.22×10^{-7}	460
A5D1.41	121	130	5.60×10^{-7}	409
A5D1.42	121	131	8.54×10^{-8}	301
A5D1.43	122	126	$1.78 \times$	150

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
			10^{-8}	
A5D1.44	122	127	1.76×10^{-8}	226
A5D1.45	122	128	1.42×10^{-8}	177
A5D1.46	122	129	1.51×10^{-8}	401
A5D1.47	122	130	1.89×10^{-8}	364
A5D1.48	122	131	1.20×10^{-8}	273
A5D1.49	123	126	6.32×10^{-9}	141
A5D1.50	123	127	5.64×10^{-9}	212
A5D1.51	123	128	4.97×10^{-9}	188
A5D1.52	123	129	4.07×10^{-9}	493
A5D1.53	123	130	6.98×10^{-9}	561
A5D1.54	123	131	4.49×10^{-9}	253
A5D1.55	124	126	6.48×10^{-9}	203
A5D1.56	124	127	8.44×10^{-9}	144

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
A5D1.57	124	128	5.59×10^{-9}	233
A5D1.58	124	129	5.37×10^{-9}	376
A5D1.59	124	130	1.05×10^{-8}	313
A5D1.60	124	131	4.57×10^{-9}	429
DNB - did not bind. N/A - Not available.				

Table 18

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
A5E8.0 (chimeric)	132	143	5.50×10^{-9}	N/A
A5E8.1	133	144	2.31×10^{-7}	267
A5E8.2	133	145	2.37×10^{-7}	459
A5E8.3	133	146	3.59×10^{-7}	281
A5E8.4	133	147	DNB	420
A5E8.5	134	144	1.75×10^{-7}	172
A5E8.6	134	145	1.57×10^{-7}	611
A5E8.7	134	146	2.58×10^{-7}	201
A5E8.8	134	147	$8.09 \times 10^{-}$	308

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
			7	
A5E8.9	135	144	1.05 × 10-8	153
A5E8.10	135	145	2.13 × 10-8	503
A5E8.11	135	146	2.69 × 10-8	372
A5E8.12	135	147	DNB	212
A5E8.13	136	144	3.98 × 10-8	301
A5E8.14	136	145	1.26 × 10-7	543
A5E8.15	136	146	1.39 × 10-7	504
A5E8.16	136	147	DNB	284
A5E8.17	137	144	2.76 × 10-8	397
A5E8.18	137	145	8.81 × 10-8	430
A5E8.19	137	146	1.09 × 10-7	220
A5E8.20	137	147	DNB	397
A5E8.21	138	144	DNB	277
A5E8.22	138	145	DNB	409
A5E8.23	138	146	DNB	339
A5E8.24	138	147	DNB	266
A5E8.25	139	144	DNB	283
A5E8.26	139	145	DNB	395
A5E8.27	139	146	DNB	277

Anti-CD38-attenuated IFN fusion protein	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:	K_D (M)	Protein A capture (RU)
A5E8.28	139	147	DNB	290
A5E8.29	140	144	3.91×10^{-8}	207
A5E8.30	140	145	5.00×10^{-8}	255
A5E8.31	140	146	6.61×10^{-8}	267
A5E8.32	140	147	DNB	42
A5E8.33	141	144	1.12×10^{-8}	134
A5E8.34	141	145	1.63×10^{-8}	301
A5E8.35	141	146	1.85×10^{-8}	177
A5E8.36	141	147	DNB	10
A5E8.37	142	144	8.19×10^{-8}	200
A5E8.38	142	145	1.55×10^{-8}	328
A5E8.39	142	146	1.74×10^{-8}	232
A5E8.40	142	147	DNB	126
DNB - did not bind. N/A - not available.				

Table 19

Anti-CD38-attenuated IFN fusion protein	SEQ ID: Variable Heavy	SEQ ID: Variable Light	K_D (M)	Protein A capture (RU)
A10A2.0 (chimeric)	148	157	5.98×10^{-10}	N/A
A10A2.1	149	158	DNB	728

Anti-CD38-attenuated IFN fusion protein	SEQ ID: Variable Heavy	SEQ ID: Variable Light	K_D (M)	Protein A capture (RU)
A10A2.2	149	159	DNB	689
A10A2.3	149	160	DNB	850
A10A2.4	149	161	DNB	996
A10A2.5	149	162	DNB	761
A10A2.6	149	163	DNB	890
A10A2.7	149	164	DNB	725
A10A2.8	150	158	7.18×10^{-7}	718
A10A2.9	150	159	6.62×10^{-7}	627
A10A2.10	150	160	9.13×10^{-7}	850
A10A2.11	150	161	2.37×10^{-7}	956
A10A2.12	150	162	1.18×10^{-6}	864
A10A2.13	150	163	6.80×10^{-7}	765
A10A2.14	150	164	DNB	645
A10A2.15	151	158	1.15×10^{-7}	488
A10A2.16	151	159	8.11×10^{-8}	759
A10A2.17	151	160	1.84×10^{-7}	684
A10A2.18	151	161	3.39×10^{-8}/	907
A10A2.19	151	162	1.84×10^{-7}	831
A10A2.20	151	163	1.23×10^{-7}	560
A10A2.21	151	164	DNB	337
A10A2.22	152	158	2.70×10^{-9}	890
A10A2.23	152	159	2.17 ×	828

Anti-CD38-attenuated IFN fusion protein	SEQ ID: Variable Heavy	SEQ ID: Variable Light	K_D (M)	Protein A capture (RU)
			10^{-9}	
A10A2.24	152	160	3.04×10^{-9}	803
A10A2.25	152	161	1.51×10^{-9}	1054
A10A2.26	152	162	3.51×10^{-9}	741
A10A2.27	152	163	2.42×10^{-9}	603
A10A2.28	152	164	3.69×10^{-8}	384
A10A2.29	153	158	2.77×10^{-8}	93
A10A2.30	153	159	2.15×10^{-8}	86
A10A2.31	153	160	5.82×10^{-8}	33
A10A2.32	153	161	8.49×10^{-9}	169
A10A2.33	153	162	5.66×10^{-8}	62
A10A2.34	153	163	3.88×10^{-8}	56
A10A2.35	153	164	DNB	DNE
A10A2.36	154	158	8.38×10^{-9}	221
A10A2.37	154	159	1.39×10^{-9}	858
A10A2.38	154	160	1.08×10^{-8}	178
A10A2.39	154	161	3.80×10^{-9}	357
A10A2.40	154	162	1.34×10^{-8}	217
A10A2.41	154	163	8.73×10^{-9}	202

Anti-CD38-attenuated IFN fusion protein	SEQ ID: Variable Heavy	SEQ ID: Variable Light	K_D (M)	Protein A capture (RU)
A10A2.42	154	164	2.09×10^{-7}	175
A10A2.43	154	158	2.45×10^{-7}	621
A10A2.44	155	159	6.23×10^{-9}	220
A10A2.45	155	160	2.84×10^{-7}	881
A10A2.46	155	161	1.39×10^{-7}	1000
A10A2.47	155	162	3.28×10^{-7}	9
A10A2.48	155	163	2.52×10^{-7}	565
A10A2.49	155	164	DNB	499
A10A2.50	156	158	1.61×10^{-9}	567
A10A2.51	156	159	2.00×10^{-7}	603
A10A2.52	156	160	1.69×10^{-9}	723
A10A2.53	156	161	1.20×10^{-9}	729
A10A2.54	156	162	1.92×10^{-9}	639
A10A2.55	156	163	1.47×10^{-9}	692
A10A2.56	156	164	1.97×10^{-7}	383
DNB - did not bind. DNE - did not express.				

[0172] For each family of humanized antibodies - 5D1, 5E8 and 10A2 - several humanized heavy and light chain combinations failed to either express protein, or to bind to human CD38. A considerable number of antibodies across all 3 families of humanized antibodies expressed

and bound to human CD38 with equilibrium dissociation constants in the nanomolar (nM) range. A10A2.53 and A10A2.25, which share a common light chain were chosen for further optimization. A10A2.53 was renamed A10.0 and A10A2.25 was renamed A10.38.

Example 2

Improved variants of A10.0

[0173] The A10.0 antibody was optimized through alterations to the variable heavy and/or light chain sequences with the aim of yielding a positive effect on the biophysical and *in silico* immunogenicity of the antibody whilst causing minimal impact on the functional activity of the antibody.

In-silico immunogenicity analysis of A10.0 heavy- and light chains

[0174] *In silico* immunogenicity analyses of the A10.0 heavy- and light chain variable regions were made using the Epibase software package. Several amino acid substitutions were introduced into the heavy- and light chain variable regions of A10.0 to remove potential immunogenic epitopes. An amino acid sequence alignment of the heavy chain variable region variants produced aligned with the humanised heavy chain (SEQ ID NO: 156) is shown in Figure 9. An amino acid sequence alignment of the light chain variable region variants aligned with the humanised light chain (SEQ ID NO: 161) is shown in Figure 10. Details of the heavy- and light chains variants co-expressed in HEK293E cells to produce proteins are summarised in Table 20.

Table 20

Anti-CD38-attenuated IFN fusion protein	VH Amino Acid Substitution (Relative to A10.0)	Variable Heavy SEQ ID NO:	VK Amino Acid Substitution (Relative to A10.0)	Variable Light SEQ ID NO:
A10.1	A40E	165	N/A	161
A10.2	A40G	166	N/A	161
A10.3	A40H	167	N/A	161
A10.4	A40Q	168	N/A	161
A10.5	A40S	169	N/A	161
A10.6	A40V	170	N/A	161
A10.7	N35E	171	N/A	161
A10.8	N35P	172	N/A	161
A10.9	N35Q	173	N/A	161

Anti-CD38-attenuated IFN fusion protein	VH Amino Acid Substitution (Relative to A10.0)	Variable Heavy SEQ ID NO:	VK Amino Acid Substitution (Relative to A10.0)	Variable Light SEQ ID NO:
A10.10	N35S	174	N/A	161
A10.11	R94E	175	N/A	161
A10.12	R94G	176	N/A	161
A10.13	R94P	177	N/A	161
A10.14	R94T	178	N/A	161
A10.15	K96G	179	N/A	161
A10.16	K96T	180	N/A	161
A10.17	N/A	156	K24E	181
A10.18	N/A	156	K24G	182
A10.19	N/A	156	K24P	183
A10.20	N/A	156	K24Q	184
A10.21	N/A	156	R54D	185
A10.22	N/A	156	I48D	186
A10.23	N/A	156	Y49E	187
A10.24	N/A	156	M89A	188
A10.25	N/A	156	M89E	189
A10.26	N/A	156	M89H	190
A10.27	N/A	156	M89K	191
A10.28	N/A	156	M89P	192
A10.29	N/A	156	M89Q	193
A10.30	N/A	156	M89S	194
A10.31	N/A	156	M89V	195
A10.32	N/A	156	Q90D	196

[0175] Each antibody generated using the heavy- and light chain pairings outlined in Table 20 was assessed for protein expression level and binding to CD38 via SPR. Furthermore, potency assays were performed using cell culture supernatants to assess the relative functional activity of each of these anti-CD38 antibody-attenuated IFN fusion proteins, Table 21.

Table 21

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V (Fold change relative to untreated cells) Assay	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay (IC_{50}pM)
A10.1	16.9	1824	1.66	3.41	4078
A10.2	16.7	1821	1.66	5.19	7622
A10.3	25.0	2166	1.63	5.46	2148
A10.4	23.7	2169	1.63	5.78	4108
A10.5	28.0	2240	1.64	5.80	3046
A10.6	31.0	2097	1.57	5.76	2283
A10.7	26.5	DNB	1.18	1.09	No IC_{50}
A10.8	2.4	DNB	N/T	N/T	N/T
A10.9	18.3	176	1.48	2.07	No IC_{50}
A10.10	32.2	1072	1.57	4.97	18870
A10.11	28.3	98	1.57	3.64	No IC_{50}
A10.12	30.7	DNB	1.22	1.99	No IC_{50}
A10.13	30.6	123	1.31	2.67	No IC_{50}
A10.14	30.5	247	1.19	5.11	68270
A10.15	41.8	1254	1.52	5.44	5169
A10.16	24.2	1210	1.70	4.57	5224
A10.17	18.2	1686	1.79	6.11	3054
A10.18	32.5	2457	1.89	6.16	2178
A10.19	1.6	DNB	1.73	2.39	No IC_{50}
A10.20	12.2	1355	4.65	7.72	564
A10.21	19.9	1837	1.84	5.56	5330
A10.22	5.5	480	N/T	N/T	N/T
A10.23	20.6	255	1.71	3.85	59720
A10.24	34.6	1943	4.14	6.75	399
A10.25	28.3	1778	1.87	6.09	4910
A10.26	5.7	706	N/T	N/T	N/T
A10.27	7.4	136	N/T	N/T	N/T
A10.28	2.2	48	N/T	N/T	N/T
A10.29	10.9	1443	N/T	N/T	No IC_{50}
A10.30	25.4	1865	1.98	6.21	1438

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V (Fold change relative to untreated cells) Assay	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay (IC_{50}pM)
A10.31	5.8	469	N/T	N/T	N/T
A10.32	34.5	615	3.80	6.97	3628
The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. DNB - Did not bind; N/T- not tested; No IC_{50} - potency not sufficient for an IC_{50} value.					

[0176] Analyses of the amino acid sequences of the variable heavy- and light chain sequences of A10.0 identified several potential deamidation sites and one potential oxidation site. Variable heavy chain substitution N98Q was prepared to remove a deamidation site from CDR3 of the heavy chain, SEQ ID NO: 197. A further variant of the A10.0 variable light chain containing the CDR2 substitution N53Q (SEQ ID NO: 198) was generated to remove this putative deamidation site. M89 within CDR3 of the light chain was also altered through amino acid substitutions at this position with the combined aims of removing this potential oxidation site and reducing the predicted immunogenicity of this region of the light chain. These substitutions are outlined in Table 22, along with the heavy and light chain pairings co-expressed to produce each anti-CD38-attenuated IFN fusion protein.

Table 22

Anti-CD38-attenuated IFN fusion protein	Amino Acid Substitution (Relative to A10.0)	Variable Heavy SEQ ID NO:	Variable Light SEQ ID NO:
A10.35	Heavy Chain N(98)Q	197	161
A10.36	Light Chain N(53)Q	156	198

[0177] Each antibody generated using the heavy- and light chain pairings outlined in Table 22 was assessed for protein expression level and binding to CD38 via SPR. Furthermore, potency assays were performed using cell culture supernatants to assess the relative functional activity of each of these anti-CD38 antibody-attenuated IFN fusion proteins, shown in Table 23.

Table 23

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR (RU) at 350 sec*	Annexin V assay (Fold change relative to untreated cells)Assay	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)
A10.35	34.5	1889	1.83	6.16	6241
A10.36	52.4	1895	3.95	5.90	534.9
The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM.					

Example 3

Generating improved variants of A10.38

[0178] A10.0 and A10.38 share a common light chain. The optimized light chain sequences of A10.0 were paired with the heavy chain of the A10.38 antibody with the aim of yielding a positive effect on the antibody's biophysical and *in silico* immunogenicity properties whilst having a minimal impact on functional activity. A summary of the changes and the pairings of heavy and light chains are described in Table 24.

Table 24

Anti-CD38-attenuated IFN fusion protein	SEQ ID: Variable Heavy	VK Amino Acid Substitution (Relative to A10.38)	SEQ ID: Variable Light
A10.38	152	N/A	161
A10.39	152	K24E	181
A10.40	152	K24G	182
A10.41	152	K24P	183
A10.42	152	K24Q	184
A10.43	152	R54D	185
A10.44	152	I48D	186
A10.45	152	Y49E	187
A10.46	152	M89A	188
A10.47	152	M89E	189
A10.48	152	M89H	190

Anti-CD38-attenuated IFN fusion protein	SEQ ID: Variable Heavy	VK Amino Acid Substitution (Relative to A10.38)	SEQ ID: Variable Light
A10.49	152	M89K	191
A10.50	152	M89P	192
A10.51	152	M89Q	193
A10.52	152	M89S	194
A10.53	152	M89V	195
A10.54	152	Q90D	196
A10.57	152	N53Q	198

[0179] Each of the above antibodies was assessed for protein expression level and binding to CD38 via SPR. Potency assays were performed using cell culture supernatants to assess the relative functional activity of each of these anti-CD38 antibody-attenuated IFN fusion proteins, Table 25.

Table 25

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR(RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)
A10.38	119.7	1540	3.22	5.17
A10.39	113.4	1444	3.39	4.79
A10.40	117.8	1562	3.28	5.12
A10.41	89.7	1459	3.27	5.12
A10.42	111.7	1443	3.32	5.60
A10.43	94.1	1426	3.21	6.15
A10.44	51.9	969	3.08	5.66
A10.45	111.7	333	2.76	5.01
A10.46	120.0	1547	3.24	4.80
A10.47	107.3	1337	3.45	4.25
A10.48	45.5	865	3.06	5.48
A10.49	55.8	213	3.46	7.63
A10.50	11.3	172	2.96	5.61
A10.51	51.6	1320	2.34	6.16
A10.52	70.0	1512	3.21	5.62
A10.53	40.0	536	3.46	4.68
A10.54	61.3	583	3.10	6.20

Anti-CD38-attenuated IFN fusion protein	Protein A HPLC (mg/L)	CD38 binding by SPR(RU) at 350 sec*	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)
A10.57	67.1	1431	3.06	6.04
The CD38 binding by SPR refers to the amount of CD38 that remains bound to the surface after 350 seconds of the dissociation phase. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM. N/T - Not Tested.				

Attenuated IFN is required for potent apoptotic and caspase activation in tumor cell lines

[0180] The relative potency of anti-CD38 antibodies A10.0 (attenuated IFN fusion) and X10.0 (no fusion) were compared using the Annexin V, Caspase and the Cell Proliferation Assays outlined in Example 1. The relative potency of A10.38 and X10.38 was also compared, Table 26.

Table 26

Anti-CD38-attenuated IFN fusion protein	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)	Figures
A10.0	2.10	4.23	2081	Fig 18
X10.0	1.27	1.70	No IC50	Fig 18
A10.38	3.22	5.17	1118	Fig 25
X10.38	1.46	2.09	No IC50	Fig 25
Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM.				

[0181] These data demonstrate the potent apoptotic activity exhibited by antibodies A10.0 and A10.38 relative to X10.0 and X10.38 respectively necessitates the presence of the attenuated IFN fusion. No anti-proliferative activity was observed with antibodies without an attenuated IFN.

[0182] A consensus sequence alignment of heavy chain variable regions from proteins with functional activity is shown in Figure 11. A consensus sequence alignment of light chain variable regions from proteins with functional activity is shown in Figure 12. It could be further envisioned that combinations of substitutions could be made such as those described for Anti-

CD38 antibodies X10.60, X10.61, X10.62, X10.63, X10.64, X10.65, X10.66, X10.67, X10.68, X10.69, X10.70, X10.71, X10.72, X10.73, X10.74, X10.75, X10.76, X10.77, X10.78, X10.79, X10.80, X10.81, X10.82, X10.83, X10.84, X10.85, X10.86, X10.87, X10.88, X10.89, X10.90, X10.91, X10.92, X10.93, X10.94, X10.95, X10.96, X10.97, X10.98, X10.99, X10.100, X10.101, X10.102, X10.103, X10.104, X10.105, X10.106, X10.107, X10.108, X10.109, X10.110, X10.111, X10.112, X10.113, X10.114, X10.115, X10.116, X10.117, X10.118, X10.119, X10.120, X10.121, X10.122, X10.123, X10.124, X10.125, X10.126, X10.127, X10.128, X10.129, X10.130, X10.131, X10.132, X10.133, X10.134, X10.135, X10.136, X10.137, X10.138, X10.139, X10.140, X10.141, X10.142, X10.143, X10.144, X10.145, X10.146, X10.147 (Figure 11, Figure 12). Further the above Anti-CD38 antibodies could also be constructed as Anti-CD38-attenuated IFN fusion proteins and tested for functional activity as described herein.

H929 multiple myeloma xenograft model

[0183] The *in vivo* potency of 10A2 variants A10.0 and A10A2.0 were evaluated in an NCI-H929 s.c. mouse multiple myeloma model, Figure 27. Both were shown to have potent antitumour activity in this model. Such a model could be used to test for anti-tumor activity of other protein constructs described within.

Off-Target Activity for the 10A2 variants

[0184] The off-target activity of the 10A2 variants A10.0, A10.38, A10A2.37 and A10A2.39 in comparison with the parental A10A2.0 chimeric antibody fused to wildtype and attenuated interferon 145D was assessed in either the iLite reporter gene assay and/or the HEK Blue assay and is shown in Figure 28 and Figure 29. The EC_{50} values are provided in Figure 28 and Figure 29. The off-target activity confirms the attenuation of the interferon and the need for the antibody to be targeted to CD38 to restore function.

Further in-vitro potency data for A10.0 and related constructs

[0185] A selection of the above Anti-CD38-attenuated IFN fusion proteins were purified and analysed for binding to CD38 positive cells in cell based assays. In addition, potency assays were repeated to more accurately determine the relative activity of each of these Anti-CD38-attenuated IFN fusion proteins. The methods for these various assays are described in Example 1. The results of each of these assays are given in Table 27.

Table 27

Anti-CD38-attenuated IFN fusion protein	H929 Flow binding (EC_{50} in µg/mL)	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)	Figures
A10.0	1.49	3.05	1.38	120.8	17,18,21,22,23, 24,25,26,27 28,29
A10.1	1.03	1.34	4.63	63.6	24, 25
A10.2	0.57	1.37	4.37	45.6	24, 25
A10.3	0.55	1.43	5.52	65.8	24, 25
A10.5	0.48	3.27	1.61	53.79	24, 25
A10.6	0.35	3.16	1.66	98.85	24, 25
A10.10	6.84	3.02	1.64	1967.00	24, 25
A10.14	2.26	2.46	1.56	2207	22, 24, 25
A10.15	2.06	3.06	2.55	174.4	22, 24, 25
A10.16	1.17	1.35	5.18	49.5	24, 25
A10.18	1.03	2.95	1.70	124.2	22, 24, 25
A10.20	2.11	2.84	1.26	656	24, 25
A10.21	0.78	3.0	1.39	147.3	22, 24, 25
A10.24	0.81	2.95	7.47	87.99	22, 24, 25
A10.25	1.37	2.75	1.38	27.69	24, 25
A10.30	0.88	3.22	1.60	18.73	24, 25
A10.32	51.69	1.93	1.19	381.4	24, 25
A10.35	1.10	2.97	2.05	93.96	22, 24, 25
A10.36	1.53	3.21	3.61	57.83	22, 24, 25
A10.37	18.57	2.53	1.40	163.5	24, 25
A10.38	1.13	3.27	1.53	36.79	23, 24, 25, 26, 29
A10.40	0.99	1.44	5.16	3.02	24, 25
A10.42	1.61	1.58	1.78	155.3	24, 25
A10.43	1.20	1.64	1.79	120.9	24, 25
A10.44	1.65	1.58	1.91	308.6	24, 25
A10.46	0.73	1.84	5.99	2.63	24, 25
A10.47	0.79	1.70	1.53	5.707	24, 25
A10.48	1.72	1.58	1.60	22.33	24, 25

Anti-CD38-attenuated IFN fusion protein	**H929 Flow binding (EC_{50} in µg/mL)**	**Annexin V Assay (Fold change relative to untreated cells)**	**Caspase Assay (Fold change relative to untreated cells)**	**Cell Proliferation Assay IC_{50} (pM)**	**Figures**
A10.53	1.32	1.56	1.67	56.01	24, 25
A10.54	5.90	1.52	1.43	2008	24, 25
A10.56	2.43	1.84	1.95	141.7	24, 25
A10.57	1.54	1.49	4.71	126.1	24, 25
A10.59	0.89	2.48	2.38	45.75	21, 24, 25
The flow binding was determined in H929 cell line. Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase activation of cells after 24 h treatment with antibody constructs at 20 nM.					

Anti-CD38-attenuated IFN fusion protein with alternative constant region

[0186] A10.0 comprises an anti-CD38-attenuated IFN fusion protein in which the constant region of the protein is HC-L0-IFN-alpha (A145D) IgG4 (SEQ ID NO: 9). Using gene synthesis, the constant region of this protein was replaced with HC-L0-IFN-alpha (A145D) IgG1 (SEQ ID NO: 10), paired with A10.0 light chain (SEQ ID NO: 161) and given the designation A10.59. The protein was expressed and was found to be potent in functional assays (Table 28). While the majority of the proteins tested in the foregoing examples were constructed on the human IgG4 constant region, these data demonstrate that other antibody constant regions, such as human IgG1, may also be used, with the resultant antibody-attenuated IFN fusion construct having potent biologic activity equivalent to constructs that utilize a human IgG4 constant region.

Table 28

Anti-CD38-attenuated IFN fusion protein	H929 Flow binding (EC_{50} in µg/mL)	Annexin V Assay (Fold change relative to untreated cells)	Caspase Assay (Fold change relative to untreated cells)	Cell Proliferation Assay IC_{50} (pM)*	Figures
A10.0	1.50	3.05	1.89	2081	21, 24, 25
A10.59	0.89	2.48	2.38	328.6	21, 24, 25
Annexin V Assay refer to cells positively stained by Annexin V-FITC after 24 h treatment with antibody constructs at 20 nM. Caspase Assay refers to caspase					

activation of cells after 24 h treatment with antibody constructs at 20 nM. N/T is Not Tested, *Data obtained from Cell Proliferation Assay assessed with cell culture supernatant.

[0187] Table 29 lists the pairing of variable heavy chain, variable light chain and constant region for each antibody described herein. Table 30 lists the sequences used in the disclosure AA refers to amino acid (sequence type) and DNA refers to polynucleotide (sequence type).

Table 29

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A02.10	19	14	9
A02.11	20	14	9
A02.112	34	65	10
A02.12	34	65	9
A02.13	35	65	9
A02.16	34	92	9
A02.17	34	93	9
A02.18	34	73	9
A02.19	34	74	9
A02.2	13	65	9
A02.20	34	75	9
A02.21	34	76	9
A02.22	34	77	9
A02.23	34	78	9
A02.24	34	79	9
A02.25	34	80	9
A02.26	34	81	9
A02.27	34	82	9
A02.28	34	83	9
A02.29	34	84	9
A02.3	17	65	9
A02.30	34	85	9
A02.31	34	86	9
A02.32	34	87	9
A02.33	34	88	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A02.34	34	89	9
A02.35	34	90	9
A02.36	34	91	9
A02.37	34	66	9
A02.38	34	113	9
A02.39	34	112	9
A02.4	18	65	9
A02.40	111	65	9
A02.41	110	65	9
A02.43	110	113	9
A02.44	111	112	9
A02.46	34	67	9
A02.47	34	68	9
A02.48	34	69	9
A02.49	34	70	9
A02.5	19	65	9
A02.50	34	71	9
A02.51	34	72	9
A02.52	34	94	9
A02.53	34	95	9
A02.54	34	96	9
A02.55	34	97	9
A02.56	34	98	9
A02.57	34	99	9
A02.58	34	100	9
A02.59	34	101	9
A02.6	20	65	9
A02.60	34	102	9
A02.61	34	103	9
A02.62	34	104	9
A02.63	34	105	9
A02.64	34	106	9
A02.65	34	107	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A02.66	34	108	9
A02.67	34	109	9
A02.8	17	14	9
A02.9	18	14	9
A10.1	165	161	9
A10.10	174	161	9
A10.11	175	161	9
A10.12	176	161	9
A10.13	177	161	9
A10.14	178	161	9
A10.15	179	161	9
A10.16	180	161	9
A10.17	156	181	9
A10.18	156	182	9
A10.19	156	183	9
A10.2	166	161	9
A10.20	156	184	9
A10.21	156	185	9
A10.22	156	186	9
A10.23	156	187	9
A10.24	156	188	9
A10.25	156	189	9
A10.26	156	190	9
A10.27	156	191	9
A10.28	156	192	9
A10.29	156	193	9
A10.3	167	161	9
A10.30	156	194	9
A10.31	156	195	9
A10.32	156	196	9
A10.35	197	161	9
A10.36	156	198	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A10.38	152	161	9
A10.39	152	181	9
A10.4	168	161	9
A10.40	152	182	9
A10.41	152	183	9
A10.42	152	184	9
A10.43	152	185	9
A10.44	152	186	9
A10.45	152	187	9
A10.46	152	188	9
A10.47	152	189	9
A10.48	152	190	9
A10.49	152	191	9
A10.5	169	161	9
A10.50	152	192	9
A10.51	152	193	9
A10.52	152	194	9
A10.53	152	195	9
A10.54	152	196	9
A10.57	152	198	9
A10.59	156	161	10
A10.6	170	161	9
A10.7	171	161	9
A10.8	172	161	9
A10.9	173	161	9
A10A2.0 (chimeric)	148	157	9
A10A2.1	149	158	9
A10A2.10	150	160	9
A10A2.11	150	161	9
A10A2.12	150	162	9
A10A2.13	150	163	9
A10A2.14	150	164	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A10A2.15	151	158	9
A10A2.16	151	159	9
A10A2.17	151	160	9
A10A2.18	151	161	9
A10A2.19	151	162	9
A10A2.2	149	159	9
A10A2.20	151	163	9
A10A2.21	151	164	9
A10A2.22	152	158	9
A10A2.23	152	159	9
A10A2.24	152	160	9
A10A2.25	152	161	9
A10A2.26	152	162	9
A10A2.27	152	163	9
A10A2.28	152	164	9
A10A2.29	153	158	9
A10A2.3	149	160	9
A10A2.30	153	159	9
A10A2.31	153	160	9
A10A2.32	153	161	9
A10A2.33	153	162	9
A10A2.34	153	163	9
A10A2.35	153	164	9
A10A2.36	154	158	9
A10A2.37	154	159	9
A10A2.38	154	160	9
A10A2.39	154	161	9
A10A2.4	149	161	9
A10A2.40	154	162	9
A10A2.41	154	163	9
A10A2.42	154	164	9
A10A2.43	154	158	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A10A2.44	155	159	9
A10A2.45	155	160	9
A10A2.46	155	161	9
A10A2.47	155	162	9
A10A2.48	155	163	9
A10A2.49	155	164	9
A10A2.5	149	162	9
A10A2.50	156	158	9
A10A2.51	156	159	9
A10A2.52	156	160	9
A10A2.53	156	161	9
A10A2.54	156	162	9
A10A2.55	156	163	9
A10A2.56	156	164	9
A10A2.6	149	163	9
A10A2.7	149	164	9
A10A2.8	150	158	9
A10A2.9	150	159	9
A5D1.0 (chimeric)	114	125	9
A5D1.1	115	126	9
A5D1.10	116	129	9
A5D1.11	116	130	9
A5D1.12	116	131	9
A5D1.13	117	126	9
A5D1.14	117	127	9
A5D1.15	117	128	9
A5D1.16	117	129	9
A5D1.17	117	130	9
A5D1.18	117	131	9
A5D1.19	118	126	9
A5D1.2	115	127	9
A5D1.20	118	127	9
A5D1.21	118	128	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A5D1.22	118	129	9
A5D1.23	118	130	9
A5D1.24	118	131	9
A5D1.25	119	126	9
A5D1.26	119	127	9
A5D1.27	119	128	9
A5D1.28	119	129	9
A5D1.29	119	130	9
A5D1.3	115	128	9
A5D1.30	119	131	9
A5D1.31	120	126	9
A5D1.32	120	127	9
A5D1.33	120	128	9
A5D1.34	120	129	9
A5D1.35	120	130	9
A5D1.36	120	131	9
A5D1.37	121	126	9
A5D1.38	121	127	9
A5D1.39	121	128	9
A5D1.4	115	129	9
A5D1.40	121	129	9
A5D1.41	121	130	9
A5D1.42	121	131	9
A5D1.43	122	126	9
A5D1.44	122	127	9
A5D1.45	122	128	9
A5D1.46	122	129	9
A5D1.47	122	130	9
A5D1.48	122	131	9
A5D1.49	123	126	9
A5D1.5	115	130	9
A5D1.50	123	127	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A5D1.51	123	128	9
A5D1.52	123	129	9
A5D1.53	123	130	9
A5D1.54	123	131	9
A5D1.55	124	126	9
A5D1.56	124	127	9
A5D1.57	124	128	9
A5D1.58	124	129	9
A5D1.59	124	130	9
A5D1.6	115	131	9
A5D1.60	124	131	9
A5D1.7	116	126	9
A5D1.8	116	127	9
A5D1.9	116	128	9
A5E8.0 (chimeric)	132	143	9
A5E8.1	133	144	9
A5E8.10	135	145	9
A5E8.11	135	146	9
A5E8.12	135	147	9
A5E8.13	136	144	9
A5E8.14	136	145	9
A5E8.15	136	146	9
A5E8.16	136	147	9
A5E8.17	137	144	9
A5E8.18	137	145	9
A5E8.19	137	146	9
A5E8.2	133	145	9
A5E8.20	137	147	9
A5E8.21	138	144	9
A5E8.22	138	145	9
A5E8.23	138	146	9
A5E8.24	138	147	9
A5E8.25	139	144	9

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
A5E8.26	139	145	9
A5E8.27	139	146	9
A5E8.28	139	147	9
A5E8.29	140	144	9
A5E8.3	133	146	9
A5E8.30	140	145	9
A5E8.31	140	146	9
A5E8.32	140	147	9
A5E8.33	141	144	9
A5E8.34	141	145	9
A5E8.35	141	146	9
A5E8.36	141	147	9
A5E8.37	142	144	9
A5E8.38	142	145	9
A5E8.39	142	146	9
A5E8.4	133	147	9
A5E8.40	142	147	9
A5E8.5	134	144	9
A5E8.6	134	145	9
A5E8.7	134	146	9
A5E8.8	134	147	9
A5E8.9	135	144	9
X02.10	19	14	3
X02.100	13	58	3
X02.101	13	59	3
X02.102	13	60	3
X02.103	13	61	3
X02.104	13	62	3
X02.105	13	63	3
X02.106	13	64	3
X02.107	13	65	3
X02.108	32	14	3

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
X02.11	20	14	3
X02.110	33	14	3
X02.114	13	660	3
X02.115	13	661	3
X02.116	13	662	3
X02.117	13	663	3
X02.118	34	700	3
X02.119	34	701	3
X02.120	728	700	3
X02.121	729	700	3
X02.122	730	700	3
X02.123	731	700	3
X02.124	728	701	3
X02.125	729	701	3
X02.126	730	701	3
X02.127	731	701	3
X02.68	21	14	3
X02.69	22	14	3
X02.70	23	14	3
X02.71	24	14	3
X02.72	25	14	3
X02.73	26	14	3
X02.74	27	14	3
X02.75	28	14	3
X02.76	29	14	3
X02.77	30	14	3
X02.78	31	14	3
X02.8	17	14	3
X02.80	13	38	3
X02.81	13	39	3
X02.82	13	40	3
X02.83	13	41	3
X02.84	13	42	3

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
X02.85	13	43	3
X02.86	13	44	3
X02.87	13	45	3
X02.88	13	46	3
X02.89	13	47	3
X02.9	18	14	3
X02.90	13	48	3
X02.91	13	49	3
X02.92	13	50	3
X02.93	13	51	3
X02.94	13	52	3
X02.95	13	53	3
X02.96	13	54	3
X02.97	13	55	3
X02.98	13	56	3
X02.99	13	57	3
X10.100	720	706	3
X10.101	721	706	3
X10.102	722	706	3
X10.103	723	706	3
X10.104	739	706	3
X10.105	740	706	3
X10.106	741	706	3
X10.107	742	706	3
X10.108	720	707	3
X10.109	721	707	3
X10.110	722	707	3
X10.111	723	707	3
X10.112	739	707	3
X10.113	740	707	3
X10.114	741	707	3
X10.115	742	707	3

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
X10.116	720	708	3
X10.117	721	708	3
X10.118	722	708	3
X10.119	723	708	3
X10.120	739	708	3
X10.121	740	708	3
X10.122	741	708	3
X10.123	742	708	3
X10.124	720	709	3
X10.125	721	709	3
X10.126	722	709	3
X10.127	723	709	3
X10.128	739	709	3
X10.129	740	709	3
X10.130	741	709	3
X10.131	742	709	3
X10.132	720	710	3
X10.133	721	710	3
X10.134	722	710	3
X10.135	723	710	3
X10.136	739	710	3
X10.137	740	710	3
X10.138	741	710	3
X10.139	742	710	3
X10.140	720	711	3
X10.141	721	711	3
X10.142	722	711	3
X10.143	723	711	3
X10.144	739	711	3
X10.145	740	711	3
X10.146	741	711	3
X10.147	742	711	3
X10.60	156	704	3

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
X10.61	156	705	3
X10.62	156	706	3
X10.63	156	707	3
X10.64	156	708	3
X10.65	156	709	3
X10.66	156	710	3
X10.67	156	711	3
X10.68	720	161	3
X10.69	721	161	3
X10.70	722	161	3
X10.71	723	161	3
X10.72	739	161	3
X10.73	740	161	3
X10.74	741	161	3
X10.75	742	161	3
X10.76	152	704	3
X10.77	152	705	3
X10.78	152	706	3
X10.79	152	707	3
X10.80	152	708	3
X10.81	152	709	3
X10.82	152	710	3
X10.83	152	711	3
X10.84	720	704	3
X10.85	721	704	3
X10.86	722	704	3
X10.87	723	704	3
X10.88	739	704	3
X10.89	740	704	3
X10.90	741	704	3
X10.91	742	704	3
X10.92	720	705	3

Antibody Name	Variable Heavy SEQ ID NO: (amino acid)	Variable Light SEQ ID NO: (amino acid)	Heavy Chain Constant Region SEQ ID NO: (amino acid)
X10.93	721	705	3
X10.94	722	705	3
X10.95	723	705	3
X10.96	739	705	3
X10.97	740	705	3
X10.98	741	705	3
X10.99	742	705	3
X910/12-HC-L0-IFN-alpha (A145D) IgG4	110	112	9
X913/15-HC-L0-IFN-alpha (A145D) IgG4	111	113	9

Table 30

SEQ ID NO:	Type	Description
1	AA	Human CD38
2	AA	Cynomolgus CD38
3	AA	Human IgG4 constant heavy chain
4	AA	Human IgG1 constant heavy chain
5	AA	Human kappa constant region
6	AA	Human lambda constant region
7	AA	IFN-alpha2b
8	AA	Intron A
9	AA	HC-L0-IFN-alpha (A145D) IgG4
10	AA	HC-L0-IFN-alpha (A145D) IgG1
11	AA	A02.1 heavy chain
12	AA	A02.1 light chain
13	AA	A02.1 variable heavy chain
14	AA	A02.1 variable light chain
15	AA	X02.1VH variable heavy chain
16	AA	IGHV4-61*01 germline sequence
17	AA	X02.8VH variable heavy chain
18	AA	X02.9VH variable heavy chain
19	AA	X02.10VH variable heavy chain

SEQ ID NO:	Type	Description
20	AA	X02.11VH variable heavy chain
21	AA	X02.68VH variable heavy chain
22	AA	X02.69VH variable heavy chain
23	AA	X02.70VH variable heavy chain
24	AA	X02.71VH variable heavy chain
25	AA	X02.72VH variable heavy chain
26	AA	X02.73VH variable heavy chain
27	AA	X02.74VH variable heavy chain
28	AA	X02.75VH variable heavy chain
29	AA	X02.76VH variable heavy chain
30	AA	X02.77VH variable heavy chain
31	AA	X02.78VH variable heavy chain
32	AA	X02.108VH variable heavy chain
33	AA	X02.110VH variable heavy chain
34	AA	A02.12VH variable heavy chain
35	AA	A02.13VH variable heavy chain
36	AA	A02.1VL variable light chain
37	AA	IGLV5-37*01 germline sequence
38	AA	X02.80VL variable light chain
39	AA	X02.81VL variable light chain
40	AA	X02.82VL variable light chain
41	AA	X02.83VL variable light chain
42	AA	X02.84VL variable light chain
43	AA	X02.85VL variable light chain
44	AA	X02.86VL variable light chain
45	AA	X02.87VL variable light chain
46	AA	X02.88VL variable light chain
47	AA	X02.89VL variable light chain
48	AA	X02.90VL variable light chain
49	AA	X02.91VL variable light chain
50	AA	X02.92VL variable light chain
51	AA	X02.93VL variable light chain
52	AA	X02.94VL variable light chain
53	AA	X02.95VL variable light chain

SEQ ID NO:	Type	Description
54	AA	X02.96VL variable light chain
55	AA	X02.97VL variable light chain
56	AA	X02.98VL variable light chain
57	AA	X02.99VL variable light chain
58	AA	X02.100VL variable light chain
59	AA	X02.101VL variable light chain
60	AA	X02.102VL variable light chain
61	AA	X02.103VL variable light chain
62	AA	X02.104VL variable light chain
63	AA	X02.105VL variable light chain
64	AA	X02.106VL variable light chain
65	AA	X02.107VL variable light chain
66	AA	A02.37VL variable light chain
67	AA	A02.46VL variable light chain
68	AA	A02.47VL variable light chain
69	AA	A02.48VL variable light chain
70	AA	A02.49VL variable light chain
71	AA	A02.50VL variable light chain
72	AA	A02.51VL variable light chain
73	AA	A02.18VL variable light chain
74	AA	A02.19VL variable light chain
75	AA	A02.20VL variable light chain
76	AA	A02.21VL variable light chain
77	AA	A02.22VL variable light chain
78	AA	A02.23VL variable light chain
79	AA	A02.24VL variable light chain
80	AA	A02.25VL variable light chain
81	AA	A02.26VL variable light chain
82	AA	A02.27VL variable light chain
83	AA	A02.28VL variable light chain
84	AA	A02.29VL variable light chain
85	AA	A02.30VL variable light chain
86	AA	A02.31VL variable light chain
87	AA	A02.32VL variable light chain
88	AA	A02.33VL variable light chain

SEQ ID NO:	Type	Description
89	AA	A02.34VL variable light chain
90	AA	A02.35VL variable light chain
91	AA	A02.36VL variable light chain
92	AA	X02.16VL variable light chain
93	AA	X02.17VL variable light chain
94	AA	A02.52VL variable light chain
95	AA	A02.53VL variable light chain
96	AA	A02.54VL variable light chain
97	AA	A02.55VL variable light chain
98	AA	A02.56VL variable light chain
99	AA	A02.57VL variable light chain
100	AA	A02.58VL variable light chain
101	AA	A02.59VL variable light chain
102	AA	A02.60VL variable light chain
103	AA	A02.61VL variable light chain
104	AA	A02.62VL variable light chain
105	AA	A02.63VL variable light chain
106	AA	A02.64VL variable light chain
107	AA	A02.65VL variable light chain
108	AA	A02.66VL variable light chain
109	AA	A02.67VL variable light chain
110	AA	910VH variable heavy chain
111	AA	915 VH variable heavy chain
112	AA	912VL variable light chain
113	AA	913VL variable light chain
114	AA	Chimeric 5D1-E2-VH
115	AA	5d1_1-f*01VH
116	AA	5d1_1-f*01VH94R
117	AA	5d1_1-18*01VH
118	AA	5d1_1-18*01VH71A
119	AA	5d1_1-24*01VH
120	AA	5d1_1-24*01VH71A
121	AA	5d1_1-24*01VH29F

SEQ ID NO:	Type	Description
122	AA	5d1_1-24*01VH94R
123	AA	5d1_1-45*01VH
124	AA	5d1_1-45*01VH71A
125	AA	Chimeric 5D1VK
126	AA	5d1_1-5*01VK
127	AA	5d1_1-9*01VK
128	AA	5d1_1-12*01VK
129	AA	5d1_1D-13*01VK
130	AA	5d1_1D-16*01VK
131	AA	5d1_3-15*01VK
132	AA	Chimeric 5E8
133	AA	5E8-1-f*01VH
134	AA	5E8-1-f*01VH301
135	AA	5E8-1-f*01VH94R
136	AA	5E8-1-18*01VH
137	AA	5E8-1-18*01VH71A
138	AA	5E8-1-24*01VH
139	AA	5E8-1-24*01VH71A
140	AA	5E8-1-24*01VH94R
141	AA	5E8-1-45*01VH
142	AA	5E8-1-45*01VH71A
143	AA	chimeric 5E8VK
144	AA	5E8-2-24*01VK
145	AA	5E8-2D-28*01VK
146	AA	5E8-2D-29*01VK
147	AA	5E8-2-30*01VK
148	AA	10A2 chimeric VH
149	AA	10A2_1-24*01VH
150	AA	10A2_1-24*01VH71A
151	AA	10A2_1-24*01VH94R

SEQ ID NO:	Type	Description
152	AA	10A2_1-24*0171A94R
153	AA	10A2_1-45*01VH
154	AA	10A2_1-45*01VH71A
155	AA	10A2_1-f*01VH
156	AA	10A2_1-f*01VH94R
157	AA	10A2 chimeric VK
158	AA	10A2_1-9*01Vk
159	AA	10A2_1-12*01Vk
160	AA	10A2_1D-13*01Vk
161	AA	10A2_1-33*01Vk
162	AA	10A2_3-11*02Vk
163	AA	10A2_3-15*01Vk
164	AA	10A2_6-21*01Vk
165	AA	10A2VH + A40E
166	AA	10A2VH + A40G
167	AA	10A2VH + A40H
168	AA	10A2VH + A40Q
169	AA	10A2VH + A40S
170	AA	10A2VH + A40V
171	AA	10A2VH + N35E
172	AA	10A2VH + N35P
173	AA	10A2VH + N35Q
174	AA	10A2VH + N35S
175	AA	10A2VH + R94E
176	AA	10A2VH + R94G
177	AA	10A2VH + R94P
178	AA	10A2VH + R94T
179	AA	10A2VH + K96G
180	AA	10A2VH + K96T
181	AA	10A2VK + K24E
182	AA	10A2VK + K24G
183	AA	10A2VK + K24P
184	AA	10A2VK + K24Q

SEQ ID NO:	Type	Description
185	AA	10A2VK + R54D
186	AA	10A2VK + I48D
187	AA	10A2VK + Y49E
188	AA	10A2VK + M89A
189	AA	10A2VK + M89E
190	AA	10A2VK + M89H
191	AA	10A2VK + M89K
192	AA	10A2VK + M89P
193	AA	10A2VK + M89Q
194	AA	10A2VK + M89S
195	AA	10A2VK + M89V
196	AA	10A2VK + Q90D
197	AA	10A2VH (AQ) + N98Q
198	AA	10A2VK (AV) + N53Q
199	AA	X02.1VH FWR1
200	AA	X02.1VH CDR1
201	AA	X02.1VH FWR2
202	AA	X02.1VH CDR2
203	AA	X02.1VH FWR3
204	AA	X02.1VH CDR3
205	AA	X02.1VH FWR4
206	AA	IGHV4-61*01 FWR1
207	AA	IGHV4-61*01 CDR1
208	AA	IGHV4-61*01 FWR2
209	AA	IGHV4-61*01 FWR3
210	AA	X02.8VH FWR3
211	AA	X02.9VH FWR2
212	AA	X02.10VH FWR3
213	AA	X02.11VH FWR3
214	AA	X02.68VH FWR1
215	AA	X02.69VH FWR1
216	AA	X02.70VH FWR3
217	AA	X02.71VH FWR1
218	AA	X02.72VH FWR3

SEQ ID NO:	Type	Description
219	AA	X02.73VH FWR1
220	AA	X02.74VH CDR3
221	AA	X02.75VH FWR3
222	AA	X02.76VH CDR3
223	AA	X02.77VH CDR3
224	AA	X02.78VH CDR1
225	AA	X02.108 FWR3
226	AA	X02.110VH FWR3
227	AA	A02.12VH FWR3
228	AA	A02.12VH CDR3
229	AA	A02.13VH FWR2
230	AA	A02.13VH FWR3
231	AA	A02.13VH CDR3
232	AA	A02.1VL FWR1
233	AA	A02.1VL CDR1
234	AA	A02.1VL FWR2
235	AA	A02.1VL CDR2
236	AA	A02.1VL FWR3
237	AA	A02.1VL CDR3
238	AA	A02.1VL FWR4
239	AA	IGLV5-37*01 FWR1
240	AA	IGLV5-37*01 CDR1
241	AA	IGLV5-37*01 CDR2
242	AA	IGLV5-37*01 FWR3
243	AA	IGLV5-37*01 CDR3
244	AA	X02.80VL CDR3
245	AA	X02.81VL FWR3
246	AA	X02.82VL FWR2
247	AA	X02.83VL FWR1
248	AA	X02.84VL FWR2
249	AA	X02.84VL CDR2
250	AA	X02.86VL CDR1
251	AA	X02.87VL CDR3
252	AA	X02.88VL CDR1

SEQ ID NO:	Type	Description
253	AA	X02.89VL CDR2
254	AA	X02.90VL CDR3
255	AA	X02.91VL CDR1
256	AA	X02.92VL CDR3
257	AA	X02.93VL CDR3
258	AA	X02.94VL CDR3
259	AA	X02.95VL FWR1
260	AA	X02.96VL FWR1
261	AA	X02.97VL FWR1
262	AA	X02.98VL CDR1
263	AA	X02.99VL CDR1
264	AA	X02.100VL CDR2
265	AA	X02.101VL FWR3
266	AA	X02.102VL FWR3
267	AA	X02.103VL FWR3
268	AA	X02.104VL CDR3
269	AA	X02.105VL CDR3
270	AA	X02.106VL CDR3
271	AA	X02.107VL FWR3
272	AA	A02.37VL FWR3
273	AA	A02.37VL CDR3
274	AA	A02.46VL FWR3
275	AA	A02.46VL CDR3
276	AA	A02.47VL FWR3
277	AA	A02.48VL FWR3
278	AA	A02.49VL FWR3
279	AA	A02.50VL FWR3
280	AA	A02.51VL FWR3
281	AA	A02.18VL FWR2
282	AA	A02.18VL FWR3
283	AA	A02.19VL FWR2
284	AA	A02.19VL FWR3
285	AA	A02.20VL FWR2
286	AA	A02.20VL FWR3
287	AA	A02.21VL FWR2

SEQ ID NO:	Type	Description
288	AA	A02.21VL FWR3
289	AA	A02.22VL FWR2
290	AA	A02.22VL FWR3
291	AA	A02.23VL FWR2
292	AA	A02.23VL FWR3
293	AA	A02.24VL FWR2
294	AA	A02.24VL FWR3
295	AA	A02.25VL FWR2
296	AA	A02.25VL FWR3
297	AA	A02.26VL FWR2
298	AA	A02.26VL FWR3
299	AA	A02.27VL CDR2
300	AA	A02.27VL FWR3
301	AA	A02.28VL CDR2
302	AA	A02.28VL FWR3
303	AA	A02.29VL CDR2
304	AA	A02.29VL FWR3
305	AA	A02.30VL CDR2
306	AA	A02.30VL FWR3
307	AA	A02.31VL CDR2
308	AA	A02.31VL FWR3
309	AA	A02.32VL CDR2
310	AA	A02.32VL FWR3
311	AA	A02.33VL CDR2
312	AA	A02.33VL FWR3
313	AA	A02.34VL CDR2
314	AA	A02.34VL FWR3
315	AA	A02.35VL CDR2
316	AA	A02.35VL FWR3
317	AA	A02.36VL CDR2
318	AA	A02.36VL FWR3
319	AA	X02.16VL CDR1
320	AA	X02.16VL FWR3
321	AA	X02.16VL CDR3

SEQ ID NO:	Type	Description
322	AA	X02.17VL CDR1
323	AA	X02.17VL FWR3
324	AA	X02.17VL CDR3
325	AA	A02.52VL CDR1
326	AA	A02.52VL CDR2
327	AA	A02.52VL FWR3
328	AA	A02.52VL CDR3
329	AA	A02.53VL CDR1
330	AA	A02.53VL CDR2
331	AA	A02.53VL FWR3
332	AA	A02.53VL CDR3
333	AA	A02.54VL CDR1
334	AA	A02.54VL CDR2
335	AA	A02.54VL FWR3
336	AA	A02.54VL CDR3
337	AA	A02.55VL CDR1
338	AA	A02.55VL CDR2
339	AA	A02.55VL FWR3
340	AA	A02.55VL CDR3
341	AA	A02.56VL CDR1
342	AA	A02.56VL CDR2
343	AA	A02.56VL FWR3
344	AA	A02.56VL CDR3
345	AA	A02.57VL CDR1
346	AA	A02.57VL CDR2
347	AA	A02.57VL FWR3
348	AA	A02.57VL CDR3
349	AA	A02.58VL CDR1
350	AA	A02.58VL CDR2
351	AA	A02.58VL FWR3
352	AA	A02.58VL CDR3
353	AA	A02.59VL CDR1
354	AA	A02.59VL CDR2
355	AA	A02.59VL FWR3
356	AA	A02.59VL CDR3

SEQ ID NO:	Type	Description
357	AA	A02.60VL CDR1
358	AA	A02.60VL CDR2
359	AA	A02.60VL FWR3
360	AA	A02.60VL CDR3
361	AA	A02.61VL CDR1
362	AA	A02.61VL CDR2
363	AA	A02.61VL FWR3
364	AA	A02.61VL CDR3
365	AA	A02.62VL CDR1
366	AA	A02.62VL CDR2
367	AA	A02.62VL FWR3
368	AA	A02.62VL CDR3
369	AA	A02.63VL CDR1
370	AA	A02.63VL CDR2
371	AA	A02.63VL FWR3
372	AA	A02.63VL CDR3
373	AA	A02.64VL CDR1
374	AA	A02.64VL CDR2
375	AA	A02.64VL FWR3
376	AA	A02.64VL CDR3
377	AA	A02.65VL CDR1
378	AA	A02.65VL CDR2
379	AA	A02.65VL FWR3
380	AA	A02.65VL CDR3
381	AA	A02.66VL CDR1
382	AA	A02.66VL CDR2
383	AA	A02.66VL FWR3
384	AA	A02.66VL CDR3
385	AA	A02.67VL CDR1
386	AA	A02.67VL CDR2
387	AA	A02.67VL FWR3
388	AA	A02.67VL CDR3
389	AA	5D1.1VH FWR1
390	AA	5D1.1VH CDR1

SEQ ID NO:	Type	Description
391	AA	5D1.1VH FWR2
392	AA	5D1.1VH CDR2
393	AA	5D1.1VH FWR3
394	AA	5D1.1VH CDR3
395	AA	5D1.1VH FWR4
396	AA	5D1.2VH FWR1
397	AA	5D1.2VH FWR2
398	AA	5D1.2VH CDR2
399	AA	5D1.2VH FWR3
400	AA	5D1.3VH FWR1
401	AA	5D1.3VH FWR2
402	AA	5D1.3VH CDR2
403	AA	5D1.3VH FWR3
404	AA	5D1.4VH FWR1
405	AA	5D1.4VH FWR2
406	AA	5D1.4VH CDR2
407	AA	5D1.4VH FWR3
408	AA	5D1.5VH FWR1
409	AA	5D1.5VH FWR2
410	AA	5D1.5VH CDR2
411	AA	5D1.5VH FWR3
412	AA	5D1.6VH FWR1
413	AA	5D1.6VH FWR2
414	AA	5D1.6VH CDR2
415	AA	5D1.6VH FWR3
416	AA	5D1.7VH FWR1
417	AA	5D1.7VH FWR2
418	AA	5D1.7VH CDR2
419	AA	5D1.7VH FWR3
420	AA	5D1.8VH FWR1
421	AA	5D1.8VH FWR2
422	AA	5D1.8VH CDR2
423	AA	5D1.8VH FWR3
424	AA	5D1.9VH FWR1
425	AA	5D1.9VH FWR2

SEQ ID NO:	Type	Description
426	AA	5D1.9VH CDR2
427	AA	5D1.9VH FWR3
428	AA	5D1.10VH FWR1
429	AA	5D1.10VH FWR2
430	AA	5D1.10VH CDR2
431	AA	5D1.10VH FWR3
432	AA	5D1.11VH FWR1
433	AA	5D1.11VH FWR2
434	AA	5D1.11VH CDR2
435	AA	5D1.11VH FWR3
436	AA	5D1.1VL FWR1
437	AA	5D1.1VL CDR1
438	AA	5D1.1VL FWR2
439	AA	5D1.1VL CDR2
440	AA	5D1.1VL FWR3
441	AA	5D1.1VL CDR3
442	AA	5D1.1VL FWR4
443	AA	5D1.2VL FWR1
444	AA	5D1.2VL FWR2
445	AA	5D1.2VL FWR3
446	AA	5D1.2VL FWR4
447	AA	5D1.3VL FWR1
448	AA	5D1.3VL FWR2
449	AA	5D1.3VL FWR3
450	AA	5D1.3VL FWR4
451	AA	5D1.4VL FWR1
452	AA	5D1.4VL FWR2
453	AA	5D1.4VL FWR3
454	AA	5D1.4VL FWR4
455	AA	5D1.5VL FWR1
456	AA	5D1.5VL FWR2
457	AA	5D1.5VL FWR3
458	AA	5D1.5VL FWR4
459	AA	5D1.6VL FWR1

SEQ ID NO:	Type	Description
460	AA	5D1.6VL FWR2
461	AA	5D1.6VL FWR3
462	AA	5D1.6VL FWR4
463	AA	5D1.7VL FWR1
464	AA	5D1.7VL FWR2
465	AA	5D1.7VL FWR3
466	AA	5E8.1VH FWR1
467	AA	5E8.1VH CDR2
468	AA	5E8.1VH FWR3
469	AA	5E8.1VH CDR3
470	AA	5E8.2VH FWR1
471	AA	5E8.2VH CDR2
472	AA	5E8.3VH FWR1
473	AA	5E8.3VH CDR2
474	AA	5E8.4VH FWR1
475	AA	5E8.4VH CDR2
476	AA	5E8.5VH FWR1
477	AA	5E8.5VH CDR2
478	AA	5E8.6VH FWR1
479	AA	5E8.6VH CDR2
480	AA	5E8.7VH FWR1
481	AA	5E8.7VH CDR2
482	AA	5E8.8VH FWR1
483	AA	5E8.8VH CDR2
484	AA	5E8.9VH FWR1
485	AA	5E8.9VH CDR2
486	AA	5E8.10VH FWR1
487	AA	58E.10VH CDR2
488	AA	5E8.11VH FWR1
489	AA	58E.11VH CDR2
490	AA	5E8.1VL FWR1
491	AA	5E8.1VL CDR1
492	AA	5E8.1VL FWR2
493	AA	5E8.1VL CDR2
494	AA	5E8.1VL FWR3

SEQ ID NO:	Type	Description
495	AA	5E8.1VL CDR3
496	AA	5E8.1VL FWR4
497	AA	5E8.2VL FWR1
498	AA	5E8.2VL FWR2
499	AA	5E8.2VL FWR3
500	AA	5E8.2VL FWR4
501	AA	5E8.3VL FWR1
502	AA	5E8.3VL FWR2
503	AA	5E8.3VL FWR3
504	AA	5E8.3VL FWR4
505	AA	5E8.4VL FWR1
506	AA	5E8.4VL FWR2
507	AA	5E8.4VL FWR3
508	AA	5E8.4VL FWR4
509	AA	5E8.5VL FWR1
510	AA	5E8.5VL FWR2
511	AA	5E8.5VL FWR3
512	AA	5E8.5VL FWR4
513	AA	10A2.1VH FWR1
514	AA	10A2.1VH CDR1
515	AA	10A2.1VH FWR2
516	AA	10A2.1VH CDR2
517	AA	10A2.1VH FWR3
518	AA	10A2.1VH CDR3
519	AA	10A2.1VH FWR4
520	AA	10A2.2VH FWR2
521	AA	10A2.3VH FWR2
522	AA	10A2.4VH FWR2
523	AA	10A2.5VH FWR2
524	AA	10A2.6VH FWR2
525	AA	10A2.7VH FWR2
526	AA	10A2.8VH CDR1
527	AA	10A2.9VH CDR1
528	AA	10A2.10VH CDR1

SEQ ID NO:	Type	Description
529	AA	10A2.11VH CDR1
530	AA	10A2.12VH FWR3
531	AA	10A2.13VH FWR3
532	AA	10A2.14VH FWR3
533	AA	10A2.15VH FWR3
534	AA	10A2.16VH CDR3
535	AA	10A2.17VH CDR3
536	AA	10A2.18VH CDR3
537	AA	10A2.19VH FWR1
538	AA	10A2.19VH FWR2
539	AA	10A2.19VH CDR2
540	AA	10A2.19VH FWR3
541	AA	10A2.19VH FWR4
542	AA	10A2.20VH FWR1
543	AA	10A2.20VH FWR2
544	AA	10A2.20VH CDR2
545	AA	10A2.20VH FWR3
546	AA	10A2.20VH FWR4
547	AA	10A2.21VH FWR1
548	AA	10A2.21VH FWR2
549	AA	10A2.21VH CDR2
550	AA	10A2.21VH FWR3
551	AA	10A2.21VH FWR4
552	AA	10A2.22VH FWR1
553	AA	10A2.22VH FWR2
554	AA	10A2.22VH CDR2
555	AA	10A2.22VH FWR3
556	AA	10A2.22VH FWR4
557	AA	10A2.23VH FWR1
558	AA	10A2.23VH FWR2
559	AA	10A2.23VH CDR2
560	AA	10A2.23VH FWR3
561	AA	10A2.23VH FWR4
562	AA	10A2.24VH FWR1
563	AA	10A2.24VH FWR2

SEQ ID NO:	Type	Description
564	AA	10A2.24VH CDR2
565	AA	10A2.24VH FWR3
566	AA	10A2.24VH FWR4
567	AA	10A2.25VH FWR1
568	AA	10A2.25VH FWR2
569	AA	10A2.25VH CDR2
570	AA	10A2.25VH FWR3
571	AA	10A2.25VH FWR4
572	AA	10A2.26VH FWR1
573	AA	10A2.26VH FWR2
574	AA	10A2.26VH CDR2
575	AA	10A2.26VH FWR3
576	AA	10A2.26VH FWR4
577	AA	10A2.27VH FWR1
578	AA	10A2.27VH FWR2
579	AA	10A2.27VH CDR2
580	AA	10A2.27VH FWR3
581	AA	10A2.27VH FWR4
582	AA	10A2.1VL FWR1
583	AA	10A2.1VL CDR1
584	AA	10A2.1VL FWR2
585	AA	10A2.1VL CDR2
586	AA	10A2.1VL FWR3
587	AA	10A2.1VL CDR3
588	AA	10A2.1VL FWR4
589	AA	10A2.2VL CDR1
590	AA	10A2.3VL CDR1
591	AA	10A2.4VL CDR2
592	AA	10A2.5VL FWR2
593	AA	10A2.6VL FWR2
594	AA	10A2.7VL CDR3
595	AA	10A2.8VL CDR3
596	AA	10A2.9VL CDR3
597	AA	10A2.10VL CDR3

SEQ ID NO:	Type	Description
598	AA	10A2.11VL CDR3
599	AA	10A2.12VL CDR3
600	AA	10A2.13VL CDR3
601	AA	10A2.14VL CDR3
602	AA	10A2.15VL CDR3
603	AA	10A2 16VL CDR3
604	AA	10A2.17VL CDR3
605	AA	10A2.18VL CDR2
606	AA	10A2.19VL CDR3
607	AA	10A2.20VL FWR1
608	AA	10A2.20VL CDR1
609	AA	10A2,20VL FWR2
610	AA	10A2.20VL CDR2
611	AA	10A2.20VL FWR3
612	AA	10A2.20VL CDR3
613	AA	10A2.20VL FWR4
614	AA	10A2.21VL FWR1
615	AA	10A2.21VL FWR2
616	AA	10A2.21VL FWR3
617	AA	10A2.21VL FWR4
618	AA	10A2.22VL FWR1
619	AA	10A2.22VL FWR2
620	AA	10A2.22VL FWR3
621	AA	10A2.22VL FWR4
622	AA	10A2.23VL FWR1
623	AA	10A2.23VL FWR2
624	AA	10A2.23VL FWR3
625	AA	10A2.23VL FWR4
626	AA	10A2.24VL FWR1
627	AA	10A2.24VL FWR2
628	AA	10A2.24VL FWR3
629	AA	10A2.24VL FWR4
630	AA	10A2.25VL FWR1
631	AA	10A2.25VL FWR2
632	AA	10A2.25VL FWR3

SEQ ID NO:	Type	Description
633	AA	10A2.25VL FWR4
634	AA	10A2.26VL FWR1
635	AA	10A2.26VL FWR2
636	AA	10A2.26VL FWR3
637	AA	10A2.26VL FWR4
638	AA	10A2.27VL FWR1
639	AA	10A2.27 VL FWR2
640	AA	10A2.27VL FWR3
641	AA	10A2.27VL FWR4
642	AA	Gly4Ser1
643	AA	Gly4Ser1 × 2
644	AA	Gly4Ser1 × 3
645	AA	Gly4Ser1 × 4
646	AA	Gly4Ser1 × 5
647	AA	IFN-alpha2b A145D
648	AA	Trunc IFN-alpha2b
649	AA	Trunc IFN-alpha2b A145D
650	AA	IFN-alpha2b A145G
651	AA	Trunc IFN-alpha2b A145G
652	AA	IgG4 IFN-alpha2b A145D
653	AA	IgG4 IFN-alpha2b A145G
654	AA	IgG4 S228P IFN-alpha2b A145G
655	AA	IgG1 IFN-alpha2b A145G
656	AA	IgG1 YTE IFN-alpha2b A145D
657	AA	IgG1 YTE IFN-alpha2b A145G
658	AA	IgG4 YTE IFN-alpha2b A145D
659	AA	A02 consensus variable heavy
660	AA	X02.114VL
661	AA	X02.115VL
662	AA	X02.116VL
663	AA	X02.117VL
664	AA	A02 consensus variable light
665	AA	A10 consensus variable heavy
666	AA	A10 consensus variable light

SEQ ID NO:	Type	Description
667	DNA	A02.12VH
668	DNA	X02.9VH
669	DNA	X02.107VL
670	DNA	A02.47VL
671	DNA	A02.31VL
672	DNA	A02.33VL
673	DNA	X02.16VL
674	DNA	X02.17VL
675	DNA	X02.114VL
676	DNA	X02.115VL
677	DNA	X02.116VL
678	DNA	X02.117VL
679	DNA	10A2VH + A40E
680	DNA	10A2VH + A40G
681	DNA	10A2VH + A40H
682	DNA	10A2VH + A40Q
683	DNA	10A2VH + K96G
684	DNA	10A2VH + K96T
685	DNA	10A2_1-f*01VH94R
686	DNA	10A2VH (AQ) + N98Q
687	DNA	10A2-1-24*0171A94R
688	DNA	10A2_1-33*01Vk
689	DNA	10A2VK + K24G
690	DNA	10A2VK + K24Q
691	DNA	10A2VK + R54D
692	DNA	10A2VK + M89A
693	DNA	10A2VK (AV) + N53Q
694	AA	IgG4 YTE IFN-alpha2b A145G
695	DNA	910VH variable heavy chain
696	AA	10A2VK + K24G CDR1
697	AA	910VH CDR1
698	AA	910VH CDR2
699	AA	910VH CDR3
700	AA	X02.118 variable light chain

SEQ ID NO:	Type	Description
701	AA	X02.119 variable light chain
702	DNA	X02.118 variable light chain
703	DNA	X02.119 variable light chain
704	AA	X10.60 variable light chain
705	AA	X10.61 variable light chain
706	AA	X10.62 variable light chain
707	AA	X10.63 variable light chain
708	AA	X10.64 variable light chain
709	AA	X10.65 variable light chain
710	AA	X10.66 variable light chain
711	AA	X10.67 variable light chain
712	DNA	X10.60 variable light chain
713	DNA	X10.61 variable light chain
714	DNA	X10.62 variable light chain
715	DNA	X10.63 variable light chain
716	DNA	X10.64 variable light chain
717	DNA	X10.65 variable light chain
718	DNA	X10.66 variable light chain
719	DNA	X10.67 variable light chain
720	AA	X10.68 variable heavy chain
721	AA	X10.69 variable heavy chain
722	AA	X10.70 variable heavy chain
723	AA	X10.71 variable heavy chain
724	DNA	X10.68 variable heavy chain
725	DNA	X10.69 variable heavy chain
726	DNA	X10.70 variable heavy chain
727	DNA	X10.71 variable heavy chain
728	AA	X02.120 variable heavy chain
729	AA	X02.121 variable heavy chain
730	AA	X02.122 variable heavy chain
731	AA	X02.123 variabel heavy chain
732	DNA	X02.120 variable heavy chain
733	DNA	X02.121 variable heavy chain
734	DNA	X02.122 variable heavy chain
735	DNA	X02.123 variable heavy chain

SEQ ID NO:	Type	Description
736	AA	910 variable heavy consensus
737	AA	X02.122VH CDR2
738	AA	X02.123VH CDR2
739	AA	X10.72 variable heavy chain
740	AA	X10.73 variable heavy chain
741	AA	X10.74 variable heavy chain
742	AA	X10.75 variable heavy chain
743	DNA	X10.72 variable heavy chain
744	DNA	X10.73 variable heavy chain
745	DNA	X10.74 variable heavy chain
746	DNA	X10.75 variable heavy chain
747	AA	X10.64VL CDR2
748	AA	910 VH FRW1
749	AA	910 VH FRW2
750	AA	X10.120VH FRW2
751	AA	910 VH FRW3
752	AA	X10.121VH FRW3
753	AA	910VH FRW4

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PATENTKRAV

1. Et polynukleotid omfattende en nukleinsyresekvens, der koder for et antistof, der specifikt binder til CD38, antistoffet omfattende en tung kæde variabel region omfattende aminosyresekvensen af SEQ ID NO: 156 og en let kæde variabel region omfattende aminosyresekvensen af SEQ ID NR: 185.

2. Polynukleotid ifølge krav 1, hvor nukleinsyresekvensen koder for et antistof omfattende en human IgG1 konstant region.

3. Polynukleotid ifølge krav 1, hvor nukleinsyresekvensen koder for et antistof omfattende en human IgG4 konstant region.

4. Polynukleotid ifølge krav 3, hvor den humane IgG4 tung kæde konstante region omfatter en prolin i position 228 ifølge EU-nummereringssystemet.

5. Polynukleotid ifølge krav 3 eller 4, hvor den humane IgG4 tung kæde konstante region omfatter en tyrosin i position 252, en threonin i position 254 og en glutaminsyre i position 256 i den konstante region ifølge EU-nummereringssystemet.

6. Polynukleotid ifølge et hvilket som helst af de foregående krav, omfattende en nukleinsyresekvens omfattende SEQ ID NO: 685, som koder for en tung kæde variabel region, og en nukleinsyresekvens omfattende SEQ ID NO: 691, som koder for en let kæde variabel region.

7. Polynukleotidet ifølge et hvilket som helst af de foregående krav, hvor nukleinsyresekvensen koder for et interferon alpha-2b fusioneret til antistoffet, hvor interferonet alpha-2b omfatter en alanin til asparaginsyre eller alanin til glycin substitution ved position svarende til position 168 i aminosyresekvensen af SEQ ID NO: 7.

8. Polynukleotid ifølge krav 7, hvor nukleinsyresekvensen koder for et interferon alpha-2b, som er et N-terminalt trunkeret interferon alpha-2b.

9. Polynukleotid ifølge krav 8, hvor det N-terminalt trunkerede interferon alpha-2b ikke har de treogtyve N-terminale aminosyrer.

10. Polynukleotid ifølge krav 7, 8 eller 9, hvor nukleinsyresekvensen koder for et interferon alpha-2b omfattende aminosyresekvensen af SEQ ID NO: 649 eller SEQ ID NO: 651.

11. Polynukleotid ifølge krav 7, hvor nukleinsyresekvensen koder for et interferon alpha-2b omfattende aminosyresekvensen SEQ ID NO: 647 eller SEQ ID NO: 650.

12. Vektor omfattende polynukleotidet ifølge et hvilket som helst af kravene 1 til 11.

13. In vitro transformeret pattedyrcelle omfattende vektoren ifølge krav 12.

DRAWINGS

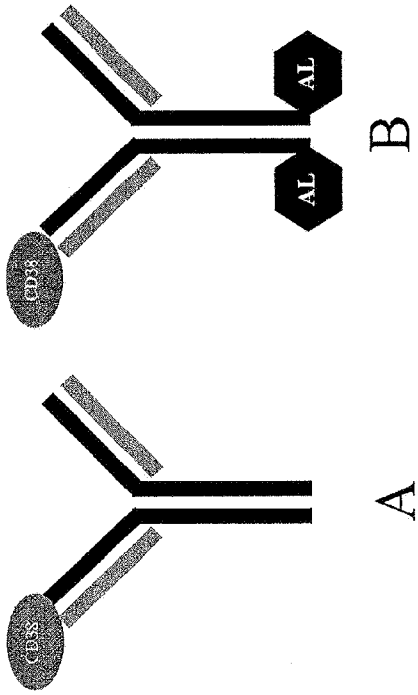

FIG. 1

FIG. 2A

SEQ ID NO: 13	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWSWIRQHPGKGLEWIGYIYYSGSTNYNPSLKS
SEQ ID NO: 16	.V.....V..G.....P.....
SEQ ID NO: 17	.....
SEQ ID NO: 18	.....P.....
SEQ ID NO: 19	.....
SEQ ID NO: 20	.....
SEQ ID NO: 21	.....K.....
SEQ ID NO: 22	.V.....
SEQ ID NO: 23	.....
SEQ ID NO: 24	.....V.....
SEQ ID NO: 25	.....
SEQ ID NO: 26	.....K.....
SEQ ID NO: 27	.....
SEQ ID NO: 28	.....
SEQ ID NO: 29	.....
SEQ ID NO: 30	.....
SEQ ID NO: 31	.....G.....
SEQ ID NO: 32	.....
SEQ ID NO: 33	.....
SEQ ID NO: 34	.....
SEQ ID NO: 35	.....P.....

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FIG. 2B

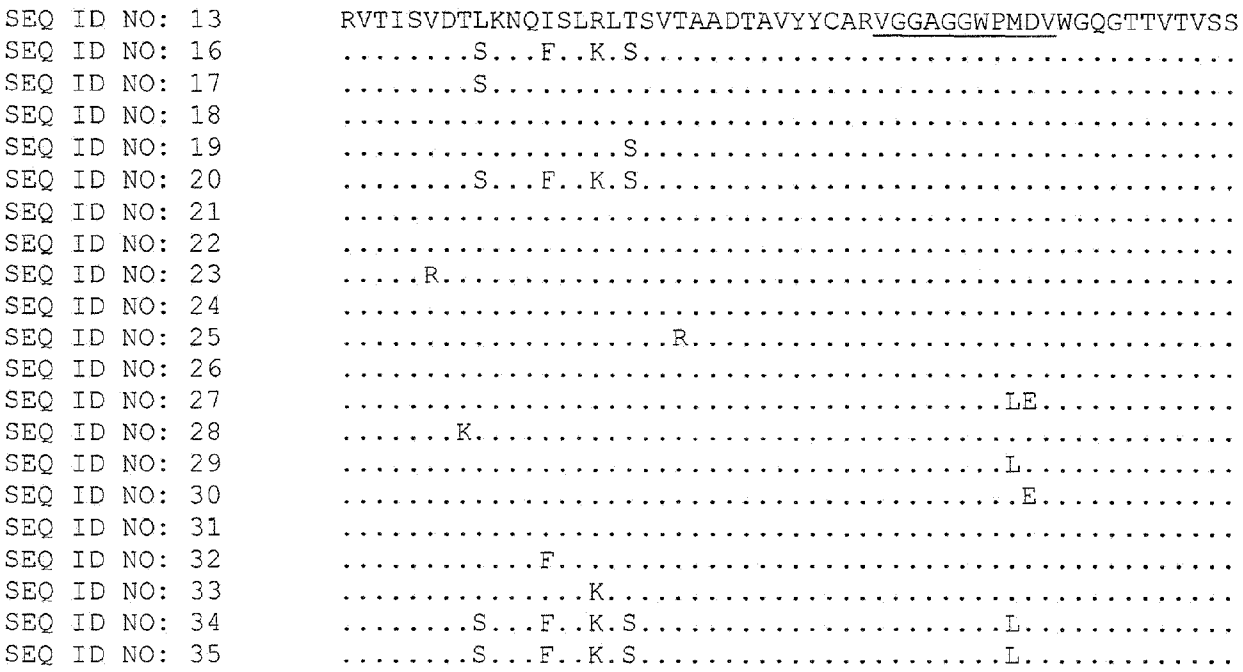

FIG. 3A

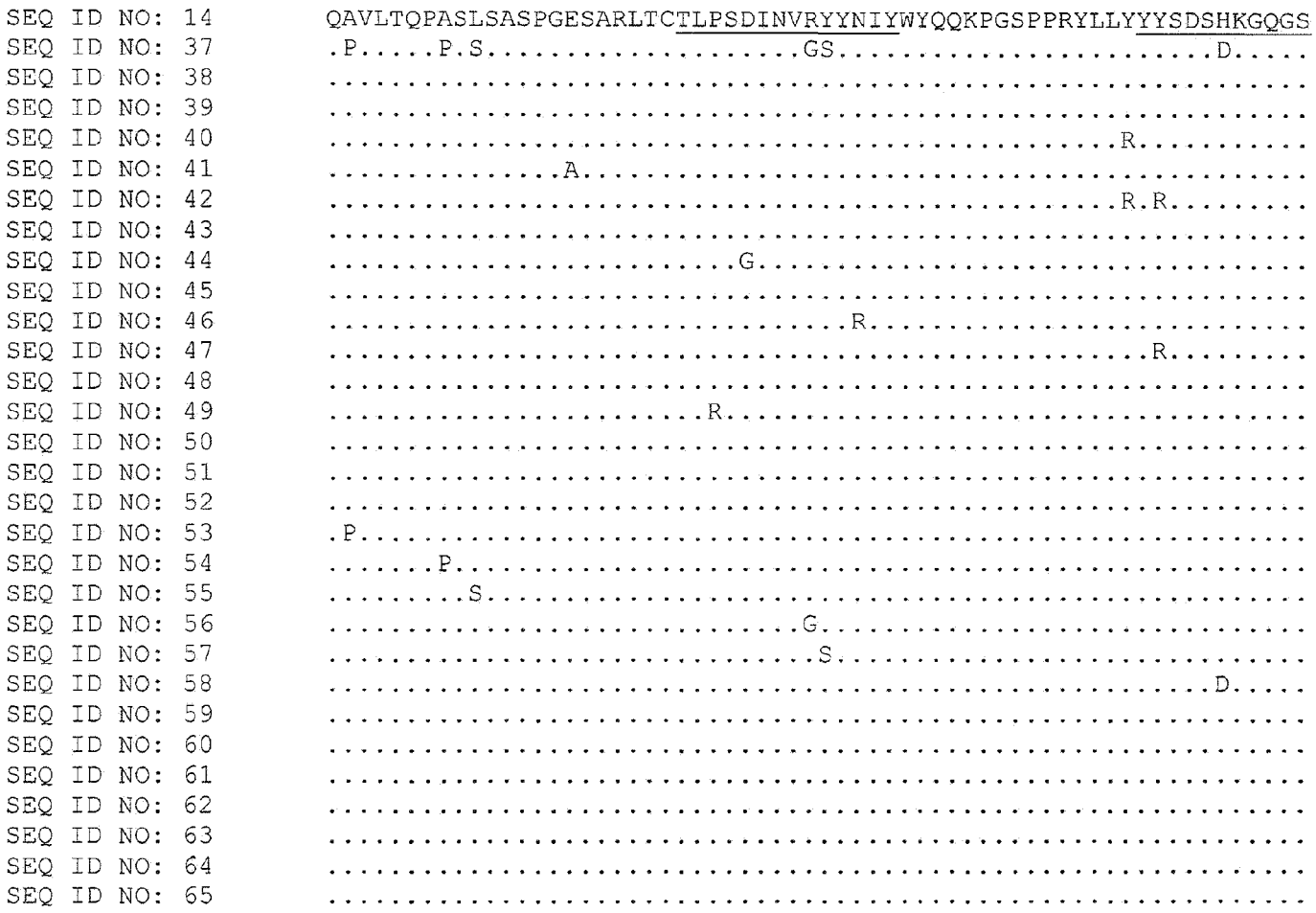

FIG. 3B

```
SEQ ID NO: 14   GVPSRFSGSKDVSTNSGILLISGLQSEDEADYYCMTWSSNGSGVFGGGTQLTVLG
SEQ ID NO: 37   ..........A.A.T..................I.P..A.............
SEQ ID NO: 38   ....................................Q.............
SEQ ID NO: 39   ............A.....................................
SEQ ID NO: 40   ............................................................
SEQ ID NO: 41   ............................................................
SEQ ID NO: 42   ............................................................
SEQ ID NO: 43   .........--..................................................
SEQ ID NO: 44   ............................................................
SEQ ID NO: 45   ....................................A.....................
SEQ ID NO: 46   ............................................................
SEQ ID NO: 47   ............................................................
SEQ ID NO: 48   ....................................T.....................
SEQ ID NO: 49   ............................................................
SEQ ID NO: 50   ....................................P.....................
SEQ ID NO: 51   ..................................I.....................
SEQ ID NO: 52   ..................................L.....................
SEQ ID NO: 53   ............................................................
SEQ ID NO: 54   ............................................................
SEQ ID NO: 55   ............................................................
SEQ ID NO: 56   ............................................................
SEQ ID NO: 57   ............................................................
SEQ ID NO: 58   ............................................................
SEQ ID NO: 59   ..........A.................................................
SEQ ID NO: 60   ............A...............................................
SEQ ID NO: 61   ..............T.............................................
SEQ ID NO: 62   ..................................I.....................
SEQ ID NO: 63   ....................................P.....................
SEQ ID NO: 64   ....................................A.....................
SEQ ID NO: 65   ............................I.T.............................
```

FIG. 4A

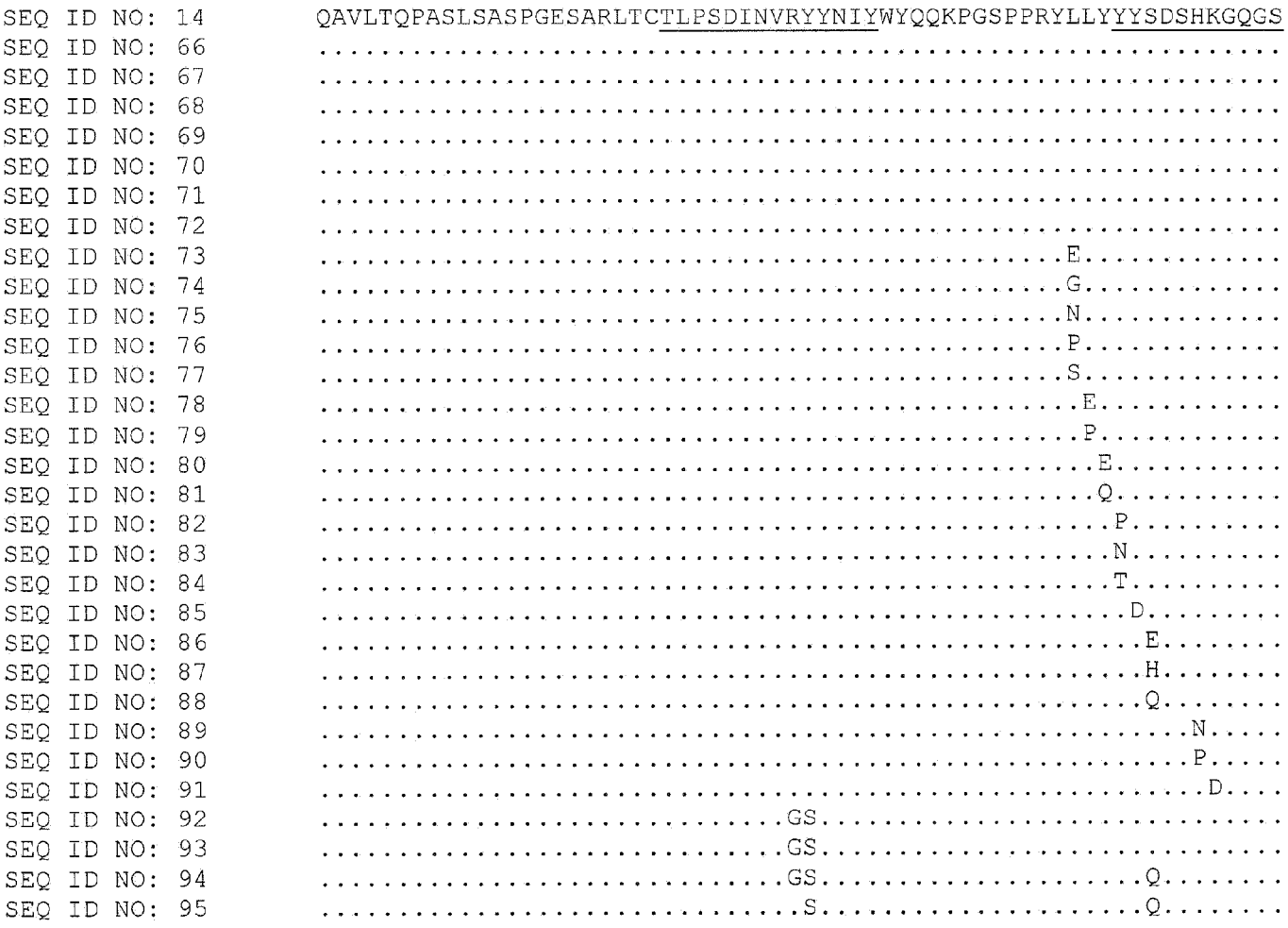

FIG. 4B

```
SEQ ID NO: 96     ..............................GS......................Q.......
SEQ ID NO: 97     ...............................S......................Q.......
SEQ ID NO: 98     ..............................GS......................E.......
SEQ ID NO: 99     ...............................S......................E.......
SEQ ID NO: 100    ..............................GS......................E.......
SEQ ID NO: 101    ...............................S......................E.......
SEQ ID NO: 102    ..............................GS......................Q.......
SEQ ID NO: 103    ...............................S......................Q.......
SEQ ID NO: 104    ..............................GS......................Q.......
SEQ ID NO: 105    ...............................S......................Q.......
SEQ ID NO: 106    ..............................GS......................E.......
SEQ ID NO: 107    ...............................S......................E.......
SEQ ID NO: 108    ..............................GS......................E.......
SEQ ID NO: 109    ...............................S......................E.......
```

FIG. 4C

```
SEQ ID NO: 14    GVPSRFSGSKDVSTNSGILLISGLQSEDEADYYCMTWSSNGSGVFGGGTQLTVLG
SEQ ID NO: 66    ............................I.T...L.....................
SEQ ID NO: 67    .............Q..............I.T.......................
SEQ ID NO: 68    .............T..............I.T.......................
SEQ ID NO: 69    .............G..............I.T.......................
SEQ ID NO: 70    .............H..............I.T.......................
SEQ ID NO: 71    .............K..............I.T.......................
SEQ ID NO: 72    .............P..............I.T.......................
SEQ ID NO: 73    ............................I.T.......................
SEQ ID NO: 74    ............................I.T.......................
SEQ ID NO: 75    ............................I.T.......................
SEQ ID NO: 76    ............................I.T.......................
SEQ ID NO: 77    ............................I.T.......................
SEQ ID NO: 78    ............................I.T.......................
SEQ ID NO: 79    ............................I.T.......................
SEQ ID NO: 80    ............................I.T.......................
SEQ ID NO: 81    ............................I.T.......................
SEQ ID NO: 82    ............................I.T.......................
SEQ ID NO: 83    ............................I.T.......................
SEQ ID NO: 84    ............................I.T.......................
SEQ ID NO: 85    ............................I.T.......................
SEQ ID NO: 86    ............................I.T.......................
SEQ ID NO: 87    ............................I.T.......................
SEQ ID NO: 88    ............................I.T.......................
SEQ ID NO: 89    ............................I.T.......................
SEQ ID NO: 90    ............................I.T.......................
SEQ ID NO: 91    ............................I.T.......................
SEQ ID NO: 92    ............................I.T...L....Q...............
SEQ ID NO: 93    ............................I.T...L....E...............
SEQ ID NO: 94    ............................I.T...L....E...............
SEQ ID NO: 95    ............................I.T...L....E...............
```

FIG. 4D

```
SEQ ID NO: 96  ..........I.T....L....Q.....
SEQ ID NO: 97  ..........I.T....L....Q.....
SEQ ID NO: 98  ..........I.T....L....E.....
SEQ ID NO: 99  ..........I.T....L....E.....
SEQ ID NO: 100 ..........I.T....L....Q.....
SEQ ID NO: 101 ..........I.T....L....Q.....
SEQ ID NO: 102 ..........I.T....L....E.....
SEQ ID NO: 103 ..........I.T....L....E.....
SEQ ID NO: 104 ..........I.T....L....Q.....
SEQ ID NO: 105 ..........I.T....L....Q.....
SEQ ID NO: 106 ..........I.T....L....E.....
SEQ ID NO: 107 ..........I.T....L....E.....
SEQ ID NO: 108 ..........I.T....L....Q.....
SEQ ID NO: 109 ..........I.T....L....Q.....
```

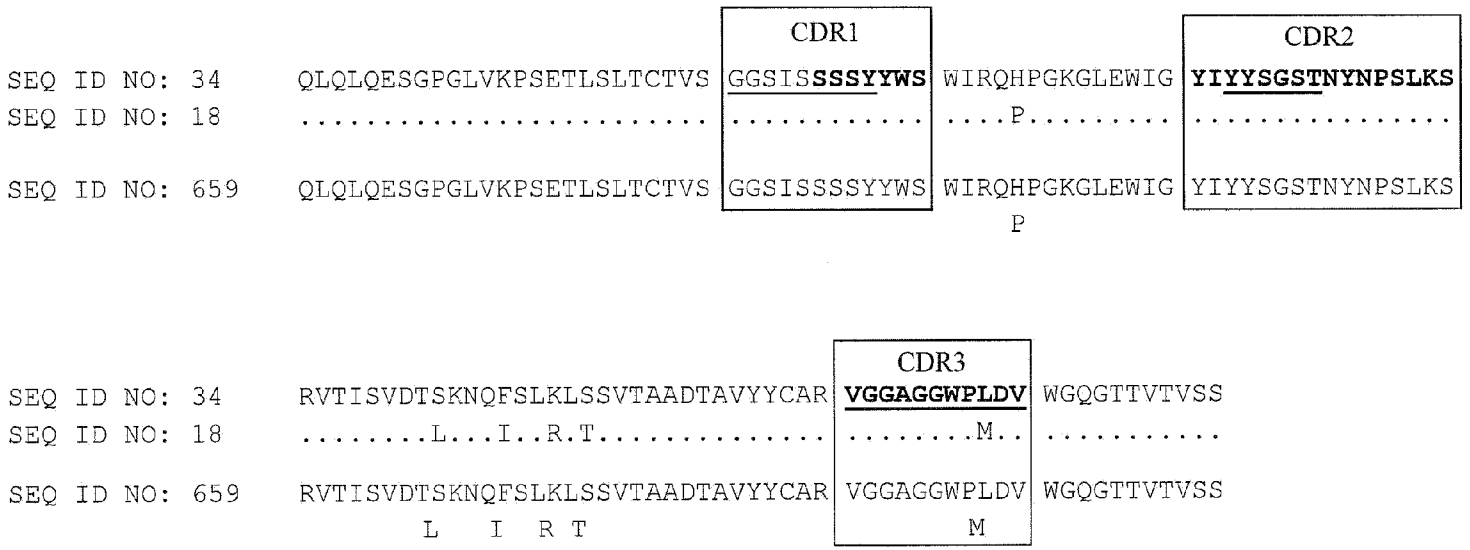

FIG. 5

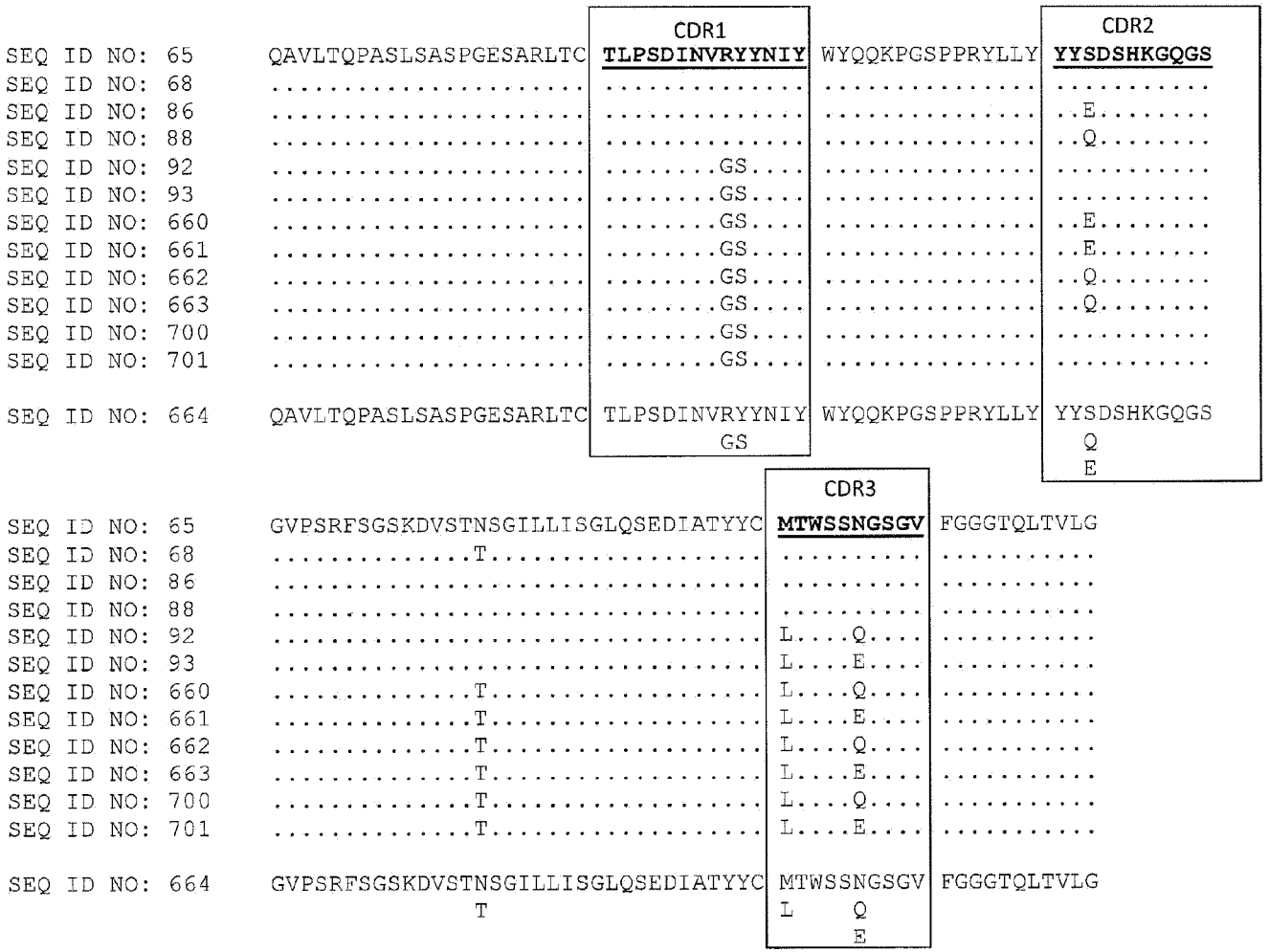

FIG. 6

FIG. 7A

```
SEQ ID NO: 114      EVQLQQSGPEVGRPGSSVKISCKASGYTFTDYIMHWVKQSPGQGLEWIGWIDPEYGRTDYAEKFKK
SEQ ID NO: 115      ....V...A..KK..AT......V.............Q.A..K....M................QG
SEQ ID NO: 116      ....V...A..KK..AT......V.............Q.A..K....M................QG
SEQ ID NO: 117      Q...V...A..KK..A...V..................R.A.......M.............Q.LQG
SEQ ID NO: 118      Q...V...A..KK..A...V..................R.A.......M.............Q.LQG
SEQ ID NO: 119      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 120      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 121      Q...V...A..KK..A...V...V.............R.A..K....M.............Q..QG
SEQ ID NO: 122      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 123      QM..V...A..KKT.....V..................R.A...A...M.............Q..QD
SEQ ID NO: 124      QM..V...A..KKT.....V..................R.A...A...M.............Q..QD

SEQ ID NO: 114      KATLTADTSSSTAYIQLSSLASEDTATYFCARSAHTTGFGFAYWGQGTLVTVSS
SEQ ID NO: 115      RV.I.....TD...ME....R.....V.Y..T.....................
SEQ ID NO: 116      RV.I.....TD...ME....R.....V.Y........................
SEQ ID NO: 117      RV.M.T...T....ME.R..R.D...V.Y........................
SEQ ID NO: 118      RV.M.....T....ME.R..R.D...V.Y........................
SEQ ID NO: 119      RV.M.E...TD...ME....R.....V.Y..T.....................
SEQ ID NO: 120      RV.M.....TD...ME....R.....V.Y..T.....................
SEQ ID NO: 121      RV.M.E...TD...ME....R.....V.Y..T.....................
SEQ ID NO: 122      RV.M.E...TD...ME....R.....V.Y........................
SEQ ID NO: 123      RV.I.R.R.M....ME....R.....M.Y........................
SEQ ID NO: 124      RV.I...R.M....ME....R.....M.Y........................
```

FIG. 7B

```
SEQ ID NO: 132      EVQLQQSGPEVGRPGSSVKISCKASGYIFTDYIMHWVKQSPGQGLEWIGWIDPEYGSTDYAEKFKK
SEQ ID NO: 133      ....V...A..KK..AT.....V...T.........Q.A..K....M.............QG
SEQ ID NO: 134      ....V...A..KK..AT.....V.............Q.A..K....M.............QG
SEQ ID NO: 135      ....V...A..KK..AT.....V...T.........Q.A..K....M.............QG
SEQ ID NO: 136      Q...V...A..KK..A...V.......T.........R.A.......M............Q.LQG
SEQ ID NO: 137      Q...V...A..KK..A...V.......T.........R.A.......M............Q.LQG
SEQ ID NO: 138      Q...V...A..KK..A...V...V...TL........R.A..K....M............Q..QG
SEQ ID NO: 139      Q...V...A..KK..A...V...V...TL........R.A..K....M............Q..QG
SEQ ID NO: 140      Q...V...A..KK..A...V...V...TL........R.A..K....M............Q..QG
SEQ ID NO: 141      QM..V...A..KKT.....V.......T.........R.A...A...M............Q..QD
SEQ ID NO: 142      QM..V...A..KKT.....V.......T.........R.A...A...M............Q..QD

SEQ ID NO: 132      KATLTADTSSSTAYIQLSSLTSEDTATYFCARVAIITTVASGGFAYWGQGTLVTVSS
SEQ ID NO: 133      RV.I.....TD...ME....R.....V.Y..T.....................
SEQ ID NO: 134      RV.I.....TD...ME....R.....V.Y..T.....................
SEQ ID NO: 135      RV.I.....TD...ME....R.....V.Y........................
SEQ ID NO: 136      RV.M.T...T....ME.R..R.D...V.Y........................
SEQ ID NO: 137      RV.M.....T....ME.R..R.D...V.Y........................
SEQ ID NO: 138      RV.M.E...TD...ME....R.....V.Y..T.....................
SEQ ID NO: 139      RV.M.....TD...ME....R.....V.Y..T.....................
SEQ ID NO: 140      RV.M.E...TD...ME....R.....V.Y........................
SEQ ID NO: 141      RV.I.R.R.M....ME....R.....M.Y........................
SEQ ID NO: 142      RV.I...R.M....ME....R.....M.Y........................
```

FIG. 7C

```
SEQ ID NO: 148      EVQLQQSGPEVGRPGSSVKISCKASGYTFTDSVMNWVKQSPGQGLEWIGWIDPEYGRTDVAEKFKK
SEQ ID NO: 149      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 150      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 151      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 152      Q...V...A..KK..A...V...V....L........R.A..K....M.............Q..QG
SEQ ID NO: 153      QM..V...A..KKT.....V.................R.A...A...M.............Q..QD
SEQ ID NO: 154      QM..V...A..KKT.....V.................R.A...A...M.............Q..QD
SEQ ID NO: 155      ....V...A..KK..AT......V.............Q.A..K....M.................QG
SEQ ID NO: 156      ....V...A..KK..AT......V.............Q.A..K....M.................QG

SEQ ID NO: 148      KATLTADSSSSTAYIYLSGLTSEDTATYFCARTKYNSGYGFPYWGQGSLVTVSS
SEQ ID NO: 149      RV.M.E.T.TD...ME..S.R.....V.Y..T...............TT.....
SEQ ID NO: 150      RV.M...T.TD...ME..S.R.....V.Y..T...............TT.....
SEQ ID NO: 151      RV.M.E.T.TD...ME..S.R.....V.Y..................TT.....
SEQ ID NO: 152      RV.M...T.TD...ME..S.R.....V.Y..................TT.....
SEQ ID NO: 153      RV.I.R.R.M....ME..S.R.....M.Y..................TT.....
SEQ ID NO: 154      RV.I...R.M....ME..S.R.....M.Y..................TT.....
SEQ ID NO: 155      RV.I...T.TD...ME..S.R.....V.Y..T...............TT.....
SEQ ID NO: 156      RV.I...T.TD...ME..S.R.....V.Y..................TT.....
```

FIG. 8A

```
SEQ ID NO: 125      DIQMTQSPASLSASLGETVTIECRASEDIYSNLAWYQQKPGNSPQLLIYDANSLAD
SEQ ID NO: 126      ........ST....V.DR...T.....................KA.K..........
SEQ ID NO: 127      ...L....SF....V.DR...T.....................KA.K..........
SEQ ID NO: 128      ........S.V...V.DR...T.....................KA.K..........
SEQ ID NO: 129      A..L....S.....V.DR...T.....................KA.K..........
SEQ ID NO: 130      ........S.....V.DR...T.....................EKA.KS.........
SEQ ID NO: 131      E.V......T..V.P..RA.LS.....................QA.R..........

SEQ ID NO: 125      GVPSRFSASGSGTQFSLKINSLRSEDVASYFCQQYNNYPYTFGAGTKLELKR
SEQ ID NO: 126      .......G.....E.T.T.S..QPD.F.T.Y............Q.....I..
SEQ ID NO: 127      .......G.....E.T.T.S..QP..F.T.Y............Q.....I..
SEQ ID NO: 128      .......G.....D.T.T.S..QP..F.T.Y............Q.....I..
SEQ ID NO: 129      .......G.....D.T.T.S..QP..F.T.Y............Q.....I..
SEQ ID NO: 130      .......G.....D.T.T.S..QP..F.T.Y............Q.....I..
SEQ ID NO: 131      .I.A...G.....E.T.T.S..Q...F.V.Y............Q.....I..
```

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FIG. 8B

```
SEQ ID NO: 143      DIVMTQGALPNPVPSGESASITCQSSESLLHSNGKTYLNWYLQRPGQSPQLLIYWMSTRAA
SEQ ID NO: 144      ......TP.SS..TL.QP...S.................LQ.....P.R..........
SEQ ID NO: 145      ......SP.SL..TP..P...S.....................K................
SEQ ID NO: 146      ......TP.SLS.TP.QP...S.....................K...P............
SEQ ID NO: 147      .V....SP.SL..TL.QP...S.................FQ.......RR.........

SEQ ID NO: 143      GVSDRFSGSGSGTDFTLTISGVEAEDVGVYYCQQFLEYPPTFGSGTKLEIKR
SEQ ID NO: 144      ..P.......A......K..R.....................Q........
SEQ ID NO: 145      ..P.............K..R.....................Q........
SEQ ID NO: 146      ..P.............K..R.....................Q........
SEQ ID NO: 147      ..P.............K..R.....................Q........
```

FIG. 8C

```
SEQ ID NO: 157      DIVMTQSPTSISISVGERVTMNCKASQNVDSDVDWYQQKTGQSPKLLIYKASNRYT
SEQ ID NO: 158      ..QL....SFL.A...D...IT.................P.KA.............
SEQ ID NO: 159      ..Q.....S.V.A...D...IT.................P.KA.............
SEQ ID NO: 160      A.QL....S.L.A...D...IT.................P.KA.............
SEQ ID NO: 161      ..Q.....S.L.A...D...IT.................P.KA.............
SEQ ID NO: 162      E..L....ATL.L.P...A.LS.................P..A.R...........
SEQ ID NO: 163      E.......ATL.V.P...A.LS.................P..A.R...........
SEQ ID NO: 164      E..L....DFQ.VTPK.K..IT.................PD.......K.......

SEQ ID NO: 157      GVPDRFTGSGSGTDFTFTISNMQAEDLAVYYCMQSNTHPRTFGGGTKLELKR
SEQ ID NO: 158      ...S..S......E..L...SL.P..F.T..................V.I..
SEQ ID NO: 159      ...S..S.........L...SL.P..F.T..................V.I..
SEQ ID NO: 160      ...S..S.........L...SL.P..F.T..................V.I..
SEQ ID NO: 161      ...S..S.............SL.P..I.T..................V.I..
SEQ ID NO: 162      .I.A..S.....R...L...SLEP..F....................V.I..
SEQ ID NO: 163      .I.A..S......E..L...SL.S..F....................V.I..
SEQ ID NO: 164      ...S..S.........L..NSLE...A.T..................V.I..
```

FIG. 9A

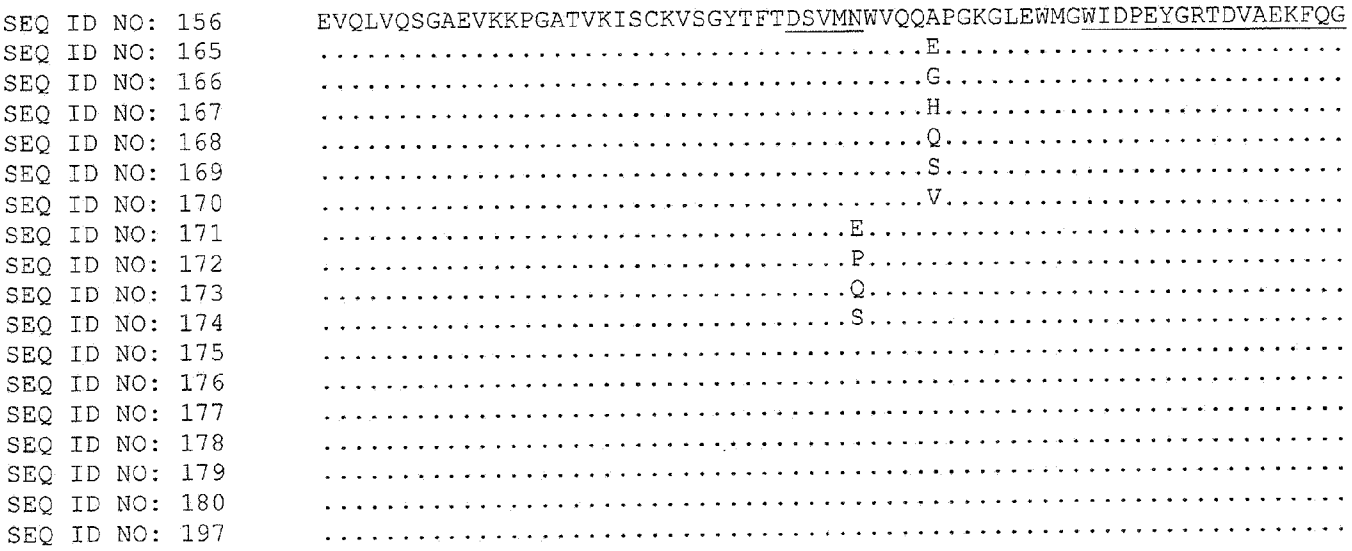


FIG. 9B

SEQ ID NO: 156	RVTITADTSTDTAYMELSSLRSEDTAVYYCARTKYNSGYGFPYWGQGTTVTVSS
SEQ ID NO: 165	.....
SEQ ID NO: 166	.....
SEQ ID NO: 167	.....
SEQ ID NO: 168	.....
SEQ ID NO: 169	.....
SEQ ID NO: 170	.....
SEQ ID NO: 171	.....
SEQ ID NO: 172	.....
SEQ ID NO: 173	.....
SEQ ID NO: 174	.....
SEQ ID NO: 175	.....E.....
SEQ ID NO: 176	.....G.....
SEQ ID NO: 177	.....P.....
SEQ ID NO: 178	.....T.....
SEQ ID NO: 179	.....G.....
SEQ ID NO: 180	.....T.....
SEQ ID NO: 197	.....Q.....

FIG. 10A

SEQ ID NO: 161	DIQMTQSPSSLSASVGDRVTITCKASQNVDSDVDWYQQKPGKAPKLLIYKASNRYT
SEQ ID NO: 183	.....P.....
SEQ ID NO: 184	.....Q.....
SEQ ID NO: 181	.....E.....
SEQ ID NO: 182	.....G.....
SEQ ID NO: 185	.....D..
SEQ ID NO: 186	.....D.....
SEQ ID NO: 187	.....E.....
SEQ ID NO: 188	.....
SEQ ID NO: 189	.....
SEQ ID NO: 190	.....
SEQ ID NO: 191	.....
SEQ ID NO: 192	.....
SEQ ID NO: 193	.....
SEQ ID NO: 194	.....
SEQ ID NO: 195	.....
SEQ ID NO: 196	.....
SEQ ID NO: 198	.....Q...

FIG. 10B

SEQ ID NO: 161	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYCMQSNTHPRTFGGGTKVEIKR
SEQ ID NO: 183	.....
SEQ ID NO: 184	.....
SEQ ID NO: 181	.....
SEQ ID NO: 182	.....
SEQ ID NO: 185	.....
SEQ ID NO: 186	.....
SEQ ID NO: 187	.....
SEQ ID NO: 188	.....A.....
SEQ ID NO: 189	.....E.....
SEQ ID NO: 190	.....H.....
SEQ ID NO: 191	.....K.....
SEQ ID NO: 192	.....P.....
SEQ ID NO: 193	.....Q.....
SEQ ID NO: 194	.....S.....
SEQ ID NO: 195	.....V.....
SEQ ID NO: 196	.....D.....
SEQ ID NO: 198	.....

		CDR1		CDR2
SEQ ID NO: 165	EVQLVQSGAEVKKPGATVKISCKVS	GYTFT**DSVMN**	WVQQEPGKGLEWMG	**WIDPEYGRTDVAEKFQG**
SEQ ID NO: 166	.....	.....	....G.....	.....
SEQ ID NO: 167	.....	.....	....H.....	.....
SEQ ID NO: 179	.....	.....	....A.....	.....
SEQ ID NO: 180	.....	.....	....A.....	.....
SEQ ID NO: 156	.....	.....	....A.....	.....
SEQ ID NO: 197	.....	.....	....A.....	.....
SEQ ID NO: 152	Q.....S..V.....	...L.....	..R.A.....	.....Q....
SEQ ID NO: 720	Q.....S..V.....	...L.....	..R.G.....	.....Q....
SEQ ID NO: 721	Q.....S..V.....	...L.....	..R.H.....	.....Q....
SEQ ID NO: 722	Q.....S..V.....	...L.....	..R.G.....	.....Q....
SEQ ID NO: 723	Q.....S..V.....	...L.....	..R.H.....	.....Q....
SEQ ID NO: 739	.....	.....	....G.....	.....
SEQ ID NO: 740	.....	.....	....H.....	.....
SEQ ID NO: 741	.....	.....	....G.....	.....
SEQ ID NO: 742	.....	.....	....H.....	.....
SEQ ID NO: 665	EVQLVQSGAEVKKPGATVKISCKVS	GYTFTDSVMN	WVQQEPGKGLEWMG	WIDPEYGRTDVAEKFQG
	Q S V	L	R G H A	Q

FIG. 11a

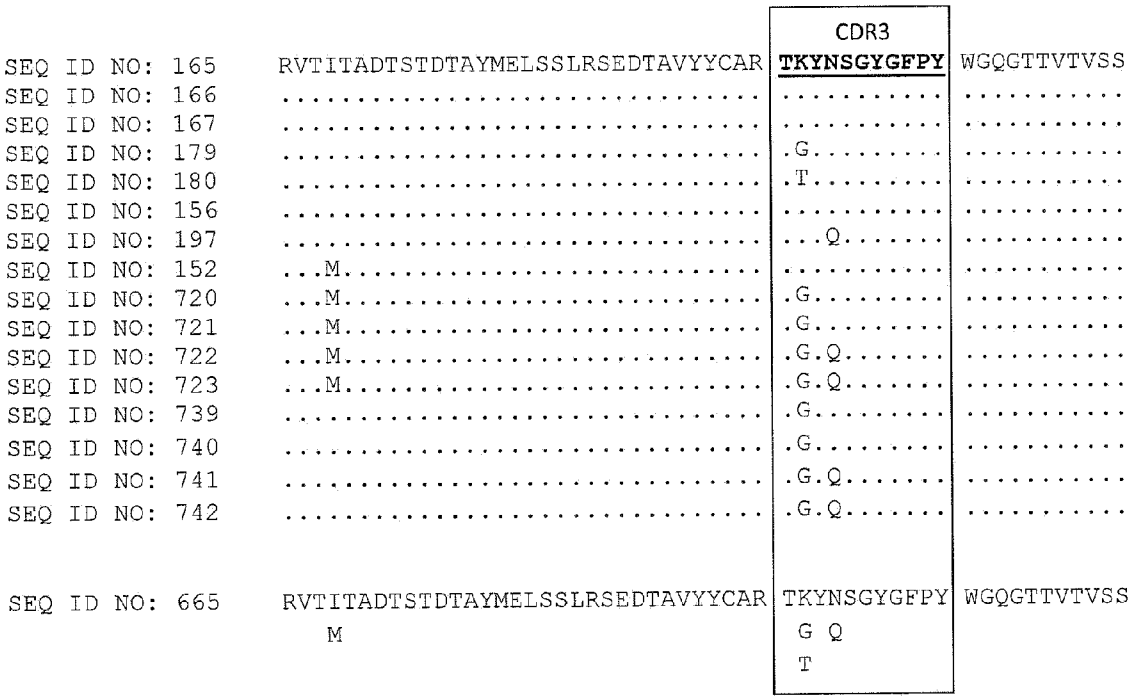


FIG. 11b

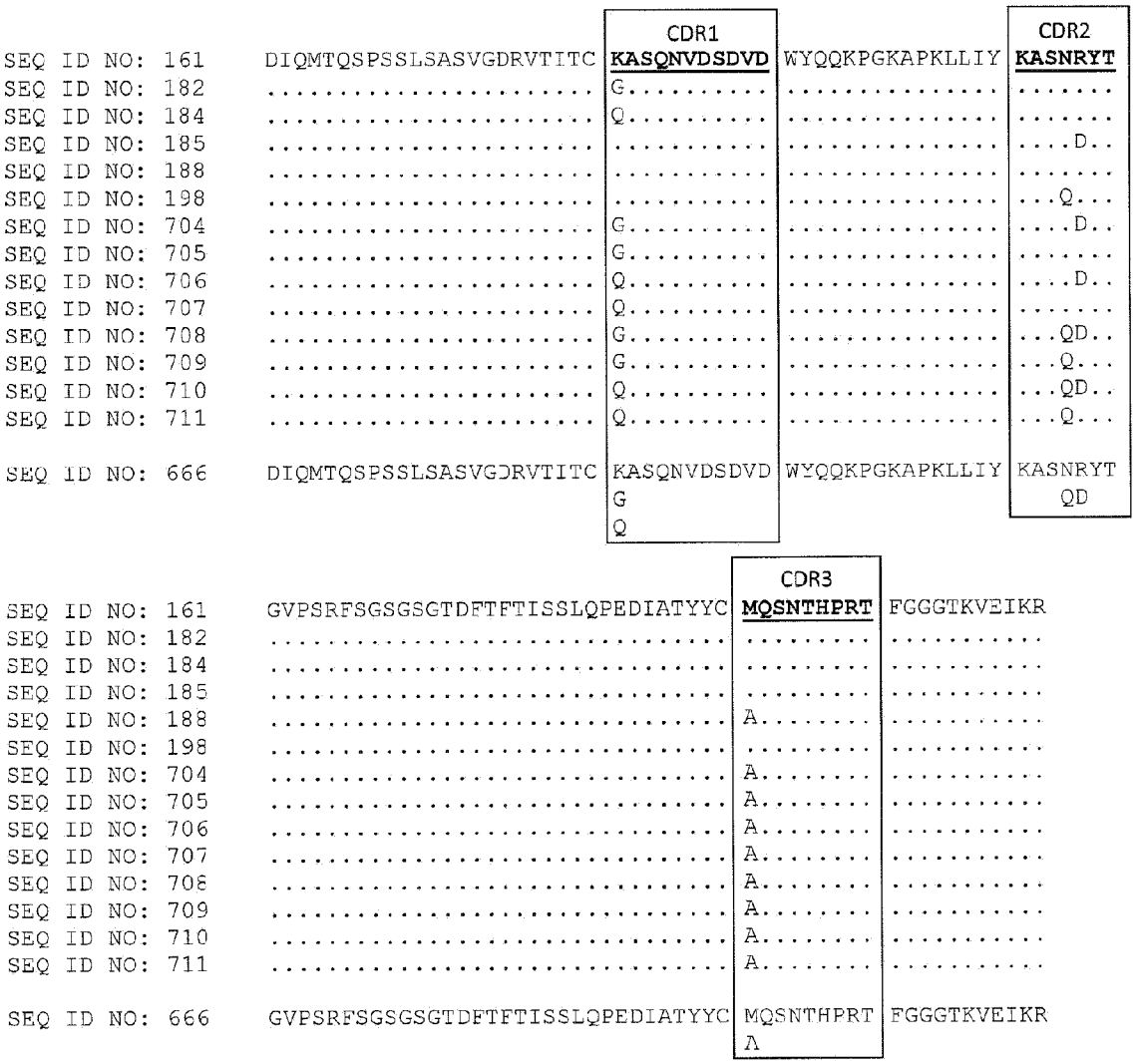


FIG. 12

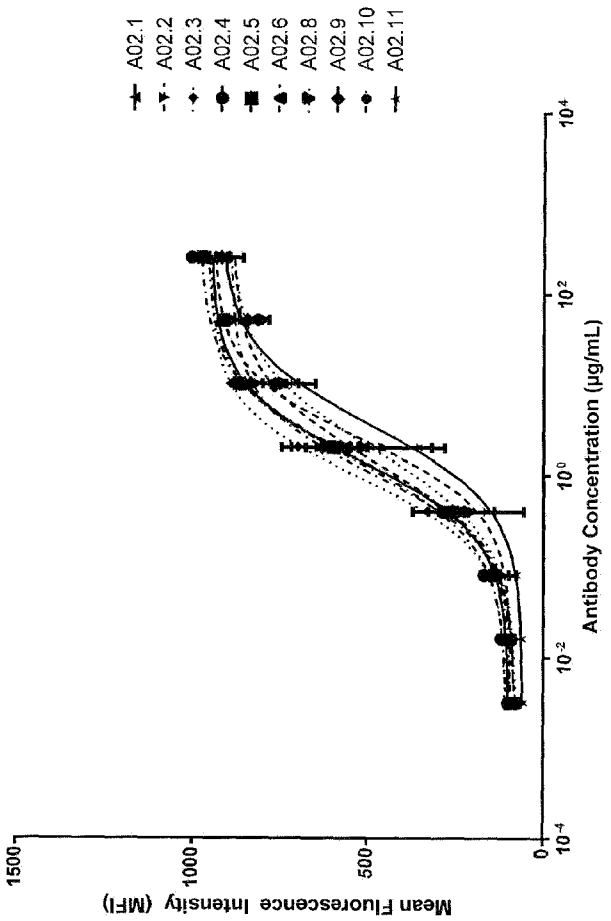

A02.1
A02.2
A02.3
A02.4
A02.5
A02.6
A02.8
A02.9
A02.10
A02.11
Mean Fluorescence Intensity (MFI)
1500
1000
500
0
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
Antibody Concentration (µg/mL)

FIG. 13

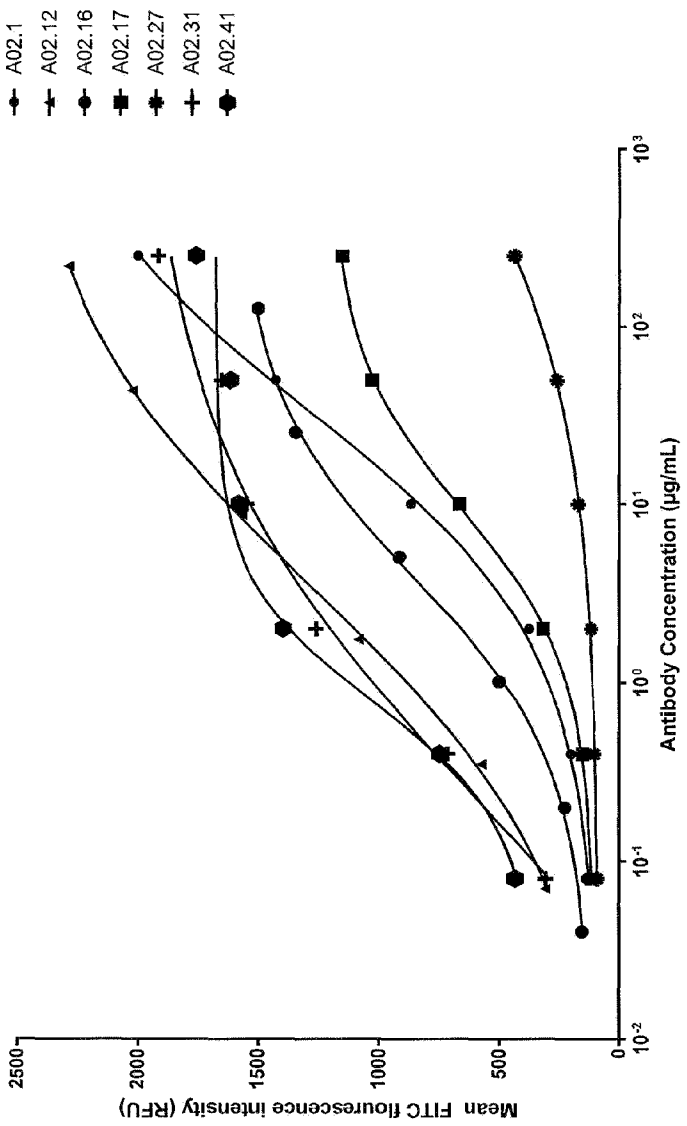

A02.1
A02.12
A02.16
A02.17
A02.27
A02.31
A02.41
Mean FITC fluorescence intensity (RFU)
2500
2000
1500
1000
500
0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Antibody Concentration (µg/mL)

FIG. 14

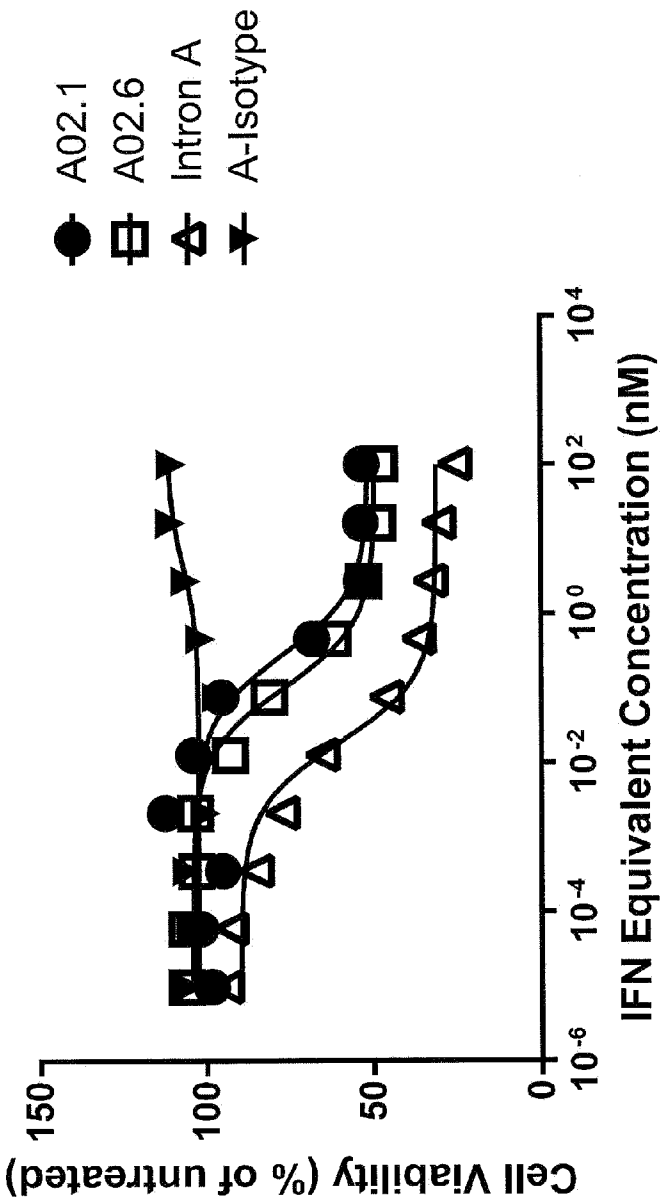

A02.1
A02.6
Intron A
A-Isotype
Cell Viability (% of untreated)
150
100
50
0
10-6
10-4
10-2
100
102
104
IFN Equivalent Concentration (nM)

FIG. 15

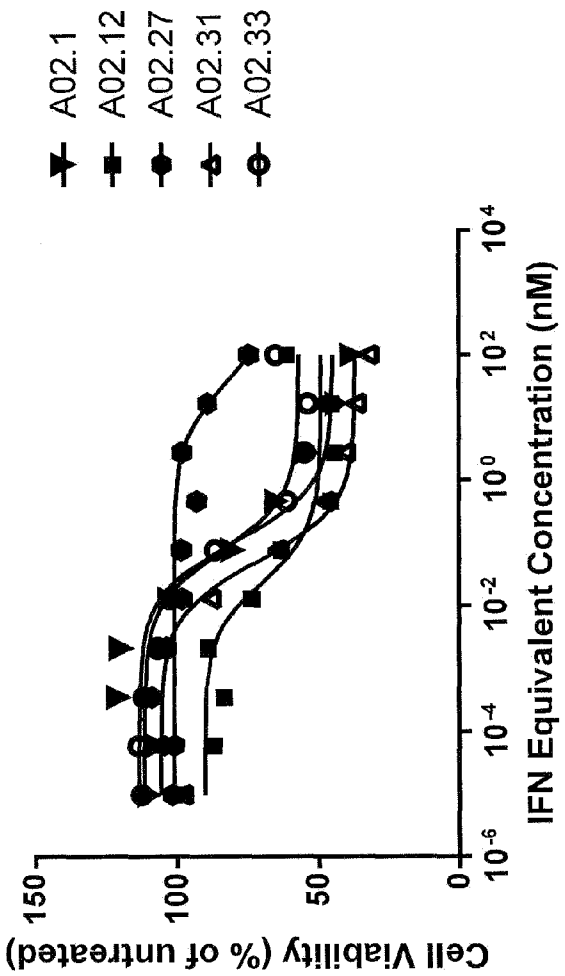

A02.1
A02.12
A02.27
A02.31
A02.33
Cell Viability (% of untreated)
150
100
50
0
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
IFN Equivalent Concentration (nM)

FIG. 16

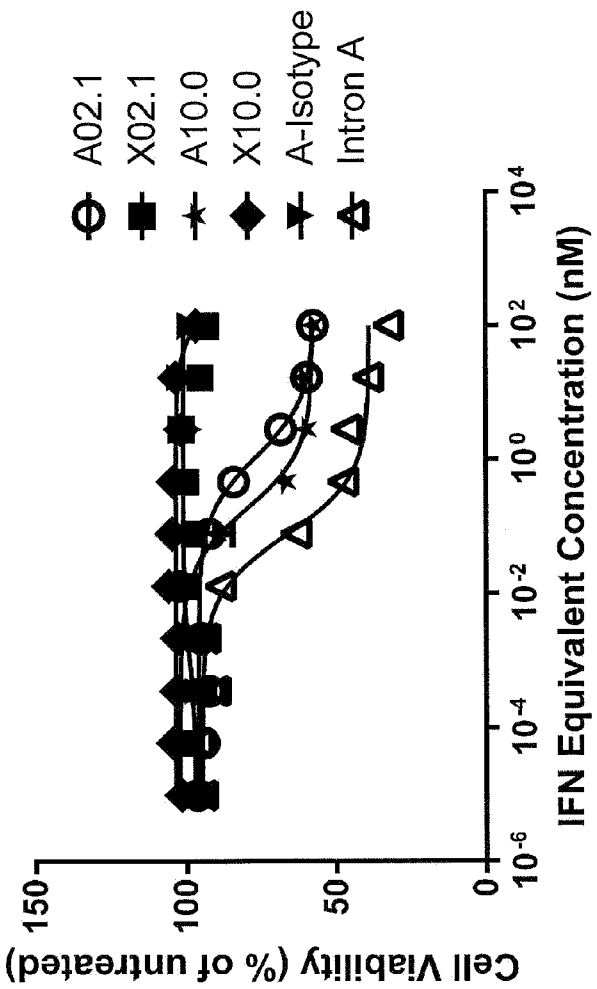

A02.1
X02.1
A10.0
X10.0
A-Isotype
Intron A
Cell Viability (% of untreated)
150
100
50
0
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
IFN Equivalent Concentration (nM)

FIG. 17

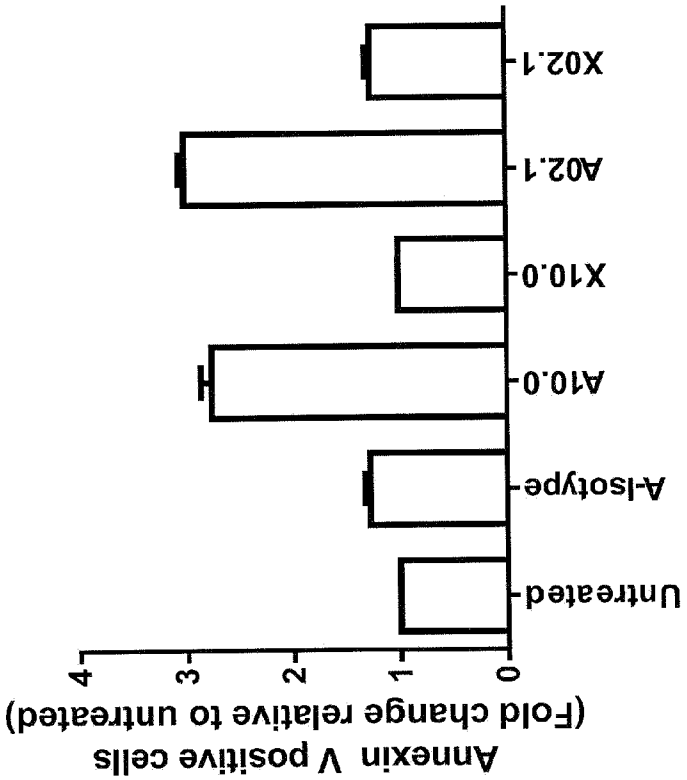

Annexin V positive cells
(Fold change relative to untreated)
0
1
2
3
4
Untreated
A-Isotype
A10.0
X10.0
A02.1
X02.1

FIG. 18

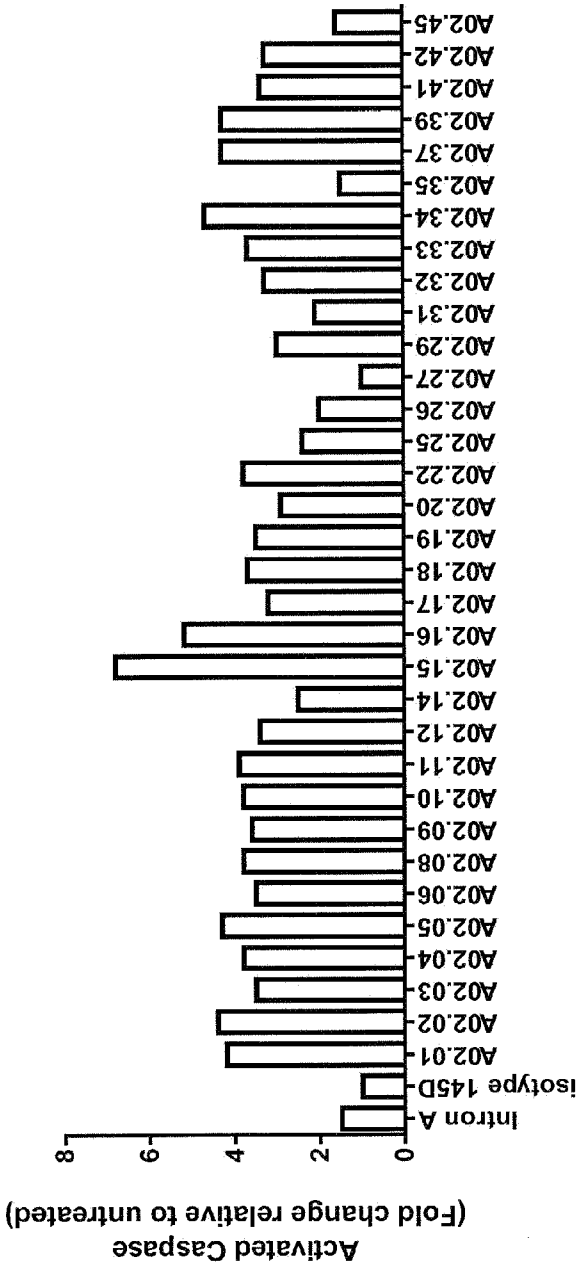
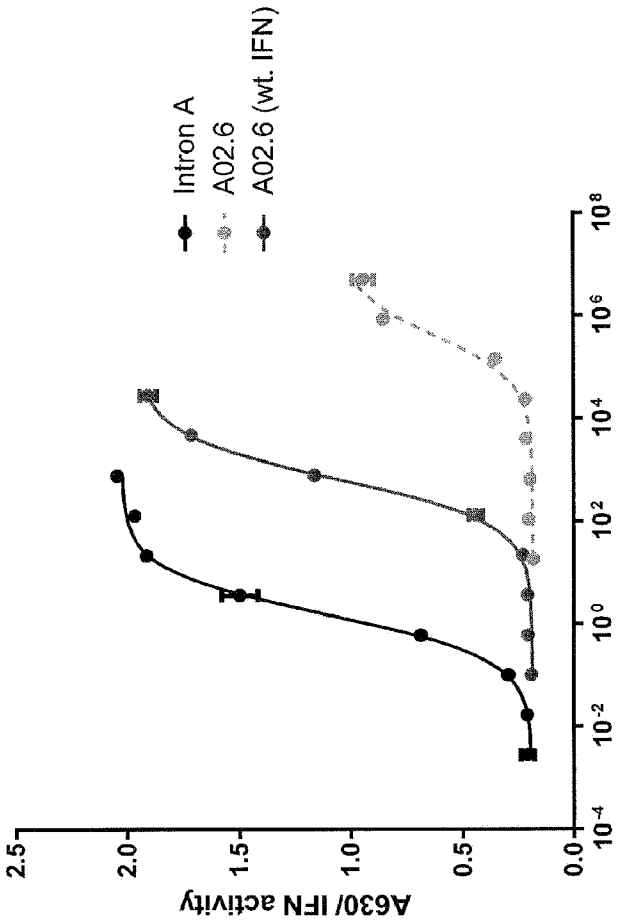

Activated Caspase
(Fold change relative to untreated)
0
2
4
6
8
Intron A
Isotype 145D
A02.01
A02.02
A02.03
A02.04
A02.05
A02.06
A02.08
A02.09
A02.10
A02.11
A02.12
A02.14
A02.15
A02.16
A02.17
A02.18
A02.19
A02.20
A02.22
A02.25
A02.26
A02.27
A02.29
A02.31
A02.32
A02.33
A02.34
A02.35
A02.37
A02.39
A02.41
A02.42
A02.45

FIG. 19



	IFNa2b	A02.6	A02.6 (wt. IFN)
EC50	1.520	371688	666.8

FIG. 20

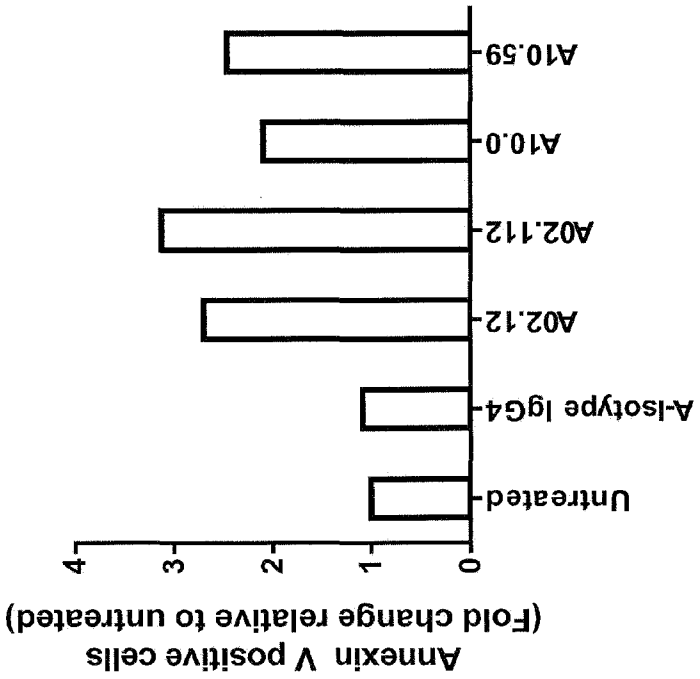

Annexin V positive cells
(Fold change relative to untreated)
0
1
2
3
4
Untreated
A-isotype IgG4
A02.12
A02.112
A10.0
A10.59

FIG. 21

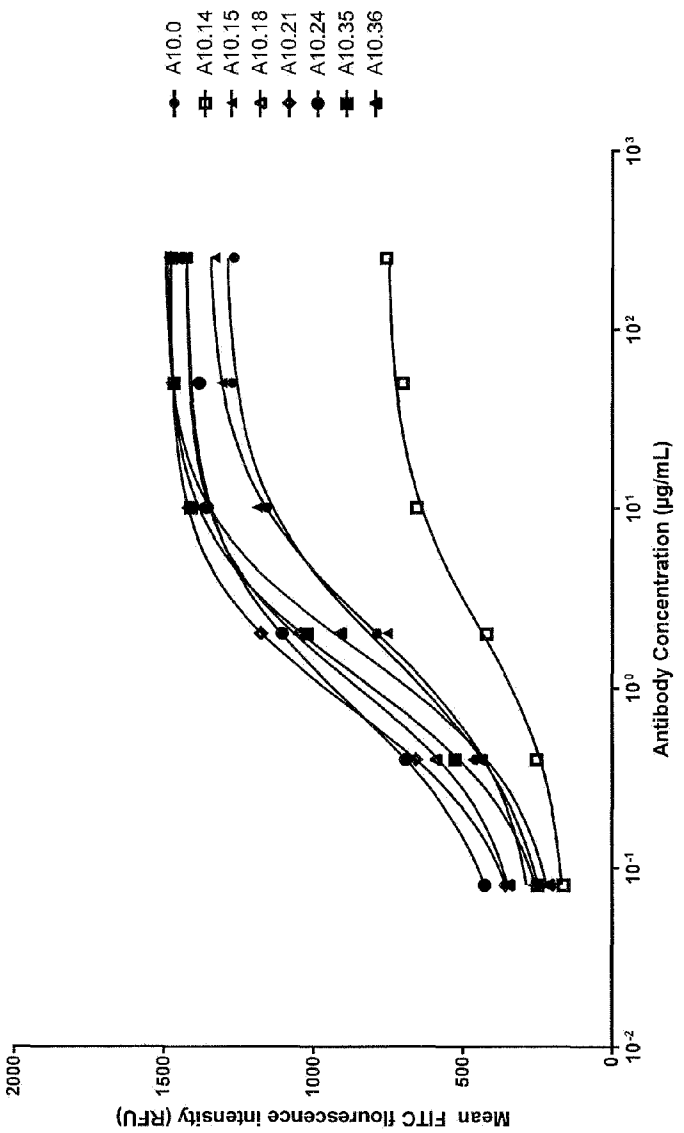

A10.0
A10.14
A10.15
A10.18
A10.21
A10.24
A10.35
A10.36
Mean FITC flourescence intensity (RFU)
2000
1500
1000
500
0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Antibody Concentration (µg/mL)

FIG. 22

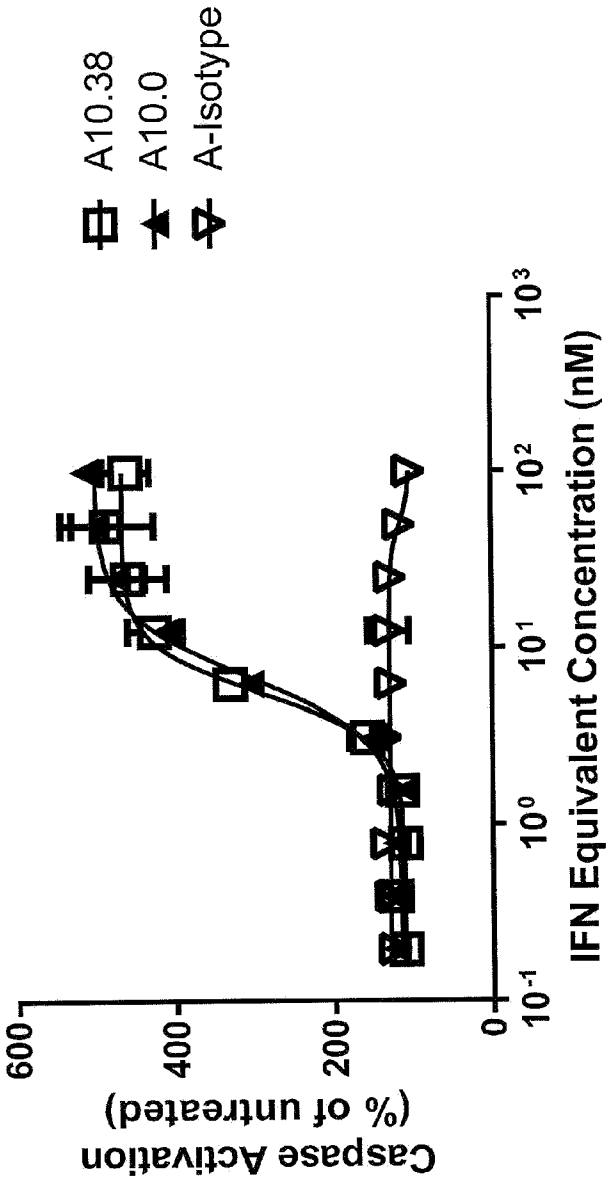

A10.38
A10.0
A-Isotype
Caspase Activation
(% of untreated)
600
400
200
0
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
IFN Equivalent Concentration (nM)

FIG. 23

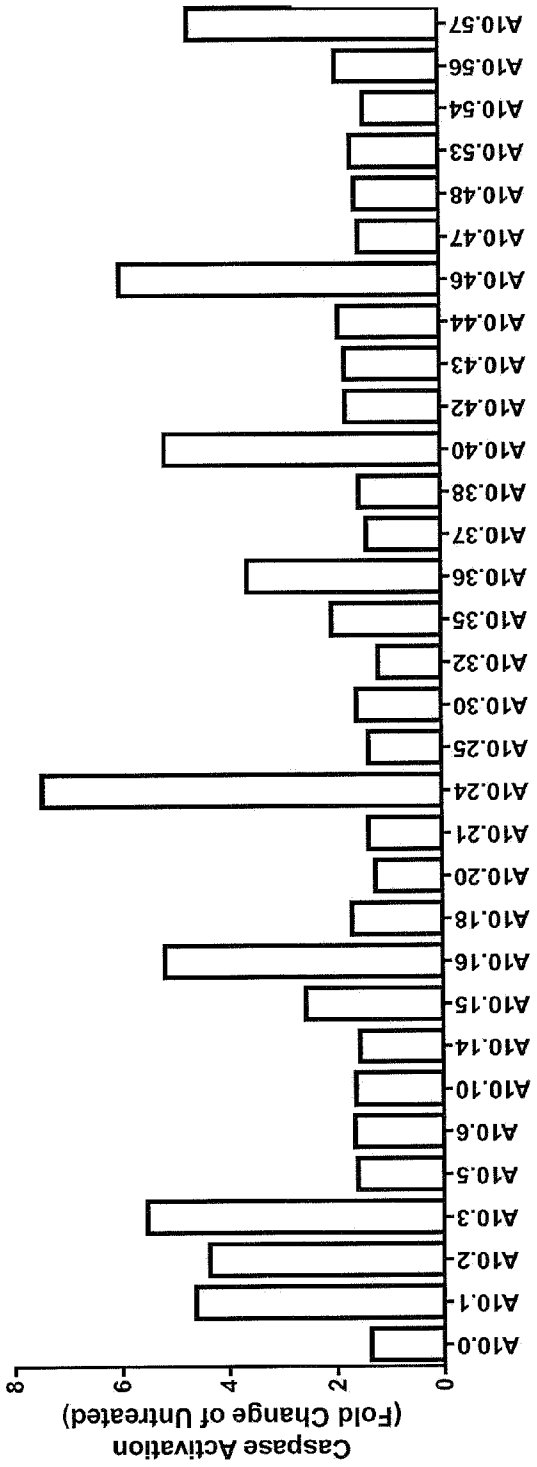

Caspase Activation
(Fold Change of Untreated)
0
2
4
6
8
A10.0
A10.1
A10.2
A10.3
A10.5
A10.6
A10.10
A10.14
A10.15
A10.16
A10.18
A10.20
A10.21
A10.24
A10.25
A10.30
A10.32
A10.35
A10.36
A10.37
A10.38
A10.40
A10.42
A10.43
A10.44
A10.46
A10.47
A10.48
A10.53
A10.54
A10.56
A10.57

FIG. 24

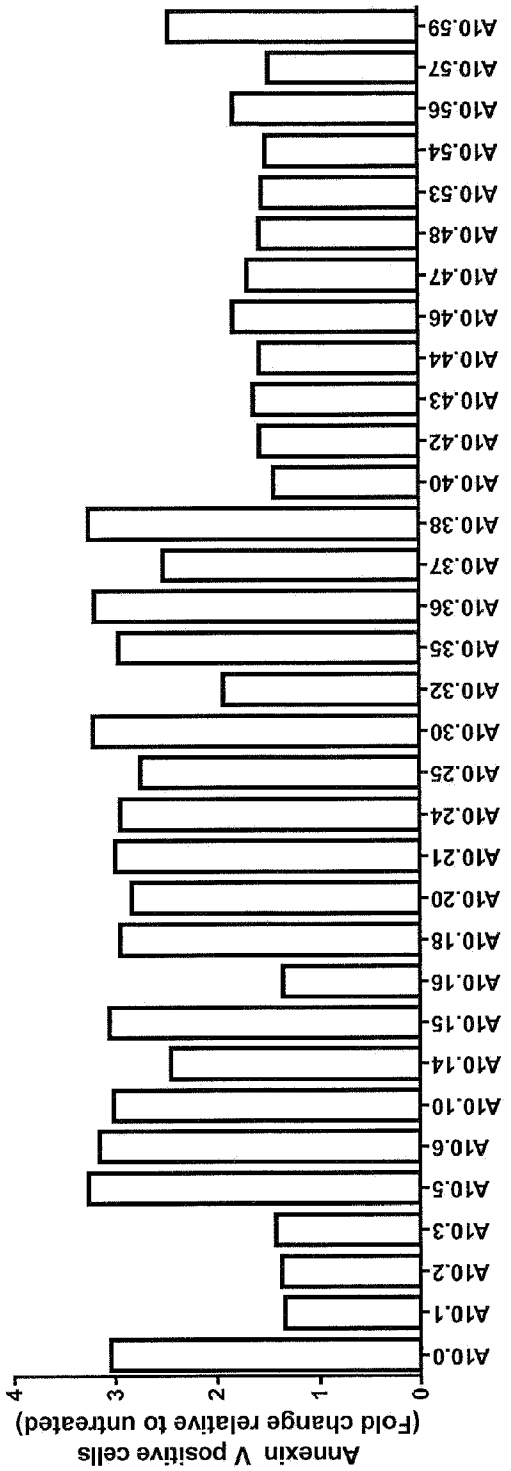

Annexin V positive cells
(Fold change relative to untreated)
0
1
2
3
4
A10.0
A10.1
A10.2
A10.3
A10.5
A10.6
A10.10
A10.14
A10.15
A10.16
A10.18
A10.20
A10.21
A10.24
A10.25
A10.30
A10.32
A10.35
A10.36
A10.37
A10.38
A10.40
A10.42
A10.43
A10.44
A10.46
A10.47
A10.48
A10.53
A10.54
A10.56
A10.57
A10.59

FIG. 25

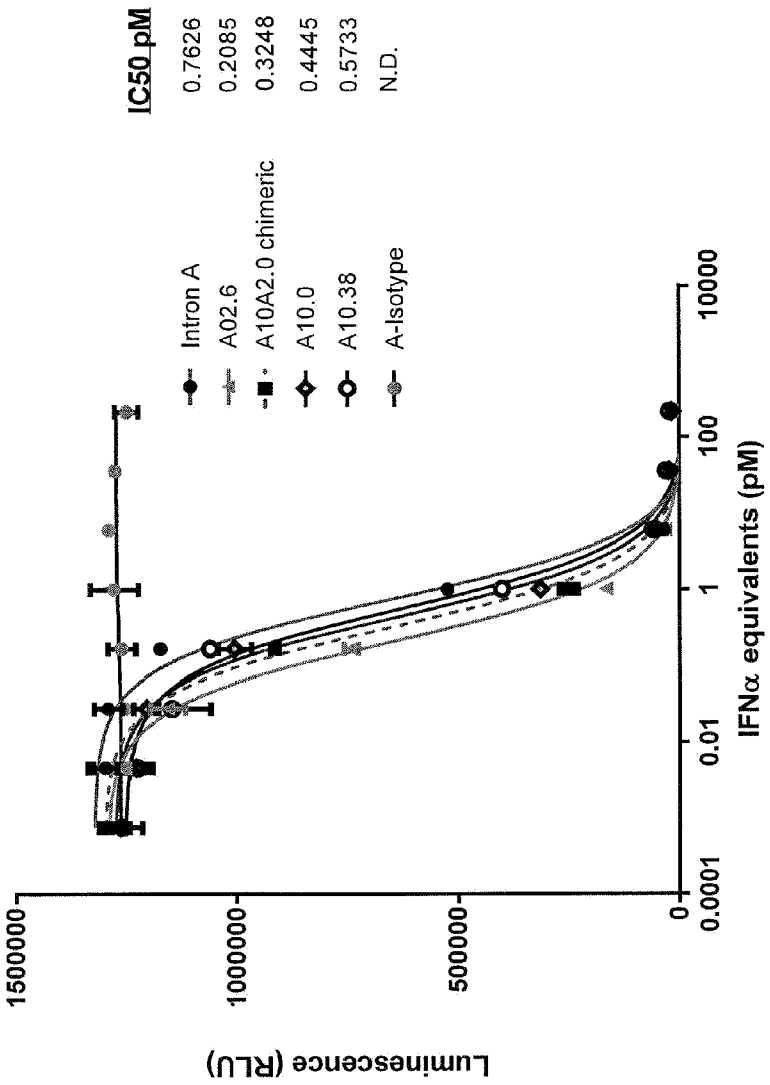

IC50 pM
Intron A 0.7626
A02.6 0.2085
A10A2.0 chimeric 0.3248
A10.0 0.4445
A10.38 0.5733
A-Isotype N.D.
Luminescence (RLU)
1500000
1000000
500000
0
0.0001
0.01
1
100
10000
IFNα equivalents (pM)

FIG. 26

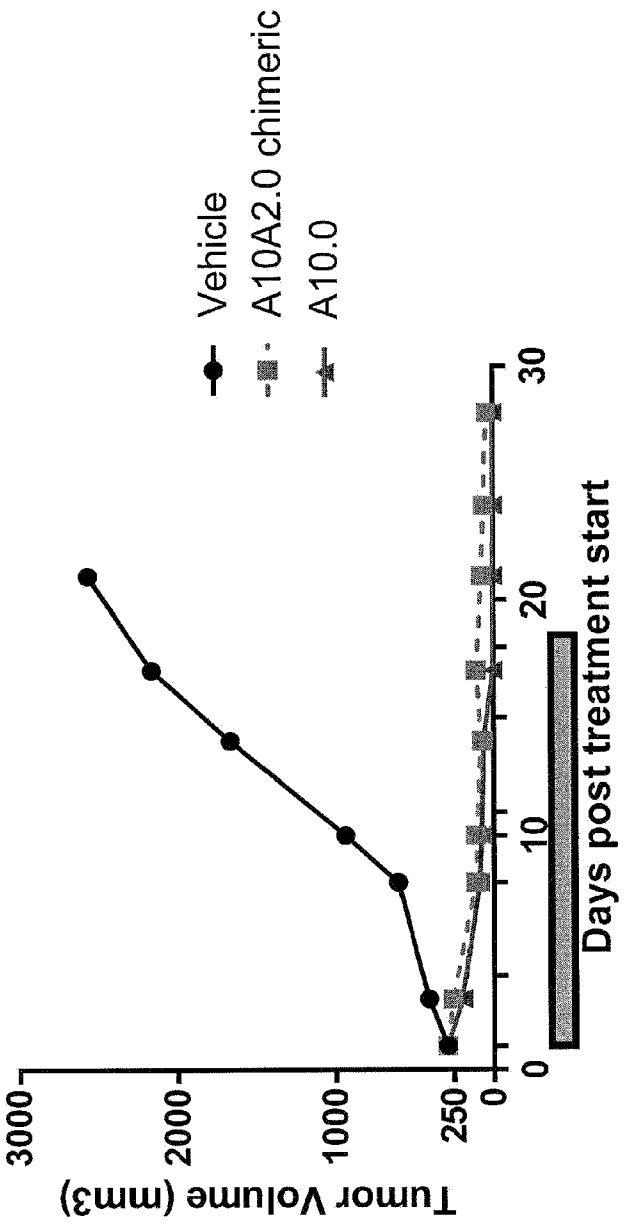

Vehicle
A10A2.0 chimeric
A10.0
Tumor Volume (mm3)
3000
2000
1000
250
0
0
10
20
30
Days post treatment start

FIG. 27

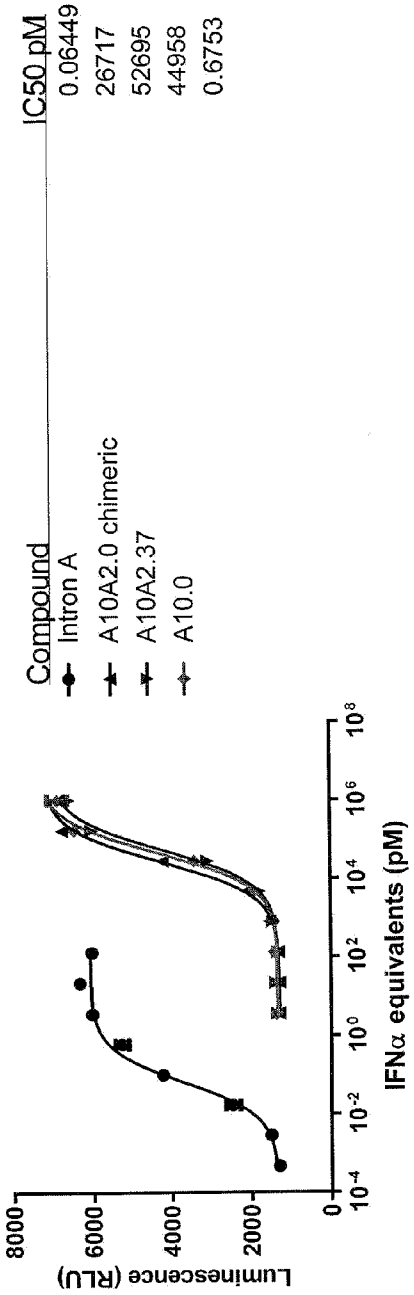
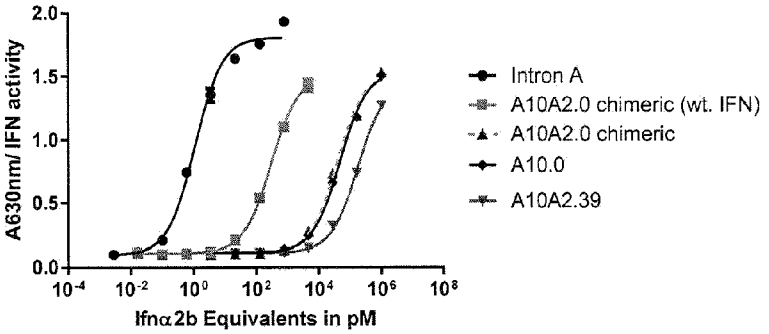
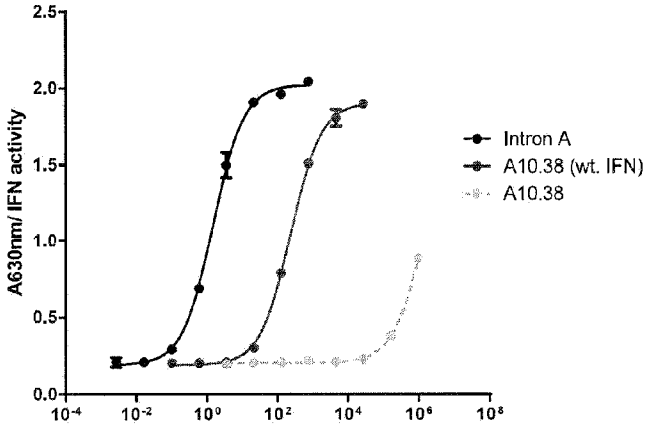

Luminescence (RLU)
8000
6000
4000
2000
0
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
10^{8}
IFNα equivalents (pM)
Compound
IC50 pM
Intron A
0.06449
A10A2.0 chimeric
26717
A10A2.37
52695
A10.0
44958
0.6753

FIG. 28



	Intron A	A10A2.0 chimeric	A10A2.39	A10.0	A10A2.0 chimeric (wt. IFN)
EC50	1.134	39843	191631	48556	295.8



	Intron A	A10.38	A10.38 (wt. IFN)
EC50	1.520	1.431e+006	244.0

FIG. 29

```
                                                   CDR1                                CDR2
SEQ ID NO: 110   EVQLVQSGAEVKKSGASVKVSCKAS GYTFTDYYIH WVRQAPGQGLEWMG WINPNNGGVTFAQKFQG
SEQ ID NO: 728   ......................... .......... ....P......... .................
SEQ ID NO: 729   ......................... .......... ....P......... .................
SEQ ID NO: 730   ......................... .......... ....P......... ........T........
SEQ ID NO: 731   ......................... .......... ....P......... .................

SEQ ID NO: 736   EVQLVQSGAEVKKSGASVKVSCKAS GYTFTDYYIH WVRQAPGQGLEWMG WINPNNGGVTFAQKFQG
                                                          P                     T

                                                  CDR3
SEQ ID NO: 110   RVTMTRDTSISTAYMDLSSLRSDDTAVYFCAR DIRMSGWLAPFDY WGQGTLVTVSS
SEQ ID NO: 728   ................................ ............. ...........
SEQ ID NO: 729   ............................Y... ............. ...........
SEQ ID NO: 730   ................................ ............. ...........
SEQ ID NO: 731   ................................ ...L......... ...........

SEQ ID NO: 736   RVTMTRDTSISTAYMDLSSLRSDDTAVYFCAR DIRMSGWLAPFDY WGQGTLVTVSS
                                             Y        L
```

FIG. 30

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.